Estimation of Trace Elements in Mixed Saliva of Caries Free and Caries Active Children

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Objective: *Composition of saliva plays an important role in defining the dental caries risk for an individual. The role of trace elements present in saliva on dental caries is still not clear. Thus this study was designed to evaluate the effect of certain salivary trace elements like copper, potassium, fluoride, selenium and lead on the caries experience of children.* ***Study Design:*** *60 subjects in the age group of 3-15 years were selected and divided into two groups, caries active and caries free. 5 ml of unstimulated saliva from each subject was collected and analyzed for the levels of trace elements using Induced Couple Plasma Spectrophotometer (ICP-OES).* ***Results:*** *Copper and Fluoride levels were significantly higher in caries free as compared to caries active individuals whereas Lead was significantly higher in caries active as compared to caries free children (p-value<0.05). Potassium and Selenium though present in appreciable amounts did not show any significant difference between the two groups (p-value>0.05).* ***Conclusion:*** *In caries free subjects, copper and fluoride levels were significantly higher while the level of lead was lower as compared to caries-active children. Potassium and selenium did not show any significant differences within the two groups.*

Key words: *trace elements, saliva, ICP-OES*

INTRODUCTION

Dental caries is a complex and dynamic process where a multitude of factors influence and initiate the progression of caries such as internal factors, saliva, tooth surface morphology, general health, and nutritional and hormonal status, and a number of external factors which include diet, the microbial flora colonizing the teeth, oral hygiene, and fluoride availability.[1]

The role of salivary components in maintaining the dental health has been lately recognized. Saliva acts as an ocean of anions, cations, nonelectrolytes, amino acids, proteins, carbohydrates, and lipids flowing in waves against and into the plaque, with a diurnal tide and varying degrees of intensity. These components play an important role in performing various functions in the oral cavity, mainly: (1) buffering ability, (2) a cleansing effect, (3) antibacterial action, and (4) maintenance of calcium phosphate in a supersaturated state. Several salivary constituents sub-serve one or more of these functions.

Research has yielded important information about organic and inorganic secretory products. Though the final result of "dental caries to be or not to be" is a complex phenomenon, focus of researchers has been on the effects of saliva and salivary constituents on cariogenic bacteria and the subsequent development of dental caries.

Trace elements are the inorganic constituents of saliva and make up for 0.2% of the total salivary composition.[2]

In analytical chemistry, a trace element is an element in a sample that has an average *concentration of less than 100 parts per million measured in atomic count or less than 100 micrograms per* gram whereas in biochemistry, a trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development, and physiology of the organism.[3]

Trace elements have long been suggested to have an impact on the demineralization-remineralization cycles of teeth. Of these, Fluoride has already been established to have remineralizing ability especially when the pH of the saliva drops. Other elements like phosphorous, copper, molybdenum, calcium, magnesium, barium, strontium and aluminum have also been associated with low levels of caries. However, some elements like iron, manganese and potassium have been associated with high incidence of dental caries.[4-5]

Although the influence of trace elements on the prevalence of caries is an unclear subject but we have sufficient evidence to justify continuing and expanding research effort in this field.

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Trace elements present in water and food exerts their effect in the oral cavity locally with saliva being the medium [6]. So it becomes essential to assess the effect of trace elements i.e. on caries activity of an individual.

Hence the present study was undertaken to estimate the levels of trace elements in mixed saliva of caries free and caries active individuals and with the hypothesis that the levels of trace elements in saliva had the potential to influence the occurrence of dental decay in children.

MATERIALS AND METHOD

The present study was conducted at Institute of Technology and Sciences (I.T.S C.D.S.R.) Muradnagar Ghaziabad,India in collaboration with National Test House (NTH) Sanjay Nagar, Ghaziabad. The study design and protocol were approved by the Institutional Ethical Board of ITS-CDSR. Consent was taken from parents/ guardians prior to the participation of the subjects in the study. All individuals that participated in this study had similar access to oral care with variable set of cultural norms related to dietary and oral hygiene habits.

Sample size was determined based on results of the pilot study using the following the formula: $N= Z^2P(1-P)/d^2$ where N= sample size, Z= statistic for a level of confidence, P= expected prevalence or proportion,d= precision (difference between expected and observed frequency).

A total of 60 children, aged 3-15 years, were divided into two groups, Group I consisting of children with dmft/DMFT >3 and Group II consisting of children with dmft/DMFT=0. The unstimulated saliva samples (5ml) were collected by passive drooling and by making the child sit in Coachman's position where in the subjects were seated with head slightly down and palms of both the hands on the thighs with a slight forward bending position. The subjects were advised to refrain from eating or drinking anything except water at least 2 hours before saliva collection. Saliva was allowed to accumulate in the mouth and was allowed to drool in the receiving vessel and the subjects were asked not to swallow or move their tongue or lips during the saliva collection.

The 5ml saliva (unstimulated) was collected in a sterile vial or plastic container and then transported to the laboratory for further investigations. The collected samples were stored under 4°C temperature until processed. The samples were transported carefully in an icebox to the laboratory.

Coding of each sample was done and the researcher carrying out the procedure of detecting the trace elements in mixed saliva was unaware of the oral health status of the subjects.

The saliva samples were run through ICP-OES (Induced Couple Plasma Spectrophotometer) Mode No Optima DU 2100, an advanced version of Atomic Absorption Spectrophotometer. The various trace elements analyzed were Copper, Lead, Potassium, Fluoride, Selenium and Cobalt. Statistical Analysis was done using SPSS version 16.0.

RESULTS

Sixty 3-15 years old (with minimum age 3years and maximum age 15 years) participated in the study for evaluation of levels of trace elements. Subjects in Group II had DMFT/dmft index of 0 while subjects in Group I had DMFT/dmft >3 (mean dmft/DMFT)

The data collected in this study were not normally distributed i.e. S.D. > Mean, therefore parametric test (t-test) could not be applied. A non- parametric test i.e. the Wilcoxon Signed Ranks test was applied for analysis of the data collected and significance level was set at 5%.

Table I shows the mean levels of all the five trace elements studied i.e Copper, Lead, Fluoride, Potassium and Selenium in caries free and caries active individuals. The levels of Copper and Fluoride were found to be significantly higher in caries free group as compared to caries active group. (p-value 027 and 0.005 respectively).

On the other hand, Lead was found to be significantly higher in caries active group (p-value 0.017). (Table I)

Table I: Distribution of trace elements in caries active and caries free children

TRACE ELEMEMTS	MEAN ±SD		p-VALUE
	CARIES ACTIVE (n=30)	CARIES FREE (n=30)	
COPPER	.002000 ±.0027889	.013800 ±.0154258	0.027*
LEAD	.004500 ±.0088851	.000000 ±.0000000	0.017*
FLUORIDE	.004200±.0023476	.037500±.0076194	0.005*
POTASSIUM	56.56000±18.7447676	69.63300±16.4907180	0.093^{ns}
SELENIUM	.000700±.0012517	.000000±.0000000	0.059^{ns}

*significant(p<0.05), ns (non-significant)

DISCUSSION

Trace elements are present in biological substances or fluids in very minute amounts i.e. in microgram per gram or less in concentration[7-9]. The influence of trace elements on the prevalence of caries is still uncertain. However, sufficient evidence has been accumulated to suggest that it is a subject that justifies a continuing and expanding research effort. A more definitive knowledge of these elements modus operandi undoubtedly will enhance our ability to use them to inhibit or to control caries. [10-11]

The conclusive demonstration of the inverse relationship between caries prevalence and fluoride (F) intake at once indicates the potential effect of a trace element on caries. If the epidemiologic studies of Arnold [12] are examined it can be seen that in addition to lower caries prevalence in localities with optimal levels of F in water, there were also substantial differences in caries prevalence between localities where the F contents of the waters were low and of the same level. [13]

Other substantial differences in caries prevalence cannot be explained on the basis of F intake, which may have a variety of causes, but evidence is available that suggests that variations in the intake of trace elements other than fluoride, in food or water, which influence caries progression. [14]

Trace elements influence susceptibility to caries, by altering the resistance of the tooth itself or its local environment.[15-20] The receptivity of the apatite lattice to a wide variety of elements facilitates their incorporation into the enamel. Such reactions occur principally in the surface layers of the enamel where the substitution of new elements can influence the solubility of the enamel, thereby

modifying the resistance to caries. It may alter the solubility of the enamel by increasing its crystallinity[21] and thereby reduce the overall surface area available for the action of acid.

The demonstration of Likins *et a1* of a reduction in the crystallinity of the mineral phase of enamel, dentin, and bone after the administration of Sr, indicates that other elements may also modify the physical composition of the teeth. The variations in the intake of different elements is reflected in the composition of saliva, and, especially, of the dental plaque.[22]

In this way accumulation of trace elements may influence susceptibility to caries by reducing acid production on caries-susceptible tooth surfaces. Bowen and Eastoe[23] showed that when solution containing 60 ppm of Mo was administered, resulted in reduction of acid production in caries susceptible tooth surfaces. Quinn[24] also suggested that variations in the intake of particular elements may influence the microbial ecology of either plaque or saliva and showed a strong correlation between K levels in saliva and the occurrence of cariogenic streptococci in caries-immune and caries-susceptible naval recruits.

In the present study copper and fluoride were found to be significantly higher in caries free group as compared to caries active group with p values 0.027 and 0.005 respectively (p-value 0.005) respectively. (Figure 1) Possible reason for this could be that most of the study population lives in high fluoride belt, therefore continuous consumption of fluoridated water could have raised its intra-oral levels.

Similarly, M. S Duggal, H.S. Chawla and M.E. Curzon in 1991 found that Cu and F had a consistent inverse relationship with caries experience whereas, Zn, Fe and Mn had no significant relationship with caries experience.[25]

P. Borella, G. Fantuzzi and G. Aggazzotti 1994 [26] analyzed the presence of trace elements and their influence on occurrence of dental caries. Their results were in accordance with the present study stating that Zn/Cu ratio in whole saliva were significantly decreased in subjects with more than three decayed teeth compared with those with no caries. Thereby, highlighting the inverse relationship between copper and occurrence of caries as the p-value was<0.05 (p-value 0.017). Hegde [27] assessed the levels of Copper and Zinc saliva samples of caries free and caries active children and reported statistically significant correlation between high levels of Copper and Zinc and caries in children.

Since copper and fluoride are the elements that influence enzyme systems involved in carbohydrate degradation (enolase activity) therefore their presence in saliva affects dental caries activity

In the present study Lead was found to be significantly higher in caries active group as compared to caries free group with p-value of 0.017. (Figure 1)

Thereby, denying the null hypothesis that, there is no significant variation in levels of trace elements in unstimulated saliva of children with caries and without caries as the p-value was <0.05.

Similar to the results of this study, F. Gil *et al* also found association of tooth lead content with dental health factors and stated that high tooth lead content lead to an increase in prevalence of caries. Papua-New Guinea[28-29] reported that soil lead had a direct association with occurrence of dental caries. Anderson *et al* [30] investigated the effect of lead on caries prevalence in children in Tamar valley in West of England and reported small reduction in caries whereas in Beara Peninsula where it led to an increased in level of caries.[31]A

Figure 1:Distribution means of trace elements in caries active and caries free children

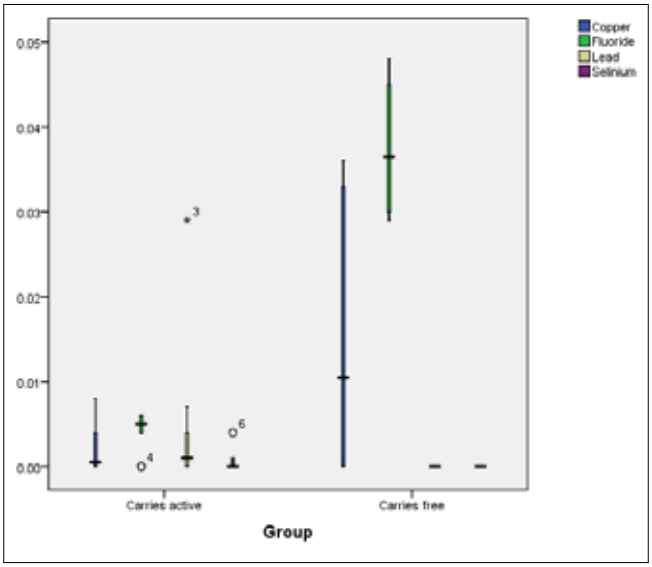
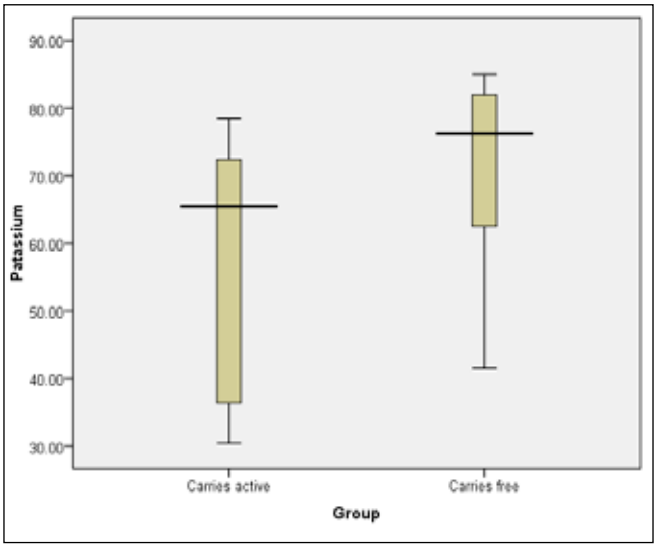


Figure 2:Distribution of mean of Potassium in caries active and caries free children



weak but significant association was found between PbB and PbSa with dental caries. Nriagu *et al* in 2007 reported that the association of PbSa was found to be marginally significant in cavitated lesions.[32]

Exposure to lead could be from the industrial waste such as people residing near a factory, incinerator or industrial plant or in the same block as a repair shop or a gas station.[33] Other sources of lead could be from foods grown in contaminated soils. It has also been reported that MS as well as other non-mutans Streptococci make a critical contribution to lower the pH in dental plaque and consequently the play a significant role in etiology of dental caries as low pH favors the exchange of enamel metal constituents with heavy metals included in contaminated foods.

In this study Potassium and Selenium did not show significant difference in values between caries free and caries active group. Selenium, though present in greater amounts in caries active group, did not show any significant difference in values between caries free and caries active group. (Figure 1 and 2)

Similarly Grad[34] reported that the concentration of potassium in saliva had no significant role in occurrence or prevalence of dental caries, as the values of Potassium were statistically non significant.

Epidemiologic studies suggested a positive correlation between selenium intake and dental caries, various authors also reported that consumption of small amounts of dietary Selenium increased the prevalence of dental caries whereas Shafer[35] reported that Se had no effect on dental caries incidence when incorporated in increasing amounts in experimental period. Storvick[36] stated that there is a direct relationship between the degree of susceptibility to dental caries and the urinary Se concentration.

Organo-selenium molecule served as a catalytic generator of superoxide radicals (O2•−) from the oxidation of thiols[37]. The superoxide radical appears to account for most of selenium's toxicity toward different bacteria, such as Staphylococcus epidermidis, Staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium, and Escherichia coli *in vitro.*

In the present study Induced Couple Plasma Spectrophotometer was used to detect trace elements in unstimulated saliva of children as it is an advanced and more sophisticated device that detects even minutest fractions of trace elements. This instrument is highly advanced in comparison to other instruments. It gives more sensitivity and repeatability. The lower detection limit is up to ppb level (software Winlab 32.)

ICP-OES DV 2100 make 'Perkin Elemer': Inductive Coupled Plasma Spectrophotometer. It has a plasma based technique. The temperature of plasma attains 10,000 K. At such a high temperature all the elements reach in excited state. With the help of this instrument we can check the concentrations at different wavelengths of a single element. Ultra pure Argon gas is used for plasma, nitrogen gas for purging and air is used as a shearing gas.

An ultrasonic nebulizer is used for detection of elements in saliva. With the help of this assembly the detection limit is highly increased.

Therefore, it becomes necessary to evaluate the level of trace elements in saliva, as it they confer susceptibility or resistance to caries.

CONCLUSION

Copper and Fluoride showed an inverse relationship with the occurrence of dental caries. Lead was found to be directly proportional to the incidence of dental caries. Its value was significantly higher in mixed saliva of caries active children.

Potassium showed high value in mixed saliva of both caries free and caries active children but was statistically non significant. Selenium, though present in higher levels in mixed saliva of caries active children in comparison to caries free children, the values were statistically non significant.

Limitations Of The Study

The change in levels of trace elements after the treatment was rendered should have been monitored in this study as they might be altered after restoring the carious lesions. Study population belonged to tropical climatic conditions therefore to rule out any discrepancy, samples should have been taken from other climatic conditions as well. As trace elements are widely distributed in foods dietary habits of children should have been recorded. Cobalt being radioactive in nature could not be detected by this method using ICP-OES, an alternative method should be recommended.

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