

ESTIMATION OF STREPTOCOCCUS MITIS IN CHILDREN SALIVA WITH AND WITHOUT BLACK STAINS

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ABSTRACT

AIM: To estimate levels of Streptococcus mitis in children with and without black stain.

OBJECTIVE: To evaluate levels of streptococcus mitis in children with black stain, in children with early childhood caries and non-carious children. To compare and correlate the level of streptococcus mitis in children with black stain, early childhood caries and non-carious children.

MATERIALS AND METHODS: The study will be done in school going children. 5ml unstimulated saliva samples were collected with and without black stains and evaluated for Streptococcus mitis colony forming units. Results were evaluated and statistics was done.

BACKGROUND: Black stain is a discolouration which mainly occurs along the cervical third of buccal or the lingual surfaces of the teeth. It most commonly affects the primary dentition, but permanent dentition may also be affected. Studies state that children with black stain are generally caries free. Thus the aim of the study was to evaluate the levels of Streptococcus mitis in children with black stain and to correlate it in children with ECC and non-carious children.

Keywords: S.Mitis, black stains, early childhood caries, saliva, streptococcus.

INTRODUCTION:

Tooth discoloration is a common dental finding and associated with clinical and aesthetic problems.⁽¹⁾ There are two types of tooth discolourations, i.e. those related to intrinsic factors such as congenital or systemic influence and inherited conditions, and those caused by extrinsic factors. Black tooth stain is an extrinsic discoloration which can occur in both primary and permanent dentition.⁽²⁾ Its clinical diagnosis 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.^(2,3)

In the oral cavity, the appearance of tooth discolourations is related to their etiology, composition, amount, hardness and attachment to the tooth enamel. According to Harald⁽⁴⁾, tooth pigmentation is formed by precipitation of chromogenic bacteria from dentobacterial plaque, deposition of coloured substances from the substrate present in the oral cavity, or formation of coloured substances due to chemical decomposition of pellicle components.

Black stain has been considered a form of dental plaque differentiated from other types by insoluble iron salt and high calcium and phosphate content. ^(3,5,6) They appear early on the tooth enamel, at the age of 2 or 3. Its negative effect on the dental aesthetic perception causes concerns in parents and can have significant effects on the personality and self-confidence of the child.

The mechanism of black stains is that the black material is a ferric compound, most likely a ferric sulphide, which arises from the interaction in the saliva or gingival fluid between hydrogen sulphide (produced by bacteria in the periodontal environment) and iron. Some hypothesis concerning the association between black stains and some bacterial strains (actinomyces, lactobacillus sp,prevotella melaninogenica) has been reported ^(7,8). The attraction of materials to the tooth surface is important to the formation of extrinsic dental stain. These attractive forces include electrostatic, van der Waals, hydration forces, hydrophobic interactions and hydrogen bonds. However, the mechanisms that determine the strength of adhesion are not perfectly understood.

Chromogenic bacteria were also proposed as an etiological factor in the production of black pigment. Periodontal pathogens such as Porphyromonas gingivalis, Prevotella intermedia, and Prevotella nigrescens are reported to be black-pigmented anaerobes in oral cavity.⁽⁹⁾

Former studies assumed Prevotella melaninogenica was closely related to black tooth stain.⁽¹⁰⁾ Traditional bacteriological examinations have implicated Actinomyces as the predominant cultivable microorganisms found in black stain.

Streptococcus mitis, previously known as *Streptococcus mitior*, is a mesophilic alpha-hemolytic species of *Streptococcus* that inhabits the human mouth.⁽²⁾ It is most commonly found in the throat, nasopharynx, and mouth. It is a Gram-positive coccus, facultative anaerobe and catalase negative. *Streptococcus mitis* are commensal bacteria that colonize hard surfaces in the oral cavity such as dental hard tissues as well as mucous membranes and are part of the oral flora.⁽²⁾

Various studies have proved *S.mitis* to be a beneficial bacteria. *Streptococcus* species such as *S.mutans*, *S.sorbenius*, *S.salivarius* are usually known to play a major role in the development of caries and its level are proved to be less in black stains tooth children. But there are no previous studies done before on role of *S.mitis* in these carious tooth children and it's levels in those children. Thus this study was done to evaluate the levels of streptococcus mitis in the oral cavity of children with and without black stains.

MATERIALS AND METHODS:

Ethical committee approval:

The study design was approved by the Ethics Committee and by the City Health Department. The goals of the study were explained in detail to the parents or caretakers of the patients who met the inclusion criteria. An informed consent was signed by the patients parents/guardian.

Diagnosis criteria for black stains:

The black stains were diagnosed based on Koch et al black stain diagnosis criteria. He considered that the presence of dark dots (diameter less than 0.5 mm) forming linear discoloration (parallel to the gingival margin) at dental smooth surfaces of at least two different teeth without cavitation of the enamel surface. Other extrinsic discoloration were excluded.

Subject recruitment:

The target population consisted of 98 children aged 6 to 12 years attending the only Rakshak tuition center, perungulathur. They included 41 females and 57 males. Clinical examinations were performed under natural light with plane mouth mirrors in the school environment. Children were screened for black stains, children with early childhood caries and children without early childhood caries.

The children were divided into three groups such as group 1, group 2, group 3 which corresponds to children with black tooth stains, children without early childhood caries and children with carious tooth respectively. The children were made to sit in a comfortable position and their oral cavity were examined. The diagnosis was done with a mouth mirror and explorer under natural daylight. Children with ECC, black stains tooth, non-carious tooth were diagnosed properly and were segregated according to the various groups to collect saliva samples. Children were asked to wash their mouth before sample collection. 5ml unstimulated saliva samples were collected from children of each group in a separate sterile container each.

Cultivation of *S.mitis*:

A *mitis salivarius* agar medium was prepared and the samples were spread and incubated for 24 hours at 37 degree celsius. Then after 24 hours, the culture plates were evaluated for streptococcus mitis colony forming units and were counted. The results were tabulated and the statistics was done using ANOVA and CHI square test.



Fig 1 : Black stain tooth

RESULTS:

The bacterial colony count was done and tabulated individually for all the three groups. The result were calculated based on ANOVA and post hoc test (Tukey HSD).

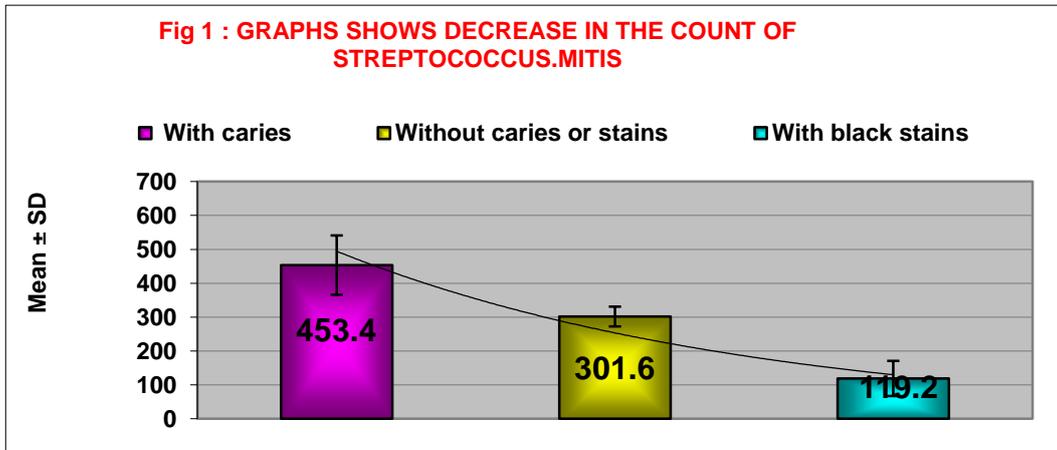


Table 1 : Mean, standard deviation and p value of 3 groups

GROUPS	MEAN ± SD	P. VALUE
GROUP A	119.20±29.18	0.00
GROUP B	301.6±77.6	
GROUP C	453.40±67.03	

Post Hoc Tests (tukey HSD)

GROUPS	GROUP A	GROUP B	GROUP C
GROUP A	-	.001	.000
GROUP B	.001	-	.006
GROUP C	.000	.006	-

Table 2 : Multigroup comparison of p value

The results show that the levels of S.mitis is low in children with black tooth stain (119.20±29.18) compared to children without early childhood caries (301.6±77.6) and the levels are high in children with caries (453.40±67.03) as tabulated in table 1. Fig 1 represents the graph which shows the decrease in the level of streptococcus mitis in each groups.

Table 2 shows the comparison of significance between multiple groups. This shows it is statistically significant between group A and group C and also between group B and group C.

This represents that levels of *S.mitis* are comparatively less in the children with black tooth stains than the other groups.

DISCUSSION:

Several studies have shown that children with black tooth stain have low incidence of caries.⁽¹¹⁾ Over the last century, the etiology of black stain has been the subject of much debate. Most of the studies concerning this issue were conducted in pediatric population. According to the reviewed articles published between 2001 and 2014, the prevalence of black stain varies from 2.4% to 18% with equal sex distribution. The majority of the authors confirm the correlation between the presence of black stain and lower caries experience. The microflora of this deposit is dominated by *Actinomyces* spp. and has lower cariogenic potential than nondiscolored dental plaque. Iron/copper and sulphur complexes are thought to be responsible for the dark color. In patients with black stain saliva has higher calcium concentrations and higher buffering capacity. Factors such as dietary habits, socioeconomic status, and iron supplementation may be contributing to the formation of black stain.

There are various studies that has proved *S. mitis* to be commensal bacteria. On records with previous studies it shows that *S.mitis* has been considered as a beneficial bacterial species.⁽¹⁷⁾ *Streptococcus* species such as *S.mutans*,*S.salivarius*,*S.sorbenius* are proved to have a role in caries development . But no previous studies are done to check the levels and presence of *S.mitis* in these caries tooth. This study was done to check the presence of *S.mitis* in children with ECC, Non carious tooth and black stains and to correlate if it's presence play a beneficial role in the prevention of caries development. Among 98 evaluated only 5 children were found to have black stain tooth. So samples of those children were collected and to compensate the sample size of black stains, 5 saliva samples of ECC children and 5 saliva samples of non carious tooth children were collected accordingly.

According to our results, there is a decrease in the levels of *S.mitis* in black tooth stain children compared to other two groups. Prevalence of dental caries in children with black stain were found to be less. Though *S.Mitis* is considered as an beneficial species, their levels were found to be decreased in children with black stains and higher in children with ECC.

Various studies have previously evaluated the correlation between the presence of black extrinsic tooth stains and caries. The study was done in 263 children aged between 6-12 years. Black stains were observed in 14.8% of the children.⁽¹²⁾ Garan found that salivary pH was not significantly different between the groups with and without black stains.⁽¹³⁾ Costa and Li report on a similar prevalence of *S. mutans* in dental biofilms of patients with and without black stain.^(14,15) The presence of black stain has been reported to be associated with a low frequency of caries, but the reasons are still unknown.⁽¹⁶⁾ Finally, all the above studies indicate a unique microbiological composition of dental plaque with black stain; however, with only a few selected species evaluated, the relationship between microorganisms and black stain still remains uncertain and incomplete. The similar bacterial composition of dental biofilms of black tooth stains and healthy tooth surfaces indicates that black tooth stains are not free of cariogenic bacteria.

This study tried to establish a relationship between *S.mitis* and its role in various children with ECC, black stains. This study can be considered as a pilot study and the sample used was a convenience sample. It is in no way to represent the entire population in general. Thus further, more studies should be done with bigger sample size to collect more evidence and to support the study.

CONCLUSION:

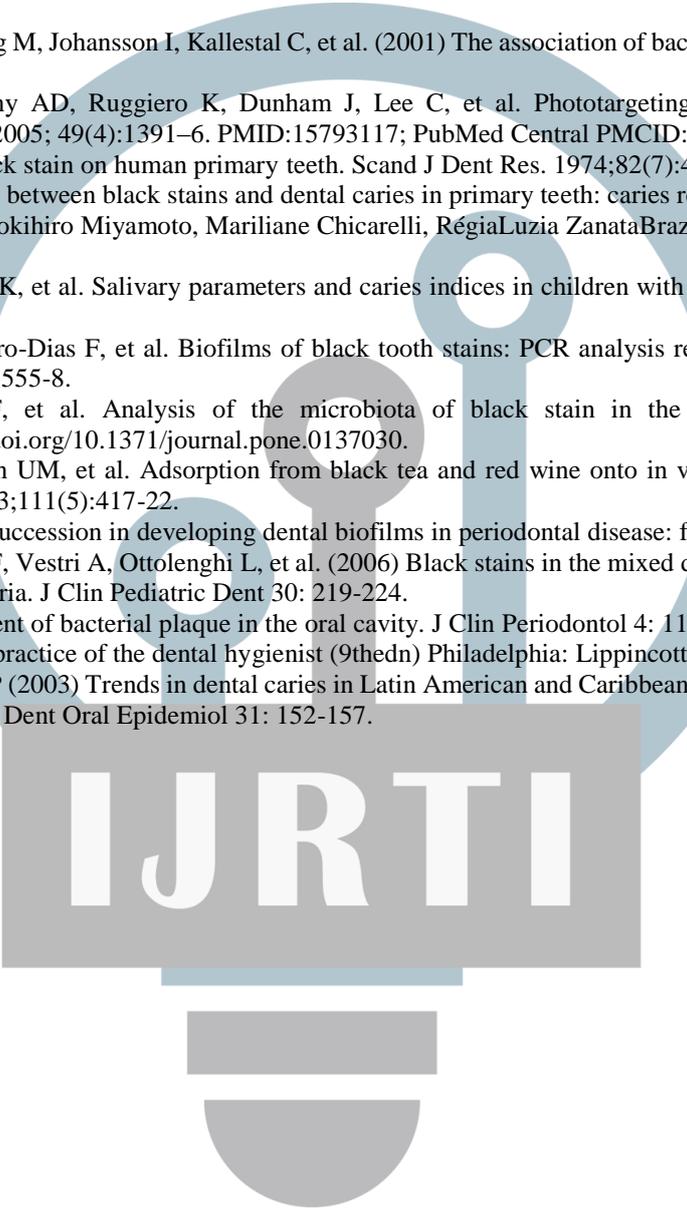
No previous studies are done about *S.mitis* since it's a normal commensal and beneficial bacteria of the oral cavity and are mentioned to have no relevant with caries development. Based on the results of this study it shows that the levels of *S.mitis* are higher in children with ECC and are low in children with black tooth stains comparatively which is in contradiction to previous studies. But no conclusion can be drawn with these results as the sample size was no sufficient. Further this research is to be done with a bigger sample size to support and provide a proper conclusion.

TREATMENT OPTIONS:

The black stain particularly poses an aesthetic problem. Daily Tooth brushing is not enough to remove this external stain. The professional cleaning is necessary to remove stains and resolve this aesthetic problem. Although a simple scaling and tooth brushing with pumice powder are usually sufficient, frequently black stain is recurrent. The ultrasonic cleaning is not recommended, this modality can lead to enamel removal; therefore, their repeated use is undesirable. Nevertheless, it can challenge the dentist, especially when it is deposited on roughened or pitted areas of the tooth. In a professional dental hygiene appointment, removal through polishing with rubber cup and fluoride pumice is possible. If the staining is resistant, the excess water can be blotted from the pumice and the tooth should be dried before the polishing procedure is performed.⁽¹⁸⁻²¹⁾

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