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Quantitative Analysis of Salivary Constituents and Their Relation with Dental Caries in Adult Population

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Abstract:
Background: *Saliva is a complex mixture of fluids which regulates oral health. Studies have stated that calcium and phosphorous content of saliva is low in caries active persons. But most investigators have been unable to confirm this finding .This is attributed to the interplay of other factors including proteins and enzymes. Thus levels and state of calcium and phosphorous in saliva may be an indicator of susceptibility to dental caries.Thus the current study aims to determine the levels of salivary constituents like calcium, phosphorous, proteins and alkaline phosphatase in caries group and caries free group and assess their relationship with prevalence of dental caries.*
Materials & Methods*: Based on the DMFS score,50 subjects in the age group of 18-25 were selected and they were divided into 2 groups. Group 1 (n=30) consisted of subjects with caries (DMFS ≥5). Group 2 (n=20) is the caries free group(DMFS <0).5ml of unstimulated saliva samples were collected from each and analysed using colorimetric method for the each constituents under study.*
Results:*The mean calcium level in group 1 (DMFS≥5) was 5.41 ± 1.09 and that of group 2 (DMFS=0) was 7.72 ± 3.2. The mean phosphorous level in group 1 (DMFS≥5) was 3.76±1.67 and that of the group 2 (DMFS=0) was 8.85 ± 5. 72. In group 1, the mean total protein was 7.02 ± 1.30 and in group 2 it was found to be 4.89 ± 2.96. The salivary alkaline phosphatase level between group 1 and group 2 was and 29.1 ± 18.79 and 20.4 ± 8.6 respectively. A negative or indirect correlationship exists between salivary calcium and phosphorus concentrations and caries. A direct positive and highly significant correlationship was shown to exist between alkaline phosphatase concentration and caries.*
Conclusion*:Conclusions that can be drawn from this study are that the level of calcium and inorganic phosphorous were high in caries free population and total proteins and alkaline phosphatase were high in caries active population. The concentration of calcium and inorganic phosphorous showed negative correlation with caries prevalence. Eventhough the salivary protein and alkaline phosphatase show a positive correlation with DMFS, it is not satistically significant (p=0.234).*
Keywords : *Saliva, dental caries, remineralization ,demineralization*

I. Introduction

Dental caries is the most prevalent dental disease affecting human race. Although the prevalence of dental caries has significantly reduced, it is still a major problem. The etiology and pathogenesis of dental caries are known to be multifactorial.

To define, dental caries is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissues[1].

Saliva is one of the most important host factors that play a role in the caries process through its organic and inorganic constituents, besides the physiological functions. The continuous flow of saliva through the mouth bathes the dentition with remineralizing ions and removes cariogenic challenges [2].Saliva has a profound influence in the prevention of dental caries in a potentially cariogenic oral environment.Studies had shown that the organic and inorganic constituents of saliva played an essential role in the caries process in addition to the other physiological functions.[3]

The concentration of inorganic calcium and phosphorus in saliva shows considerable variation depending on a number of factors. Previous studies, such as the one reported by Karshan (1939) stated that the calcium and phosphorus content of saliva is low in caries-active persons.He interpreted his findings as indicating that "the saliva of persons free from dental caries would protect enamel against solution by acids to a greater extent than would the saliva of persons with active decay."[4].

But, most investigators have been unable to confirm this finding. The levels and state of calcium and phosphate in saliva may be related to the susceptibility to dental caries [5]. Hence, the current study is carried out to assess the relationship of salivary calcium, phosphorous, proteins, and alkaline phosphatase with the prevalence of dental caries in adult population.

II. Materials & Methods

The study was conducted among the dental students of Amrita School of Dentistry and patients who reported for treatment to the Department of Conservative Dentistry and Endodontics, Amrita School of Dentistry.Population in the age group of 18-25 years with good general health and no history of intake of antibiotics for past 3 months were included in the study. Medically compromised patients and those undergoing orthodontic treatment were excluded in the study.

The study subjects were selected on the basis of the DMFS index and categorized into control group (Group I) and case group (Group II). Study subjects were seated comfortably in a dental chair. A thorough oral hard and soft tissue examination was done with sterilized mouth mirror and
probe. DMF surfaces index (DMFS) was used to measure the severity of dental caries.
Subjects with DMFS score ≥ 5 were grouped under caries group whereas subjects with DMFS score =0 were categorized as caries free group.

Group 1 (n=30) consisted of subjects with caries (DMFS ≥5) (control group) Group 2(n=20) is the caries free group. (DMFS = 0) (case group)

All the selected study subjects were then subjected to salivary sample collection. Subjects were refrained from eating, drinking, and oral hygiene procedures for a minimum of one and a half hour before salivary collection.

Unstimulated, directly expectorated, whole saliva samples were collected in clean, dry, sterile vacuettes. Saliva samples were collected one and a half hours after the last meal. The subjects were asked to allow saliva to drool from the oral cavity into the sterile vacuettes . A total of 5ml of saliva was collected from each. The saliva was then transported to the biochemistry lab within 1 hour where it was stored in the freezer at -20 ◦ C. The vacuettes were then placed in a centrifuge (Beckman Coulter centrifuge) and centrifuged at 3000 rpm for 5 min to remove the particulates in the collected saliva. Estimation of calcium, phosphorous, proteins and alkaline phosphatase were done using the kit provided by Beckman Coulter.

III. Statistical Analysis

The collected data was tabulated and analysed using IBM SPSS Version 20. All the continous parameters were presented as mean ± standard deviation.

In the present study, it was seen that Calcium, Phosphorous and Protein levels followed a normal distribution, so the Independent sample t -test was used to compare between Group 1(caries active) and Group 2(caries free). For alkaline phosphatase, Mann –Whitney U-Test was used as the data did not follow normal distribution. p-value <0.005 was considered as statistically significant.

IV. Results

Results from Independent sample t -test for comparing salivary calcium, phosphorous, proteins are given in Tab/Fig-1.

There is significant difference between all the tested parameters in both groups (Group 1 & Group 2) at $p < 0.005$

The relation between the tested parameters (Ca,Ph,Pr,ALP) and caries prevalence was done by correlating the values with DMFS score. The result is given in Tab/Fig-2

The result can be inferred as follows:-

A negative or indirect correlationship exists between salivary calcium and phosphorous concentrations and caries activity but it is not satistically significant. ($r = -0.078$ & $p = 0.681$ and $r=-0.014$ & $p=0.942$, respectively)
Eventhough the salivary protein and DMFS show a low positive correlation, it is not satistically significant ($r=0.224$ & $p=0.234$)

A direct positive correlationship exists between alkaline phosphatase and caries activity but is not satistically significant. ($r=0.036$ & $p=0.850$).

None of the tested parameters show any significant correlation with DMFS .Eventhough the level of calcium and phosphorous were high in caries group, it cannot be statistically correlated to the prevalence of dental caries.

V. Discussion

Oral cavity is a distinctive ecosystem, which performs a wide range of functions, harbours a plethora of microorganisms and is unique in accommodating exposed mineralized tissues. The saliva bathes this ecosystem and possesses a large number of components, plays a major role in the etiopathogenesis of dental caries [6,7]. Our understanding of etiological factors, the progress of the disease, and the effectiveness of prophylactic procedures have led us to believe that we understand the disease. However, the reason for not being immunized,

predictability of the disease, amount of saliva needed, its protective constituents and the constituents which predispose to caries are still unknown. It is generally accepted, however, that saliva secretion and salivary components secreted in saliva are important for dental health [8]. Alteration in physiochemical properties of saliva such as decreased salivary flow rate, pH, buffering capacity and calcium play a major role in the development of dental caries[9].The fact that the teeth are in constant contact with saliva suggests that this environmental agent would profoundly influence the dental caries process.

The complex nature of saliva and great variations in its composition are premonitory of the difficulties involved in establishing the causative factors, which may directly influence the dental caries process.Saliva by constantly bathing the teeth and oral mucosa, functions as a cleansing solution, a lubricant, a buffer, and an ion reservoir of calcium and phosphate which are essential for remineralization of initial carious lesions. The levels and state of calcium and phosphate in saliva may be related to susceptibility to dental caries. Saliva is also rich in enzymes, and the role of alkaline phosphatase (ALP) plays an important role in the mineralization process. 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 [10]. A recent study by Vijayaprasad et al [5] cited that the mean salivary calcium content in the control group (4.29 mg%) is found to be slightly higher when compared to the minimal caries group (3.95 mg%) as well as the rampant caries group (3.94 mg%).For each cycle of acid production, the amount of mineral that dissolves will be proportional to the calcium and phosphate concentrations within the lesion, as determined by Ph. Similarly, Tulunoglu et al [11] indicated that salivary calcium concentration values were found to be higher in caries free groups.The present study found that the mean calcium level in group 1(caries active) (DMFS $\geq$5) was 5.41±1.09 and that of Group 2 (caries free) (DMFS=0) was 7.72 ± 3.2($p<0.005$). Salivary calcium level in caries active group was lower compared to caries free group. This is in accordance with the study conducted by B.P. Preeti[12] and M. Shahrabi [13] where they attributed that the decrease in caries experience in children with high calcium concentration to the process of remineralization of the incipient caries lesions.Another important salivary constituent is the inorganic phosphorous which also helps in maintaining the integrity of enamel.

In a study carried out by GandhyM andDamle S G [14], the saliva collected from children with rampant 55 caries contain higher levels of inorganic phosphorous(26.33mg%) compared to the saliva collected from caries free children(14.22mg%).The present study found that the mean phosphorous level in Group 1 (DMFS $\geq$5) was found to be 3.76±1.67 and in Group 2 (DMFS=0) was 8.85±5.72 ($p<0.005$).Salivary proteins possess antimicrobial, lubricative and digestive properties and play an important role in modulating the microbial colonization of teeth and soft tissues. In a study by O. Tulunoglu et al [15], the mean total protein level in caries active children was found to be 0.45. They found that total protein increased with caries activity. Similar result was observed in a study conducted by B P Preeti et al [12] where total protein increased significantly in caries active children.The mean salivery total protein level in the present study in Group 1 (caries active) was 7.02 ± 1.30 and that of the Group 2 (caries free) was found to be 4.89 ± 2.96 ($p<0.005$). In caries active group (group 1), the salivary protein level was higher compared to caries free group(group 2) which is in accordance with Tulungolu et al.[15] and Kargul et al. [16] who observed an increase in the salivary protein concentration with increased caries prevalence.The salivary alkaline phosphatase is one of the factors governing the calcium and phosphorous levels in saliva.A study by Vijayaprasad KE et al [5],stated that the ALP activity in the rampant caries group was found to be much higher (18.66 K.A) than that for the caries free group (4.68 K.A).The present study found that the mean alkaline phosphatase level in Group 2 (caries free) (DMFS=0) was 20.4 ± 8.6 and that of the Group 1 (caries active) (DMFS $\geq$5) was found to be 29.1 ± 18.79 ($p<0.052$). The level of alkaline phosphatase for caries active (Group-1) was higher when compared to caries free (Group-2) which is in contrast to the findings reported by Shahrabi et al [13].In the current study, the study population were selected on the basis of DMFS score. DMF surfaces index (DMFS) measures the severity of dental caries. All the tested parameters were correlated with DMFS score to evaluate any relationship between them and caries prevalence.Negative or indirect correlationship was seen to exist between salivary calcium and phosphorous concentrations and caries. However, it was not satisticallysignificant. ($r = -0.078$ & $p = 0.681$ and $r=-0.014$ & $p=0.942$, respectively).The salivary protein and DMFS showed a poor positive correlation ($r=0.224$ & $p=0.234$), which was not statistically significant. A direct positive correlationship between alkaline phosphatase and caries was also observed but with no statistical significance. ($r=0.036$ &$p=0.850$).

VI. Conclusion

Thus the present study reinforces the argument in favour of saliva as a diagnostic tool and a probable predictor for dental caries, but is limited in being able to unequivocally establish a specific parameter for such a prediction.

This may be regarded as a limitation of the methodology used in the current study and may in fact, open new avenues for further research on thetopic. The accessibility of saliva and the non-invasive manner of

obtaining the specimen are major advantages of using saliva as a diagnostic tool, and hence, salivary diagnostics remains a field of immense untapped potential.

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Tables

Table/Fig1- Result of mean levels of Ca,Ph,Pr& ALP in Group 1 and Group 2

Variables	Group 1 (caries group)	Group 2 (caries free group)	p-value
	Mean ± SD	Mean ± SD	
Ca(mg/dl)	**5.41±1.09**	**7.72±3.2**	0.005
Ph(mg/dl)	**3.76±1.67**	**8.85±5.72**	0.005
Pr(g/dl)	**7.02±1.30**	**4.89±2.96**	0.005
Alk(IU/L)	**29.1±18.79**	**20.4±8.6**	0.052

Table/Fig 2- Correlation of DMFS vs other parameters

Details	r-value
DMFS vs Calcium	-.078
DMFS vs phosphorous	-.014
DMFS vs proteins	.224
DMFS vs alkaline phosphatase	.036