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Mineral Composition of Saliva in Children with Black Stain

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Abstract

Introduction: The global prevalence of black stain is around 2.4%-21%. Though studies confirm decreased prevalence of caries in children with black stain there is a lacunae of knowledge with respect to the reason behind it. Teeth are composed of minerals and its composition determines the amount of demineralisation and remineralisation. Hence the aim of this study was to compare and assess salivary Copper, Iron, Sodium, Calcium and Phosphorous in children with and without chromogenic stains**.**

Methods: 5 ml unstimulated saliva was collected from 24 subjects;12 subjects with black stains and 12 subjects without black stains respectively. Salivary sample was subjected to laboratory testing for mineral estimation. Minerals assessed were salivary copper, iron, sodium, calcium, and phosphorous.

Results: Mean salivary copper levels were significantly higher whereas mean salivary Iron and sodium levels

were significantly lower in children with black stains. Mean salivary calcium and phosphorous were higher in children with black stain.

Conclusion: Low caries tendency seen in children with black stain may be associated with high salivary copper, calcium and phosphorous levels.Copper can be considered as caries inhibiting element.

Keywords: Black stain, children, Dental caries, Saliva

Introduction

Black stain is a characteristic, extrinsic dental discoloration that occurs on the cervical third of the tooth and follows the contour of the gingival margin. Though it can occur at any age, there is a higher prevalence in children which decreases in adolescence and adulthood.[1]

Black stain deposits are made of an iron compound-ferric sulfide, which is formed as a result of the reaction between hydrogen sulfide (a bacterial byproduct) and the iron in saliva or crevicular fluid.[2]

Black stains have been associated with decreased dental caries [3,4]. There is a lacunae in the knowledge of relation between dental caries and black stain. In addition there is only limited data on the chemical composition of saliva in subjects with black stain. Studies have reported a significantly higher content of total calcium, inorganic phosphates, copper and sodium in children with black stain compared to those without black stain.[5,6], They suggested that the higher salivary levels of calcium and phosphorus in children with black stain were responsible for the decreased caries experience.[5]Spatial chemical analysis using wavelength dispersive spectrometry showed high concentration of copper/iron in black stain suggesting that metal ions form these intensely colored compounds.[7] Thus the aim of this study was to compare and assess salivary Copper, iron, sodium, calcium and Phosphorous in children with and without black stain.

Materials and Method

120 children aged between 5 -12 years were screened and 24 children fulfilling the selection criteria were selected and divided into 2 groups.

Group 1:12 children with black stain

Group 2:12 children without black stain

Thus the aim of this study was to compare and assess salivary Copper, iron, sodium, calcium and Phosphorous in children with and without black stain.

Selection of children with black stain was made based on Lobene stain index.8

Score 0: No stain detected

Score 1: Stain covering up to 1/3rd of region

Score 2: Stain covering >1/3rd to 2/3rd of region

Score 3: Stain covering>2/3rd of region

Selection Criteria

- Children without any systemic diseases.
- Children without any medication including chlorhexidine, iron preparate and antibiotics for at least 1 month.

Saliva sample was collected and the DMFT and dft indices were scored for all 24 children. For primary dentition, the dft index and for permanent dentition DMFT index were used, in accordance with the recommended codes, criteria and biosafety standards of the World Health Organization (WHO).[8]

Dental examinations for the presence of black stains and dental caries was performed by a single trained examiner.

Saliva sample

5ml of unstimulated saliva sample was collected two hours after the last meal. The subjects were asked to rinse thoroughly with distilled water before the collection of salivary samples. Five minutes after the oral rinse, subjects were asked to swallow any residual saliva that may be present in their mouth. Un-stimulated

whole saliva was collected by making the patient to sit in upright position at rest and bow their head. The saliva samples were transported to the laboratory for estimation within 24 hrs using standard gel coolant pack in order to maintain the temperature between 2 ºC to 4ºC.

Laboratory analysis

Estimation of iron was done by Ferrozine method, Copper by Di-Br-PAESA method, sodium by Phosphanazo Method, inorganic salivary calcium by OCPC method and salivary phosphorous by Molybdate U.V. method.[9,10,11,12,13]

Statistical Analysis

Comparison of mean Salivary Copper, sodium and phosphorous levels between children with & without Chromogenic Stain was done using Independent Student t Test. Comparison of mean Salivary Iron and calcium levels between Children with and without Chromogenic Stain was done using Mann Whitney Test.

Results

Results are shown in Table 1

The mean Salivary Copper levels in Children with Chromogenic Stain group was significantly higher [173.405 ± 39.598] as compared to Children without Chromogenic Stain group [98.187 ± 22.563]. This difference in the mean Salivary Copper levels between 2 groups was statistically significant at p;<0.001.

The mean Salivary Iron levels in children without Chromogenic stains was significantly higher [1.1513 ± 0.1954] when compared to Children with Chromogenic Stain [0.8021 ± 0.2449]. The difference in Salivary Iron levels between 2 groups was statistically significant at p=0.007.

The mean Salivary Sodium levels in Children without Chromogenic Stain was significantly higher [102.431 ± 16.644] as compared to Children with Chromogenic Stain [62.889 ± 29.412]. The difference in mean Salivary Sodium levels between 2 groups was statistically significant at p<0.001.

The mean Salivary Calcium and Phosphorous levels in Children with Chromogenic Stain group was relatively higher [Calcium-5.958 ± 2.726; phosphorous-65.673 ± 32.435 respectively] as compared to Children without Chromogenic Stain [calcium-5.639 ±3.384; phosphorous-59.201± 29.102]. However, this difference in the Salivary Calcium and phosphorous levels between 2 groups was not statistically significant [calcium- p=0.80; phosphorous- p=0.45].

Table 2 shows the percentage of children with caries among black stain group and black stain free group.

Discussion

Black stain is a common discoloration in children. Its prevalence is reported to be 2.4-21% in literature.[14] Bhat et al in their study have showed a prevalence of 18% in Indian population among the children aged between 5-12 years. Data shows that there is decreased caries experience in children with black stains but there is a lacunae of knowledge with respect to the reason behind it.[15,16,17,18,19,20] The current study also showed decreased caries incidence in children with black stains.

Saliva plays an important role in maintaining oral health and protecting the teeth from dental caries. Salivary parameters such as pH, buffering capacity, and calcium and phosphate ion concentrations are well-known caries-protective factors.[21]But only limited studies have associated mineral composition of saliva in children with black stains and their effect on caries.

Surdacka et al have reported salivary mineral levels in children with black stain compared to those without black stain.[5] They found higher content of total calcium, inorganic phosphates, copper and sodium in the saliva of children with black stains[11]. This is in accordance with the results of present study.

In the current study salivary copper levels were significantly higher in children with black stains.

Klimuzko et al in their study stated that in the presence of copper ions. dissolution of enamel is reduced.[22] The protective copper phosphate phase formed on the tooth surface decreases and inhibits demineralization through stabilization of the crystal lattice of enamel surface.[23] Furthermore, copper exerts a cariostatic effect through inhibition of the bacterial growth and the various bacterial metabolic enzymes as given below.[24,25]

1. Copper ions act on bacterial cytoplasm causing changes in its redox potential resulting in nitrative stress leading to cell death
2. Copper ions inhibit the expression of biofilm-forming related gene, GTF genes (gtfB, gtfC, gtfD) and *GBP* genes (gbpB, gbpC) of S. mutans.
3. Copper ions irreversibly suppress the activity of S. mutans F-ATPase, affecting the glycolysis ability of bacteria in an acidic environment, resulting in cell death

Khan et al in their systematic review explained that Copper inhibits acid formation in plaque biofilm. It was found that acid production in dental plaque following a mouth rinse with copper sulfate was significantly lower.[26]These findings were supported by randomised control trials conducted by Afseth et al.[27]

Cross sectional studies by Duggal et al and Bhandary et al supports that salivary concentrations of copper were found to be significantly low in high caries activity group.28 Studies by Hussein et al showed that salivary concentrations of copper were low in children with ECC compared to caries free children.29 The above study results regarding copper is in accordance with the results of the present study which showed significantly higher levels of copper in children with black stains with decreased caries activity.

Salivary iron levels were significantly lower in children with chromogenic stain in the present study.

Ferritin being acidic can reduce the salivary pH and the buffering capacity of saliva; this can in turn lead to an increased incidence of dental caries.[31] Low pH has been proposed to cause a shift in acid-tolerant and acid-producing bacterial consortia, favoring the formation of caries lesions(30)

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In the current study, salivary sodium levels were significantly reduced in patients with black stain.

Siddharths chandel et al[31] stated that bacterial acids attack mineral phase of teeth causing sodium to diffuse out from crystal apatite resulting in increased salivary levels. This suggests that sodium increase by itself is not a causative factor, rather its resultant of carious process.

In the present study, salivary calcium and phosphorous levels in children with black stain were higher.

Surdacka et al in their study suggested that the higher salivary levels of calcium and phosphorus in children with black stain were characteristics of subjects with low susceptibility to caries.[12] Salivary calcium and phosphorous indirectly regulates the aggregation of microorganism in saliva to help maintaining the globular structure of salivary micelles." The micelles are composed of salivary immune system proteins such as low molecular weight mucin, sIgA, lactoferrin, amylase, glycosylated proline rich proteins and lysozyme."32 In this present study, the increase in salivary calcium and

phosphorous levels in children with black stain may contribute to form more salivary micelles and a reservoir for remineralization.

Conclusion

Among various minerals assessed Copper levels were significantly high in saliva among children with black stain. The results of present study suggested that low caries tendency seen in children with black tooth stain may be associated with significantly high copper levels which precipitates a protective copper phosphate phase on the tooth surface decreasing and inhibiting demineralization as well as high salivary calcium and phosphorous levels which enhances buffering capacity. More studies needs to be conducted on Copper as this study shows that copper is not a caries promoting trace element as it was considered earlier.

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Legend Tables

Table 1: Summary of comparison of mean salivary mineral levels between children with and without chromogenic stain

Element	Groups	N	Mean	SD	Mean Diff	p-value
Copper (in ng)	Children with chromogenic stain	12	173.405	39.598	75.218	<0.001*
	Children without chromogenic stain	12	98.187	22.563		
Iron (in µg)	Children with Chromogenic Stain	12	0.8021	0.2449	-0.349	0.007*
	Children without Chromogenic Stain	12	1.1513	0.1954		
Sodium (in µg)	Children with Chromogenic Stain	12	62.889	29.412	-39.542	<0.001*
	Children without Chromogenic Stain	12	102.431	16.644		
Calcium (in µg)	Children with Chromogenic Stain	12	5.958	2.726	0.319	0.80
	Children without Chromogenic Stain	12	5.639	3.384		
Phosphorous (in µg)	Children with Chromogenic Stain	12	65.673	32.435	6.472	0.45
	Children without Chromogenic Stain	12	59.201	29.102		

Table 2: Percentage of children with and without caries among those with black stain and without black stain

CARIES ACTIVITY	WITH BLACK STAIN	WITHOUT BLACK STAIN
Children with caries	25%	84%
Children without caries	75%	16%