

Extrinsic black dental stains in relation to dental caries and mutans streptococci in saliva.

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Abstract:

The severity of extrinsic black dental stain in relation to dental caries and levels of mutans streptococci in saliva was studied. The study population consisted of 23 children with mean age of 8 ± 2.15 years. A lower caries experience was recorded among children with black stain than those with out, however a statistical difference was seen only among permanent dentition $P < 0.05$. A lower level of bacteria was recorded among children with the stain compared to other group, but difference was not significant $P > 0.05$. A strong and highly significant inverse correlation coefficient was seen between the severity of dental stain and DMFS, $r = -0.53$ $P < 0.01$. As for dmfs and levels of bacteria for each an inverse correlation was seen but statistically not significant $P > 0.05$.

Keywords:

Dental caries, mutans streptococci, saliva, extrinsic stain.

Introduction:

Deposits of pigmented plaque and /or calculus on the tooth surfaces can cause extrinsic black dental stain. It can be removed with an abrasive material^(1,2). Epidemiological studies often recorded a lower prevalence and severity of dental caries among children with this type of stain⁽³⁻⁶⁾. While other failed to detect such an observation⁽⁷⁾. The low caries experience was attributed to a diversity of microbial composition of the black deposit from what is considered normal. In addition a low number of cariogenic bacteria, especially Streptococci was reported in comparison to other types oral bacteria^(8,9).

Mutans streptococci are the most important in relation to dental caries due to their cariogenic traits.

They can be found in saliva as well as dental plaque and other pigmented deposits on teeth^(10,11,12). Levels of mutans streptococci in saliva are positively correlated with their number in deposits on teeth, as saliva baths the whole teeth surfaces^(11,12).

As far as it is known no previous study in Iraq was present to correlate between the severity of black dental stain and both dental caries and mutans streptococci counts in saliva. For this reason this study was conducted.

Materials and Method:

In this study and within a 2-week's period a group of children attending the Paedodontic Clinic/ College of Dentistry were included. Their age range was 6-10 years, recorded according to the last birthday.

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Those children were healthy looking with no history of serious medical problem. In addition they did not receive any medicament or antimicrobial agent in the last 2 -3 weeks to the examination.

Diagnosis and recording of dental caries were achieved following the criteria of WHO, ⁽¹³⁾. Extrinsic dental stain were assessed according to the index of Leung, ⁽¹⁴⁾. The extension of the stain on tooth surface i.e. the width was scored as followed:

1. Less than 1 mm. of tooth surfaces
2. 1/3 of tooth surfaces
3. 2/3 of tooth surfaces
4. All tooth surfaces.

Prior to clinical examination, stimulated saliva was collected for bacteriological analyses. Collections of samples were performed under standard conditions according to recommendations cited by Tenovuo and Legerlof ⁽¹⁵⁾. After breakfast by 1-2 hours each child was asked to chew a piece of Arabic gum for a minute then expectorates to remove remnant of food debris. Chewing was then continued for two minutes and saliva collected in a sterial screw capped bottle. After disappearance of foam of saliva, 0.1ml was transferred to 0.9 ml of sterial phosphate buffer saline (pH 7.0). Ten fold dilutions were carried out using phosphate buffer saline. From dilutions 10^{-3} and 10^{-6} of samples, 0.1 was taken and spread in duplicate on Mitis Salivarius Bacitracin Agar, a selective media for mutans streptococci. Plates were incubated anaerobically for 48 hours at 37°C, then further aerobically for 24 hours at room temperature ⁽¹⁶⁾.

Identification of bacteria was performed depending on their microscopic appearance and ability to

ferment carbohydrate. These Gram-positive bacteria appear as irregular colonies with rough or frosted - glass surface or circular convex colonies with smooth glistening surface. They were violet or deep blue in color in similarity to descriptions of Edwardsson ^(17, 18) and Hoffman ⁽¹⁹⁾. Mutans streptococci were able to ferment mannitol and sorbitol used in concentration of 1% of Brain Heart Infusion broth in a presence of bromocrsol purple ⁽²⁰⁾. The number of colonies was recorded and expressed as colony forming unit per ml saliva (CFU/ml).

Data processing and analysis were carried out using SPSS package version 10. Student t - test, Pearson's and Spearman's correlation coefficient were applied for statistical differences at 95% confidence limit.

Results:

The sample consisted of 23 children, of both sexes, with a mean age of 8.7 ± 2.15 years. Ten subjects (43.48%) demonstrated an extrinsic black dental stain, compared to thirteen with out (56.52 %). The severity of dental stain by mean number of affected sextants was 2.83 ± 2.48 , with a mean width of color equal to 0.65 ± 1.02 .

Caries experience as recorded by means of dmfs and DMFS of the total sample were 5.52 ± 4.7 and 2.09 ± 3.1 respectively. Results showed lower values of these indices among children with stain compared to the other group. However, differences were statistically significant for permanent dentition and not for primary ones (Table 1).

Table (1): Caries – Experience and Levels of Mutans Streptococci among Children with and with out Black Stain.

Variables	With out Stain Mean ± SD	With Stain Mean ± SD	t Value	P- Value
Dmfs	7.77 ± 4.62	3.80 ± 4.32	1.61	NS*
DMFS	3.93 ± 3.45	0.50 ± 1.08	2.41	0.025*
CFU/ml [‡]	3.10 ± 4.50	0.90 ± 1.17	1.47	NS

Not significant Highly significant.

‡CFU/ ml x 10⁶

The CFU of mutans streptococci per ml saliva was $2.14 \pm 0.35 \times 10^6$ of the total sample. Table 1 shows a lower number of bacteria isolated from saliva of children with black stain compared to those with out,

differences however were statistically not significant.

The correlation coefficient between severity of staining and both caries – experience and level of mutans streptococci are seen in .

Table (2): Correlation Coefficient between Severity of Black Stain and Dental Caries and Levels of Bacteria.

Variable	Sextants*		Width		
	R	P	R	P	
D	-0.54	NS	-0.43	0.04	S**
DMFS	-0.54	NS	-0.53	0.01	HS***
D	-0.42	NS	-0.32	NS	
Dmfs	-0.43	NS	-0.44	0.04	S**
CFU/ml [‡]	-0.40	NS	-0.23	NS	

*Sextants: Affected sextants by stain.

**Significant

***Highly significant

‡Levels of mutans streptococci.

A negative correlation were recorded for all variables i.e. a decrease in dental caries and numbers of bacteria with the increase of affected sextants and width of black stain. For permanent teeth a strong and highly significant correlation was recorded. Where as for primary teeth, the correlation was weak and statistically not significant. In relation to bacterial

counts, correlations were weak and not significant.

Discussion:

Dental caries is a microbial disease as found by different epidemiological, experimental animals and human studies^(2,19,11). Mutans streptococci are the prime role in the initiation of caries lesion^(2,19,21). The quantitative evaluation of their number in dental plaque and saliva has been used for identification and /or predication of high-risk groups. Children with a threshold value of 2.5

10^6 CFU/ml saliva have been suggested to be at high risk to dental caries⁽²⁾. In the present study a relatively high levels of bacteria was recorded for the total sample. This was expected as the sample studied here composed from patients attended the clinic for treatment of carious teeth. Although statistically not significant, a lower value of CFU/ml of bacteria was recorded among children with extrinsic black stain, compared to those with out. At the same time an inverse correlation coefficient was seen between the increase severity of black stain and number of bacteria.

The relatively lower number of *mutans* streptococci of children with the black stain may be related to their lower number in the black deposit it self on surfaces of teeth. Previous studies of the microflora of the black deposit reported a predominance of Gram - positive filamentous microorganisms with a mixture of Gram- positive cocci and rods^(3, 9). Slots⁽⁸⁾ showed that Streptococci averaged only 5% of the black deposit, while Gram-positive rods averaged 90 % of the cultivable organisms.

Data of the present study demonstrated a lower caries - experience among children with black dental stain compared to those with out. This was in agreement with Iraqi epidemiological studies among children and adolescents⁽⁴⁻⁶⁾. The severity of black staining was found to be an affecting factor, as an inverse correlation coefficient was reported between severity of staining and dental caries.

The differences in caries - experience between groups with and with out black stain may be related to the microbial diversity of the black deposit it self as explained above. This in turn may affect the number of bacteria not only in saliva as seen here but may be in dental plaque as well.

One thing must not be ignored that dental caries is a multi factorial disease, i.e. the microbial diversity may not be the seldom factor responsible for the variation in caries severity between the two groups. Previous studies showed that children with extrinsic black stain had also a high concentration of calcium and phosphate ions in the gingival debris⁽²²⁾. These two elements may increase the resistance of teeth to acid attack as they may increase buffer capacity and enhance remineralization⁽²¹⁾. Ried and Beeley⁽²²⁾ reported that phosphate ions were about six fold more in children with black stain. These ions may play an important role in buffer system in un stimulated saliva⁽²¹⁾.

Further studies are needed concerning the microbial composition of the black deposits it self, and the chemical composition of the gingival fluids as well.

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