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RESEARCH ARTICLE

CORRELATION OF TRACE ELEMENTS IN SALIVA WITH DENTAL CARIES ACTIVITY

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ABSTRACT

Saliva is the first line of defence against caries. Hence it is very important to understand the physiochemical nature of saliva so as to use its beneficial effect to combat dental caries. The saliva contains a lot of organic and inorganic substances, but in addition to this there are trace elements. The present study is concentrated on the trace elements zinc and copper present in saliva and the correlation of these elements with dental caries activity. The study included 30 individuals; 15 with low caries activity and 15 with high caries activity. Every patient was screened and unstimulated saliva was collected. The samples were centrifuged and the supernatant fluid was subjected to atomic absorption spectrometry. The results from the experiment revealed that the amount of copper and zinc in saliva of low caries individuals was higher than the high carious individuals. When the data was subjected to statistical analysis it was evident that zinc had a high significant value whereas copper was not so significant. Hence it is concluded that copper and zinc has negative correlation on dental caries, but only zinc has a significant effect.

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INTRODUCTION

Dental caries is a multifactorial disease. Classic Venn diagram schematically sums of the factors i.e, Diet, host (saliva and tooth), bacteria and time are responsible for the development of dental caries. The first line of defence against dental caries is saliva (Zahir *et al.*, 2006). The general term "saliva" refers to the fluid that surrounds all oral hard and soft tissues. This oral fluid (that is, whole saliva) represents the mixture of individual fluids and components derived from several sources. Major and minor salivary glands make the bulk contribution to whole saliva, with minor contributions from nonglandular sources such as crevicular fluid, oral microorganisms, host derived cells, and cellular constituents, as well as diet-related components. Basic salivary research relevant to the development of caries has provided considerable understanding of various salivary anticariogenic mechanisms. Saliva plays a fundamental role in maintaining the physical-chemical integrity of the tooth enamel by modulating remineralisation and demineralization (Cataldo *et al.*, 2008). It consists of approximately 99% water, containing variety of electrolytes (sodium, potassium,

calcium, chloride, manganese, bicarbonate, phosphate) and protein represented by enzymes, immunoglobulin etc. In addition to these organic and inorganic components there are some trace elements. Trace elements are present in saliva in very minute amounts that is in microgram/gram or less in concentration. Six of this namely, iron, iodine, copper, cobalt, manganese and zinc, have been shown to be essential to animal life. Investigations conducted on the bacterial level using chemically defined synthetic media indicate not all species of organisms require the same trace minerals, and that in some instances those which are beneficial to the host may be detrimental to bacteria. As producers of acids capable of decalcifying tooth structure, certain bacteria have been long identified with the pathogenesis of human dental caries. Extensive studies have been made in an effort to elucidate the factors restricting the growth of such organisms in human saliva.

That some of the trace elements might affect dental caries activity is suggested by the findings of MacLeod and Snell and of Stephan (Patricia Del Vigna de Almeida, 2008; Samuel Dreizen *et al.*, 1951). Various investigations suggest that trace elements influence the susceptibility of tooth to dental caries (Curzon *et al.*, 1970; Derise *et al.*, 1974; Maltz *et al.*, 1988 and Rosalen *et al.*, 1996). Many authors have suggested that concentration of zinc is higher in non carious teeth as compared to carious teeth. Also it has

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been suggested that remineralisation effect can be achieved in the initial stages of caries by oral applications of zinc preparations (Khrosh *et al.*, 1966; Fang *et al.*, 1980). Many observations suggest that presence of copper in teeth may be important. A number of investigators have studied copper ion or copper salt and its influence on dental caries (Brookes *et al.*, 2003; Abdulla *et al.*,). There is also some clinical indication that high concentration of copper in enamel may produce resistance to dental caries (Brookes *et al.*, 2003; Brudevold *et al.*, 1955 and Abdulla *et al.*,). Very few studies are present about trace elements present in saliva. Present study is an attempt to make an estimation of trace elements- copper and zinc in saliva of patients and to correlate it with dental caries activity.

MATERIALS AND METHODS

The present study was conducted on the patients visiting the OPD of Department of conservative dentistry and endodontics, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore. The patient was screened for dental caries activity. The procedure was explained to the patients to be analyzed and informed consent was obtained from them. Healthy adults, not taking any medication that would affect the microflora or the salivary flow rate, and with minimum unstimulated salivary flow of 0.25ml/min were included in the study. Patients with epilepsy, risk of endocarditis, or hemophilia and patients with silver amalgam restorations were excluded from the study.

The grouping for the studies was as follows:

- Group 1. Low caries activity (2 or less decayed teeth)
- Group 2. High caries activity (3 or more decayed teeth)

15 patients was allotted to each above mentioned group. Patients were allowed to sit on the chair in a relaxed position.

transferred to test tubes and then loaded onto the laboratory centrifuge. Then it is centrifuged at the 2000 rpm. After the centrifuge the saliva is divided into 2 layers. The upper supernatant layer was collected. And then stored in the refrigerator. About 0.5 ml of filtered saliva was collected in a test tube. About 1 ml of HNO₃ measured with the help of the pipette was then added to the saliva. The volume is made upto 10ml by adding double distilled water to the collected sample. A total of 10ml of each sample was analyzed. All 30 samples were scrutinized for estimation of accurate percentage of zinc and copper present in saliva will be estimated by atomic absorption spectrometer in Nitte Mahalinga Adyanthaya Memorial Institute of Technology, Nitte and the analysis was done. Data was obtained which was subjected to the statistical analysis using Student 't' test.

RESULTS

According to the statistical analysis, it was found that zinc and copper were found to be comparatively higher in patients with low caries activity. The mean values for zinc was 118.1867 and 60.4267 in low and high caries respectively. The mean values for copper was 0.5600 and -3.5733 for low and high caries activity respectively (from table-1). And the significance values for zinc and copper are given in table-2.

Between 2 groups Zinc had a high significant value (P=0.005) whereas copper did not have significant variation (P=0.478). Henceforth, a negative correlation was found between copper and zinc in saliva and dental caries activity.

Table 1. Mean values of zinc and copper

	1-low caries activity	N	Mean	Std. Deviation	Std. Error Mean
	2-high caries activity				
Zn(µg/dl)	1.00	15	118.1867	45.28787	11.69328
	2.00	15	60.4267	58.78376	15.17790
Cu(µg/dl)	1.00	15	.5600	12.75241	3.29266
	2.00	15	-3.5733	17.93733	4.63140

Table 2. Independent Samples Test for significance values of Zn & Cu

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower		Upper
Zn	Equal variances assumed	1.33	.258	-3.015	28	.005	-57.7600	19.1598	-97.0072	-18.5127
	Equal variances not assumed			-3.015	26.29	.006	-57.7600	19.1598	-97.1226	-18.3973
Cu	Equal variances assumed	1.84	.185	.727	28	.473	4.1333	5.6825	-7.50686	15.7735
	Equal variances not assumed			.727	25.27	.474	4.1333	5.6825	-7.56372	15.8303

The use of a suction apparatus that collects saliva from a specific salivary gland was rejected in favor of a method that would produce a mixed saliva sample contributed by each of the three salivary gland. Unstimulated saliva was collected in marked test tubes. The collected sample was

DISCUSSION

Human saliva is an unique secretion of major and minor salivary glands to maintain normal physiological functions of oro-biological structures. Apart from the organic and

inorganic components there are trace elements in it. Trace elements are present in biological substances and fluid in minute amounts i.e, microgram per gram or less in concentration. Very few studies were undertaken about inorganic and trace elements present in human saliva. Present study is attempted to make an assay of zinc and copper in saliva. The composition of saliva is potentially of great importance to the prevention of caries (Zahir *et al.*, 2006). The relationship that trace elements in saliva might have to dental caries activity has interested scientists for many years. Conflicting reports from researchers who have investigated copper and zinc in saliva indicate that the nature of the role that these elements play in the carious process remains undefined. An intimate and perhaps significant relationship between the trace elements copper and zinc and the number of decayed tooth surfaces is indicated by the statistical results obtained in this investigation (Irving Green *et al.*, 2010).

In the present study copper was chosen because many observations suggest that its presence in teeth may be important. For instance, enamel with brown coloration has been found to have a high copper content. This observation, together with the finding that trace amounts of copper in protein may catalyze enzymatic pigment formation, suggests that the presence of copper may affect the coloring of teeth. There is also some clinical indication that high concentrations of copper in the enamel may produce resistance to dental caries. Further support for this theory is provided by the laboratory findings that copper ions in concentrations of less than 5 ppm will exert an inhibitory effect on salivary acid production and that certain copper salts will reduce the solubility of powdered enamel. However, with regard to the latter observation there is still some uncertainty, because no reduction in solubility was observed after application of a copper salt to intact enamel. It should be noted that copper has been found to be normally present in small amounts in saliva so that a continuous pick up of copper by the enamel is possible (Abdulla *et al.*). Many nutritional deficiencies such as protein, vitamin A and certain minerals have profound effects on tooth development and on susceptibility of teeth to caries. In a tentative classification suggested by Navia *et al.*, zinc was considered to be a doubtful element among trace minerals that inhibit caries development. However, Lin and Liu observed increased dental caries along with a decrease in plasma zinc level in contraceptive steroid treated rats.

Furthermore, zinc deficiency has been related to certain oral diseases such as taste impairment in humans and parakeratosis in the buccal epithelium of the experimental animals. Other researchers have shown that certain subjects with hypogeusia (loss of taste acuity) exhibited lower zinc content in serum and in parotid saliva. Serum and parotid saliva levels, taste perception and taste bud anatomy returned to normal in some of these patients after zinc treatment (Khrosh *et al.*, 1966; Derise *et al.*, 1974). The role of zinc in development and mineralization of tooth; and the effect of this trace element on dental decay is the matter of curiosity (Fang *et al.*, 1980). In the present

study it was found that concentration of copper in saliva with low caries activity was high. This is in accordance with Derise_ *et al.*, 1974; Duggal *et al.*, 1991. Also, the ability of Cu^{2+} to inhibit cariogenesis in animals has been well-documented Maltz and Emilson, 1988; Rosalen *et al.*, 1996a,b. The mechanism involved has been attributed to the antimicrobial properties of Cu^{2+} , which include acute loss of bacterial intracellular K^+ and inhibition of H^+ -ATP synthase, inhibition of various bacterial metabolic enzymes through oxidation of key thiol groups (Maltz and Emilson, 1988), and formation of insoluble Cu-P salts on the tooth surface, thereby increasing its acid resistance. In addition, Brookes *et al.* (2003) reported that Cu^{2+} directly inhibited the acid dissolution of human enamel *in vitro*, suggesting that the anti-caries properties of Cu^{2+} could be due to a combination of its antimicrobial effects and its ability to inhibit demineralization directly (Abdulla *et al.*)

However, Some investigators, such as Brudevold and Steadman (Brudevold *et al.*, 1955), have claimed that copper has no inhibitory effect on dental decay. They found that the addition of copper ion concentration in amounts far greater than the amounts present in saliva did not depress the acid production in saliva (Irving Green *et al.*, 2010). Present study showed Zn concentration in saliva was 60-118 $\mu\text{g}/\text{dl}$. Percentage of salivary Zn was detected to be higher by various investigators (Zahir *et al.*, 2006). Zinc deficiency can also reduce the concentration of zinc at the enamel surface to such an extent that the susceptibility of the tooth to dental caries was increased.

Thus dietary zinc may be a very important trace element in the process of post-eruptive mineralization of the enamel and may also be responsible for reducing the susceptibility of tooth to dental caries (Fang *et al.*, 1980). In comparing the values obtained by other investigators for copper and zinc in saliva with the values obtained from this experiment, the following factors should be considered: the method implemented to quantitatively determine the concentration of the trace elements in saliva; the number of saliva samples used in the various studies; and the age of the subjects who provide the saliva samples for analysis. The exact mechanism of the interrelationship between copper and zinc in saliva and dental caries is still a matter of conjecture (Irving Green *et al.*, 2010). Extensive research *in vivo* under various conditions are required to understand more the subject.

Conclusion

By the above study it is concluded that zinc and copper has a negative correlation with dental caries activity. That is the amount of zinc and copper in low carious individuals is high whereas it is low in case of high caries individuals. This implies that zinc and copper has a negative effect on dental caries. But by statistical analysis it is understood that zinc has higher significance than copper in this study.

Implications

Dental caries is among the important public health problem. Understanding the etiology and factors affecting the dental caries activity requires a multidisciplinary approach. By analyzing the elements affecting caries activity as is done in this study, it can be derived that these elements (zinc and copper) can be incorporated into oral supplementation and applications like mouth rinse and toothpaste to produce an inhibitory effect on caries activity and beneficial effect on tooth structures.

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