Differences in Calcium and Phosphate Levels in the Saliva of Children with and without Black Dental Stain

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Abstract

Black dental stain is a discoloration of the teeth, which appears as a black-pigmented line or collection of black dots on the cervical third of the tooth crown. Tooth plaque with black dental stain contains higher calcium and phosphate concentrations compared to tooth plaque without black dental stain. The calcium and phosphate composition of saliva is suspected to be the cause of calcium and phosphate level elevation in black stained dental plaque. This study aimed to determine the difference in calcium and phosphate levels of saliva from children with black dental stain and without black dental stain. The subjects were children aged 4–8 years, and as many as 30 children, consisting of 15 children with black dental stain and 15 children without black dental stain, were included in the study. The samples of calcium and phosphate were obtained from the children's saliva. The levels of calcium and phosphate were measured using a UV-Vis spectrophotometer. The results showed, that the levels of calcium and phosphate in the saliva of children with black dental stain were significantly higher compared to the levels found in the saliva of children without black dental stain.

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Introduction

Tooth discoloration is considered one of the most common problems in human dentition. The problems it causes are related to both clinical and esthetic aspects. Tooth discoloration is caused by food deposits and drinks, other chemical substances, necrotic pulp, and systemic disorders.[1] Black dental stain is a type of tooth discoloration caused by extrinsic factors. This stain can be diagnosed by the presence of a black-pigmented line parallel to the gingival margin or black spots on the cervical third of the tooth crown.[2,3,4]

Black dental stain is different than dental plaque which appears in the oral cavity without black dental stain. The difference lies in the chemical composition and the type of bacteria. Black dental stain consists of insoluble ferric salts and high concentrations of calcium and phosphate.[2,3] The bacterial composition of black dental stain is dominated by Actinomyces spp. and Prevotella melaninogenica.[2,3]

The levels of calcium and phosphate in plaque on teeth with black dental stain are known to be higher when compared to plaque on teeth without black dental stain. The cause of the higher levels of calcium and phosphate, in plaque on teeth with black dental stain, is still unknown.[5] In several studies, however, the occurrence happened because of the overpressure on the surface of the tooth enamel, causing the enamel surface to lift, and resulting in high levels of calcium and phosphate in the black dental stain.[6] One of the sources of calcium and phosphate in the human oral cavity is saliva.[6-8]

The composition of saliva in the oral cavity can affect the condition of the plaque and also cause stains on human dentition.[8] It was concluded that high levels of calcium and phosphate, in black dental stain, were probably affected by the level of calcium and phosphate in the saliva. This study aimed to determine the level difference of calcium and phosphate in the saliva of children with black dental stain and without black dental stain.

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Materials and methods

This research was laboratory observational research. Research samples were the saliva of children, obtained from children in the age range of 4–8 years, with black dental stain and without black dental stain. The variables were the levels of calcium and phosphate in the saliva of children with black dental stain and without black dental stain. The numerical data collected for the study was in the form of concentrations (ppm). Normality tests were carried out on the data using the Saphiro-Wilk test, and after this a significance test was done using either the Mann-Whitney U test or the independent t-test, depending on the result of the normality test. Samples were collected from five primary schools and one kindergarten in Jakarta. These schools were SD Strada Van Lith I, SD Jubliee, SD St. Cicila Ancol, SD Ibnu Sina, SDIT Insan Mandiri, and TK Tunas Bangsa. Furthermore, sampling was also undertaken in the subjects' houses. Laboratory procedures were carried out in the Centre for Nano Biotechnology Laboratory, University of Indonesia. UV-Vis spectrophotometer was used in this research. Materials used for the research were the saliva from children with and without black dental stain on the enamel, CaCl2.2H2O, KH2PO4, HNO3, HClO4, H2SO4, NaOH, murexide, ammonium molybdate, SnCl2.2H2O, and aquabides. Before sampling, informed consent was given by the parents of the subjects. Afterwards, an intraoral examination was undertaken for subject selection. The sampling was done by asking the subjects to spit into sterile containers. The sterile containers, that contained saliva, were then brought to the laboratory to be analyzed.

Before the sample analysis took place, the samples were destructed by first using nitrate acid and perchlorate acid to remove organic elements in the saliva samples. Afterwards, masking agents were added to the samples so they could be analyzed using the UV-Vis spectrophotometer. The masking agents used were: murexide for calcium, and ammonium molybdate and tin chloride for phosphate. A standard solution was made using a standard dilution method for calcium and phosphate until 100 ppm standard solutions were obtained. Later, a maximum wavelength was measured to find the wavelength that would be used in the measurement by the UV-Vis spectrophotometer. Next, a variation of standard solutions was made by diluting the 100 ppm-standard solutions into 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm-standard solutions. The absorbance of the standard solution variations was measured, using the maximum wavelength, in order to find the standard curve, for each element. Afterwards, the samples, that had been prepared, were measured using the UV-Vis spectrophotometer so that the absorbance data, which were later converted into concentration indexes (ppm) using the linear equation of the standard curve, could be obtained.

Results

From the population of 378 children, 32 children matched the criteria for the study. These consisted of 16 children with black dental stain and 16 children without black dental stain. After obtaining the informed consent approvals and biographical data of the children who matched the criteria for the subject, the subject's saliva was collected. Measurement of the maximum wavelength resulted in 511 nm for calcium and 649 nm for phosphate. Calcium and phosphate level data was first tested using the normality test. The normality test used for the study was the Shapiro-Wilk analytical test. From the normality test, it was found that the calcium levels of children with black dental stain and without black dental stain were not distributed normally, so for further data analysis the Mann-Whitney test was used. On the other hand, the phosphate levels of children with black dental stain and without black dental stain were distributed normally, so for further data analysis the independent t-test was used.

Element	Group	Mean±SD	p-value
Calcium	Black dental stain	91.133±5.052	0.000
	Without black dental stain	82.445±2.937	

Table 1. Mean, standard deviation, and differential test values of calcium levels.

From the results, shown in Table 1 it was found that the quantity of calcium in the saliva of children with black dental stain was higher than children's saliva without black dental stain. To test this hypothesis, the Mann-Whitney test was carried out and a p value of < 0.05 was obtained for the calcium levels. This result showed that

there was a significant difference between the calcium levels in children with black dental stain and children without black dental stain.

Element	Group	Mean±SD	*p*-value
Phospate	Black dental stain	12.996±1.848	0.000
	Without black dental stain	9.435±1.286	

Table 2. Mean, standard deviation, and differential test values of phosphate levels.

The result in Table 2 showed that the quantity of phosphate in the saliva of children with black dental stain was higher than that of children without black dental stain. To test this hypothesis, an independent t-test was carried out and a *p* value of <0,05 was obtained for the phosphate levels. This result showed that there was a significant difference between phosphate levels in saliva of children with black dental stain and children without black dental stain.

Discussion

This research was a study concerning the difference between calcium and phosphate levels in the saliva of children with black dental stain and without black dental stain. Finding the difference between calcium and phosphate levels in the saliva of the children could be initial research into the etiology of black dental stain. Previous studies have proved that plaque with black dental stain contains higher calcium and phosphate levels compared to plaque without black dental stain.[6] However, the real cause of this occurrence is still unknown.[6] Therefore, this study used saliva samples that could affect the composition of plaque and stains which appear in the human dentition. Samples for this study were obtained from children in the age range of 4–8 years, because, based on previous studies, black dental stain is predominantly found on children's teeth during the stage of primary and mixed dentition.[4] This is related to the primary tooth's characteristic of having higher permeability and porosity compared to permanent teeth.[9] The collected saliva was unstimulated so that the concentrations of calcium and phosphate in the saliva was not affected. The subjects were not allowed to eat or drink for at least two hours before sampling. This aimed to maintain the real concentration of calcium and phosphate in the saliva because saliva secretion tends to be normal two hours after eating.[10]

A UV-Vis spectrophotometer was used to measure the calcium and phosphate levels in the saliva. The UV-Vis spectrophotometer was used for the quantitative analysis of calcium and phosphate because this tool has high precision, is fast, is an accurate process, and can be used for many organic and inorganic substrates. Before measuring the calcium and phosphate levels, samples were destructed by a wet destruction method to remove organic substrates which could disrupt the reading process of calcium and phosphate levels by the spectrophotometer. As discussed above, the UV-Vis spectrophotometer is a tool that can detect elements that have colors with wavelengths ranging from 130 nm–780 nm.[10] Therefore, complexing agents were applied in order to produce different colors for calcium and phosphate that could be distinguished during the reading process by the UV-Vis spectrophotometer.[10] The results showed that calcium and phosphate levels in the saliva of children with black dental stain were significantly different compared to the saliva of children without black dental stain. This result showed that high calcium and phosphate levels in plaque with black dental stain might have been affected by the saliva condition in the oral cavity, which has higher calcium and phosphate levels than saliva of children without black dental stain. As stated before, the plaque composition is affected by the condition of the saliva in the oral cavity.[8]

Calcium and phosphate, in saliva, are elements that contribute to the process of tooth remineralization. High calcium and phosphate levels in the saliva of children with black dental stain could increase the rate of tooth remineralization. Moreover, phosphate also has a role in maintaining pH in the oral cavity because of its buffering function, so that the acidic condition can be avoided.[11] Organic and inorganic elements can enter dental plaque during the formation of plaque.[8] High calcium and phosphate levels in saliva can also enter plaque during its formation, so that the plaque has a relatively high pH. Calcium and phosphate minerals could adhere during the initial phase of plaque formation in the pellicle formed on the surface of the tooth. Furthermore, calcium and phosphate could adhere directly to the plaque surface or through fluid channels, which are located on intermicrobial matrix.[8] High pH levels can affect the bacterial composition of plaque, which

commonly has an acidic condition. Streptococcus mutans is a bacteria known to cause dental caries, and it is one of the most dominant bacteria in plaque. High pH levels can inhibit the growth of bacteria because Streptococcus mutans optimally grows in acidic environments.[12] On the other hand, high pH levels can have a positive effect on the growth of Actinomyces spp. in plaque, which is known to not be resistant to acid.[12] Actinomyces spp. also has the ability to inhibit the attachment of Streptococcus mutans.[12] Presumably, this could explain the dominance of Actinomyces spp. bacteria which are assumed to be the main bacteria involved in the formation of black dental stain.

Conclusion

Based on the results and discussion above, it was concluded that the levels of calcium and phosphate in the saliva of children, with black dental stain, were significantly higher than saliva of children without black dental stain. These higher levels of calcium and phosphate created an increase in the pH of the oral cavity that could modulate the growth of Actinomyces spp., which was assumed to be the main bacteria involved in the formation of black dental stain. Further study is required to discover more about the formation of black stained dental plaque, so that the cause of high calcium and phosphate levels in black stained dental plaque can be discovered. Furthermore, more study is required into diet or other nutritional intakes of children with black dental stain, in order to find an explanation of the high calcium and phosphate levels in the saliva of children with black dental stain in comparison to children without black dental stain.

Declaration of Interest

The authors report no conflict of interest.

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