Original Research

Salivary Total Protein Levels and their correlation to Dental Caries

Pavitra Vibhakar A, Sangeeta Patankar R, Monica Yadav R, Parag Vibbhakar A

Abstract

Background: Saliva is essential for a lifelong conservation of dentition; its various functions are implicated in the maintenance of oral health and the protection of teeth. Saliva contains a large number of proteins that participate in the protection of the oral tissues, for instance lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinin and mucin. Because, all these proteins and peptides have a broad spectrum of antimicrobial activity there seems to be a considerable overlap in their functionality. **Aims**: Analysing variability of naturally occurring total protein concentration in unstimulated whole human saliva of patients with dental caries and hence to correlate the levels of total salivary protein with DMFT index. **Methods and Materials:** Thirty nine patients were randomly selected and informed consent was obtained. Saliva was collected by the spitting method with all the necessary precautions. It was collected on ice, preserved at -20°C, centrifuged and speed evaporated. The salivary total protein levels were estimated by Lowry's method after reconstitution with Lamelli's Buffer. Statistical analysis: The data thus obtained was subjected to Pearson's Correlation Test and p value below 0.05 was considered significant. **Results**: A significant correlation was obtained between the salivary total protein levels and dental caries; also protein levels with age. **Conclusion**: The total salivary proteins levels show a linear increase with the DMFT index, thus establishing a correlation between the parameters under our study. This study adds to better understanding of salivary components and their role in dental caries and in the future, modulation of such parameters could play an important role in controlling formation of carious lesion, opening an entirely new avenue of carious prevention.

Keywords: Dental Caries; Immunoglobulin; Lactoferrin; Lactoperoxidase; Proteins; Saliva.

Introduction

Saliva is one of the most important protective body fluids but remains the least understood[1]. Saliva has various functions in the oral environment like clearing of food debris and bacteria, buffering capacity on tissue damaging strong bases and acids, providing saturated solution of calcium needed in remineralisation of teeth; it also has antibacterial, antifungal and antiviral capacity[2]. The amount saliva secreted in the oral cavity and its constituents have a role in the incidence of dental caries[3]. However, we still have too few answers to many questions:"Why can we not predict who will get the disease?" "Why do we not become immunized?" "How much saliva is enough?" or "Which salivary components are protective?" and "Which salivary components predispose for caries?" It is generally accepted, that certain salivary components especially proteins have protective effects against dental caries[4]. These proteins act directly and indirectly through various methods on plaque and bacteria modulating susceptibility of the

tooth to dental caries[5]. Hence a study was undertaken to evaluate the changes in the levels of total salivary proteins with increase in dental caries, to throw light on the role of salivary protein component in the multifactorial aetiology of dental caries.

Materials and Methods

Thirty nine patients were randomly selected, clinically examined, given a detailed explanation about the study and their consent was obtained. A detailed case history and the evaluation of number of carious teeth carried out using simple clinical examination techniques with regular diagnostic instruments i.e. mouth mirror, probe, explorer. The Decayed Missing Filled teeth Index (DMFT Index) was then calculated and noted. The patients selected were free from all systemic diseases and oral diseases except dental caries if present. Patients currently on medication or indulging in any adverse habits like tobacco chewing, smoking, etc were excluded and those with space infection due to caries were also excluded. Within this random sample group,

13 patients presented with a zero score on the DMFT index and were included to be employed as controls for the study.

Saliva was collected only between 9:00am and 11:00am. Patients were requested not to consume food or drink at least two hours prior to the collection. They were instructed to avoid all major oral and body movements even talking during the collection process and saliva was collected by spitting method into a sterile labelled container placed on ice and then stored at -20°C followed by centrifugation using the Rota 4R - V/Fm, Plasto Crafts® centrifuge at 4°C at 4000rpm for 45 minutes. The 500µl of the supernatant was transferred into the Eppendorf tube, sealed with punctured Parafilm®, dried in Labconco® speed evacuator and stored at -20°C.

Salivary Total Protein was estimated by means of Lowry's method. Firstly, Five standards of Bovine Serum Albumin (BSA) (Sigma)- 0ug, 20ug, 40ug, 60ug and 80ug were prepared at 1mg/ml of concentration and other tubes were marked the protocol number of each sample. All test tubes were prepared in doublets. 900µl of (double distilled water) DDW was added to all the tubes. In the first five tubes marked as 0, 20, 40, 60, 80 corresponding µls of BSA was added and in the following tubes 10µl of the saliva was added. The volume in all tubes was made to 1000µl by adding DDW. In each test tube 1ml of freshly prepared CTC solution [20% sodium carbonate (Qualigens), 0.2% copper sulphate (Sarabhai Chemicals), 0.4% potassium tartarate (Sarabhai Chemicals)] was added, followed by 500µl of Folin Ciocalteu's Phenol Reagent (FC reagent- Sigma). The test tubes were then vortexed for a few seconds and incubated for 30 minutes in dark. The readings were taken at 750Å in the Uvikon® spectrometer.

The data obtained was subjected to a statistical analysis, Pearson's correlation test was applied (software) to compare the values obtained, where values of $p<0.05$ were considered significant.

Results
Of the given samples 39, 53.85% (21) are male and 46.15% (18) are female (Table 1). The age distribution of the patients is as follows: 2.56% (1) is between 11-15 years, 15.38% (6) are between 16-20 age group, 48.72% (19) fall under 21-25 years, 20.51%

(8) belong to 26-30 years and 12.82% (5) belong to >30 years of age (Table 2). Correlation between DMFT and Salivary Total Proteins was evaluated by Pearson's correlation test. The Pearson's coefficient (r) = 0.405, p-value = 0.011 were obtained; hence a positive correlation was noted. (Graph-1)

Pearson's correlation coefficient (r) = 0.334, p-value = 0.038 was calculated between age of subjects (years) and Total Proteins. The p-value being <0.05, the result is significant. (Graph-2) Comparison of Protein Levels to gender was done by applying two independent sample t-tests; mean value of proteins was 39.80 ± 14.04 µg/10µl in male and 34.23 ± 12.28µg/10µl in female patients respectively. The p-value= 0.194 (p-value > 0.05) therefore, there is no significant difference between gender with respect to total proteins (Graph 3). DMFT and age significantly related as the Pearson's correlation coefficient (r) = 0.445, p-value = 0.005 were seen. (Graph 4)

Gender wise distribution of patients		
Gender	Number of patients	Percentages
Male	21	53.85
Female	18	46.15
Total	39	100.00

Table 1: Table represents the gender wise distribution of the samples.

Age wise distribution of patients		
Age group	Number of patients	Percentages
11 - 15	1	2.56
16 - 20	6	15.38
21 - 25	19	48.72
26 - 30	8	20.51
> 30	5	12.82
Total	39	100.00

Table 2: Table represents the age wise distribution of the samples.

Discussion
The correlation between the DMFT index and the Total Salivary Protein has been studied by several researchers and the results are mixed. De Farias, et al have studied the mean protein levels between caries free and early childhood caries patients each 20 in sample size. They found no significant difference between the total protein content between the two groups. Similar results have been obtained by S. Bhalla, et al in similar groups with a sample size of 50 each[6]. Roa, et al also reported no

correlation between the total protein concentration and the caries activity[7]. Total salivary proteins may have both protective and detrimental properties. Thus, salivary proteins can be known as "double edged" swords. Functions of salivary proteins may depend on the molecule's location or site of action. Some proteins such as antimicrobial and pH modulating proteins play a protective role in the oral cavity, while adhesins and agglutinins play a detrimental role by increasing the colonization of microorganisms.[8] In our study a significant linear correlation has been established but, the p value being 0.011 it is a poor positive correlation.
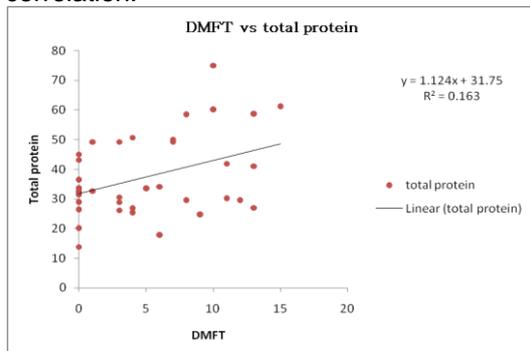
Graph 1: Concomitent increase of Total Proteins with increasing DMFT score.
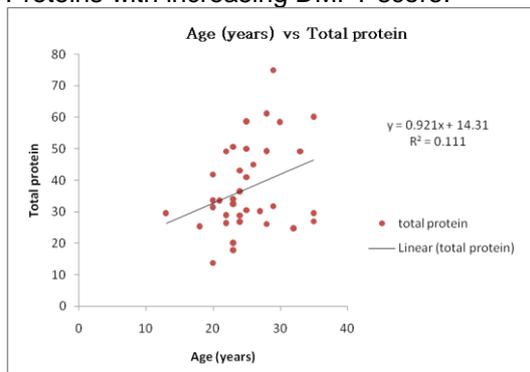
Graph 2: Corelation of Salivary Total protein levels and Age of the patients.
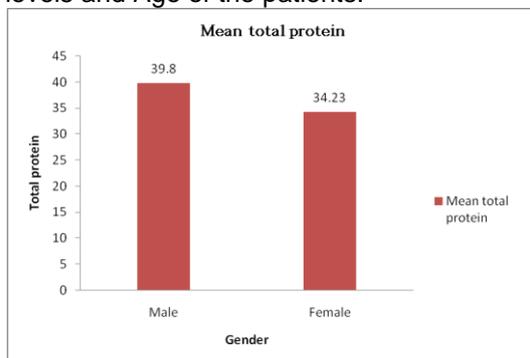
Graph 3: Difference in levels in total protein levels according to gender.

There are only a few studies on salivary composition of healthy children as well as adults available. Hence this study may help

in analyzing the physiologic variability of naturally occurring total protein concentration in unstimulated saliva as a function of age.[8] Here Age and Total Protein in our study, show a positive correlation which is statistically significant. Deshpande et al have shown a significant correlation between total protein and age in patients between 3-16 years of age[8]. M. Morzel et al state, salivary profiles were modified substantially between the ages of 3 and 6 months due to eruption of teeth and change in diet[7].
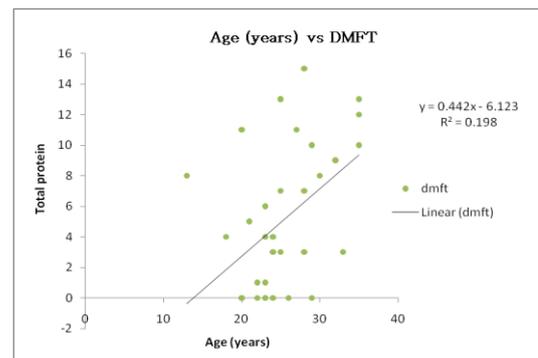
Graph 4: Increase in DMFT score with Age is illustrated.

Taking in to account Sex and Total Protein Content of saliva, Dodds et al, studied the protein profile of parotid fluids in caries free and caries active individuals and found women had significantly higher proteins than males[9]. Roa, et al too reported similar results in the Colombian population[7]. Banderas-Tarabay, et al reported that females had more protein concentration (1.406 mg/ml) than males (1.342 mg/ml); however, this difference was not statistically significant[10]. In our study the mean protein concentration in females was 34.23 ± 12.28 µg/10µl lower than that in males i.e. 39.80 ± 14.04 µg/10µl. However, this difference was not statistically significant.

The mean total protein values obtained in our study were 37.23µg/10µl. This is drastically different from reported studies and can be due to the methodology used in which 1ml of saliva was speed vacuumed and reconstituted by adding using Lammeli's Buffer (1x concentration) until the mixture was homogenous and transparent (non turbid), resulting in a concentrated solution. Several researchers have evaluated the Total Protein Content of Saliva in which C. A. Bonilla, reported the concentration of protein in human mixed saliva normally as about 124 to 206 mg/100 ml and that these values were not modified significantly by

stimulation[11]. However, L. Becerra et al, analyzed the secretion patterns of these parotid saliva proteins under resting and stimulated conditions and reported that the total protein was significantly different under the two conditions. The average mean value of total protein exhibited a similar pattern in which the mean resting value was 151.1mg% and the mean stimulated value was 322 mg%[12]. Banderas-Tarabay, et al reported a mean total protein of 1.375 mg/ml[10].

Considering the relationship between the Age of the patient and Caries Experience; Shafer states caries prevalence increases with age of the patient; as the carious lesions are irreversible they are cumulative with age. It would be beyond the scope of our study to comment on the caries experience in relation to age, as the study group under consideration is non representative due to the stringent exclusion criteria.

Saliva plays an important role in the maintenance of oral environment and its role in etiopathogenesis is gradually being unravelled as its non-invasive techniques are making it the fluid in vogue for research. The importance of the protein component in saliva has been highlighted in our study. Further studies with larger sample sizes; evaluation of individual proteins and their specific roles should provide valuable insight into the multifactorial etiology of dental caries. The artificial modulation of salivary protein levels can be considered in the future for caries prevention, once enough information on this illusive fluid is unravelled, salivary diagnostics will not revolutionize the medical, but also the dental community.

Author Affiliations
1.Dr.Pavitra Vibhakar, Lecturer, Terna Dental College, 2.Dr.Sangeeta R. Patankar, Professor and Head, Yerala Dental College, 3.Dr.Monica Yadav R, Professor and Head, Department of Oral Pathology, Terna Dental College, 4.Dr.Parag Vibhakar A, Plastic Surgeon, E.S.I.C. Model Hospital, MIDC, Andheri (East), Mumbai, India.

References
1. Bhalla S, Tandon S, Satyamurthy K. Salivary Proteins and Early Childhood Caries: A Gel Electrophoretic Analysis. Contemp Clin Dent 2010;1(1):17-22.
2. Kauffman E, Lamster IB. The Diagnostic Applications of Saliva- A Review. Crit Rev Oral Biol Med 2002;33:499-505.
3. Segal A, Wong DT. Salivary Diagnostics: Enhancing Disease Detection and Making Medicine Better. Eur J Dent Educ 2008;12(1):222-92.
4. Lenander-Lumikari M, Loimaranta V. Saliva and Dental Caries. Adv Dent Res 2000;14:40-47.
5. Mandel ID. Electrophoretic Studies of Saliva. J Dent Res 1966;45:634.
6. Mandel ID. The Diagnostic Uses of Saliva. J Oral Pathol Med 1990;19:119-25.
7. Roa NS, Chaves M, Gómez M, Jaramillo LM. Association of salivary proteins with dental caries in a Colombian population. Acta Odontol Latinoam 2008;21(1):69-75.
8. Deshpande RR, Panvalkar PS, Kulkarni AA, Gadkri TV. Age-related changes of the human salivary secretory total protein complex and trace elements in children between the age group of 3-16 years. J Biomed Sci Res 2011;3:362-7.
9. Dodds MW, Johnson DA, Mobley CC, Hattaway KM. Parotid saliva protein profiles in caries-free and caries-active adults. Oral Surg Oral Med Oral Pathol 1997;83:244-51.
10. Banderas-Tarabay JA, Zacarías-D'oleire IG, Garduño-Estrada R, Aceves-Luna E, González-Begné M. Electrophoresis Analysis of Whole Saliva and Prevalence of Dental Caries: A Study in Mexican Dental Students. Arch Med Res 2002;33:499–505.
11. Bonilla CA. Human Mixed Saliva Protein Concentration. J Dent Res 1972;51:664.
12. Becerra L, Soares RV, Bruno LS, et al. Patterns of secretion of mucins and non-mucin glycoproteins in human submandibular / sublingual secretion. Arch Oral Biol 2003;48:147-54.
13. Sivapathasundaram B, Raghu AR. Dental Caries. In: Rajendran R, Sivapathasundram, editors. Shafer's textbook of oral pathology. 6th edition. Elsevier Publications: India; 2009. p 441.

Corresponding Author
Dr.Pavitra Vibhakar,
501, Amartaru-4, New Nagardas Crs Rd,
Andheri East, Mumbai-400069.
Ph: 022-28270079
E-mail: pavitravibhakar@gmail.com