ESTIMATION OF LEVELS OF STREPTOCOCCUS SALIVARIUS IN CHILDREN WITH BLACK STAIN:

TYPE OF MANUSCRIPT: Research
RUNNING TITLE:Levels of Streptococcous salivarius in children with black stains.

Aishwaryah Ravisankar, Dr.Dhanalakshmi Ravikumar, Dr.Deepa Gurunathan

Undergraduate Student, Senior Lecturer, Professor
Saveetha Dental College and Hospitals, Saveetha University,
Chennai,India.

ABSTRACT:

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 is to evaluate the levels of Streptococcus salivarius in children with black stain and to correlate it in children with dental and non carious children.

AIM: To estimate levels of Streptococcus salivarius in children with black stain.

OBJECTIVE:. To compare and correlate the level of streptococcus salivarius in children with black stain, dental caries and non carious children.

MATERIALS AND METHODS: The study was done in school going children. 5ml of unstimulated saliva samples were collected in children with and without black stains and evaluated for Streptococcus salivarius colony forming units. Results were tabulated and statistics was done using SPSS software.

RESULTS:
The results showed that mean value of streptococcus salivarius is highest in the saliva samples collected from children with dental caries (591+ or – 87) and least in the children with black stains.The post hoc results showed that children with black stains was significant in comparison with without dental caries and in children with dental caries.

CONCLUSION:
The study shows that the levels of S.salivarius are higher in children with dental caries and are low in children with black tooth stains comparatively which is in correlation with previous studies.

KEYWORDS:
S.salivarius, black stains, dental caries, colony count, non carious children,saliva.

INTRODUCTION:

The colour and appearance of teeth is a complex phenomenon, which is affected by various factors such as translucency, lighting conditions, light scattering, opacity, gloss and the efficiency of the human eye and brain influencing the overall perception of tooth colour. [1]Tooth discolouration is a frequent dental finding associated with clinical and esthetic problems. It differs in aetiology, appearance, composition, location, severity and degree of adherence. Tooth stains consist of compounds that have darker shades which are called chromogens that when accumulated in the tooth is called intrinsic stain or on the tooth reffered to as extrinsic stain. [2]

Intrinsic discolouration occurs as a result of change to the structural composition or thickness of the dental hard tissues. The normal colour of teeth is measured by the pink, green and blue tints of the enamel and is reinforced by the yellow through to brown shades of dentine beneath. Intrinsic stains are caused due to a number of metabolic diseases and systemic factors such as fluorosis, amelogenesis imperfecta, dentinogenesis imperfecta, trauma to developed teeth, root canal theraphy,tetracycline staining root resorption, aging etc are known to affect the dentition and cause discolouration as a consequence. Bleaching agents such as hydrogen peroxides which are strong oxidising agents are used for lightening and whitening of discoloured teeth.[3]

Extrinsic discolouration is formed when an outside tooth substance which is present on the tooth surface or in the acquired pellicle. Extrinsic discolouration is outside the tooth substance and lies on the tooth surface or in the acquired pellicle. Extrinsic discoloration is determined by the attractive force between the teeth surface and the materials. The types of attractive force include electrostatic and van der Waals forces, hydration forces, hydrogen bonds and hydrophobic interactions. Extrinsic stains

should be carefully examined for the distribution of the stain along with its postion on the tooth surface.[4] The enamel rougness and defect on the area of the stain is to be noted. Extrinsic discoloration is influencd by age,home care, habits of the indivual and various other factors. The most common extrinsic stain Indian population is tobacco stain. Metallic stains, green stain, brown stain, black stain are few examples of extrinsic stains. Extrinsic stains can be removed by oral prophylaxis while certain forms of extrinsic strains require advanced treatments.

Black stain is a special form of dental plaque that differs from other types because it contains insoluble iron salt and a high content of calcium and phosphate. Black stains are present as wide or thin black lines on the lingual and facial surfaces of the tooth near to the gingival margin and extends to the proximal surface. The black stain is a ferric compound, most often presented as ferric sulphide. These black stains are formed when saliva or gingival crevicular fluid interacts with hydrogen sulfide (produced by bacteria in the periodontal environment) and iron.[5] Some hypothesis have found an significant association between black stains and some bacterial strains (actinomyces, lactobacillus sp, prevotella melaninogenica).[6] Black stains cannot be removed from the tooth surface with tooth brush as the stains are firmly adhered to the tooth surface.[7] It has higher incidence in females than in males. Black stains can be removed by microabrasion technique.[5] Porcelien dental veneer crowns or Zirconium crowns can be used as these stains to occur even after removal. Black stains can be diagnosed clinically as lines with incomplete coalescence of dots, continuous lines or pigmented dots [5,6], which rarely go beyond the cervical third and contour the crown around the gingival third, does not extend to the proximal areas. Prescence of these stains have proven low caries risk in children.

Dental caries is a multifactorial chronic disease which results in demineralisation of tooth structure. [7] It is one of the most common important global oral health problems in the world. Worldwide, approximately 2.43 billion people (36% of the population) are affected with dental caries in their permanent teeth. In primary teeth it affects about 620 million people or 9% of the population today. Socio economic and demographic conditions play a major role in incidence of dental caries.[8] Four things are required for caries formation: a tooth surface (enamel or dentin), caries-causing bacteria, fermentable carbohydrates (such as sucrose), and time.[9]

S. salivarius is a spherical, Gram-positive bacteria. S. salivarius belong to the Lancefield group K. Their genomic GC content is 39–42%, and the type strain is ATCC7073. S. Salivarius is a facultative anaerobic commensal bacterium that is both catalase and oxidase negative.[10] The optimal atmosphere condition for bacterial cultures should have a bacterial content less than 5 to 10 % carbon dioxide. S. Salivarius grows grows at 37 °C, As one of the first colonizers of the human oral cavity, upper respiratory tract, and gut after birth. [11]Streptococcus salivarius causes abundant dense plaque formation in fissures which in turn leads to dental caries formation. .Streptococcus salivarius is a frequent cause of endocarditis. The pathogen is generally associated with infection following rheumatic fever and unrepaired congenital heart disease, and with late postoperative endocarditis. Lewis C. Jones and David Shafer demonstrated that S.salivarius is responsible for a large portion of dental caries.[12]

The prevelance of back tooth stain is found to be 18% in both male and female children. Black tooth stain is influenced by dietary habits, iron supplements and socioeconomic status.[5] Various studies show that black stain teeth children have higher levels of Actinomyces spcc but there is no literature study on correlation between S.salivarius and black stains.[6] Further it has been proved in various researches that S.salivarius plays a major role in development of dental caries. [7,8] Hence this study aims to find out the levels of S.salivarius levels in children with dental caries, black stains and children without dental caries.

MATERIALS AND METHODS:

Ethical committee approval:

The study design was approved by the Ethics Committee of Saveetha dental college. The goals of the study were explained in detail to the parents or caretakers of the patients who met the inclusion criteria. The patients caretaker signed an informed consent form.

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. Cavitation on the tooth due to caries was the criteria used for diagnosis of dental caries.[11]

Subject recruitment:

The target population consisted of all 90 children (52) aged 3 to 12 years attending the only Rakshak tuition center, perungulathur. Clinical examinations were performed under natural light with plane mouth mirrors in the school environment. Children were screened for black stains, children with dental caries and children without dental caries.

Sampling:

The children were divided into group 1, group 2, group 3 which corresponds to children with black tooth stains , children without dental caries and children with carious tooth respectively. 5ml unstimulated saliva samples were collected from children of each group in a separate sterile container each.

Cultivation of S.salivarius:

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 salivarius colony forming units and were counted. The results were tabulated and the statistics was done using SPSS software version 17.

RESULTS:

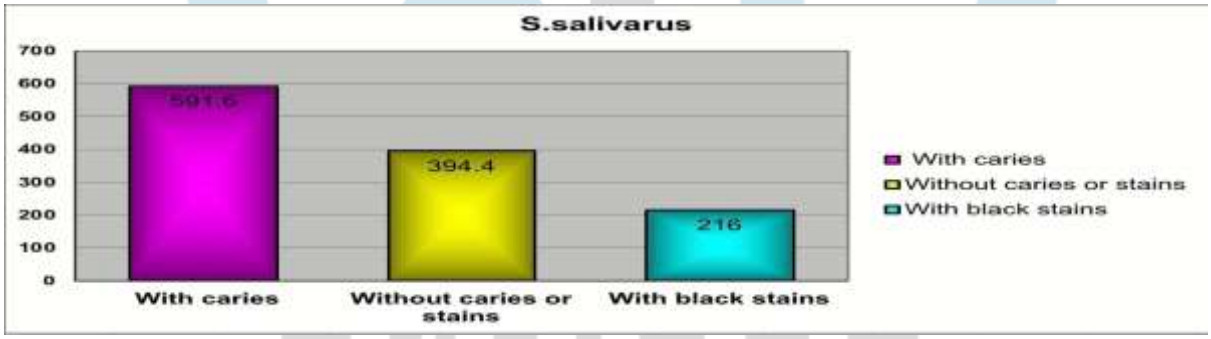
TABLE 1: S.Salivarius colony count in each group.

Groups	Mean	Standard deviation
Group 1(black stains)	216	52
Group 2(without early childhood caries)	394	29
Group 3(with early childhood caries)	591	87

TABLE 2: Intergroup comparison – Post HOC results.

Inter group comparison	Group 1(with black stains)	Group 2(without ECC)	Group 3(with caries)
Group 1(with black stains)	Nil	0.002	0
Group 2(without ECC)	0.002	Nil	0.001
Group 3(with caries)	0	0.001	Nil

FIGURE 1: Graphical representation of S.salivarius colony forming unit in the three groups.



The table 1 show that mean value of streptococcus salivarius is highest in the saliva samples collected from children with dental caries (591+ or – 87) and least in the children with black stains. A value less than 0.05 was considered significant. Table 2, post hoc results showed that children with black stains had significant bacterial count in comparison with without dental caries and in children with dental caries. The inter group comparison of bacterial colony count between children with dental caries showed significant results in comparison with stains and without dental caries. There was significant difference between the bacterial colony count results on comparison of without dental caries and children with dental caries and with black stains.

DISCUSSION :

The study was aimed to estimate the levels of S.salivarius in children with black stains and compare it with children with dental caries and without dental caries. The results showed that the average values of the colony count of S.salivarius in group 1 was 216, group 2 was 394.4 and in group 3 was 591which indicates that levels are lower in black tooth stain children. The results show that S.salivarius levels are highest in children with dental caries which is similar with previous studies which shows that S.salivarius has been considered to cause increased dental caries. [14] The majority of the authors confirm the correlation between the presence of black stain and lower caries experience.[15] The microflora of children with black stains is dominated by Actinomyces spp. and showed lower cariogenic potential due to higher calcium concentrations and higher buffering capacity. [16,17]

J.P. Burton,C, did a invitro study to find out the action of S.salivarius on black stains on teeth. The study was done with blood agar vancomycin medium which is semiselective and supports the growth of Actinomycis spc. One half of the agar had been impregnated with both salivaricin A and salivaricin B was used to detect inhibitory action against black-pigmented bacteria. The numbers of black-pigmented bacteria grown from the saliva were decreased by 92% on comparison to control counts which is in correlation with the results of our study. [18]

A study was done by Erin L gross on dental caries and its link with multiple microbial species which was assessed by 16s RNA community analysis. The results showed S. salivarius levels remained high providing support for a role in caries which in turn is in accordance with the results of this study where S.salivarius colony count was highest in children with dental caries.[19]

A Study by André Gasparetto, evaluated the correlation between black extrinsic tooth stains and caries in Brazilian school children. The study was done in 263 children aged between 6-12 years. Black stains were observed in 14.8% of the children. The mean DMFT was 1.46 +/- 1.39 for children with black stains and 2.42 +/- 2.09 for children without black stains. [20]

A Study by André Gasparetto, evaluated the correlation between the presence of black extrinsic tooth stains and caries in Brazilian school children. The study was done in 263 children aged between 6-12 years. Black stains were observed in 14.8% of the children. The number of children with caries-free permanent dentition was not statistically different between groups. The mean DMF-T was 1.46 +/- 1.39 for children with black stains and 2.42 +/- 2.09 for children without black stains. [21]

CONCLUSION

The study shows that the levels of S.salivarius were higher in children with dental caries and found to be low in children with black tooth stains.

LEGENDS

TABLE 1: S.Salivarius colony count in each group
TABLE 2: Intergroup comparison – Post HOC results.
FIGURE 1: Graphical representation of S.salivarius colony forming unit in the three groups.

REFERENCE ARTICLES:

[1] AndrewJoiner. Tooth colour: a review of the literature. j.jdent.2003.10.013
[2] Fawzi Rachid1* and Hariri El Mehdi. Black Stains in Primary Teeth: Overview. Journal of Neonatal and Pediatric Medicine; November 16, 2016 Citation 1:123.
[3] Shah HN, Bonnett R, Mateen B, Williams RA. The porphyrin pigmentation of subspecies of Bacteroides melaninogenicus. Biochem J;(1979) 180: 45-50.
[4] Stenudd C, Nordlund A, Ryberg M, Johansson I, Kallestal C, Stromberg N. The association of bacterial adhesion with dental caries. J Dent Res; 80: 2005-2010.
[5] Koch MJ, Bove M, Schroff J, Perlea P, Garcia-Godoy F, Staehle HJ. Black stain and dental caries in schoolchildren in Potenza, Italy. ASDC J Dent Child;2001- 68: 353-355.
[6] Gasparetto A, Conrado CA, Maciel SM, Miyamoto EY, Chi- carelli M et.al. (2003) Prevalence of black tooth stains and dental caries in Brazilian schoolchildren. Braz Dent J 14: 157-161.
[7] S.Mounika , Nithya Jagannathan, Murali, Association of S.Mutans and S.Sanguinous in dental caries, S.Mounika et al /J. Pharm. Sci. & Res. Vol. 7(9), 2015, 764-766.
[8] Sri Sakthi, Joseph John, Dental caries experience and treatment needs in Chennai workers, johr.in index.php JOHR issue view3.
[9] Southam JC, Soames JV (1993). Dental Caries. Oral pathology (2nd ed.). Oxford: Oxford Univ. Press. ISBN 0-19-262214-5.
[10] Shiere FR, Georgi CE, Ireland RL. A study of Streptococcus salivarius and its relationship to the dental caries process. J Dent Res; 1951 Feb;30(1):116-25.
[11] Jan Kok, ... Véronique Monnet, in Handbook of Proteolytic Enzymes (Third Edition), 2013
[12] Supragingival Microbes In Atlas of Oral Microbiology, 20153.1.6.1
[13] Amid I. Ismail BDS, MPH, DrP Woosung Sohn DDS, P. A Systematic Review of Clinical Diagnostic Criteria of Early Childhood Caries. Journal of public health dentistry;September 1999 1752-7325.
[14] V Paredes Gallardo,C Paredes Cencillo. Black stain: a common problem in pediatrics. J dent Res;2005 Mar;62(3):258-60.
[15] J.van HouteR.J.Gibbons. Adherence as a determinant of the presence of Streptococcus salivarius and Streptococcus sanguis on the human tooth surface. Swed Dent J;10.1016/0003-9969(70)90115-9
[16] Xi Chen,Jing-Yu Zhan,Hai-Xia Lu,Wei Ye,Wei Zhang,Wen-Jie Yang,Xi-Ping Feng Clin. Factors associated with black tooth stain in Chinese preschool children.,. Epub 2014 Jan 16.;18(9):2059-66.
[17] Carlsson, J., Grahnen, H., Jonsson, G. and Wikner, S. (1970) Early establishment of Streptococcus salivarius in the mouth of infants. J Dent Res 49, 415–418.
[18] Wim van Palenstein Helderman Black stain and dental caries in Filipino schoolchildren. Community Dent Oral Epidemiol; 2009 Apr 14;37(2):182-7. Epub 2009 Feb 14.
[19] Erin L. Gross,1 Clifford J. Beall,1 Stacey R. Kutsch,2,3 Noah D. Firestone,1 Eugene J. Leys,1 and Ann L. Griffen.Beyond Streptococcus mutans: Dental Caries Onset Linked to Multiple Species by 16S rRNA Community Analysis. journal.pon;.0047722,PMCID: PMC3472979
[20] André Gasparetto, Ernesto Yiokihiro Miyamoto, Mariliane Chicarelli, Régia Luzia ZanataBraz Dent to J 2003 29;14(3):157-61. Epub 2004 Mar 29.
[21] Drucker DB, Shakespeare AP, Green RM.Head, Neck, and Orofacial Infections in Pediatric PatientsLewis C. Jones, David Shafer, in Head, Neck, and Orofacial Infections, 2016