How to Cite:
Ambreen, F., Ali, D. E. S., Faiz, S., Shah, I., Majid, B., & Khan, Z. (2022). Evaluation of salivary parameters (flow rate, pH, amylase and total protein) in periodontitis patients based on CPITN criteria. *International Journal of Health Sciences*, *6*(S9), 1286–1295.
https://doi.org/10.53730/ijhs.v6nS9.12520

Evaluation of salivary parameters (flow rate, pH, amylase and total protein) in periodontitis patients based on CPITN criteria

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***Abstract*---**Introduction: The available literature on the topic is inconclusive and sometime taking less parameters in consideration. Moreover, there is no published data available in local population. Objectives: To determine and compare salivary flow rate, pH, total proteins, and alpha amylase in periodontitis patients based on community periodontal index of treatment needs (CPITN) score. Materials & Methods: A cross sectional study on 80 participants selected by purposive sampling was completed in 6 months. Participants were divided into 5 groups by CPITN scoring. Individuals more than 30 years with at least 20 teeth having calculus/staining were included. The study was conducted at Dental department of Civil Dispensary Peshawar city, Khyber College of Dentistry (periodontology ward) and Khyber Medical University (Biochemistry lab). After explaining the procedure and taking consent, saliva was collected from the participants following aseptic technique and analyzed through ELISA. Data was analyzed using SPSS version 22. P value

<0.05 was considered to be significant in our study. Results: Among 80 subjects, 51 were females and 29 were males with mean age 35.73±7.05 years and BMI 26.47±5.23. Significant difference for mean Salivary flow rate (P Value = 0.037), Mean salivary total protein (P value <0.01) and mean Salivary amylase (P Value <0.001) was observed based on CPITN scoring. A gender-based difference was observed for Salivary amylase (P value <0.01). However, mean pH concentration did not seem to be altered by gender or CPITN Scoring. In addition, Salivary amylase showed a strong correlation with age, height and total salivary protein. Conclusion: Salivary amylase and total protein concentration increase with severity of the periodontitis and both of them can be used as prognostic tools to evaluate the disease progress.

***Keywords*---**salivary parameters, dental, treatment.

Introduction

Saliva is a protective fluid of oral cavity produced by three pairs of major (parotid, submandibular, sublingual) and minor glands of lips, palate, cheek, lingual and labial mucosa. (1) Submandibular gland forms the main contribution of saliva (65-70%), followed by parotid gland (20%), sublingual gland (7-8%) and 5% by minor salivary glands. (2) Saliva is mainly composed of water, organic and inorganic components including enzymes, mucin, electrolytes, antibacterial factors, proteins, food debris, secretions from upper respiratory tract. (3) Main functions of saliva are lubrication, digestion, mastication, speech, maintenance of tooth structure and innate immunity of the oral cavity. (4) Normal pH of saliva is 6.5 to 7.(1) Daily production of saliva in humans is 500-1000ml/day (2), with an average flow rate of 0.3-0.5 ml/minute for un- stimulated and 1-3ml/minute for stimulated saliva. (1) Sympathetic stimulation increases salivary flow while parasympathetic stimulation decreases salivary flow. (1) Hypo salivation increases susceptibility to dental caries and periodontal diseases. (5)

Periodontitis is an inflammatory dental condition frequently associated with plaque involving the periodontium (gingiva, cementum, periodontal ligament, alveolar bone) which results in bleeding from gums, pocket formation, gum recession, bone loss and ultimately tooth loss. (6) Salivary α amylase and total protein have been shown to have antibacterial activities and therefore play a role in maintaining the internal environment of the oral cavity. Salivary amylase is a digestive enzyme of oral cavity produced by the salivary glands (55-60%) and pancreatic amylase (45-50%) by pancreas. (7) It has also got antibacterial action by binding with lipopolysaccharide component of bacteria *(Aggregatibacteractinomycetum comitans)* present in plaque (8) and inhibits the attachment of bacteria on tooth pellicle and colonization of bacteria *(Porphyromonas gingivalis).* (9)

Total protein is an important component of saliva and its main role is lubrication, protection, buffering, maintenance of tooth integrity, digestion and antibacterial activity. (10)

Periodontitis is classified into many types on the basis of severity and extent (6), however, it is diagnosed on basic periodontal examination based on Community Periodontal Index of Treatment Need (CPITN) criteria that includes three parameters i.e. bleeding on probing, supra or sub gingival plaque, pocket depth as given in Table 1. (11) Main bacteria involved in periodontitis are *A. comitans* and *P. ginigivalis.* (9)

Table 1: CPITN Scoring. (9) CPITN Scoring Criteria. It should be noted that Score Zero denotes healthy gums and score 5 denotes advance disease state

CPITN score	Periodontal condition clinically	Criteria
0	Normal healthy periodontium	No treatment needed
1	Bleeding on probing	OHI
2	Presence of plaque and calculus	Scaling and polishing. OHI
3	Pocket depth is 4-5mm	Deep scaling with OHI
4	Pocket depth is more than 6 mm	Scaling, root planning, surgical intervention

Changes in the composition of salivary components are thought to play a role in the pathogenesis of periodontitis i.e. inflammation leads to increased capillary permeability and leakage of proteins. (4) Similarly, low pH of saliva favours the growth of acidogenic bacteria (*Prevotella ginigivalis*, *Prevotella intermidia*) that causes periodontitis. (12, 13) High salivary amylase level increases the protective action of saliva seen in periodontitis. High level of total proteins is associated with increased inflammatory response due to sympathetic activation in periodontitis. (4)

There are reported studies on the changes in composition of these parameters in different reference population, however, results are often contradictory. (4, 9, 12) Moreover, there are no reported studies on these parameters in periodontitis patients based on CPITN score in Pakistan. Therefore, this study has been designed to detect and compare the differences in salivary flow rate, pH, total proteins and alpha amylase in periodontitis patients based on CPITN score.

Materials & Methods

This cross-sectional study included 80 participants selected by purposive sampling after approval from the ethics review committee for human subjects of Khyber Medical University, Peshawar and was conducted in accordance with the revised (2013) declaration of Helsinki 1975. Before recruitment into the study the purpose of the study was explained to the participants and written informed

consent was taken. Participants were divided into 5 groups (16 in each group) on the basis of CPITN scoring mentioned in Table 1. Individuals more than 30 years with at least 20 teeth having calculus/staining, coming for routine visits at Dental department of Civil Dispensary Sheikhabad No 3 Peshawar city, KCD (Periodontology ward), KMU (Biochemistry lab) were made part of the study. The study spanned over 6 months (October 2018 to March 2019). Procedure and study questionnaire were clearly explained, and consent was taken. Saliva from the participants was collected in falcon tubes by aseptic technique, followed by anthropometric measurements and general physical examination.

Oral examination including periodontal examination with CPITN probe were followed by CPITN scoring and allocation of the patient to the groups. Partipciapnt were advised to spit out saliva every 30 seconds for 5 minutes for determination of Salivary flow rate. Later on a total of 5 to 7 ml of unstimulated saliva was collected in falcons tubes by direct spitting method. Saliva was centrifuged, pH was determined, and aliquots were made in Eppendorf tubes and stored at -80°C. The aliquots were labelled as A, B, C & D. Sodium azide was added in A and B aliquots for protein analysis by Benedicts method whereas aliquots C and D were stored for measurement of salivary amylase by ELISA.

Results

Among 80 subjects 51 were females and 29 were males with mean age 35.73 ± 7.05 years and BMI 26.47 ± 5.23. The mean diastolic B.P was 71 ± 4 mmHg and that of systolic B.P was 112 ± 6.26 mmHg. Variables related to oral hygiene are represented in Table 2.

Table 2: Determinants of Oral Hygiene

Variable	Frequency	Percent %
Chief complaints		
Extraction	8	10
Filling	1	1.25
Polishing	12	15
Scaling	37	46.25
Routine check up	15	18.75
Other	7	8.75
Brush daily times		
0	5	6.33
1	41	50.63
2	31	39.24
3	3	3.80
Bleeding gums		
Yes	34	42.50
No	46	57.50
Halitosis		
Yes	34	43.04
No	46	56.96
Plaque/calculus/stained		
Plaque	20	25

Calculus	23	28.75
Stained teeth	37	46.25
Oral ulcer		
Yes	5	6.25
No	75	93.75
Ill-fitting		
Yes	11	13.75
No	69	86.25
Presence of pockets		
Yes	32	40
No	48	60
Total	80	100.00

Descriptive analyses were done for all participants. Gender based difference were determined through independent sample t-test which shows significant difference for total salivary protein (p=0.001) and salivary α amylase (p = 0.003) as given in Table 3.

Table 3: Gender Differences of Salivary Parameters in Periodontitis Patients

	Total	Female	Male	P-Value
Sample size (n)	80	51	29	
Age (Years)	35.6 ± 7.2	34.98 ± 6.9	36.8 ± 7.7	0.29
Height (m)	1.61 ± 0.09	1.58 ± 0.08	1.64 ± 0.9	**0.004**
Weight (kg)	67.04 ± 14.9	66.2 ± 15.07	68.4 ± 14.9	0.53
BMI (kg/m^2)	25.95 ± 5.24	26.38 ± 5.09	25.2 ± 5.5	0.39
Heart Rate	71 ± 4	71 ± 4	71 ± 4	0.14
SBP	112 ± 6	112 ± 07	112 ± 5	0.14
DBP	74 ± 6	73 ± 6	73 ± 6	0.73

Group wise analysis based on CPITN score analyzed through ANOVA followed by Post hoc tests, yielded significant difference in all parameters except pH of the saliva as shown in Table 4.

Table 4: Salivary parameters in periodontitis patients categorized on CPITN score

		Sum of squares	df	Mean Square	*p-value*
Salivary Flow rate 5 min	Between groups Within Groups Total	11.4 77.6 89.1	5 75 80	2.8 1.0	0.03
pH	Between groups Within Groups Total	0.2 9.2 9.4	5 75 80	0.56 0.13	0.76
Total Protein mg/mL	Between groups Within Groups	90052 72330	4 75	22513 964	<0.001

	Total	162383	79		
Salivary α amylase U/ml	Between groups Within Groups Total	294907 422360 717268	4 75 79	73726 5631	<0.001

Moreover, salivary amylase concentration shown a significant correlation with age (r = 0.366, p = 0.001) Figure 1, height (r = 0.256, p = 0.022), and total protein concentration (r = 0.486, p = 0.001) Figure 2 as determined by Pearson correlation.

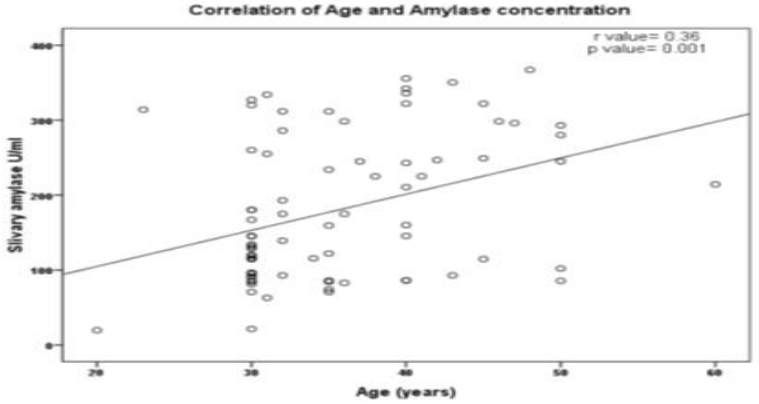

Figure 1: Correlation between age and salivary amylase concentration

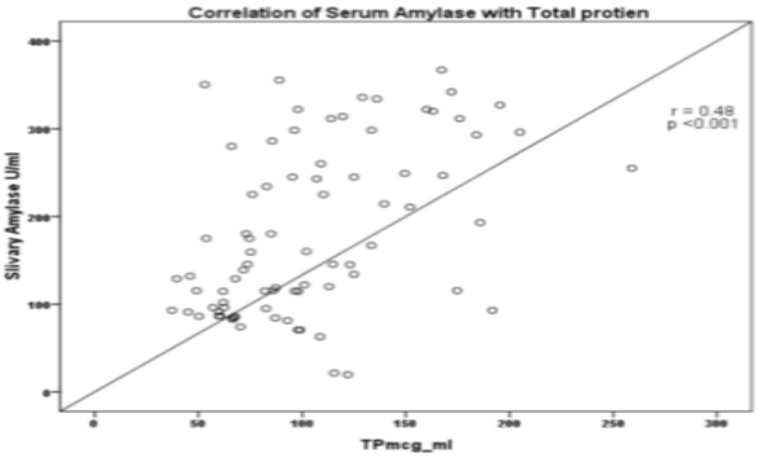

Figure 2: Correlation between total salivary protein and salivary amylase concentration

Discussion

The salivary flow rates for all the patients were determined with no gender difference. Mean salivary flow rate for all participant reported were 2.10 ml/5min. This is in accordance with the published literature (4) where the authors has reported a salivary flow rate of 0.5 to 1 ml per minute. In our study the mean salivary flow rate per minute is slightly less than 0.5 ml (0.42 ml/min). However,

in the previous studies the collection of the saliva was done for 1 minute only. The salivary glands production exhausts with repeated spitting of saliva.

The mean salivary flow rate for males in this study was 2.13 ± 1.04 ml/ min and for females it was 2.03 ± 1.09 ml/ min. Similar results has been reported previously.(6) Though the difference in the salivary flow rate between females and males are not significant, however, the high salivary flow rate in males could be attributed to the increase salivary gland size.(13) Moreover, increasing age of the females especially after menopause increase the chances of xerostomia increases.(14) The mean age of females in our study was 34.98 ± 6.9 years which can also be attributed to the decrease salivary flow rate observed in females.

In addition, the analysis revealed that salivary flow rate increased with increasing severity of the disease (Anova P value=0.037). Changes in salivary flow rate with diseased gums has been reported before which shows that salivary flow rate increase in local periodontal conditions.(8) It is a well-known hypothesis that periodontitis being an inflammatory conditions triggered by the microbes causes an increase salivary flow rate. The flow of saliva is directly related to the cleaning and diluting capacitates and an increase in salivary flow in such conditions means a homeostatic effort of the body to add to the healing process to overcome the inflammation. (10)

pH of the saliva shows no difference based on gender or CPITN categories. This could be due to the fact that means pH concentration of all the sample populations (6.89 ± 0.35) points towards an acidic environment as inflammation favors academia. Normal pH of saliva is 7.4 ranging from 7.1 to 7.5 and recruitment of normal people could have pointed out the real difference which is a limitation of this study. (15) Moreover, an estimation of the proper difference between the means of the pH could not be concluded with low sample size (n=16 in each category), as pH tends to remain stable most of the time. (16) Salivary pH below 7 usually indicates acidemia and in the presence of a chronic conditions the susceptibility of the oral environment increases to different local (Halitosis, periodontitis, dental decay) and systemic manifestations. (15) Oral fluid has hydrogen ions from many sources including glandular production, end product of degradation by microbiata and food particles from outside which disturbs the equilibrium of the oral environment. (17) A low pH favours the growth of bacteria such as *P.gingivalis and B. intermedius* as reported by Takahasi. The same bacteria play a significant role in chronic tooth conditions including periodontitis. (18) With some exceptions most of the bacteria involved in the pathogenesis of the periodontitis uses or forms end product that are mildly acidic in nature. For example, *Fusobacterium* species depends on glutamic acid for food with producing acetic and butyric acids as end product. Moreover, P. *gingivalis*, *P. intermedia* and *C. rectus* require formic acid as a reducing agent for mobilization of aspartic acid to succinic acid. Similarly, presence of Isobutyric acid are prerequisite for growth of Oral *Treponemal* species. (19)

The mean total protein concentration found in our study was almost similar to that reported by Walsh and his colleagues. (18) The salivary total protein concentration depends on factors like salivary flow rate, protein concentration from the glands and gingival cervicular fluid (GCF). (6) Inflammatory process

initiates the protein leakage along with increases in the glandular protein excretion. Salivary albumin concentration enhances as an immune response in chronic inflammation thus increasing overall protein concentration. (2) Thus salivary protein can be easily used as a biomarker of chronic oral problems. Salivary amylase catalyzes the α-glycosides bonds of glycogen and starch. Like proteins it is also involved in the oral defense system because of its anti-microbial properties thus leading to raised serum amylase levels. (8) The mean concentration of serum amylase was higher than healthy volunteers probably because of increase in total protein concentration as shown by the positive correlation of the two. Kejriwal et al also reported a strong correlation between serum amylase and protein concentration, as amylase is proteinaceous in nature.

Periodontitis is an inflammatory condition caused by microbes such as *A. actinomycete comitans* and P. *gingivalis.* Amylase acts as a major binding protein interfering with pathogens adhesion. This study reports a marked difference between categories (CPITN score) justifying severity of disease which is in accordance with findings reported elsewhere. (2, 20) The probable reasons of raised salivary amylase concentration are inflammatory response to the ongoing inflammation and direct stimulation and release of inflammatory products. (17).

This study suggests a dependence of increase in salivary amylase and total protein concentration on the inflammatory condition of the oral cavity especially periodontitis. Both of the parameters can be used as prognostic tools for the disease progression. It is recommended that the observed gender-based difference for salivary amylase and total protein requires a further large sample-based study in future for probing deep with emphasis on potential causes for this difference.

Acknowledgements

The authors acknowledge the support and assistance provided by all participating patients, laboratory staff of KMU, KCD and dispensary staff. The study was partially funded by Office of Research, Innovation and Commercialization (ORIC), Khyber Medical University.

References

1. De Almeida PDV, Grégio AMT, Machado MÂN, De Lima AAS, Azevedo LR. Saliva composition and functions: A comprehensive review. Journal of Contemporary Dental Practice. 2008.
2. Henskens YMC, Van Den Keijbus PAM, Veerman ECI, Van Der Weijden GA, Timmerman MF, Snoek CM, et al. Protein composition of whole and parotid saliva in healthy and periodontitis subjects: Determination of cystatins, albumin, amylase and IgA. J Periodontal Res. 1996;
3. Humphrey SP, Williamson RT. A review of saliva Normal composition, flow, and function. Humphrey, Williamson. 2001. Journal of Prosthetic Dentistry.pdf. J Prosthet Dent. 2001;
4. Pandey AK. Physiology of Saliva: An Overview. J Dent Indones. 2014;
5. Jawed M, Khan RN, Shahid SM, Azhar A. Protective effects of salivary factors in dental caries in diabetic patients of Pakistan. Exp Diabetes Res. 2012;

6. Shaila M, Pai Gp, Shetty P. Salivary protein concentration, flow rate, buffer capacity and pH estimation: A comparative study among young and elderly subjects, both normal and with gingivitis and periodontitis. J Indian Soc Periodontol. 2013;
7. Cutress TW, Ainamo J, Sardo-Infirri J. The community periodontal index of treatment needs (CPITN) procedure for population groups and individuals. Int Dent J. 1987;
8. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;
9. Sánchez G, Miozza V, Delgado A, Busch L. Relationship between salivary mucin or amylase and the periodontal status. Oral Dis. 2013;
10. Mese H, Matsuo R. Salivary secretion, taste and hyposalivation. Journal of Oral Rehabilitation. 2007.
11. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontology 2000. 2004.
12. 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.
13. Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. Journal of dental research. 1987;66(1_suppl):648-53.
14. Parvinen T, Larmas M. Age dependency of stimulated salivary flow rate, pH, and lactobacillus and yeast concentrations. Journal of dental research. 1982;61(9):1052-5.
15. Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker. Journal of Indian Society of Periodontology. 2013;17(4):461.
16. Cogulu D, Sabah E, Kutukculer N, Ozkinay F. Evaluation of the relationship between caries indices and salivary secretory IgA, salivary pH, buffering capacity and flow rate in children with Down's syndrome. Archives of oral biology. 2006;51(1):23-8.
17. 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.
18. Takahashi N, Schachtele C. Effect of pH on the growth and proteolytic activity of Porphyromonas gingivalis and Bacteroides intermedius. Journal of dental research. 1990;69(6):1266
19. Takahashi N, editor Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. International Congress Series; 2005: Elsevier.
20. Baik JE, Hong SW, Choi S, Jeon JH, Park OJ, Cho K, et al. Alpha-amylase is a human salivary protein with affinity to lipopolysaccharide of Aggregatibacter actinomycetemcomitans. Mol Oral Microbiol. 2013; 28(2):142-53
21. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. International Journal of Health Sciences, 5(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
22. Perez, A. V., Briones, V. V., Viteri, C. G. V., & Gamez, M. R. (2017). Iberoamerica in network, GIS & TIC. International Journal of Social Sciences and Humanities, 1(3), 108–117. https://doi.org/10.29332/ijssh.v1n3.63
23. Sembiring, T. B., Maruf, I. R., Susilo, C. B., Hidayatulloh, A. N., & Bangkara, B. M. A. S. A. (2022). Health literacy study on approaching forest and

boosting immune system strategy. International Journal of Health Sciences, 6(1), 40–49. https://doi.org/10.53730/ijhs.v6n1.3145