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ASSOCIATION OF SALIVARY CALCIUM, INORGANIC PHOSPHOROUS AND ALKALINE PHOSPHATASE LEVELS IN RELATION TO DENTAL CARIES STATUS- A CROSS SECTIONAL STUDY

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ABSTRACT

Dental caries is a complex multifactorial disease. Saliva plays a central role in the maintenance of oral homeostasis and its constituents influence the development of dental caries. Aim of our study was to assess the association of salivary calcium, inorganic phosphorous and alkaline phosphatase levels in relation to dental caries status. The study was carried out on 50 subjects with dental caries experience and 50 subjects without caries experience based on DMFS score and analyzed for calcium, phosphorus and alkaline phosphatase using gold standard methods. In our study the mean Alkaline Phosphatase(ALP) level in caries subjects was found to be statistically higher compared to caries free subjects and also a statistically significant positive correlation was established with ALP and caries status. Saliva is a clinically informative, body fluid and can be obtained non invasively offers its utility as a diagnostic tool.

KEYWORDS: Dental caries, Saliva, Alkaline phosphatase, calcium and phosphorous.

INTRODUCTION

Dental caries is as ancient as mankind and still remains a tormenting factor in the face of several efforts towards its eradication. It is a complex multifactorial disease caused by the interaction between a susceptible host, fermentable substrate and microflora.[1] Saliva as a biological fluid has growing interest as a diagnostic tool over the past decade. Saliva is the product of multiple glandular secretion which lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears.[2] Saliva contains a wide spectrum of proteins/peptides, nucleic acids, electrolytes and hormones that originate from multiple local and systemic sources.

Lack of saliva predisposes the development of atypical or unusual dental decay. In this era of genomic medicine, sialochemistry plays an increasingly important role in the early detection, monitoring and progression of systemic and oral diseases.[3] The complex mixture of proteins, glycoproteins, mucins, and ions present in the saliva helps to prevent many oral diseases including dental caries. Level of salivary components (immunoglobulins, salivary protein, salivary calcium, and inorganic phosphorous and alkaline phosphatase levels), its flow rate, viscosity, buffering capacity, pH etc influence initiation and progression of dental caries.

A major salivary enzyme Alkaline phosphatase (ALP) functions as a Calcium and phosphate binding protein and a phospho-hydrolytic enzyme.[4] A variation in the level of alkaline phosphatase affects the ionic concentration of phosphate and calcium, which in turn can alter the equilibrium of demineralization and remineralization process of enamel and thereby in the process of Dental caries.[5]

So this study has been designed to estimate the levels of salivary calcium, inorganic phosphorous and alkaline phosphatase levels, its association with dental caries and to confirm their use as diagnostic biomarker.

MATERIALS AND METHOD

The study was carried out among100 participants (17 -60 years of age) in the Department of Biochemistry, and the Department of Public health dentistry, Vivekanandha Dental college for Women, Tiruchengode. To ensure a representative sample, a simple random sampling approach was employed. Subjects were selected based on their caries status and recorded as per WHO criteria (1997).[6] The selected subjects were divided into two groups. Group I – 50 participants with dental caries experience and Group II- 50 age and sex matched participants

without caries experience. Cases presenting with known syndromes and bone disorders, cases received drug treatment in the form of bisphosphonates, fluoride, calcium or hormone replacement therapy were excluded as it impede with salivary biochemical estimation. Patients with any systemic diseases, periodontal disease, root caries were also excluded.

An ethical clearance was obtained from the Institutional Ethics Committee, prior to the commencement of study. The present study was approved and supported as an Indian Council of Medical Research (ICMR) (STS-2015-04097) project. Written consent was obtained from the study subjects prior to commencement of oral examination and relevant medical and dental history were elicited from the subjects.

The unstimulated whole saliva samples were collected. Participants were advised to refrain from intake of any food or beverages (Water exempted) one hour before collecting saliva. Before collection, the participants rinsed their mouth with water. The participants were then instructed to sit in a relaxed position and passively drool the saliva into a pre-weighed container. Finally, the collected samples were transported in a thermal insulation box maintaining a constant temperature of 4°C and transferred to the clinical laboratory within 30 minutes for further analysis.[7] The samples were investigated for the estimation of activity of salivary alkaline phosphatase andcalcium by Autoenzyme method[8],[9] and phosphorous by Molybdate U.V. method.[10]

Statistical analysis

Data obtained was analyzed using Statistical package for Social Sciences (SPSS) software version 17(SPSS, ver. 17.0; SPSS Inc., Chicago, IL, USA). All values are to be expressed as means ± standard deviation (SD). The collected data was assessed for normality using Shapiro Wilk's test. Based on the distribution of data Unpairedt-test was performed to compare Salivary alkaline phosphatase, calcium and phosphorus level between study and control group. Pearson correlation was employed to find correlation between caries status and salivary Calcium, Phosphorus and ALP.

RESULTS

Table I: Comparision of concentration of salivary calcium, phosphorous and salivary alkaline phosphatase between group I and group II.

Variables	Subjects with caries experience Group I (n=50)	Subjects without cariesexperience Group II (n=50)	p value
Calcium (mg/dl)	4.04±0.97	6.06±1.22	0.11
Phosphorus (mg/dl)	7.99±2.40	8.23 ±2.75	0.82
Alkaline phosphatase(U/L)	16.57 ±6.6*	5.01 ± 1.52	0.03*

Data are expressed as (mean ± S.D).Statistical analysis was done by unpaired t test. * Statistically significant($p<0.05$).

Table II: Correlation of salivary Calcium, Phosphorus and ALP with DMFS status.

Variables	DMFT status in dental caries subjects	
	r value	p value
Calcium	-0.1290	0.115
Phosphorus	0.1506	0.12
ALP	0.5678	0.006**

Statistical analysis was done by Pearson correlation. ** denoted significant at $p<0.001$.

Table 2. depicts a negative correlation was established with calcium (r=.-0.129, *P*>0.01) and a statistically significant positive correlation with ALP (r=.0.567, *P*<0.001).

DISCUSSION

It has been accepted that salivary secretion and components such as enzymes, immuno-globulins, inorganic materials and ions have different effects on mouth homeostasis are important for dental health and any changes may compromise the integrity of soft and hard tissues in the oral cavity.[11]

In our study no significant (*P*>0.05) difference was observed between the mean salivary calcium level and salivary inorganic phosphate level between the caries free subjects and caries subjects two groups. There was significant (*P*<0.05) increase in the ALP level in caries subjects when compared to caries free group as given in table 1.

Changes in salivary components are in connection with caries formation and it may be used for prevention and to make out risk in patients.[12] Though the etiology of dental caries is rationally studied, the chemical and physical process and the mechanism that results in the demineralization of enamel and dentin has been less appreciated.

Studies have proved that the oral milieu is in a constant dynamic equilibrium maintaining demineralization-remineralization process at all times. Any change in this equilibrium, initiates the caries process. Unstimulated whole saliva contains Calcium at 1.4 mmol/l and has been found to be one of the efficient buffers for regulating the body fluids unlike phosphates which are more resistant to depression of plaque pH towards the critical pH.[13] Caries process requires diffusion of solubilizing ions into and solubilized products out of the leisons. The dissolution of hard tissues of tooth in the state of calcium occurs in the oral cavity in the presence of saliva. The ionic concentration of calcium in saliva helps to maintain equilibrium between dissolution and remineralisation of enamel. This balance between remineralization and demineralization depends on the concentration of salivary calcium, phosphate and the level of salivary alkaline phosphatase.[14] The formation of carious lesions has been proved to be a relatively complex physio-chemical process. According to law of saturation a dynamic equilibrium exists between the mineral contents in the hydroxyapatite of the tooth and the oral fluid Earlier studies have proved that the rate of lesion formation strongly depend on diffusion, but the significance of various phenomenon over riding diffusion rates are still unknown. The tooth surface may be an important factor controlling the relative rates of ion diffusion.[15]

Also it has been found that for each cycle of acid production, the amount of calcium and phosphate ions that dissolve depends on the concentration of these ions within the lesions and the volume of lesion. The dissolution of calcium and phosphorus and hence, diffusion of these ions into and out of the lesion is influenced by alkaline phosphatase which leads to the initiation and progression of dental caries. Thus the rate of caries formation accelerates as the volume of the carious lesion grows.[16] This may be the reason for decreased level of salivary calcium in caries subjects compared to the caries free subjects and this difference was statistically not significant which are at odds with the results of previous study.[17] Studies of Kanshan *et al* and Ashley *et al* also revealed that salivary calcium concentration decreased with increasing caries activity confirming our finding that the level of calcium in saliva may be related to dental caries risk.[18] Therefore a high- salivary calcium level has to be maintained to avoid carious attacks and supersaturate the saliva favouring remineralization.

Another study had shown that salivary calcium increases with decreasing caries activity.[19] A similar study in children also found an inverse relationship between parotid saliva and Decay, Missing, Filling Surface score (DMFS).[20] Hedge et al found an inverse relationship between

the levels of salivary and serum Calcium and dental caries confirming the importance of Calcium levels in inhibiting caries progression. The salivary and serum Alkaline Phosphatase levels also showed significant correlation and their levels increased as caries progressed. Hence saliva may be used as a biochemical indicator in evaluating the susceptibility of caries.

The levels of alkaline phosphatase for caries active subjects were found to be higher as compared to caries free group in the current study. The salivary alkaline phosphatase level has been found to have a positive correlation with caries. Few other studies have also reported a positive correlation between salivary alkaline phosphatase activities with dental caries.[21] In the present study, mean of inorganic phosphorous in caries subjects were higher than the caries free group, but this difference was not statistically significant. Other studies also investigated the relationship between alkaline phosphatase and inorganic phosphorous with decay, filling, surface and they observed the level of alkaline phosphatase and inorganic phosphorous in rampant caries children was higher than the caries free children.[22]

Correlation analysis have shown that the level of salivary ALP activity was not related to the concentrations of salivary calcium and phosphate. Hence, this study shows that other controlling mechanism might be responsible for maintaining calcium and phosphate in a normal range. Alkaline phosphatase activity was found to have highly significant positive correlation with dental caries. Hence the diffusion of inorganic ions in progression of caries lesion is influenced by alkaline phosphatase which demonstrated a strong positive correlation with levels of alkaline phosphatise and caries status. A study on Brazilian population have shown that the stability of salivary calcium and phosphate in different time and temperature was studied and was found to be stable under different conditions.[23,24] Hence various studies reinforces the argument in favour of saliva as a diagnostic tool and a probable predictor for dental caries.

CONCLUSION ST

To stumble on and comprehend the role of salivary Alkaline phosphatase, Calcium and Phosphate ions in the development of dental caries, the following study was undertaken. Saliva offers an alternative to serum as an important body fluid for diagnostic purposes. The ease with which saliva can be collected in non-laboratory settings, and the fact that no special equipment is required, it is an ideal medium for use in the diagnostic field to screen for various diseases. Whole saliva can be used for diagnosis of systemic diseases, because it contains serum constituents that are derived from the local vasculature of the salivary glands

and gingival cervicular fluid. With this study, gap is about to close between the use of saliva and other biofluids for disease diagnostics. However further studies conducted on a wider population with regards to sex, age and race are strongly recommended.

LIMITATION OF THE STUDY

The limitation of the present study is that a factor with a small effect will require a bigger sample size, so the total number of the subjects included in the study may not be sufficient to demonstrate the role of saliva as a diagnostic marker for dental caries. Also the other etiological factors for dental caries like p H, buffering capacity were not included in the study.

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