Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Relationship between salivary and serum alpha amylase and the periodontal status

Fatemeh Ahmadi- Motamayel[1], NasrinRafieian[2] , Mohammad Taghi Goodarzi[3] , Mina Hamian*[4], Zohreh Jamshidi[5], Soheila Mesgaran[6]

1 Associate professor, Dental Research Center and Research Center for Molecular Medicine, Department of Oral Medicine, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.
2 Assistant professor, Department of Oral Medicine , Faculty of Dentistry, Alborz University of Medical Sciences, Karaj, Iran.
3 Professor, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
4 Assistant professor, Department of Oral Medicine , Faculty of Dentistry, Alborz University of Medical Sciences, Karaj, Iran.
5 Oral medicine specialist
6 Dental faculty nurse assistant, Hamadan University of Medical Sciences, Hamadan, Iran.

ARTICLE HISTORY

Received: 11.09.2017

Accepted: 13.12.2017

Available online: 31.12.2017

Keywords:

Alpha amylase; Chronic periodontitis; saliva; Serum

*Corresponding author:

Email : hamian.mina@gmail.com
Tel.: 00989195314856, 00982633531614

ABSTRACT

The composition of whole salivary proteins can be changed in many diseases, such as periodontal disease, compare to healthy people. In fact, host response against periodontal microbial causes these changes in profile of salivary proteins.This study investigated the relation between level of salivary and serum alpha- amylase (AA) and chronic periodontitis (CP In this case-control investigation, un-stimulated whole saliva and serum sample was collected from 111 patients between 30-60 years old (including 55 females and 56 males). They were divided intofour groups: CP females, Healthy females (controls), CP males and Healthy males considering age and gender match ($p> 0.05$). Mean levels of salivary and serum AA was assayed by spectrophotometric method to assay enzyme kinetics. Data were analyzed using student's t-test and chi-square test. Salivary AA was significantly higher in CP group compare to control group ($P<0.0001$). Serum AA was not statistically significant in CP group as compared to healthy control group ($P=0.07$). In healthy group mean salivary AA levels were not statistically significant in males than females ($P=0.34$). A significant association was noticed between salivary AA and CP. However, there was not significant association between serums AA with CP. More research to demonstrate the real relation between AA and CP is recommended.

INTRODUCTION

Chronic periodontitis (CP) are inflammatory disorders that occur as a result of the interaction between a pathogenic bacterial biofilm and host-derived inflammatory cells and molecules [1,2,3]. The traditional methods for diagnosis of these diseases, clinical measurements, and radiographic assessments, not often are able to diagnose CP diseases in primary stage, also these methods have possibility to measurement errors to some extent [1,3]. Hence, today various studies are trying to evaluate different compounds in the salivary fluids that help clinicians to primary diagnosis of diseases before their clinical appearance. Also, these markers might help to identify at risk patients simply and rapidly [1,3].

Over 2,000 proteins has identified in the saliva, which approximately 2530% of them are shared with blood [4,5].Most enzymes, specific and nonspecific proteins, antibodies, and other substances infiltrate passively into saliva from plasma and their salivary levels are reflect their levels in plasma. Using saliva, as a diagnostic laboratory tests is noninvasive, convenient and repeatable[4,6]. 50 - 60% of salivary protein consists Alpha amylase (AA) [7]. The serous cells of the parotid gland are the main cells to produce Salivary AA but also this protein is produced by other salivary glands[8]. One of AA roles in saliva is performance a direct inhibitory effect on the growthof certain bacteria and also binds to bacterial surface structure and bacterial toxin that lead to tissue destructive inflammatory reactions[9,10]. Also extracted AA from human saliva, has shown a significant

cell growth inhibitory activity against Porphyromonas gingivalis species, and interfere with the adherence and biofilm formation of Aggregatibacter actinomycemcomitans, so there is a hypothesis that AA could have preventingrole against periodontal diseases [11]. Also, several studies have reported that the levels of distinct salivary proteins like AA are altered in individuals with periodontal disease. Therefore, it seems these salivary constituents may play a role in the etiopathogenesis of this disease [1,3,4,9].

So far there are a few studies regarding the relationship between AAand chronic periodontitis, especially there is sparse studies compare the level of AA between saliva and blood in patients with CP disease [12,15]. This case-control study was designed to assess the amount of salivary AA activity and compare it with serum AA level in healthy and CP subjects.

MATERIALS AND METHODS

This case-control study was approved by the ethics committee of Hamadan University of Medical Sciences, Hamedan, Iran and the procedures followed were in accordance with the Helsinki Declaration of1975 that was revised in 2000. Informed consent was obtained from each patient. Participants:111 patients between 30-60 years old (including 55 females and 56 males) were selected and divided into four groups with age match**:** CP females (N= 28),Healthy females (controls) (N=28), CP males (N= 28) and Healthy males (N= 27).

Medical and dental histories were recorded prior to the study and subjects with the following criteria were excluded from the study:

1.Positive history of any systemic orsalivary gland disease

2.Apparent oral infections (i.e. herpes labialis or oral candida)

3.Regular users of medications within the past 6 months

4.Positive history of smoking or any form of tobacco and alcohol use

5.Pregnant or breast-feeding mothers

6.Receiving any periodontal treatment in the past six months [18-21].

Clinical examination of CP disease:

One oral medicine specialist carried out all clinical examination. CP disease was evaluated. Probing pocket depth (PPD), clinical attachment loss (CAL), gingival index (GI) were used as a complete periodontal examination. PPD and CAL were measured at six sites per tooth (mesio-, mid-, disto-buccal/palatal or lingual. Patients with clinical attachment loss of ≥ 4mm were selected for CP group. Control groups included subjects with minimum complement of 20 natural teeth, GI score ≤ 1 and absence of loss of attachment.[1]

Saliva Collection:

Whole unstimulated saliva was collected within 5 minutes (10) between 8 to 11AM. We advised the participants to brush their teeth and do not use any oral stimulation such as eating and drinking for 90 min prior to collection of saliva, then we asked them to sit on a chair and lean their head forward over the test tube whole saliva samples were obtained by expectorating into polypropylene tubes. The saliva samples were kept at 4° C and were transferred to the laboratory up to 20 minutes and stored at -20^{0} C for final of measure of AA level[32].

Blood collection:

Blood samples were obtained by venous arm puncture and collected in heparinized tubes.

Sialochemical analysis

To achieving pure saliva, saliva samples were centrifuged (KD2-TDSA, Nantong Hailun Bio-medical Apparatus Manufacturing C., Ltd., Haimen city, Jiangsu, China) for 3 - 5 minutes. In order to assay the salivary AA activity, a biochemical kit (EPS-G7, Pars Azmoon Co, Karaj, Iran) and a spectrophotometer (6300, Jenway, Staffordshire, UK) at a wavelength of 590 nm was used. In the above-mentioned method, the reaction of salivary AA on a chromogenic substrate makes a colored solution of chloro-p-nitrophenol, and its darkness was proportional to the level of enzyme activity. Serum alpha amylase activity also was measured similarly using commercially available kit (Pars-Azmoon Co., Iran) that used to measure amylase activity.[32]

Statistical analysis:

Data were analyzed using student's t-test and chi-square test with Stata.11 software. The values are expressed as mean ± SD. A P value of <0.05 was considered statistically significant.

RESULTS

There was no statistical difference between groups considering the participant´s age and gender ($p> 0.05$). The comparison between salivary AA levels in different groups is shown in table 1. Salivary AA was significantly higher in CP group compare to control group ($P<0.0001$). Also mean salivary AA levels were significantly higher in both male and female CP group compare to control group ($P<0.0001$). Although the mean salivary AA was higher in CP males compared to CP females but this difference was not significant ($P=0.08$). In healthy group mean salivary AA levels was not different statistically in males than females ($P=0.34$).

The comparison between serum AA levels in different groups is shown in table 2. Serum AA was not statistically significant between CP group and healthy control group ($P=0.07$). Also, mean serum AA levels were not statistically significant in males and females CP groups compare to control groups ($P=0.08$ and $P=0.40$ respectively). The mean serum AA was not statistically significant between females and males nor between CP and healthy control groups ($P=0.85$ and $P=0.60$ respectively).

DISCUSSION

Recent studies have been shown inflammatory markers to be useful for evaluating of progression of periodontal diseases [15,16] but there are no reliable specific markers that can help clinicians to predict the onset or progress of these diseases [15] due to multiple factors that have roles on etiopathology of CP diseases.

Salivary AA is calcium containing metallo enzyme with anti-microbial properties that seems participate in the oral defense mechanism [17].

The results of this study demonstrated that salivary AA was significantly higher in CP group compare to control group. However, Serum AA was not significantly higher in CP group as compared to healthy control group. We evaluated unstimulated salivary AA because oral cavity is in resting condition most of the time during 24-hour period [9,17]. Also, chronic periodontitis

Table 1 : Comparison of salivary AA in patients with chronic periodontal (CP) disease, according to gender and periodontal status (by using T-Test)

Variable	Number	Mean Salvia α-Amylase	SD	Std. Err.	[95% Conf.	Interval	P value
Sex							***P***=0.14
Male	56	157.46	11.92	1.59	154.27	160.66	
Female	55	154.17	11.59	1.56	151.04	157.30	
Periodontal							***P***<0.0001
No	56	149.72	12.56	1.67	146.35	153.08	
Yes	55	162.06	6.76	0.91	160.23	163.89	
Sex/male							***P***<0.0001
Healthy	28	151.31	12.52	2.36	146.46	156.17	
CP	28	163.61	7.38	1.39	160.75	166.48	
Sex/female							***P***<0.0001
Healthy	28	148.12	12.62	2.38	143.23	153.02	
CP	27	160.45	5.7	1.10	158.18	162.72	
Healthy							***P*** =0.34
Male	28	151.31	12.52	2.36	146.46	156.17	
Female	28	148.12	12.62	2.38	143.23	153.02	
Periodontal							***P*** =0.08
Male	28	163.61	7.38	1.39	160.75	166.48	
Female	27	160.45	5.74	1.10	158.18	162.72	

were chosen because most of patients who are referred for periodontal treatment had the criteria of chronic period ontitis.The results were in accordance with other studies which reported an increase in AA activity in patients with periodontitis [1,22-28]. According to Sa´nchez et al (2011) [22], Goncalvez et al (2010) [23], and Miozza et al (2009) [24], salivary AA activity was significantly higher in unstimulated whole saliva of CP patients compared to control healthy group. Also Acquier et al (2015) [9] indicated a higher concentration of amylase in saliva from patients with aggressive and chronic periodontitis compared with healthy subjects. In another research, Sa´nchez et al (2013) [17] have evaluated salivary amylase in patients with mild, moderate and severe periodontal. They have shown patients with positive bleeding on probing had significantly higher salivary proteins concentration such as amylase, while there was a decrease in salivary AA level after treatment with improvement of clinical parameters. Also according to Kejriwal et al (2014) [1] there was a significant increase in the levels of salivary amylase in gingivitis and chronic periodontitis patients as compared to healthy individuals.

In contrast to this study, Harieian et al(2012)[15] demonstrated no statistically significant difference in the activity of salivary AA in stimulated whole saliva among patients with aggressive and chronic periodontitis as well as healthy control. However, they found a positive correlation between salivary AA activity and the number of periodontally disease teeth. We determined the salivary AA level in unstimulated whole saliva but Harieian evaluates stimulated whole saliva.

Previous study has shown proteins like Cystatins and IgA as well as mucin and AA act as first line of protective roles in the oral cavity against inflammatory diseases [17]It is supposed that the secretion of the proteins like AA controlled directly under the stimulation of the inflammatory process, besides through the activation of the sympathetic nervous system under stress loading from the infection process[9,15, 17,22].This process increase the protective potential role of saliva against accumulation of plaque-derived substances and inflammatory products involved in periodontitis [9,17]. The results of this study confirm the increase in the rate of secretion of AA when the periodontal disease occure.

Besides, in the study of Harririan et al, they supposed in periodontitis AA is a component in saliva that involved in the oxidation process and play an inhibitory function against microorganisms. They suggested the production of pro-inflammatory and anti-inflammatory mediators might be modified by catecholamines or AA. Thus these salivary proteins influence on disease activity [15]. Also other studies showed that AA is a major lipopolysaccharide protein that bind to microorganismslike Aggregatibacter actinomycetemcomitans or Porphyromonas gingivalis and interferes with bacterial adherence and biofilm formation [25-28]. Thus, the high concentration of salivary AA can be an important defense molecule essential for the innate immunity in the oral cavity.

All groups were matched in terms of age and sex in the present

Table 2 : Comparison of serum AA in patients with chronic periodontal (CP) disease, according to gender and periodontal status (by using T-Test)

Variable	Number	Mean serum Amylase	SD	Std. Err	[95% Conf.	Interval]	P value
Sex							P=0.96
Male	56	229.05	32.76	4.38	220.28	237.83	
Female	47	229.45	39.08	5.70	217.98	240.93	
periodontal							P=0.07
No	48	222.53	45.91	6.63	209.20	235.86	
Yes	55	235.08	22.01	2.97	229.13	241.04	
Sex/male							P=0.08
Healthy	28	221.48	42.28	7.99	205.08	237.87	
CP	28	236.62	16.73	3.16	230.13	243.11	
Sex/female							P=0.4
Healthy	20	224.00	51.68	11.56	199.80	248.19	
CP	27	233.49	26.65	5.12	222.94	244.03	
Health							P=0.85
Male	28	221.48	42.28	7.99	205.09	237.88	
Female	20	224.00	51.68	11.56	199.81	248.19	
periodontal							P=0.60
Male	28	236.62	16.73	3.16	230.13	243.11	
Female	27	233.49	26.65	5.13	222.95	244.03	

study. According to Arhakis and et al (2013), the level of AA activity could be influenced by age. They reportedvery low activity of AA in newborns. Over the adulthood until older ages the salivary AA activity seems to remain unchanged. However, salivary AA concentration is higher in elderly compared to young adults [29].Inukai et al (2010)[30] and Ahmadi et al (2013)[7],found that there was a positive relationship between age and salivary AA activity.

The mean salivary AA level was not significantly different between males and females in this study. A similar finding was reported in studies of Bos and co-workers (2014) [31] and Ahmadi et al (2016) [32]. They did not observe any difference in AA levels at the different time point across the day between males and females. However, Ahmadi et al(2013)[7], reported differed AA activity between male and female.

Also we evaluated serum AA activity in CP and healthy control groups and compared it with salivary AA. Although, we found higher serum AA activities in CP comparing to control group, this difference was not significant. The parallel of this study, Haririan reported no differences in serum AA activities among groups with periodontitis and healthy control[15] .

Although Sanchez (2013) [17] reported salivary proteins in patients with periodontal disease not only derived from salivary glands and crevicular fluid, but also may derive from blood and from inflammation. But according to another study AA has been determined in saliva has different origin and produce by salivary glands [32].

Our study showed salivary AA not serum AA activity alter in CP patient. In fact our result supported this theory that the special process of local immune (including increase salivary AA) is responsible for defense against CP disease[15].

CONCLUSION

According to the results of the present study, the level of salivary AA was significantly correlated in patients with the CP. However, there was not significant association between serum AA with CP.More studies need to understanding real role of salivary or serum AA in onset or progress of CP disease and effect of altering in AA activity in treatment process of CP.

REFERENCE

1. Kejriwal S, Bhandary R, Thomas B, Kumari S Estimation of Levels of Salivary Mucin, Amylase and Total Protein in Gingivitis and Chronic Periodontitis Patients J ClinDiagn Res. 2014; 8(10): ZC56ZC60.
2. Rocha Dde M, Zenóbio EG, Van Dyke T, Silva KS, Costa FO, Soares RV. Differential expression of salivary glycoproteins in aggressive and chronic periodontitis. J Appl Oral Sci 2012;20(2):1 80-85.
3. GonçalvesLda R, Soares MR, Nogueira FC, et al. Comparative proteomic analysis of whole saliva from chronic periodontitis patients. J Proteomics 2010; 73:133441.
4. Podzimek S,Vondrackova L, Duskova J, Janatova T, Broukal Z. Salivary Markers for Periodontal and General Diseases. Dis Markers. 2016; 2016: 9179632.
5. Loo J. A, Yan W, Ramachandran P, Wong D. T. Comparative human salivary and plasma proteomes. J Dent Res 2010; 89(10):1016-1023.
6. Ahmadi-Motamayel F, Goodarzi M. T. Hendi S, Abdolsamadi H , Rafieian N. Evaluation of salivary flow rate, pH, buffering capacity,calcium and total protein levels in caries free and caries active adolescence. JDOH2013; 5(4): 35-39.
7. Ahmadi-Motamayel F, Shahriari S, Goodarzi M. T, Moghimbeigi A, Jazaeri S. Babaei P. The relationship between the level of salivary alpha amylase activity and pain severity in patients with symptomatic irreversible pulpitis.Restor Dent Endod 2013;38(3):141-5.
8. Nikitkova A E, Haase E M., and Scannapieco F A. Taking the Starch out of Oral Biofilm Formation: Molecular Basis and Functional Significance of Salivary α-Amylase Binding to Oral Streptococci. Appl Environ Microbiol 2013; 79(2): 416-423.
9. Acquier AB, De Couto Pita AK, Busch L, Sánchez GA.Comparison of salivary levels of mucin and amylase and their relation with clinical parameters obtained from patients with aggressive and chronic periodontal disease. J Appl Oral Sci 2015; 23(3): 288-294.
10. Fábián TK, Hermann P, Beck A, Frejérdy P, Fábián G. Salivary defense proteins: their network and role in innate and acquired oral immunity. Int J MolSci 2012; 13: 4295-4320.
11. Baik JE, Hong SW, Choi S, et al. Alpha-amylase is a human salivary protein with affinity to lipopolysaccharide of Aggregatibacteractinomycemcomitans. Mol Oral Microbiol 2013;28:142-153.
12. Fábián TK, Fejérdy P, Csermely P. Saliva in health and disease, chemical biology of. In: Begley TP, editor. Wiley encyclopedia of chemical biology. Vol. 4. Hoboken: John Wiley & Sons, Inc.; 2008. pp. 19.
13. Chaudhuri B, Rojek J, Vickerman MM, Tanzer JM, Scannapieco FA. Interaction of salivary alpha-amylase and amylase-binding-protein A (AbpA) of Streptococcus gordoniiwith glucosyltransferase of S. gordonii and Streptococcus mutans. *BMCMicrobiol* 2007 ;25;7:60
14. Rohleder N, NaterUMDeterminants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology 2009; 34(4): 469-85.
15. Haririan H, Bertl K, Laky M, et al. Salivary and Serum Chromogranin A and a-Amylase in Periodontal Health and Disease. J Periodontol2012;83(10):1314-21.
16. Kinney JS, Morelli T, Braun T, et al. Saliva/pathogen biomarker signatures and periodontal disease progression. J Dent Res 2011;90:752-58 .
17. Sanchez GA, Miozza VA, Delgado A, Busch L.Relationship between salivary mucin or amylase and the periodontal status. Oral Diseases 2013; 19: 585-91 .
18. Abou-Seif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. ClinChimActa 2004; 346:161-70.
19. Aydin S. A comparison of ghrelin, glucose, alpha amylase and protein levels in saliva from diabetics. *J BiochemMolBiol*2007; 40:29-35.
20. Uesato M, Nabeya Y, Akai T, et al. Salivary amylase activity is useful for assessing preoperative stress in response to pain in patients undergoing endoscopic submucosal dissection of gastric tumors under deepsedation. *Gastric Cancer* 2010;13: 84-89.
21. Robles TF, Shetty V, Zigler CM, e. The feasibility of ambulatory biosensor measurement of salivary alpha amylase: Salivary alpha amylase and pain in pulpitis relationships with self-reported and naturalistic psychological stress. *BiolPsychol*2011; 86:50-56.
22. Sa´nchez GA, Miozza V, Delgado A, Busch L. Determination of salivary levels of mucin and amylase in chronic periodontitis patients. J Periodont Res 2011; 46: 221227.
23. Da RosGoncalvez L, Soares MR, Nogueria FCS, et al. Comparative proteomic analysis of whole saliva from chronic periodontitis patients. J Proteomics 2010; 73:13341341
24. MiozzaV, Borda E, Sterin-Borda L, Busch L. Experimental periodontitis induces a cAMP-dependent increase in amylase activity in parotid glands from male rats. Inflammation 2009; 32(6): 35763.
25. Sánchez GA, Miozza VA, Delgado A, Busch L. Relationship between salivary mucin or amylase and the periodontal status. Oral Dis. 2013; 19(6): 58591.
26. Henskens YM, van den Keijbus PA, Veerman EC, 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;31(1):5765.
27. Baik JE, Hong SW, Choi S, et al. Alpha-amylase is a human

salivary protein with affinity to lipopolysaccharide of *Aggregatibacteractinomycetemcomitans*. Mol Oral Microbiol 2013;28(2):14253.

28. Choi S, Baik JE, Jeon JH, et al. Identification of Porphyromonasgingivalis lipopolysaccharide-binding proteins in human saliva. MolImmunol. 2011;48(15-16):220713.

29. Arhakis A, Karagiannis K, Kalfas S. Salivary alpha-amylase activity and salivary flow rate in young adults. Open Dent J. 2013;7:7-15.

30. Inukai K, Shinada M, Tanida S, et al. Salivary alpha-amylase levels and big five personality factors in adults. *NeuroEndocrinolLett*2010;31:771-74.31- van den Bos R, Taris R, Scheppink B, et al. Salivary cortisol and alpha-amylase levels during an assessment procedure correlate differently with risk-taking measures in male and female police recruits. Front BehavNeurosci. 2014 ; 16:7:219.

32. Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Mahdavinezhad A, Rafieian N. Evaluation of salivary and serum alpha amylase level in dental caries of adolescence. Braz Dent Sci 2016;19(2):40-46.