Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 3, 3756 –3766

"A Comparitive Study Of Salivary Protein Concentration, Flow Rate, Buffer Capacity And Ph Among Young And Elderly Subjects With Gingivitis And Periodontitis"-A Biochemical Study

Dr.V.T.Hemalatha[1]

MDS.,PhD.,Dr.ManiSundar.N[2]MDS.,PhD.,Dr.Julius.A[3]PhD.,

Dr.Sivaranjini.P[4]

MDS., Dr.V.Aarthi Nisha[5] MDS.,PhD.,Dr.Sankar Narayanan R[6] MDS.,

1.Dr.V.T.Hemalatha

MDS.,PhD., Associate Professor, Department Of Oral Medicine And Radiology, SreeBalaji Dental College And Hospital, Bharath Institute Of Higher Education And Research , Chennai, TamilNadu,India.

2.Dr.ManiSundar.N

MDS.,PhD., Associate Professor, Department Of Periodontics, Sri Venkateshwara Dental College And Hospital, Ariyur, Puducherry, Tamilnadu, India.

3.Dr.Julius.A

PhD., Professor and Head, Department Of Biochemistry, SreeBalaji Dental College And Hospital, Bharath Institute Of Higher Education And Research,Chennai, TamilNadu,India.

4.Dr.Sivaranjini.P

MDS., Reader, Department Of Periodontics,Priyadarshini Dental College And Hospital,Pandur, Thiruvallur.

5.Dr.V.Aarthi Nisha

MDS.,PhD., Assistant Professor, Department Of Oral Medicine And Radiology, Government Dental College, Chennai, TamilNadu,India.

6.Dr.Sankar Narayanan R

MDS.,Reader,Department Of Oral Medicine And Radiology, Sree

Balaji Dental College And Hospital, Bharath Institute Of Higher Education And Research , Chennai, TamilNadu,India.

ABSTRACT

Background:

To evaluate the salivary protein concentration in gingivitis and periodontitis patients and compare the parameters like salivary total protein, salivary albumin, salivary flow rate, pH, buffer capacity and flow rate in both young and elderly patients with simple biochemical methods.

Materials and Methods:

One hundred and twenty subjects were grouped based on their age as young and elderly. Each group was subgrouped (20 subjects) as controls, gingivitis and periodontitis. Plaque index, gingival index, modified sulcular bleeding index, probing pocket depth and clinical attachment level were recorded. The unstimulated whole saliva was collected from patients and flow rate was noted down during collection of the sample. Estimation of total salivary protein and albumin was performed by colorimetric method. The pH estimation was done using pH meter and buffering capacity was analysed with the titration method. Student's t-test, Fisher's test (ANOVA) and Tukey HSD (ANOVA) tests were used for statistical analysis.

Results:

A very highly significant rise in the salivary total protein and albumin concentration was noted in gingivitis and periodontitis subjects of both young and elderly. An overall decrease in salivary flow rate was observed among the elderly, and also the salivary flow rate of women was significantly lower than that of men.

Conclusion:

Significant associations between salivary total protein and albumin in gingivitis and periodontitis were found with simple biochemical tests. A decrease in salivary flow rate among elderly and among women was noted.

Keywords: Saliva, salivary protein, gingivitis, periodontitis.

INTRODUCTION

Periodontitis, a chronic inflammatory disease of the periodontal tissues, is a multifactorial disease of bacterial origin.[1] Saliva may contain biomarkers which are specific for the unique physiological aspects of periodontitis, and the qualitative changes which occur in the composition of these biomarkers could have diagnostic and therapeutic significance.[2]

Human salivary proteins have a wide range of functional properties including the immune response, inhibition of calcium precipitation, taste perception, digestion, cell proliferation, signal transduction, chemotaxis and cell motility.[2] In the oral cavity, proteins, especially albumin, are considered as a serum ultrafiltrate to the mouth.[3] Salivary proteins have been shown to be increased in medically compromised patients whose general conditions get worse. Elderly subjects usually show less-effective immune response than the young ones. Therefore, salivary total normal protein concentration is vital for good oral health and a sustained change in it for any reason adversely affects the oral health of these patients. [4]

Gingivitis and periodontitis are oral diseases that are characterized by chronic inflammation. Here, salivary protein and albumin concentrations were determined as markers for plasma protein leakage, occurring as a consequence of the inflammatory process. Saliva as a mirror of oral and systemic health is a valuable source for clinically relevant information because it contains biomarkers specific for the unique physiological aspects of periodontal ⁄ peri-implant disease, and

qualitative changes in the composition of these biomarkers could have diagnostic value by identifying patients with enhanced disease susceptibility, identifying sites with active disease, predicting sites that will have active disease in the future and ⁄ or serving as surrogate end points for monitoring the effectiveness of therapy.[5]

Hence, the aim of the present study was to analyze and compare the salivary total protein, albumin, pH, buffering capacity and flow rate in young and elderly subjects, both normal and with gingivitis and periodontitis, using simple biochemical methods.

MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology, Sree Balaji Dental College And Hospital,TamilNadu,Chennai,India.The institutional ethical committee of the faculty approved the study

One hundred and twenty subjects were included of which Eighty subjects were chosen on the basis of presence of gingivitis and periodontitis under two different age groups.

Criteria for gingivitis was based on the National Institute of Dental Research (NIDR) criteria

NIDR – Gingival Inflammation Index (Bleeding index)

0=No bleeding

1 = Bleeding after probe is placed in gingival sulcus up to 2 mm and drawn along the inner surface of the gingival sulcus.

Criteria for periodontitis was based on loss of attachment with pocket depth of >/=5 mm in at least eight sites.

The first group comprised of forty young subjects between twenty and thirty five years of age with no systemic diseases and not on medication. The second group comprised of forty elderly subjects of sixty five years and above, with no systemic diseases and not on medication. Twenty control samples for each group were collected on the basis of presence of healthy periodontium with no systemic diseases and not on medication.

All the patients were subjected to routine examinations and case history was recorded. Based on the above-mentioned criteria, subjects were subgrouped under six groups as:

1. Young – Control (number of subjects = 20)
2. Young – Gingivitis (number of subjects = 20)
3. Young – Periodontitis (number of subjects = 20)
4. Elderly – Control (number of subjects = 20)
5. Elderly – Gingivitis (number of subjects = 20)
6. Elderly – Periodontitis (number of subjects = 20).

COLLECTION OF SALIVA

Subjects were instructed not to brush their teeth, or eat or drink one hour before the time of saliva collection.Human whole unstimulated saliva was collected by spitting method without swallowing, with the subject seated in an upright position between 11 am and 12 noon, after they had refrained from oral intake, tooth brushing and smoking for 2 h before saliva collection. Approximately, 5 mL of saliva was collected. Flow rate was calculated as volume collected divided by the time required for the collection.

Salivary protein estimation was done based on the Biuret method. Protein forms a colored complex with cupric ions in alkaline medium. Based on this principle, salivary protein estimation was done by mixing undiluted saliva with the reagent (45 g of Rochelle salt and 15 g of copper sulfate in 400 mL of 0.2 N sodium hydroxide. Five grams of potassium iodide was added to make up to 1 L with 0.2 N sodium hydroxide) and measuring the colored product

using a photoelectric colorimeter at a wavelength of 546 nm. Standard solution of 6 g of bovine albumin dissolved in 100 mL of normal saline containing 0.1 g/dL sodium azide was used.

Salivary albumin was estimated using the Bromocresol green method (albumin colorimetric test). The reaction between albumin in saliva and the dye Bromocresol green (prepared by mixing 8.85 g succinic acid, 108 mg Bromcresol green, 100 mg sodium azide and 4.0 mL Brij-35 in 900 mL of distilled water) produces change in color, which is proportional to the albumin concentration in the saliva. It was estimated using a photoelectric colorimeter at wavelength of 630 nm. Standard solution of 6 gm of bovine albumin dissolved in 100 mL of normal saline containing 0.1 g/dL sodium azide was used.

Salivary pH was estimated electrometrically with the help of a pHmeter. A pair of electrodes (glass electrode and calomel electrode) was dipped in saliva, whereby potential developed across the thin glass of the bulb (of glass electrode). Variations of pH with electromotive force (E) were recorded directly on the potentiometer scale graduated to read pH directly.

Then, a titration method was used to determine the buffering capacity. One milliliters of saliva of known pH was taken in a test tube to which was added phenol red indicator. It was titrated against 0.1 N sodium hydroxide till the pH was raised by one unit. The color was compared with the standard buffer. Then, saliva was titrated against 0.1 N hydrochloric acid using methyl red indicator to lower the pH by one unit. The titer values were noted down. The buffering capacity of the saliva toward acidic and alkaline side was calculated. Buffering capacity = titer value × normality × 1000/1000 × 10.

RESULTS

The biochemical values of this study were subjected to statistical analysis to specify the statistical differences between the groups and subgroups. Student's t-test, Fisher's test (ANOVA) and Tukey HSD (ANOVA) tests were used to compare and correlate different parameters in subgroups among the young and the elderly.

Table 1 correlates the parameters of the subgroups among the young and elderly using *t*-test. Of all the parameters, flow rate in all the three subgroups was shown to be higher in the younger group (*P*=0.001, very highly significant) as shown in the above-mentioned graphs. Buffer capacity of the elderly controls was significantly (*P*=0.054) higher than that of the young control group. pH, salivary total protein and albumin estimations did not show any significant changes.

Table 1

Correlation of the parameters in the subgroups among the young and elderly (*t*-test)

Sub Group	Class	N	MEAN	S.D	P VALUE
Ph	Young	20	6.580	0.4666	1.1500
	Old	20	6.740	0.4001	-0.264 *

Buffering Capacity	Young	20	6.810	0.3291	1.9670
	Old	20	6.015	0.3233	-0.064 †
Salivary total protein	Young	20	0.8960	0.2012	0.8630
	Old	20	0.8360	0.2368	-0.390 *
Salivary albumin	Young	20	0.1000	0.0636	1.2510
	Old	20	0.0000	0.0142	-0.219 *
Flow rate	Young	20	0.5400	0.1314	3.6570
	Old	20	0.3960	0.1191	-0.001 † (Very Highly Significant)

* - Nothing significant † - Highly significant

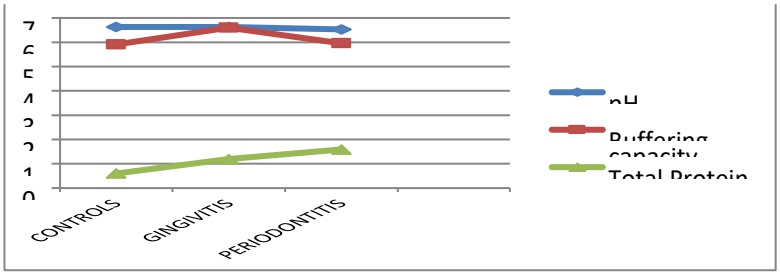
Table 2 :Estimate the significance of different parameters in combined young and elderly groups using Fisher's test. Salivary total protein and albumin estimation were shown to be very highly significant (*P*=0.001) biochemical markers. Salivary total protein in the controls, gingivitis and periodontitis subgroup was 0.86 g/mL (SD=0.21), 1.19 g/mL (SD=0.23) and 1.59 g/mL (SD=0.48). Total mean salivary albumin for controls, gingivitis and periodontitis patients was 0.09 (SD=0.04), 0.24 (SD=0.09) and 0.44 (SD=0.12) mg/mL. pH and buffer capacity did not show any significant changes among the control, gingivitis and periodontitis groups. There was no significant difference in all the parameters except flow rate, which was found to be higher in males (*P*=0.001, very highly significant) than in females.

Table 2: Estimating the significance of the parameters in the study

	N	MEAN	S.D	F	P VALUE
Ph					
Control	40	6.6600	0.4390	1.072	0.34 *
Gingivitis	40	6.6300	0.3743		
Periodontitis	40	6.5325	0.4060		
Buffering Capacity					
	40	5.9125	0.3363	1.070	0.34 *
Control	40	6.6025	0.8638		
Gingivitis	40	5.9610	0.3997		
Periodontitis					
Salivary total protein					
	40	0.6060	0.2190	46.674	0.001†
Control	40	1.1900	0.2362		(vh sig)
Gingivitis	40	1.5909	0.4951		
Periodontitis					

* - Nothing significant † - Highly significant

Graph 1. Comparison of variables LIKE SALIVARY Ph, buffering capacity and salivary total protein AMONG three groups namely: control, gingivitis and chronic periodontitis (CP).



Graph 1 shows on comparison of three groups,Salivary total protein were shown to be very highly significant. pH and buffer capacity did not show any significant changes among the control, gingivitis and periodontitis groups

DISCUSSION

There was a rise in the total salivary protein concentration in the gingivitis and periodontitis subgroup in both the groups. In total, the mean values in the controls, gingivitis and periodontitis subgroups were 0.86 g/mL (SD=0.21), 1.19 g/mL (SD=0.23) and 1.59 g/mL (SD=0.48). The rise in these values was statistically very highly significant (*P*=0.001). The study conducted by Henskens, *et al*.[6] showed the mean values in the controls, gingivitis and periodontitis subgroup as 1.06 mg/mL (SD=0.25), 1.49 mg/mL (SD=0.58) and 2.21 mg/mL (SD=1.0). Both the groups showed 1.8 and 1.3 times value rise in the periodontitis and gingivitis subgroups, respectively, when compared with that of the controls.

In general, the major factors affecting the protein concentration and composition of whole saliva are the salivary flow rate, protein contributions of the glandular saliva and crevicular fluid proteins.

Thus, the elevated protein levels are most likely due to enhanced synthesis and secretion by the individual glandular saliva. Also, glandular-derived proteins, Cystatin C and amylase showed significant rise in periodontitis subjects, proving the glandular origin of these proteins.[7] In addition, the rise in salivary albumin also plays a role in the rise of total proteins. Thus, in the present study, salivary total protein concentration was proved to be a valuable biochemical marker of periodontal disease using the Biuret method.

Albumin was formerly detected as a minor component of whole, parotid, submandibular and sublingual saliva. Notable rise in albumin concentration in the gingivitis and periodontitis subgroups was noted when compared with the controls using the Bromocresol green method. The groups showed around four to five and two to three times rise in the periodontitis and gingivitis subgroups, respectively, when compared with that of the controls. Total mean salivary albumin for controls, gingivitis and periodontitis patients was 0.09 (SD=0.04), 0.24 (SD=0.09) and 0.44 (SD=0.12) mg/mL. The rise in these values was statistically very highly significant (*P*=0.001). There are reports of studies in which increased albumin concentrations during inflammation and periodontal breakdown were found in saliva and gingival crevicular fluid (GCF).[8,9] It showed the mean values in the controls, gingivitis and periodontitis subgroups as 0.08 mg/mL (SD=0.05), 0.30 mg/mL (SD=0.30) and 0.67 mg/mL (SD=0.50), which are slightly on the higher side than that found in the present study. Comparison of the data between the groups showed that there was no significant difference. It signifies the fact that old age as such does not affect the composition of saliva. Saliva from totally edentulous patients contained five to six times less albumin than saliva from the controls, confirming the sulcular origin for albumin.[4] This suggests the role of this parameter as a marker for gingivitis and periodontitis where plasma protein leakage occurs as a consequence of the inflammatory process.

GCF is both a physiological fluid as well as an inflammatory exudate, originating from the gingival plexus of blood vessels in the gingival corium, subjacent to the epithelium lining of the dentogingival space. As GCF traverses through inflamed periodontal tissues en route to the sulcus, biological molecular markers are gathered from the surrounding areas and are subsequently eluted into whole saliva.[8]

The hypothesis that periodontal microbes trigger inflammatory response and results in higher levels of salivary albumin and total protein is well known. Therefore, proteins are considered potential markers for plasma protein leakage. Subjects with periodontitis had significantly more *P. gingivalis, P. intermedia*and *T. denticola* when compared with the controls. *T. denticola* increased the levels of salivary albumin and total protein as the proteins existing in the periodontal pocket, including immunoglobulins and albumin, are potential energy sources for *T. denticola*. Hollman and Van Der Hoeven (1999) [10] have reported that degradation of albumin by *T. denticola* strains was not detected, but suggested that *T. denticola* occurs in close association with strong proteolytic bacteria such as *P. gingivalis* and *T. forsythia* in the subgingival plaque. Thus, controlling the microbes in turn decreases the inflammatory response, which in turn decreases the plasma leakage in the saliva through GCF.[10]

Salivary values have been published for 629 patients from the University of Lund, Sweden, where the majority of the subjects had resting flow rates of 0.1-0.5 mL/min.[11] It is in accordance with the present study, where the mean flow rate in the young subjects was around 0.5 mL/min. Flow rate did not alter with the periodontal status of the subjects in both the groups. Salivary flow rates in the elderly were lower than in adults in general. It seems that old age as such does not cause diminished salivary flow.[12,13] It has also been suggested that there are some age-related alterations in salivary function.[14]

As shown in the present study, there was a significantly decreased flow rate in females when compared with males (P=0.001). This difference has been suggested to be due to the size of the salivary gland.[14]Also, in menopause, many women seem to suffer from xerostomia, which then ameliorates in older age.[15]

The salivary flow is directly related to the salivary consistency. Thus, greater the salivary flow, greater the consistency and greater the cleaning and diluting capacities; therefore, if changes in health cause a reduction in salivary flow, there would be a drastic alteration in the level of oral cleaning.[15] The normal salivary pH is from 6 to 7, and varies in accordance with the salivary flow from 5.3 to 7.8. There are various sources of hydrogen ions in oral fluids: secretion by the salivary glands in the form of organic and inorganic acids, production by the oral microbiota or acquisition through foods. These ions influence the equilibrium of calcium phosphates in the enamel. The higher the concentration of hydrogen ions, the lower the pH, and vice versa.[16]

An interesting finding was significant rise in the buffering capacity among the elderly controls when compared with the young. Galgut[17] in his study had tried to correlate between pH and gingivitis and periodontal pockets. Statistically significant correlations between gingivitis and pH did not exist, but significant correlations did exist between pH and periodontal pockets. Our finding correlates with Sewon, *et al*. [18] studies, which suggests that pH and buffer capacity changes in gingivitis and periodontitis are not as consistent as GCF changes. This may be due to the dilution of the GCF contents in total saliva.

The development of new separation techniques and different mass spectrometry instrumental devices, as well as the great availability of specific reactants, offers ample choice to scientists for carrying out high-throughput proteomic studies and being competitive in the field today. Koss, *et al*. analyzed total saliva for its physical and chemical properties. The aim of their study was to identify salivary parameters

that could identify different stages of the periodontal disease. Dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used for protein detection and zymography for type IV collagenase identification. Salivary flow rate, pH and buffer capacity showed similar values in all groups. Proteins were augmented in severe periodontitis, as also shown by SDS-PAGE. Hydroxyproline rose significantly in all periodontal groups as secretory immunoglobulin A significantly diminished compared with the control group. An increase in peroxidase was detected in moderate and severe periodontitis. All salivary samples contained 200116-92 kDa gelatinases; minor bands at 66-31 kDa were also present in all periodontitis groups. Calcium levels showed significant differences between all periodontitis groups compared with the control group. Thus, they claimed that quantitative changes in the chemical composition of the saliva of patients with periodontal disease could be of significance in the diagnosis and progression of periodontal disease.[19]

Total protein is a vital component of saliva and is responsible for most of its functions like lubrication, physical protection, cleansing, buffering, maintenance of tooth integrity, taste and digestion and antibacterial activity. **[7]** The major factors affecting the protein concentration and composition of whole saliva are the salivary flow rate, protein contributions of the glandular saliva and crevicular fluid proteins. [21]

Quantitative proteomics (two-dimensional SDS-PAGE) was used to investigate whole saliva from individuals with severe periodontitis and their proteomic profiles before and after periodontal treatment were compared. Results highlighted the predominant involvement of S100 proteins in the host response during periodontitis, identifying host defence components that have not been linked previously to this disease and suggesting new potential biomarkers for monitoring disease activity in periodontitis.[20]

CONCLUSION

The present finding suggests A very highly significant rise in the salivary total protein levels are considerably associated with clinical parameters of gingivitis and PPD in periodontitis. Salivary protein levels can also reflect gingival inflammation, periodontal pocket formation, and severity of periodontal conditions. Longer studies should be conducted in relationship of identified marker to nature of periodontal disease. Although the saliva-based diagnostic method has been still challenged, these will be promising as a future application in diagnostic for periodontal diseases and to prognosticate periodontal treatment outcomes.

ACKNOWLEDGMENT

The authors would wish to extend grateful acknowledgment to Dr. Julius.A, Professor, Department of Biochemistry, for his guidance in the Biochemistry Laboratory.

Source of Support: Nil

Conflict of Interest: None declared

REFERENCES

1. Deepti Wadhwa et al., Determination of levels of nitric oxide in smoker and nonsmoker patients with chronic periodontitis. J Periodontal Implant Sci 2013;43:215-220. [PubMed] 2. Manisundar N et al., Comparison of the Salivary and the Serum Nitric Oxide Levels in Chronic and Aggressive Periodontitis: A Biochemical Study. Journal of Clinical and Diagnostic Research. 2013 April, Vol-7(4): 000-0002. [PubMed]
3. Yugantar N. Wakde et al. Comparative Evaluation of Salivary Flow Rate, Ph, Buffering Capacity, Total Protein and Albumin Levels in Chronic Periodontitis Patients: A ClinicoBiochemical Study International Journal of Health Sciences & Research 2018: Vol.8; Issue: 5; May 2018[PubMed]

4. Terrapon B, Mojon P, Mensi N, Cimasoni G. Salivary albumin of edentulous patients. Arch Oral Biol. 1996;41:1183–5. [PubMed]
5. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: Current state and future directions.Periodontology 2009;50:52–64. [PubMed]
6. Henskens YM, van der Velden U, Veerman EC, Nieuw Amerongen AV. Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis or periodontitis. J Periodontal Res. 1993;28:43–8. [PubMed]
7. Henskens YM, van der Velden U, Veerman EC, Nieuw Amerongen AV. Protein composition of whole and parotid saliva in healthy and periodontitis subjects. J Periodont Res. 1996;31:57–65. [PubMed]
8. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: Current state and future directions.Periodontology 2000. 2009;50:52–64. [PubMed]
9. Yakob M, Kari K, Tervahartiala T, Sorsa T, Söder PÖ, Meurman JH, et al. Associations of periodontal microorganisms with salivary proteins and MMP-8 in gingival crevicular fluid. J Clin Periodontol. 2012;39:256–63. [PubMed]
10. Hollman R, Van Der Hoeven HJ. Inability of intact cells of Treponema denticola to degrade human serum proteins IgA, IgG and albumin. J Clin Periodontol.1999;26:477–9. [PubMed]
11. Shern RJ, Fox PC, Li SH. Influence on age on the secretory rates of the human minor salivary glands and whole saliva. Arch Oral Biol. 1993;38:755–61. [PubMed]
12. Närhi TO, Meurman JH, Ainamo A, Nevalainen JM, Schmidt-Kaunisaho KG, Siukosaari P, et al. Association between salivary flow rate and the use of systemic medication among 76-, 81-, and 86-year old inhabitants in Helsinki, Finland. J Dent Res. 1992;71:1875–80. [PubMed]
13. Yeh CK, Johnson DA, Dodds MW. Impact of aging on human salivary gland function: A community based study. Aging. 1998;10:421–8. [PubMed]
14. Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. J Dent Res. 1987;66:648–53. [PubMed]
15. Shaila M, Pai GP, Shetty P. Salivary protein concentration, flow rate, buffering capacity and pH estimation: A comparative study among young and elderly subjects, both normal and with gingivitis and periodontitis. J Indian Soc Periodontol. 2013;17:42-46 [PubMed]
16. Kudva P, Tabasum ST, Sharma S, Gupta S. Role of Saliva as a Diagnostic Tool in Periodontal Disease. Arch Dent Sci Orig Res. 2010;1:16–20.
17. Galgut PN. The relevance of pH to gingivitis and periodontitis. J Int Acad Periodontol. 2001;3:61–7. [PubMed]
18. Sewon L, Soderling E, Karjalainen S. Comparative study on mineralization-related intraoral parameters in periodontitis-affected and periodontitis-free adults.Scand J Dent Res. 1990;98:305–12. [PubMed]
19. Koss MA, Castro CE, Salúm KM, López ME. Changes in saliva protein composition in patients with periodontal disease. Acta Odontol Latinoam.2009;22:105–12. [PubMed]
20. Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose,

salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci. 2010; 52:359-68 [PubMed]

21. Haigh BJ, Stewart KW, Whelan JR, Barnett MP, Smolenski GA, Wheeler TT. Alterations in the salivary proteome associated with periodontitis. J Clin Periodontol.2010;37:241– 7. [PubMed]