

A CLINICAL INVESTIGATION OF THE CORRELATION OF BLACK STAINS WITH ORAL CAVITY PH AND DENTAL CARIES PRESENCE IN CHILDREN: A CROSS-SECTIONAL STUDY

Maguy Sfeir¹ / Nada Chedid²

Introduction: Black stains (BS) on teeth often lead to dental visits in children due to their esthetic impact. Beyond cosmetic concerns, black stains may play a role in oral health, potentially suggesting a lower risk of dental caries through the regulation of oral microbiota and pH levels. Understanding the association of black stains with oral pH and caries presence can offer insights into protective and preventive strategies in pediatric dentistry.

Objectives: the purpose of this study is to assess the correlation between black stains, oral cavity pH, and the presence of caries in a sample of 6-to 8- year-old Lebanese children.

Methods: In this clinical comparative study, 80 healthy children aged from 6 to 8 years were selected from Lebanese schools, the Department of Pediatric Dentistry at the Saint Joseph University Faculty of Dental Medicine in Beirut, and a private practice in Rayfoun (Mount Lebanon). Participants were classified into two groups: 40 children with and 40 without black stains on their teeth. Data including the presence or absence of black stains on teeth surfaces, oral pH, age, gender, and carious teeth number were registered for each participant. Clinical examination for black stain presence and caries detection was conducted through visual and tactile inspection without radiographs. Black stain were recorded as present or absent and carious teeth count was scored. Salivary samples were obtained following standard protocols, with at least a 30-minute interval away from eating, drinking, or tooth brushing. Oral pH was determined by placing salivary samples on litmus paper (TRIXES® pH Test Paper Book); this test was performed twice per subject and obtained values were added then divided by 2, and the mean value obtained was recorded as the subject's salivary pH.

Results: Children with black stains on teeth surfaces had a higher oral cavity pH and fewer dental carious teeth compared to those without black stains. No meaningful differences in age or gender were detected between groups.

Conclusions: Black stains' association with a higher oral cavity pH and a lower number of carious teeth may imply a potential protective role in children's oral health against the development of caries.

Keywords: Bacteria, Buffer Solutions, Carious Teeth, Dental Caries, Hydrogen-Ion Concentration, Pigmentation; Saliva, Tooth Discoloration.

Corresponding author:

Dr Maguy Sfeir, e-mail: sfeirmaguy@gmail.com

Conflicts of interest:

The authors declare no conflicts of interest.

1. Resident, Pediatric Dentistry Department, Faculty of Dental Medicine, Saint Joseph University of Beirut.

2. Professor, Pediatric Dentistry Department, Faculty of Dental Medicine, Saint Joseph University of Beirut Arabia.

UNE ETUDE CLINIQUE SUR LA CORRELATION ENTRE LES TACHES NOIRES, LE PH DE LA CAVITÉ BUCCALE ET LA PRESENCE DE CARIES DENTAIRES CHEZ LES ENFANTS: UNE ÉTUDE TRANSVERSALE

Introduction: Les taches noires (TN) sur les dents conduisent souvent à des visites chez le dentiste chez les enfants en raison de leur impact esthétique. Au-delà des préoccupations cosmétiques, les taches noires peuvent jouer un rôle dans la santé bucco-dentaire, pouvant suggérer un risque moindre de caries dentaires par la régulation du microbiote oral et du pH. Comprendre l'association entre les taches noires, le pH buccal et la présence de caries peut offrir des aperçus sur des stratégies protectrices et préventives en odontologie pédiatrique.

Objectifs: Le but de cette étude est d'évaluer la corrélation entre les taches noires, le pH de la cavité buccale et la présence de caries dans un échantillon d'enfants libanais âgés de 6 à 8 ans.

Méthodes: Dans cette étude clinique comparative, 80 enfants en bonne santé âgés de 6 à 8 ans ont été sélectionnés dans des écoles libanaises, au Département d'Odontologie Pédiatrique de la Faculté de Médecine Dentaire de l'Université Saint-Joseph à Beyrouth, ainsi que dans un cabinet privé à Rayfoun (Mont-Liban). Les participants ont été répartis en deux groupes : 40 enfants avec des taches noires et 40 sans taches noires sur leurs dents. Les données recueillies comprenaient la présence ou l'absence de taches noires sur les surfaces dentaires, le pH buccal, l'âge, le sexe et le nombre de dents cariées. Un examen clinique pour détecter la présence de taches noires et de caries dentaires a été réalisé par inspection visuelle et tactile, sans radiographies. Les taches noires ont été enregistrées comme présentes ou absentes, et le comptage des dents cariées a été noté. Des échantillons salivaires ont été prélevés selon des protocoles standard, avec un intervalle d'au moins 30 minutes après manger, boire ou se brosser les dents. Le pH buccal a été déterminé en plaçant les échantillons salivaires sur du papier de tournesol (TRIXES® pH Test Paper Book); ce test a été effectué deux fois par sujet, les valeurs obtenues ont été additionnées puis divisées par 2, et la valeur moyenne ainsi calculée a été enregistrée comme le pH salivaire du sujet.

Résultats: Les enfants ayant des taches noires sur les surfaces dentaires présentaient un pH buccal plus élevé et moins de dents cariées par rapport à ceux sans taches noires. Aucune différence significative en âge ou en sexe n'a été détectée entre les groupes.

Conclusions: L'association entre les taches noires, un pH buccal plus élevé, et un nombre réduit de dents cariées pourrait impliquer un rôle protecteur potentiel dans la santé bucco-dentaire des enfants contre le développement des caries.

Mots-clés: Bactéries, Solutions tampon, Dents cariées, Carie dentaire, Concentration en ions hydrogène, Pigmentation, Salive, Coloration des dents.

Introduction

Dental discoloration is a common challenge in clinical practice, particularly among young patients [1]. Recent studies report an increasing prevalence of chromatic alterations in children's anterior teeth, posing significant esthetic concerns [2]. Such discolorations can have psychological and social impacts, including bullying and reduced resilience during childhood and adolescence [3]. Therefore, early identification of the underlying etiology and the implementation of preventive strategies are essential to minimize recurrence after treatment [2].

Black stain (BS) is a form of extrinsic dental discoloration frequently observed in children, characterized by black deposits appearing as dots or lines parallel to the gingival margin, predominantly on the cervical third of teeth, though other surfaces can be affected as well [2, 4]. It is considered a significant aesthetic concern, particularly when visible on vestibular surfaces [2]. The formation of black stains is linked to chromogenic bacteria within the oral biofilm, notably gram-positive rods such as *Actinomyces naeslundii* [4, 5] and gram-negative rods like *Prevotella melaninogenica* [6]. The formation of black stains (BS) occurs on the tooth surface or the acquired pellicle, a thin, acellular, organic layer that forms after exposure to saliva and plays essential roles in remineralization and hydration [7], by promoting mineral deposition, buffering pH changes, facilitating fluoride uptake for fluorapatite formation, and preserving enamel moisture [5, 7-8]. Transmission of BS-associated bacteria can occur vertically, from mother to child, or horizontally, between children through saliva-contaminated objects, emphasizing the need for preventive measures such as avoiding the sharing of utensils and direct mouth-to-mouth contact [8-10].

In addition to bacterial factors, other contributors to BS formation include iron supplementation, dietary habits, oral hygiene status and fluoride exposure. Iron from supplements can accumulate in saliva and, through bacterial metabolism, react with hydrogen sulfide to form ferric sulfide, contributing to BS development [6-8]. Dietary factors, such as the consumption of pigmented drinks like tea and coffee, can promote staining, although the addition of milk significantly reduces tea's staining potential due to casein content [11, 12]. Poor oral hygiene leads to plaque accumulation, which can darken over time and must be distinguished from black stains to ensure appropriate management [7, 8]. Furthermore, dental fluorosis, resulting from excessive fluoride intake during enamel formation may cause intrinsic discolorations ranging from white to dark brown spots [8, 13]. Accurate differential diagnosis between BS, iron stains, and fluorosis is critical, as they differ in origin, appearance, and treatment approaches [2-4, 8, 11, 12, 14]. Although BS often regresses with age, early dental intervention using ultrasonic scaling, polishing, or lactoferrin-based treatments, such as Forhans Gengi-For tablets, can significantly improve aesthetic outcomes and children's psychological well-being [8, 15].

Dental caries is among the most common chronic diseases in childhood, with a notable rise in incidence during the mixed dentition phase due to the eruption of permanent incisors and molars, the latter being difficult to clean [16-18]. This period presents a crucial opportunity for pediatric dentists to implement preventive measures and educate families to reduce caries presence [16, 17, 19]. An important clinical challenge during this period is the differentiation between arrested caries and black stains in children. Arrested caries represents a halted demineralization

process, typically characterized by dark brown to black lesions that are hard, smooth, and shiny due to surface remineralization [14, 20, 16]. In contrast, black stains are extrinsic discolorations caused by pigmented bacterial activity or external factors like food, beverages, or iron supplements, without any underlying loss of tooth structure [18, 16]. Although both conditions may appear similar visually, their etiology, clinical implications, and management strategies differ significantly. Therefore, a thorough clinical examination, patient history, and when necessary, radiographic evaluation are essential to accurately distinguish between these two conditions and ensure the appropriate treatment approach [2, 3, 6, 14, 16].

The pH of the oral cavity plays a critical role in maintaining dental health, with normal values ranging from 6.2 to 7.6, and an average of around 7.4 under typical conditions [21, 22]. Oral cavity pH can fluctuate based on factors such as dietary intake, salivary flow, and bacterial activity, often dropping below 5.5 after the consumption of fermentable carbohydrates, which can initiate enamel demineralization [21, 22]. Saliva's buffering capacity, primarily mediated by bicarbonate ions, acts to neutralize acids and maintain a stable pH, protecting against caries development [22]. Studies have demonstrated a significant association between higher oral cavity pH values and the presence of black stains in children, suggesting that a more alkaline environment may contribute to a lower prevalence of dental caries in these individuals [2, 3, 17]. The present study aims to explore the relationship between black stains, oral pH, and dental caries in children aged 6 to 8 years. The null hypothesis (H0) purports no correlation between oral cavity pH, black stains, and the presence of dental caries.

Materials and methods

This clinical comparative study was conducted in accordance with ethical research guidelines and was approved by the Ethics Committee of Saint Joseph University of Beirut (reference number USJ-2024-154). Prior to participation, informed consent was obtained through a detailed parental consent form, which was thoroughly reviewed and signed by the parents or legal guardians of each child.

To determine the minimum required sample size, a power analysis for the comparison of two independent means was performed using G*Power software version 3.1.9.7 (Heinrich Heine, Universität Düsseldorf, Düsseldorf, Germany). The analysis was based on a power of 95%, an alpha level of 0.05, and an effect size of 1.31 derived from Mousa et al's study. (2022). These parameters yielded a minimum required total sample size of 34 participants, with 17 subjects in each group assuming an allocation ratio of 1:1. However, to increase the robustness of the study and allow for greater statistical validity, a total of 80 children aged 6 to 8 years were recruited—40 with black stains and 40 without.

Participants were selected from Lebanese schools, the Department of Pediatric Dentistry at Saint Joseph University of Beirut's Faculty of Dental Medicine, and a private pediatric dental practice in Rayfoun, Mount Lebanon. Inclusion criteria required children to be systemically healthy, within the 6–8-year age range, and capable of providing saliva samples. Exclusion criteria included children with systemic health conditions or those outside the specified age range.

For each participant the following factors were recorded:

- Presence or absence of black stains, the presence of black stains on at least one tooth surface qualifying the subject for inclusion in the black stain group
- Oral pH

- Age
- Gender
- Number of caries.

After obtaining written parental consent, oral saliva samples were collected from healthy children aged between 6 and 8 years, with no current diseases, allergies, daily medications, heart problems, chronic diseases, or infections. The collection was carried out by the same researcher under standard conditions to ensure both accuracy and hygiene. The process involved preparing the necessary materials, including gloves, saliva collection tubes, and a clean table. To avoid contamination, children were instructed to refrain from eating, drinking, or brushing their teeth for at least 30 minutes before the sample collection. A screening procedure was performed to detect dental caries in each participant using a mirror and probe, employing non-invasive methods, with examinations carried out while each child was seated in a regular chair under natural light, occasionally supplemented by flashlight when necessary. Occlusal and smooth surfaces were assessed for signs of color changes, translucency, texture alterations, white spot lesions, cavitations, and discolorations, which are primary indicators of caries and caries presence. In this study, caries was recorded as carious teeth number per subject, regardless of the type of tooth (primary or permanent), rather than the number of affected surfaces.

For each patient, the pH test was performed twice using the TRIKES® Litmus pH 1 to 14 Test Paper Book (80 strips, Trikes®, South Essex Coast, England) (Figure 1) and a mean pH value was calculated by adding both results and dividing them by 2, as a single measurement was not considered sufficient due to natural variations in salivary pH within the oral cavity. A thirty-minute interval was maintained between the two measurements for each participant to ensure greater accuracy and reliability of the recorded pH value.

During sample collection, care was taken to avoid contact between the pH paper detector and any obstacles that could cause contamination. Each sample was labeled with the participant's name, date, and relevant information. The samples were then stored on a clean surface and exposed to normal air for approximately 30 minutes. After this interval, the pH was measured using the Trikes® pH chart, and the mean pH value was calculated and recorded alongside the participant's information. Figure 2 illustrates all the steps of the procedure.



Figure 1. TRIKES® Litmus pH 1 to 14 Test Paper Book (80 strips, Trikes, South Essex Coast, England) (copyright: amazon.fr)

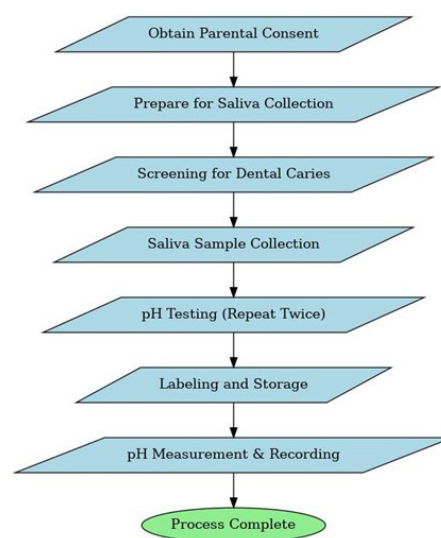


Figure 2. Flowchart showing a summary of the steps conducted during the specimen collection

Statistical analysis

For statistical analysis, univariate analysis was performed to describe quantitative continuous variables using means, standard deviations, and medians, while categorical variables were expressed as frequencies and percentages. Normality of the data within groups was assessed using the Shapiro-Wilk test, and equal variances between groups were tested with Levene's test. To compare the two groups, independent samples t-tests were applied for normally distributed data, with the Levene test determining whether to use the standard or adjusted t-test. If normality was not assumed, the Mann-Whitney U test was employed as a non-parametric alternative.

Results

A total of 80 children, evenly divided between Group A (without black stains) and Group B (with black stains), participated in the study. The sample included 40 boys (50%) and 40 girls (50%), ensuring a balanced gender distribution (table 1). The mean age of the participants was 6.89 years (SD = 0.81), with a median of 7 years. Regarding socioeconomic status, although not directly quantified in table 1, all children were recruited from similar school environments, suggesting a relatively homogeneous socio-economic background.

Descriptive analysis revealed that the mean oral pH of the study population was 7.43, with values ranging from 5.0 to 10.0, indicating a predominantly neutral to slightly alkaline oral environment (Figure 3). The Mann-Whitney U test was performed to compare pH levels between children with black stains (Group B) and those without (Group A). The results showed a significant difference between the two groups, with Group B exhibiting a higher mean rank (59.58) compared to Group A (21.43), suggesting that

Table 1. Demographic and clinical characteristics of the study population (N = 80), including age, gender, salivary pH, and caries prevalence by group
Total sample (n=80)

Characteristic	Frequency	Percentage (%)	Mean (SD)	Median (IQR)	Shapiro-Wilk
Gender					
Boys	40	50			
Girls	40	50			
Age	N/A	N/A	6.89 (0.811)	7.00 (2)	
pH					
Group A (n=40)			6.33 (0.79)	6 (1.0)	0.04*
Group B (n=40)			8.51 (1)	8.50 (1.0)	0.10**
Tooth decay					
Group A (n=40)			3.60 (1.64)	4.00 (2)	0.06**
Group B (n=40)			1.48 (0.90)	1.00 (1)	0.001*
pH in the total sample			7.42 (1.32)	7.50 (2.5)	0.004*
Number of decayed teeth in the total sample			2.54 (1.69)	2.00 (3)	<0.001*

N/A: not applicable; * significant if $p < 0.05$; ** not significant

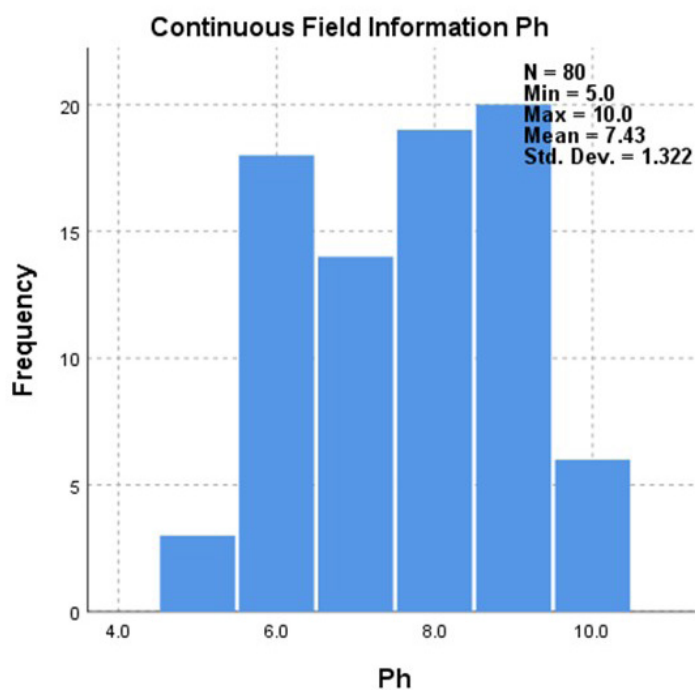


Figure 3. Histogram showing the frequency distribution of pH values among the study participants, indicating minimum, maximum, mean, and standard deviation

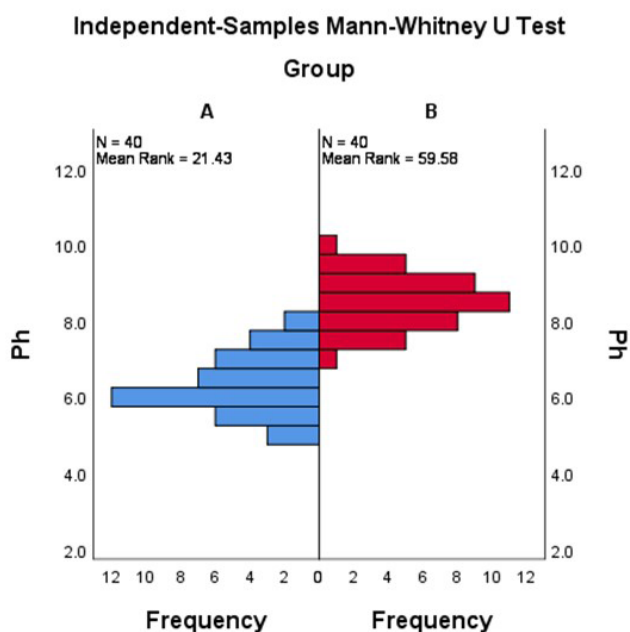


Figure 4. Histogram showing the frequency distribution of pH values among the study participants, indicating minimum, maximum, mean, and standard deviation

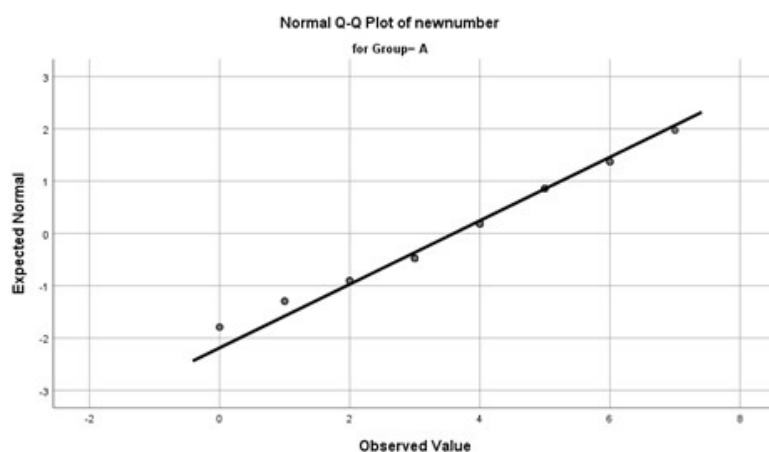


Figure 5. Normal Q-Q plot assessing the normality of data distribution in group A

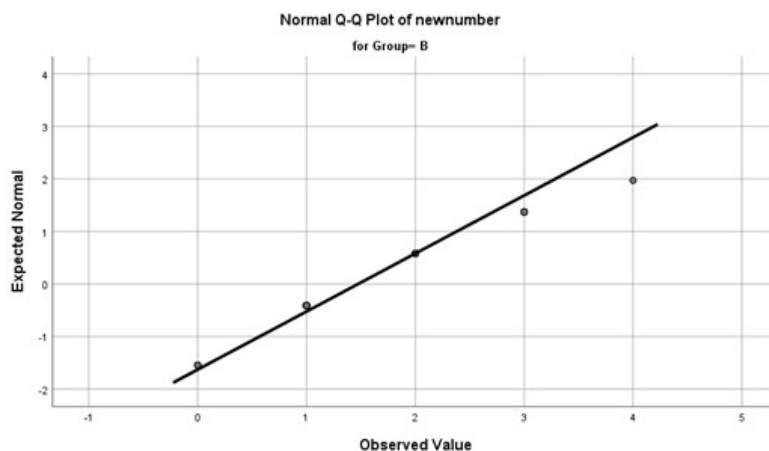


Figure 6. Normal Q-Q plot assessing the normality of data distribution in group B

children with black stains have a more alkaline oral pH (Figure 4).

Additionally, the normal Q-Q plots for both groups (Figures 5 and 6) revealed deviations from the expected normal distribution, indicating variability in pH levels, particularly in Group A. Pearson's correlation analysis revealed a strong, statistically significant negative correlation between oral pH and the number of carious teeth ($r = -0.746$, $p < 0.001$) as shown in Table 1.

Correlation between pH and the number of carious teeth was analyzed using Pearson's correlation coefficient. The results as seen in tables 2 and 3 revealed a strong, statistically significant negative correlation between pH and the number of caries ($r = -0.746$, $p < 0.001$, $N = 80$). Figure 7 revealed consistent results illustrated through mean values displayed in a box-plot format. This indicates that as the pH level decreases (becomes more acidic), the number of carious teeth increases. Additionally, table 2 showed that among 80 participants, 7.5% had no carious teeth, while the majority presented with at least one. Specifically, 27.5% had one carious tooth, 20.0% had two, and 13.8% had three. The number of carious teeth showed significant higher levels in group A compared to group B (figure 7). The distribution showed a gradual decline in frequency as the number of carious teeth increased, with only 1.3% of children having seven carious teeth. Overall, 93.8% of children had between 0 and 5 carious teeth, highlighting a moderate spread of dental decay within the sample. One case (1.2%) had missing data, which was excluded from the valid analysis (table 2).

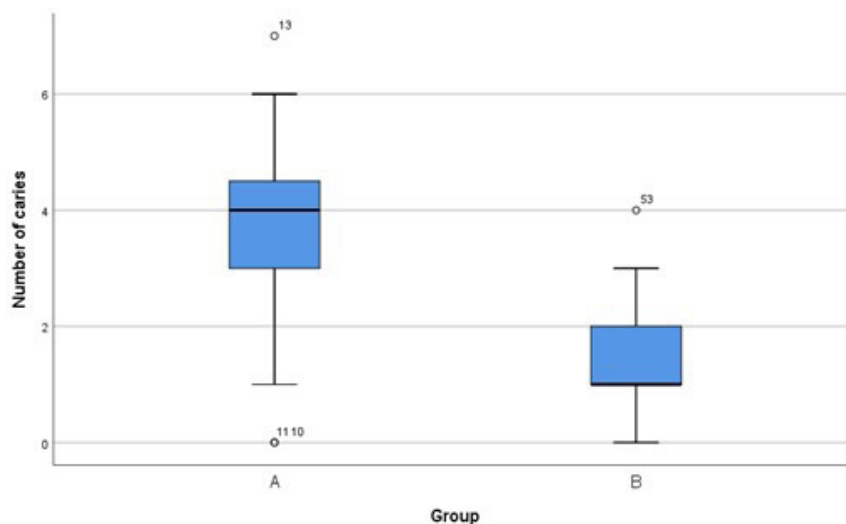


Figure 7.Box-plot expressing the difference in terms of means of the number of carious teeth between both groups

Table 2. Distribution of the number of carious teeth among study participants

Number of carious teeth					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	6	7.4	7.5	7.5
	1	22	27.2	27.5	35.0
	2	16	19.8	20.0	55.0
	3	11	13.6	13.8	68.8
	4	15	18.5	18.8	87.5
	5	5	6.2	6.3	93.8
	6	4	4.9	5.0	98.8
	7	1	1.2	1.3	100.0
	Total	80	98.8	100.0	
Missing	System	1	1.2		
Total		81	100.0		

Table 3. Descriptive statistics for number of carious teeth, salivary pH, and gender in the study population

Descriptive Statistics			
	Mean	Std. Deviation	N
Number of carious teeth	2.54	1.698	80
pH	7.425	1.3219	80
Gender	1.50	0.503	80

Table 4. Pearson correlation analysis between number of carious teeth, salivary pH, and gender in the study population

Correlations				
		Number of caries	pH	Gender
Pearson Correlation	Number of carious teeth	1.000	-.746	-.081
	pH	-.746	1.000	.057
	Gender	-.081	.057	1.000
Sig. (1-tailed)	Number of carious teeth	.	.000	.236
	pH	.000		.307
	Gender	.236	.307	
N	Number of carious teeth	80	80	80
	pH	80	80	80
	Gender	80	80	80

Discussion

The findings of this study confirm that the presence of black stains in children aged 6 to 8 years is associated with a higher salivary pH and a lower prevalence of dental caries, probably due to the reaction of chromogenic bacteria secretions with hydrogen sulfide to form ferric sulfide [6]. Children with black stains exhibited significantly more alkaline oral environments compared to those without, as shown by a higher mean rank in the Mann-Whitney U test. This suggests that children with black stains may have a more stable and alkaline oral pH, potentially making them less susceptible to caries formation, in line with previous literature (Mousa et al., 2022). A strong negative correlation between salivary pH and carious teeth prevalence ($r = -0.746$, $p < 0.001$) highlights the protective role of higher pH against caries development. This indicates that as the pH level decreases (becomes more acidic), the number of carious lesions increases, supporting the theory that lower pH environments are associated with higher caries prevalence. These results align with previous studies by Mutsaddi et al.

and Lihala et al., which reported fewer caries among children with black stains and elevated pH levels. This association may be explained by the presence of less acidogenic bacteria, such as *Actinomyces* spp. [2-5, 23]. In the present study, gender did not significantly influence pH or caries presence, although different results were reported by Wang et al. Study limitations should be considered, however they are not exclusive to the present study, as others have worked under comparable conditions, these limitations include the absence of radiographic evaluation (Mousa et al.) and potential variability in pre-sampling conditions (Bhayat et al.). The 30-minute interval for pH sampling chosen for feasibility due to the screening environment and conditions may also have been insufficient, and the conditions for food intake prior to the measurements may not have always been respected by the subjects, an issue that was difficult to strictly enforce from a practical standpoint. Future research involving larger cohorts, microbiome profiling, and standardized caries detection methods is recommended to confirm and expand upon these findings.

Conclusion

Black stains have become a frequent reason for dental consultations among children, mainly due to growing esthetic concerns. This study confirmed that the presence of black stains is associated with higher salivary pH levels and a lower prevalence of carious teeth in children aged 6 to 8 years. The more alkaline oral environment observed in children with black stains appears to protect against enamel demineralization and caries development. No significant gender differences were found regarding pH or caries presence, suggesting that the protective effect is independent of gender. These findings highlight black stains as a potential natural indicator of lower caries presence and emphasize the need for future longitudinal studies to explore the microbial and functional characteristics of black stain biofilms.

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