

Estimation of Copper Levels in Saliva and Its Relation to Dental Caries and Hemoglobin Levels

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ABSTRACT

Context: A very small quantity of trace elements will be present in saliva. Salivary composition influences caries inhibition and promotion based on the different organic, inorganic, and trace elements.

Aim and objective: To evaluate the levels of copper in saliva in children with caries-active and caries-free group and to associate copper levels of saliva with hemoglobin (Hb) levels of blood.

Materials and methods: Sixty subjects between the ages of 6 and 14 years were divided into group I—caries-free and group II—caries-active. The caries-active group was further subdivided into group IIa (deft/DMFT < 3) and group IIb (deft/DMFT > 3). To maintain the uniformity of salivary composition, unstimulated saliva (5 mL) was collected in noontime before food from all the participants. The copper levels were estimated by atomic absorption spectrophotometry. Hemoglobin levels in the blood and their relation with copper levels in saliva were estimated.

Statistical analysis: The results thus obtained were analyzed by *t*-test and Karl Pearson's correlation coefficient. It was considered significant if the "*p*" value is 0.05 or less.

Results: Copper levels were higher in group II showing statistically highly significant results compared with that of group I with $p \leq 0.00001$. The results were no significant with respect to Hb scores among the groups.

Conclusion: A definite positive correlation is seen with dental caries and levels of copper in saliva. Hence, the amount of copper in saliva can be considered as a caries risk assessment tool.

Keywords: Caries, Copper levels, Hemoglobin, Saliva.

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INTRODUCTION

Dental caries is a challenging disease that affects regardless of age, gender, and ethnicity of world's population. While this is not the case of a life-threatening condition, it can cause significant pain, misery, and tooth loss which has for reaching impact in terms of eating, social behavior, and speaking. Dental caries is a multifactorial disease in etiology and depends on the interaction of three main factors; microbial, substrate, and host (tooth and saliva).¹

The key elements of saliva are mainly 99% of water and 1% of organic, inorganic, and trace elements. These trace elements are found in relatively very low concentrations.² The existence of salivary components specifically the concentration of trace elements present in saliva has been proven to have a direct impact on caries. Previous studies have shown the positive as well as the negative correlation of copper levels in saliva and caries-active children.³⁻⁵

Although the influence of salivary copper levels and blood hemoglobin (Hb) on caries prevalence an inconclusive subject, we have quite enough data to justify the effort in continuing and increasing the research in this area. Hence, the present study was undertaken to evaluate the copper level in individuals mixed saliva to determine possible effect on caries as well as to correlate copper levels of saliva with Hb levels in the blood.

MATERIALS AND METHODS

Research Design

The research was carried out in Sri Siddhartha Dental College and Hospital, Karnataka. The institutional ethical board approved the

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research design and protocol. The consent for participation in the study was taken from the parents or legal guardians of the subjects. All participants were healthy with no history that affects salivary secretions due to local and systemic disease. The subjects had different cultural norms related to diet and similar oral hygiene habits. The criteria to evaluate the caries status were done according to the dental caries index (WHO 1997).

The sample size was calculated using this formula $N = Z^2 P(1 - P) / d^2$ based on results of the pilot study, where *N* = sample size, *Z* = statistic for a level of confidence, *P* = expected prevalence or proportion, *d* = precision. Data and sample collection were carried out during school

hours over a period of 3 months (May–August 2019). Before the study inter- and intra-examiners calibration were conducted. Kappa statistics revealed that the inter- and intra-examiners agreement were good (0.7) and excellent (0.85), respectively. Children between the ages of 6 years and 14 years meeting inclusion criteria were screened by one examiner at their schools. Sixty subjects were selected for this cross-sectional study, the subjects were divided into group I—caries-free and group II—caries-active, further caries-active group was subdivided into two groups (group IIa and group IIb) based on the deft/DMFT score as, group IIa ($n = 15$, deft/DMFT < 3) and group IIb ($n = 15$, deft/DMFT > 3). Assessment and recording of caries experience were done using deft and DMFT indices for primary and permanent dentition, respectively.

Saliva Collection

Unstimulated saliva of 5 mL was collected by another examiner from all the participants in the noontime before food to prevent or minimize the effects of diurnal variability in salivary composition. Children were provided with distilled water to rinse their mouth thoroughly to minimize food debris and they were asked to sit in a relaxed position on a chair to avoid anxiety. After resting for 5 minutes with minimized orofacial movements children spit gently into clean, sterile tubes. From each child, 5 mL of unstimulated whole saliva was collected without repeating the procedure. Each sample was coded and the researcher performing the trace elements detection method in mixed saliva was uninformed about the individual dental health status. Once samples arrived at the laboratory, with the help of the pipette 0.5 mL of concentrated nitric acid (HNO_3) was measured and added to the saliva to drop the pH < 2 and prevent microbial growth and enzymatic alteration. The saliva was centrifuged for 10 minutes at 4,000 rpm to eliminate any food debris or mucin clot that could clog the atomic absorption spectrophotometer (AAS) capillary tube. The obtained sample was added with 5 mL of deionized redistilled water, in sterile containers, a total of 10 mL of saliva sample taken from a test tube was stored at 4°C for subsequent analysis.²

Copper Estimation

Copper value was calculated using atomic absorption spectrophotometry (Avanta GBC, Hampshire, USA). In an experimental solution, a known concentration of the element created a concentration curve, whereas an unknown concentration of the element was obtained from a calibration curve.

Hb Estimation

Hemoglobin levels in the blood of the children were assessed by a fingerprick test. The Hb levels in the blood and their relation with copper levels in saliva were estimated.

Statistical Analysis

The test used to correlate copper levels in saliva and dental caries between the group is the one-way ANOVA test. It was considered significant if the “ p ” value is 0.05 or less. The data are presented as a mean \pm standard deviation. Karl Pearson’s correlation coefficient method was used to correlate copper levels in saliva and Hb.

RESULTS

The mean copper levels in saliva of group I ($n = 30$) were 0.06 ± 0.02 and those of group IIa ($n = 15$) and IIb ($n = 15$) were 0.18 ± 0.10 and 0.37 ± 0.17 , respectively, and are expressed in $\mu\text{g/dL}$. Statistically

highly significant ($p < 0.05$) “ p ” value was obtained. The copper levels in saliva increased with caries prevalence (Table 1). The mean values of Hb levels were non-significant when compared among the groups (Table 2). The correlation between DMFT/def, copper ($\mu\text{g/mL}$), and Hb (g%) in total samples by Karl Pearson’s correlation coefficient showed a positive correlation with caries and copper levels in saliva, there was a negative correlation between copper and Hb levels (Table 3).

DISCUSSION

Dental caries is a multifactorial disease. Normal physiologic activity of oro-biological structures is maintained by salivary fluids secreted from salivary glands. The presence of various types of organic, inorganic, and trace elements in saliva that affect dental caries has been reported in previous studies.^{1–5}

Unstimulated whole saliva was sampled in this study. Unstimulated whole saliva has many advantages over-stimulated gland-specific saliva which makes it more convenient and preferable and it also fulfills demand that diagnostic aids for oral and systemic diseases be acceptable, simple to use, and non-invasive. Also, unstimulated whole saliva is the predominant condition in terms of salivary gland activity and is the primary determinant for oral clearance which gives a strong protective effect against caries. Hence, unstimulated whole saliva is more important than stimulated saliva in the development of various processes.⁵

In this study, we have emphasized the role of trace element copper in the prevalence of caries and also evaluate its relation with Hb. The study showed that the increase in copper levels in saliva was directly proportional to caries experience and Hb levels do not influence caries. The finding is in accordance with Hussein et al.’s research, where they found that levels of zinc and copper in saliva were significantly greater in children with dental caries

Table 1: Comparison of group I, group IIa, and group IIb with respect to copper ($\mu\text{g/mL}$) scores by one-way ANOVA test

Groups	N	Copper ($\mu\text{g/dL}$)	p value
Group I	30	0.06 ± 0.02	$p < 0.00001^*$
Group IIa	15	0.18 ± 0.10	
Group IIb	15	0.37 ± 0.17	

* $p < 0.05$, significant

Table 2: Comparison of group I, group IIa, and group IIb with respect to Hb (g%) scores by one-way ANOVA test

Groups	N	Hb (g%)	p value
Group I	30	12.17 ± 1.11	$p < 0.4158$
Group IIa	15	12.35 ± 1.37	
Group IIb	15	11.81 ± 0.83	

* $p < 0.05$, significant

Table 3: Correlation between DMFT/def, copper ($\mu\text{g/mL}$), and Hb (g%) in total samples by Karl Pearson’s correlation coefficient

Variables	DMFT/def	Copper ($\mu\text{g/mL}$)	Hb (g%)
DMFT	–		
Copper ($\mu\text{g/mL}$)	$r = 0.8900^*$	–	
Hb (g%)	$r = -0.0827$	$r = -0.0485$	–

* $p < 0.05$

compared with those who were not.² A study was done by Hedge et al. in caries-free and caries-active children reported that there is a statistically significant association with copper and zinc levels in saliva and dental caries in children. He concluded that higher copper and zinc levels in saliva will lead to dental caries.⁶ Watanabe et al.'s study further showed that the levels of copper in saliva were substantially greater for children with caries-active than that of the caries-free group children.⁵ Interestingly, some of the studies have highlighted the inverse relationship between copper and the occurrence of caries, this could be possible due to high fluoride levels in saliva.^{2,4}

The presence of copper and fluoride in saliva influences the enzyme system involved in carbohydrate degradation (enolase activity) and dental caries activity. Also, there are limited studies about Hb level and dental caries in children. Investigations are done in children with early childhood caries were found to be in association with serum iron levels. Iron deficiency is responsible for salivary function impairment, which in turn reduces salivary secretion, salivary flow rate, and low buffering capacity. It has been suggested that variations in the levels of free radicals, saliva antioxidants, and reactive oxygen species have all been attributed to the initiation and progression of dental caries.^{7,8} In our study, the mean values of Hb levels were non-significant when compared among the groups, also there was a negative correlation between copper and Hb levels.

Salivary components released in saliva are thought to be vital for oral and dental health. Internal defense components such as saliva, tooth surface morphology, overall health, nutritional and hormonal state, as well as many external elements such as fluoride sources, oral hygiene, and diet are all involved in the complex process of dental caries. Quantitative X-ray fluorescence imaging of sections of human teeth indicated an increase in copper concentration in various regions of dentine compared with the sound tooth.^{9,10} The limitation of the study is children from one particular school were included in this study. Research on a large group is required in this subject, which helps in early diagnosis, caries risk assessment in children, and prevention of caries.

CONCLUSION

From the above study, it can be concluded that copper has a positive correlation with dental caries activity suggesting the possibility

of their effect in the formation of decay. There is an association between high salivary copper levels and greater caries experience. Copper levels in saliva can be used as a caries risk assessment tool. However, more studies are required on this subject among multi-ethnic group children to conclude the same.

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