Salivary Parameters and Caries Indices in Children with Black Tooth Stains

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Objective: *Black tooth stain in children has been associated commonly with a low caries experience. The present study aimed to to compare salivary factors and caries indices in children with and without black tooth stain and to investigate the relationship between caries and caries associated salivary factors in these children.* ***Study Design:*** *Salivary flow rate, pH, buffering capacity, total calcium and phosphorus were determined. Calcium and phosphorus levels were assayed by Inductive Coupled Plasma with Atomic Emission Spectrometry. DMFT and dft indices were evaluated according to WHO criteria.* ***Results:*** *Significantly higher levels of salivary buffering capacity and calcium, and lower flow rate were found in children with black tooth stain compared with those of without black tooth stain ($p < 0.01$, $p = 0.044$ and $p = 0.037$, respectively). The differences in phosphorus and pH were not significant between the groups. The dft index was found to be significantly lower in children with black tooth stain than children without black tooth stain ($p = 0.030$). However, DMFT did not change between the groups. There is no relationship between salivary parameters and caries indices in children with black tooth stain.* ***Conclusion:*** *It is suggested that low caries tendency seen in children with black tooth stain may be associated with high salivary calcium and buffering capacity.*

Keywords: *black tooth stain, dental caries, saliva, children*
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INTRODUCTION

Tooth discoloration is a frequent dental finding associated with clinical and aesthetic problems. It can have significant effects on the personality and self confidence of the child, and even cause stress within the family. In addition, discoloration was suggested to be a sign of systemic diseases.[1] It differs in etiology, appearance, composition, location, severity and degree of adherence.[2] Basically, there are two types of tooth discolorations: those related to intrinsic factors such as congenital or systemic influence and inherited conditions, or those caused by extrinsic factors, related to metallic or nonmetallic stains.[1-3] However in recent studies,[2,4,5] three types of tooth discoloration are mentioned: extrinsic, intrinsic and internalized. Extrinsic dental stains may be caused by predisposing factors, and other factors such as dental plaque, foods and beverages, chromogenic bacteria, metallic compounds and medications. It is located on the outer surface of the teeth. Intrinsic stains result from the incorporation of pigmented materials into dentinal tissues. The third category of stain internalization has been recently described as to include those circumstances where extrinsic stain infiltrates through the defects in the tooth structure.[2,4,5]

Black stain is one of the extrinsic dental stains.[1,3,6] It is defined as dark pigmented exogenous substance in lines or dots parallel to the gingival margin and firmly adhered to the enamel at the cervical third of the tooth crowns in the primary and permanent dentition.[3,6] This particular type of pigmentation has been considered to be a special form of dental plaque that differs from the other types because it contains insoluble iron salt and a high content of copper, calcium and phosphate. The black material is a ferric salt, probably ferric sulfide, formed by the reaction between the hydrogen sulfide produced by bacterial action and iron in the saliva or gingival exudates.[1,3]

Salivary parameters like salivary flow rate, buffering capacity, pH and calcium and phosphorus concentrations play a major role in the development of caries.[7] The presence of black tooth stain in children has been commonly associated with a low caries experience.[1,3,6,10] However, little is

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known about caries associated salivary factors and their relationship with caries experience in children with black tooth stain.[8,9]

Thus the aim of this study was to compare salivary factors and caries indices in children with and without black tooth stain and to investigate the relationship between caries and these caries associated salivary parameters in these children.

MATERIALS AND METHOD

A total of 50 children (age range 7 and 12) with and without black stain who visited the Dental Clinic of Pediatric Dentistry, Marmara University, Istanbul, Turkey were selected. However twelve of them were excluded from the study because of insufficient saliva collection. The study groups consisted of 23 and 15 children with and without black tooth stain, respectively.

The study protocol was approved by the Research Ethics Committee of Marmara University. Before the study was initiated, all parents or guardians were informed of the objective of the investigation and a written consent was obtained for the participation of each children in this study.

None of the children had systemic diseases nor took any medication including chlorhexidine, iron preparate and antibiotics for at least 1 month. Dental examinations were conducted by one experienced dentist for the presence of black stain and dental caries (A.G.). The clinical diagnosis of the black stain was made based on the criteria of Koch *et al*[11] as: score 1: the presence of pigmented dots or thin lines parallel to gingival margin; score 2: continuous pigmented lines, which were easily observed and limited to half of the cervical third of the tooth surface; score 3: the presence of pigmented stains extending beyond half of the cervical third of the tooth surface. Examples of black tooth stain with scores 1, 2 and 3 are seen in Figure 1.

Decayed, missing and filled permanent teeth (DMFT) and decayed and filled primary teeth (dft) indices were evaluated according to the criteria recommended cayed and filled primary teeth by the World Health Organisation.[12]

Saliva Samples

Unstimulated whole saliva samples were collected for 5 min into test tubes from all children between 09:00 and 12:00 am to minimize the circadian rythm. Each child was instructed not to eat or drink anything for 2 h preceeding before the appointment. The average salivary flow rate was calculated from the total volume divided by the time taken to obtain the sample.

Immediately after collection, saliva samples were analyzed for pH and buffering capacity using pH paper (GC

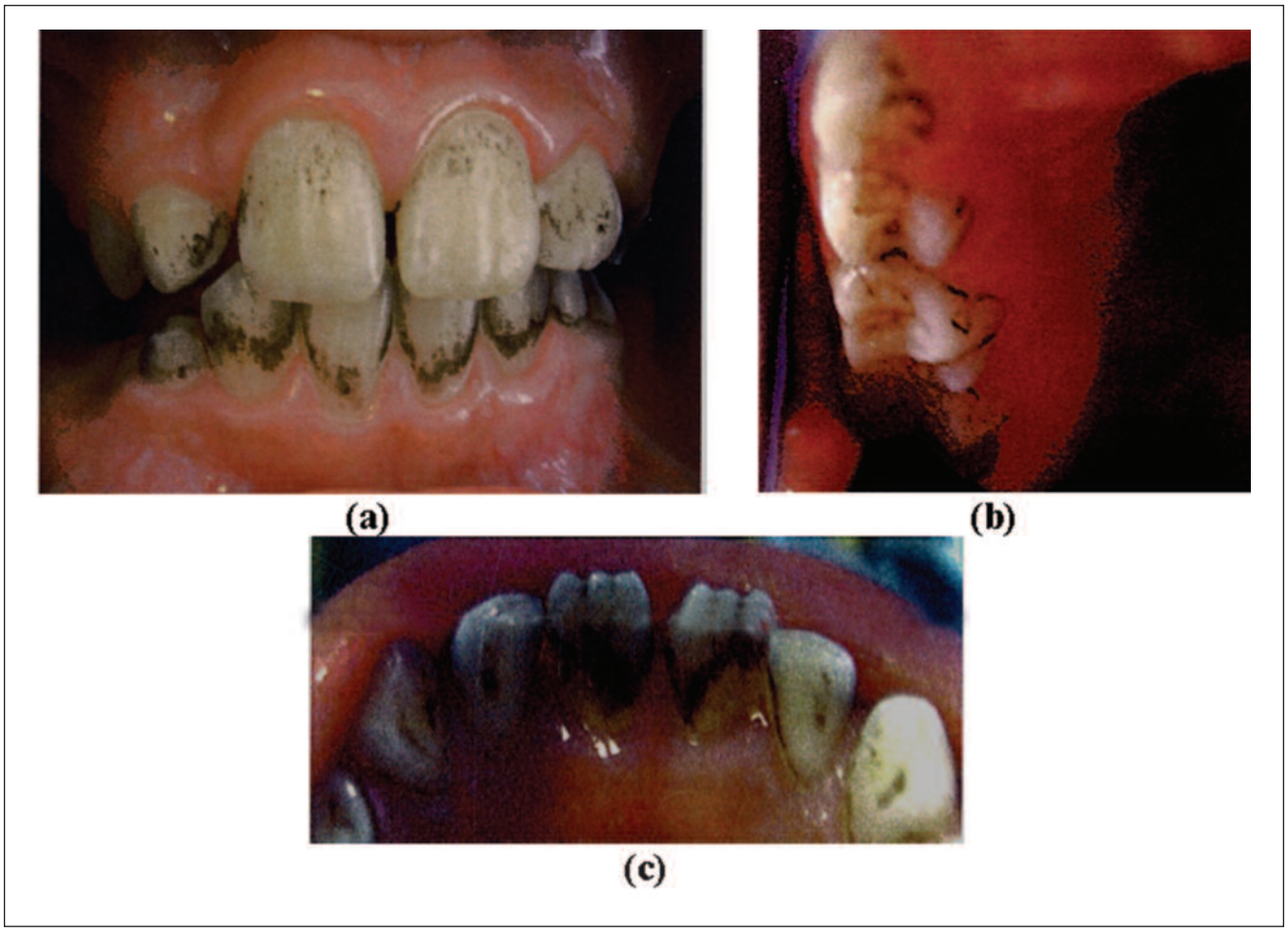

Figure 1. Represantative views of score 1 (a), score 2 (b), score 3 (c) black tooth stain.

America). Each pH test strip was compared to the reference standard color chart to assess the pH level in 0.2 pH increments. Buffering capacity was determined by Ericsson method.[13] 0.5 mL of collected saliva were placed in a sterile tube containing 1.5 mL of 0.0033 N HCl. The tube was shaken for 1 min and opened immediately to remove out CO_2. The final pH was determined after 10 min by pH paper.[13] One mililiter of these saliva samples were stored at -20ºC until used. Salivary total calcium and phosphorus were analyzed by Inductive Coupled Plasma with Atomic Emission Spectrometry (Perkin Elmer Optima 2100 DV). Calcium and phosphorus determinations were determined in dublicated saliva samples.

Statistical Analysis

All data was analyzed using NCSS (Number Cruncher Statistical System) 2007 PASS 2008 Statistical Software (Utah, USA). Salivary parameters and caries indices between the groups were compared using Student t-test. The correlation between salivary and dental parameters was performed using Pearson's correlation test. Spearman's correlation test were done to the relationship between the presence of black tooth stain and caries indices. $p<0.05$ were considered to be significant.

RESULTS

Gender and age between the children with and without black tooth stain were not significantly different ($p = 0.120$).

Salivary buffering capacity and calcium of children with black stain were significantly higher ($p < 0.01$ and $p = 0.044$, respectively) and flow rate was significantly lower ($p = 0.037$) than those of the children without black stain. pH and phosphorus levels were similar in both groups ($p = 0.318$ and $p = 0.415$, respectively) (Table 1). The dft index of the children with black stain group was significantly lower than that of without black stain ($p = 0.030$). The difference in DMFT indices between the groups was not statistically significant ($p = 0.873$) (Table 1).

The negative significant correlations were found between the flow rate and dft ($n = 23$, $r = -0.533$, $p = 0.041$) or DMFT ($n = 23$, $r = -0.588$, $p = 0.021$) indices in children without black tooth stain. However, no correlations were found between salivary parameters and caries indices in children with black tooth stain. A significant negative correlation was found between the presence of black tooth stain and DMFT ($n = 38$, $r =- 0.297$, $p = 0.037$).

Table 1. Caries indices and salivary parameters in children with and without black stain

	Children		
	With Black Stain (n=23)	**Without Black Stain (n=15)**	***p* value***
DMFT	1.60±1.26	1.53±1.59	0.873
dft	1.21±2.04	3.60±3.60	<0.05
Phosphorus (mM)	4.3±1.5	4.6±1.1	0.415
Calcium (mM)	2.53±1.1	1.94±0.7	<0.05
Flow rate ($ml.min^{-1}$)	0.45±0.13	0.62±0.29	<0.05
Buffering Capacity	3.85±0.53	3.23±0,7	<0.01
pH	7.73±0.10	7.69±0.13	0.318

Values were given as mean±standard deviation, DMFT: decayed, missing, filled teeth in permanent dentition, dft: decayed filled teeth in primary dentition. *Unpaired Student t-test

DISCUSSION

In literature, there is no consensus between the studies on the prevalence of black stain in children.[1,3,6,10,14,15] The prevalence of black staining was reported to be 17% in Turkish children aged between 5 and 12 years old.[16] Similar prevalence was also reported by Bhat *et al* in Indian children at similar ages.[3] Additionally, the presence of black stain is commonly associated with a low caries experience[1,3,6,10] which is similar to our results. Heinrich-Weltzien *et al* in Filipino children[6] also reported significantly lower caries prevalence in children with black stain aged between 11 and 13 years. They proposed that the lower caries experience in children with black stain reflect a general lower caries activity rather than a localized effect.[6] Koch *et al*[10] and Bhat *et al*[3] also found significantly lower caries incidence in children with black stain aged between 6-12 years. As reported by Gasperetto *et al*,[1] the presence of black tooth stain was significantly correlated to DMFT which is confirmed by present finding. They also reported that the children with largest area effected by stains demonstrated less caries. However, they found no difference in DMFT levels between groups. Our DMFT results were consistent with the results of Gasperetto *et al*,[1] but not with Koch[10] Bhat *et al*[3] findings Sample size, oral hygiene and dietary habits could be some factors influencing the different DMFT results. The presence of black stain has been shown to be also associated with low cariogenic oral microflora with predominance of *Actinomyces* and low number of *Streptococci.*[6]

In the present study, salivary flow rate was significantly lower in children with black stain which is in contrast with the study conducted by Surdacka *et al*.[9] These authors used stimulated saliva. On the other hand, there was no significant correlation between flow rate and DMFT or dft indices in children with black stain, whereas in the present study, the negative correlations were found between flow rate and DMFT of dft indices in children without black stain.

In Surdacka's study, salivary pH levels were found to be significantly higher in children with black stain than those of without black stain.[9] In this present study, there was no significant change in pH between groups. However, buffering capacity was significantly higher in children with black stain than those of without black stain. The higher buffering

capacity may be related to low caries tendency in children with black stain.

Only Surdacka reported salivary mineral levels including calcium and phosphorus in children with black stain compared to those without black stain.[8] They suggested that the higher salivary levels of calcium and phosphorus in children with black stain were characteristics of subjects with low susceptibility to caries.[8] In this present study, higher salivary calcium levels in children with black stain are compatible with the result of Surdacka, whereas phosphorus levels are not.[8] Salivary calcium indirectly regulates the aggregation of microorganism in saliva to help maintaining the globular structure of salivary micelles.[17] The micelles are composed of salivary immune system proteins such as low molecular weight mucin, sIgA, lactoferrin, amylase, glycosylated prolin rich proteins and lysozyme.[17] In this present study, the increase in salivary calcium levels in children with black stain may contribute to form more salivary micelles and a reservoir for remineralization.

CONCLUSIONS

Low caries tendency seen in children with black stain may be associated with high salivary total calcium and buffering capacity. Actually, the relationship between black stain and dental caries cannot be explained only with the biochemical parameters, but could be also related to dietary habits, caries activity of the children and predominant microorganisms of biofilm such as *Actinomyces* and *Prevotella melaninogenica*. Therefore, more comprehensive further studies are needed to clarify the relationship between caries and caries associated salivary factors in children with black tooth stain.

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