



Evaluation of the Etiological Factors of Black Tooth Stain in Children

● Gülşen İlgen¹, ● Dilşah Çoğulu², ● Ege Uçan³, ● Ataç Uzel³

¹Erzurum Oral Health Center, Erzurum, Turkey

²Ege University Faculty of Dentistry, Department of Pedodontics, İzmir, Turkey

³Ege University Faculty of Science, Department of Biology, Division of Basic and Industrial Microbiology Section, İzmir, Turkey

ABSTRACT

Aim: Tooth discoloration is a common clinical finding which is considered primarily as an aesthetic problem. Black stain (BS) is a specific type of extrinsic tooth discoloration mostly seen in children, but also in adults and it is not dependent on gender. The present study aimed to investigate the relationships between the presence of BS and dental caries incidence, dental plaque scores and to examine the colonization of *Streptococcus mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. in dental plaque samples with or without BS. The socioeconomic status of the family, the oral hygiene and dietary habits of the children, and the medical and dental history of the children were also compared between the two groups.

Materials and Methods: A total of 1000 children aged 3-12 years were evaluated to take part in this study. From this group, those children with BS (n=44) were selected as the study group. With the same number as the study group, and with a same age and gender profile, 44 children without BS were selected as a control group. Dental examinations including the presence of BS, dental caries incidence and dental plaque scores were performed by the same investigator. Structured questionnaires were completed by the parents. The levels of *S. mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. were determined from dental plaque samples. All data were analyzed by SPSS 25.0 using Student's t-test, the Mann-Whitney U, Fisher's exact and the chi-squared tests.

Results: BS was detected in 4.4% of the patients in the present study. DMFT and DMFS scores were significantly lower in those children with BS than in those without BS ($p=0.001$ and $p=0.010$). However, no statistically significant difference was found between dmft and dmfs scores and the presence of BS ($p>0.05$). Lower numbers of *S. mutans* and *Lactobacillus* spp. and greater numbers of *Actinomyces* spp. and *Capnocytophaga* spp. were found in those children with BS. There was no statistically significant relationship between *S. mutans* and *Actinomyces* spp. and the presence of BS ($p>0.05$). Colonizations of *Lactobacillus* spp. were statistically significantly lower, while colonizations of *Capnocytophaga* spp. were significantly higher in the BS group than in the control group ($p<0.05$).

Conclusion: It could be suggested that the different microbial composition of BS might be associated with lower caries experiences in affected subjects.

Keywords: Black stain, extrinsic, microbiology, dental caries

Introduction

Tooth discoloration is a common clinical finding which is considered primarily as an aesthetic problem. It can be influenced by many different factors and usually differs in

etiology, appearance, composition, location and degree of adherence (1). According to its location, it is divided into three groups, namely intrinsic, extrinsic or internalized stains. Intrinsic stains occur when the pigmented material

Address for Correspondence

Dilşah Çoğulu, Ege University Faculty of Dentistry, Department of Pedodontics, İzmir, Turkey
Phone: +90 532 430 33 31 E-mail: dilsah.cogulu@gmail.com ORCID: orcid.org/0000-0002-3156-9801

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penetrates into the tooth structure. Extrinsic stains are inserts on the tooth surface or on the acquired pellicle. Internalized stains are formed by the inclusion of extrinsic stains on the tooth surface together with tooth development (2,3).

Black stain (BS) is a characteristic extrinsic discoloration which is defined as a dark line or incomplete coalescence on the tooth surface following the gingival margin in the cervical third of the tooth. BS is a common finding in children; however, it can also be seen in adults. Studies have shown equal prevalence in both sexes. The prevalence of BS varies between 1.6% and 21% because of the unspecified criteria used for diagnosis and the different populations included in studies (4-6).

Black tooth stains are considered as a dental plaque form which contains insoluble iron salt and high calcium and phosphate content. BS material is a ferric sulfate formed by the interaction of iron in saliva or gingival fluid with hydrogen sulfide produced by bacteria in the periodontal ligament (1,2,4).

Dietary habits may also play a role in its etiology. The consumption of vegetables, fruits, dairy products, eggs, and soy sauce promotes BS development. Drinking tap water instead of bottled mineral or natural spring water also seems to be associated with higher prevalences of BS (4).

Gram-positive rods and chromogenic bacteria have often been found to be associated with BS (1,2). In terms of microbial diversity and abundance, *Actinomyces*, *Cardiobacterium*, *Haemophilus*, *Corynebacterium*, *Capnocytophaga*, *Tannerella* and *Treponema* spp. levels were higher in those children with BS than in those without BS (2,4).

Most of the authors showed that the presence of BS is associated with a lower caries experience. The causative factors of BS are not fully understood. Certain types of bacteria seem to be involved in its etiology. It is not clear how the presence of BS on the tooth surface reduces susceptibility to caries. The relationship between the presence of BS in children and their experience of low dental caries has made the characterization of factors which contribute to the formation and nature of BS more important. When studies on the etiology of caries are examined independently from BS, it is noteworthy that there is an inverse correlation between cariogenic *Streptococci* and *Capnocytophaga* spp. Oral *Capnocytophaga* might prevent the proliferation of certain caries-causing organisms such as *S. mutans* (7,8).

However, despite this inhibitory activity, the relationship between the presence of *Capnocytophaga* spp. and the low caries indices of children with BS has not been investigated to date.

Although BS does not cause any pathology, treatment is usually carried out for aesthetic reasons. The professional polishing process on the tooth surface eliminates the BS of the teeth, but these stains can re-occur over time.

The aim of this study was to evaluate the prevalence of black tooth stain among children aged 3-12 years who applied for routine dental examination and to determine any relationship between the presence of BS and dental caries incidence, dental plaque scores and to examine the colonization of *Streptococcus mutans*, *Lactobacillus* spp., *Actinomyces* spp. and *Capnocytophaga* spp. in dental plaque samples with or without BS. The socio-economic status of the families, the oral hygiene and dietary habits of the children, and the medical and dental history of the children were also compared between the two groups.

Materials and Methods

A total of 1000 children aged 3-12 years who applied to the Department of Pedodontics, Ege University Faculty of Dentistry, for routine dental examination were enrolled in this study. From this group, those children with BS (n=44) were selected as the study group. With the same number as the study group, and with the same age and sex profile, 44 children without BS were selected as a control group. Ethical approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: 17-4/18, date: 20.04.2017) and written informed consent was acquired from each parent. The parents were interviewed based on a structured questionnaire including questions on the socio-economic status of the family, the oral hygiene and dietary habits of the children, and the medical and dental history of the children.

Those children who had taken antibiotic therapy within the 3 months prior to the dental plaque sampling and those children with any systemic disease were not included in this study.

Dental Examination

The presence and grading of BS, dental caries incidence and dental plaque scores were recorded by the same pediatric dentist (G.İ.). The dental examinations of the children were conducted under natural light with the aid of a dental mirror and explorer. The dental caries scores were recorded according to World Health Organization criteria using DMFT/DMFS and dmft/dmfs indices (9). All of the children were classified according to their DMFT/dmft scores; Group-1: caries active (dmft+DMFT \geq 1), Group-2: caries-free (dmft+DMFT=0). The dental plaque scores were recorded according to the Sillness and L oe (10) index.

The BS scores were classified according to the work of Gasparetto et al. (8).

The classifications of Gasparetto et al. (8):

Score 1: Presence of pigmented dots or thin lines with incomplete coalescence parallel to the gingival margin

Score 2: Continuous pigmented lines, which are easily observed and limited to half of the cervical third of the tooth surface

Score 3: Presence of pigmented stains extending beyond half of the cervical third of the tooth surface.

Dental Plaque Sampling

Dental plaque samples from those children with BS (study group) and those without BS (control group) were taken. The sampling was performed 2 hours after breakfast between 9.00-11.00 a.m. The dental plaque samples were gently collected with a sterile dental curette and placed into Eppendorf tubes containing Stuart transport medium. All Eppendorf tubes were weighed before and after sampling (Ohaus, Adventurer, AR3130). The samples were transferred to a laboratory on ice. All microbiological procedures were performed at Ege University Faculty of Science, Department of Basic and Industrial Microbiology Department Laboratory.

Isolation and Identification of Microorganisms

The plaque samples were dispersed in a vortex mixer in order to obtain a homogeneous suspension and cultivated on selective VCAT medium (Oxoid) for *Capnocytophaga* spp., mitis salivarius agar (Difco) with 15% sucrose (Difco) and 0.2 units/mL of bacitracin (Sigma, Sigma-Aldrich Co., St Louis, MO, USA) for *S. mutans*, MRS Agar was used for the isolation of *Lactobacilli* spp. and Actinomyces Selective Agar was used for the isolation of *Actinomyces* spp.

All of the plates were incubated for 2-5 days at 37 °C in 8% CO₂. Suspected colonies were counted, and two bacterial isolates were recovered from each of the cultivation media for the identification and verification of the isolates. Suspected *Capnocytophaga* colonies were then transferred to McConkey agar plates and incubated under the same conditions. The identification of the representative isolates were performed using the VITEK II identification system (Biomèrioux, France) in addition to microscopic and cultural examinations. VITEK NH card panels (Biomèrioux) and VITEK GP Card panels were used for the identification of *Capnocytophaga* spp. and *S. mutans* isolates, respectively.

Statistical Analysis

All data were analyzed by SPSS 25.0 (SPSS Statistics for Windows, Armonk, NY: IBM Corp.). In the analysis of

data, the t-test and Mann-Whitney U test were used for the comparison of the two groups. Categorical data were analyzed by Fisher's exact test and the chi-squared test. For the significance level of the tests, $p < 0.05$ and $p < 0.01$ were accepted.

Results

In this study, 480 girls and 520 boys aged between 3-12 years who attended the clinic for routine dental examination were evaluated. From these 1000 children, BS was detected in 44 children (4.4%) and this group was categorized as the study group. The control group ($n=44$) was randomly selected from the same group with the same age and gender profile.

No statistically significant relationship was found between the presence of BS and gender, sex and the plaque scores ($p > 0.05$).

No statistically significant relationship was found between socio-economic factors (education levels of the mother and father, family income) and the presence of BS ($p > 0.05$).

No statistically significant relationship was found between medical history (systemic disease, drug usage, vitamin intake, breastfeeding and formula intake in infancy) and the presence of BS ($p > 0.05$).

No statistically significant relationship was found between dental history (previous dental visits, previous dental treatment, tooth brushing habits, usage of toothpaste, fluoride content of the toothpaste, professional fluoride applications) and the presence of BS ($p > 0.05$).

No statistically significant relationship was found between dietary habits (eating before sleeping, keeping food in the mouth, the number of main and intermediate meals, drinking water source, frequency of the consumption of milk, yogurt, buttermilk, probiotics, meat, chicken, fish, eggs, vegetables, fruit, bread, biscuits, wafers and chocolates) ($p > 0.05$). A negative correlation was found between the presence of BS and fizzy drink consumption ($p = 0.035$).

The caries scores of those children with or those without BS is presented in Table I. DMFT and DMFS scores were lower in those children with BS than in those without BS and this relation was statistically significant ($p = 0.001$ and $p = 0.010$). However, no statistically significant difference was found between the dmft and dmfs values and the presence of BS ($p > 0.05$) (Table I).

Bacterial counts are shown in Table II. A lower number of *S. mutans* and *Lactobacillus* spp. and a greater number of *Actinomyces* and *Capnocytophaga* spp. were found in those children with BS. However, there was no statistically significant relation between *S. mutans* and *Actinomyces* spp. and the presence of BS ($p>0.05$). The colonizations of *Lactobacillus* spp. were statistically significantly lower while the colonizations of *Capnocytophaga* spp. were significantly higher in the BS group than in the control group ($p<0.05$). No significant relationship was found between the caries scores and colonizations of *S. mutans*, *Lactobacillus* spp., *Actinomyces* and *Capnocytophaga* spp. ($p>0.05$).

Table I. The relationship between the presence of black tooth stain and caries index scores

	BS (-)		BS (+)		p-value
	Average	SD	Average	SD	
DMFT	2.67	2.98	0.89	1.43	0.001*
dmft	4.60	4.23	3.86	3.83	>0.05
DMFS	4.65	6.31	2.23	4.25	0.01*
dmfs	10.74	9.60	7.95	8.59	>0.05

* $p<0.05$ Statistically significant
SD: Standard deviation, BS: Black stain

Table II. The relationship between the presence of black tooth stain and microorganism

CFU/mL	BS (-)	BS (+)	p-value
<i>S. mutans</i>	$6.8 \times 10^5 \pm 1.0 \times 10^5$	$4.3 \times 10^5 \pm 1.8 \times 10^5$	>0.05
<i>Lactobacillus</i> spp.	$1.1 \times 10^5 \pm 8.0 \times 10^4$	$4.0 \times 10^4 \pm 2.0 \times 10^4$	0.002*
<i>Actinomyces</i> spp.	$2.0 \times 10^7 \pm 1.1 \times 10^7$	$2.4 \times 10^7 \pm 1.7 \times 10^7$	>0.05
<i>Capnocytophaga</i> spp.	$4.2 \times 10^6 \pm 2.3 \times 10^6$	$2.2 \times 10^6 \pm 1.3 \times 10^6$	0.01*

* $p<0.05$ Statistically significant
BS: Black stain

Discussion

Tooth discoloration is a common dental finding and it is associated with clinical and esthetic problems. The clinical diagnosis of BS is based on the presence of pigmented dark lines parallel to the gingival margin or an incomplete coalescence of dark dots rarely extending beyond the cervical third of the crown (1-3).

The prevalence of BS ranges from 1.6% to 21% in the literature (4-6). The prevalence of BS was found to be 4.4% ($n=44/1000$) in the present study. The differences in the prevalences of BS between studies may be due to differences

in age, diet and oral hygiene habits, microbiological differences and diagnostic criteria and also the quantitative characteristics of the groups.

In this study, no statistically significant relationship was found between gender and black tooth stain prevalence. Garcia Martin et al. (11), Akyüz et al. (12), Chen et al. (6), and França-Pinto et al. (13) also did not find any relationship between gender and black tooth stain.

In the present study, there was no statistically significant difference between the socio-demographic factors and the presence of black tooth stain, dental caries and dental plaque scores ($p>0.05$). Akyüz et al. (12) also did not find any relationship between the socio-demographic factors and the presence of BS. Chen et al. (6) reported a negative correlation between the education levels of the parents and the presence of BS.

No statistically significant correlation was found between the medical and dental history of the children and the presence of BS ($p>0.05$). The results of the study of Chen et al. (6) were also in accordance with our findings. They did not report any significant correlation between the systemic disease and drug usage and the presence of BS.

It has been reported that dietary habits may also play a role in the etiology of BS. The consumption of vegetables, fruits, dairy products, eggs, and soy sauce promotes BS development. No significant correlation was detected between dietary habits and the presence of BS in the present study ($p>0.05$). A positive correlation was detected between the consumption of fizzy drink and the presence of BS ($p=0.035$). Children who had been fed with formula during infancy tend to have higher BS occurrence (6,11). No significant correlation was found between feeding with formula during infancy and the presence of BS in the present study ($p>0.05$). Drinking tap water instead of bottled mineral or natural spring water also seems to be associated with a higher prevalence of BS (13). No significant correlation was found between the source of drinking water and the presence of BS in the present study ($p>0.05$). The content of the formula and tap water might explain the different results of these different studies.

The DMFT and DMFS scores in those children with BS were lower than in those without BS and this relationship was found to be statistically significant in the present study ($p=0.001$ and $p=0.010$). However, there was no statistically significant relationship between dmft and dmfs scores and BS ($p>0.05$). Most of the authors have shown that the presence of BS is associated with a lower caries experience. It has been assumed that the presence of BS is associated

with a predominance of *Actinomyces* spp.. Immunological studies and investigations on bacterial adhesion found that high levels of *Actinomyces naeslundii* in biofilms on teeth correlated with lower caries experiences and lower *Streptococcus mutans* adhesion. This may explain from another perspective why those children with BS had lower caries prevalences (3,14).

A lower number of *S. mutans* and *Lactobacilli* spp. and a greater number of *Actinomyces* spp. were found in those children with BS. There was no statistically significant relationship between *S. mutans* and *Actinomyces* spp. and the presence of BS ($p>0.05$). However, the relationship between *Lactobacilli* spp. and BS was statistically significant ($p=0.002$). No significant relationship was found between the caries scores and colonization of *S. mutans*, *Lactobacilli* spp. and *Actinomyces* spp. ($p>0.05$).

It has been reported that those children with BS have a lower caries incidence, but the cause of this phenomenon is still not fully understood. The thin black-brown lines on the teeth were observed and it has been suggested that this is a sign for a low caries index (3,8). Many other recent studies also support an inverse relationship between BS and tooth caries (5,7,8).

Capnocytophaga spp. are prevalent members of the BS microbiota but their role in the etiology of BS or their relation between the low caries frequency in children with BS has not been investigated yet. *Capnocytophaga* spp. can produce bacteriocins which can inhibit the growth of other bacteria including oral *Streptococci* which cause caries (15,16).

A lower caries index was found in those children with BS in the present study. *Capnocytophaga* spp. levels were statistically higher in those children with BS ($p=0.01$). Although *Capnocytophaga* members themselves are opportunistic pathogens, they can show an antagonist activity against persistent *S. mutans*, and thus, may reduce the caries index. Furthermore, co-aggregation studies between *Streptococcus* spp. and *Actinomyces* spp. showed that the interbacterial adhesion between these two bacteria improved earlier dental plaque biofilm formation. Earlier colonizers provide specific binding sites for other bacteria and can improve biofilm progress directly or by the saliva glycoproteins which bind to the pioneer organisms (17). *Capnocytophaga* members also exhibits a co-aggregation property, this co-aggregation may also prevent the attachment of persister *S. mutans* to the BS. Consequently, the *Capnocytophaga* abundance in BS may affect *S. mutans*

cells through proposed or unknown mechanisms and prevent caries progress.

Study Limitations

In the present study based on 1000 children, BS was detected in 44 children (4.4%). The microbiological evaluation was determined in these patients alone. Further studies with a larger number of subjects may support our results.

Conclusion

Capnocytophaga spp. levels in children with BS were significantly higher when compared to the control group. Higher levels of *Capnocytophaga* directly or indirectly may prevent caries formation. This should be clarified by further studies which can reveal whether *S. mutans* in children with BS are persisters or whether *Capnocytophaga* members actually inhibit the proliferation of *S. mutans*. The identification of *Capnocytophaga* isolates at species level with metagenomic studies may also contribute to identify this link.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval no: 17-4/18, date: 20.04.2017).

Informed Consent: Written informed consent was acquired from each parent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.İ., D.Ç., Concept: G.İ., D.Ç., A.U., Design: G.İ., D.Ç., A.U., Data Collection and/or Processing: G.İ., E.U., Analysis and/or Interpretation: G.İ., D.Ç., E.U., A.U., Literature Search: G.İ., D.Ç., A.U., Writing: G.İ., D.Ç., A.U.

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