Journal of International Oral Health 2015; 7(9):54-57

Received: 22th March 2015 Accepted: 23th July 2015 Conflicts of Interest: None

Source of Support: Nil

Original Research

Estimation of Specific Salivary Enzymatic Biomarkers in Individuals with Gingivitis and Chronic Periodontitis: A Clinical and Biochemical Study

Roji Luke[1], S Nubesh Khan[2], P Safar Iqbal[3], Rino Roopak Soman[4], Jithesh Chakkarayan[5], V Krishnan[6]

Contributors:

[1]Assistant Professor, Department of Periodontics, Educare Institute of Dental Sciences, Kerala, India; [2]Assistant Professor, Department of Periodontics, Sri Sankara Dental College, Varkala, Kerala, India; [3]Assistant Professor, Department of Periodontics, Malabar Dental College, Edappal, Kerala, India; [4]Associate Professor, Department of Periodontics, Pushpagiri College of Dental Sciences, Thiruvalla, Kerala, India; [5]Assistant Professor, Department of Orthodontics, Kannur Dental College, Anjarakandy, Kannur, Kerala, India; [6]Professor, Department of Periodontics, Rajah Muthiah Dental College & Hospital, Chidambaram, Tamil Nadu, India.

Correspondence:

Dr. Luke R. Mallel Bunglow, Kurumpakara P.O, Pathanamthitta-691 523, Kerala, India. Phone: +91-9946199658. Email: roji.luke@gmail.com

How to cite the article:

Luke R, Khan SN, Iqbal PS, Soman RR, Chakkarayan J, Krishnan V. Estimation of specific salivary enzymatic biomarkers in individuals with gingivitis and chronic periodontitis: a clinical and biochemical study. J Int Oral Health 2015;7(9):54-57.

Abstract:

Background: Host response to periodontal disease includes the release of different enzymes from stromal, epithelial or inflammatory cells. The enzymes which are produced from these cells are associated with cell injury and cell death like: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Blood urea nitrogen (BUN). Normal enzymatic activity of these enzymes is necessary for healthy functioning of gingiva and periodontium. The aim of the study is to estimate the levels of enzymes AST, ALT, ALP and BUN and to correlate the level of estimated enzymes with that of clinical parameters in the saliva of Healthy subjects, Gingivitis patients and patients with chronic periodontitis.

Methods: The study included a total of 40 male subjects within the age group of 21 to 50 years, and examined the activity of enzymes AST, ALT, ALP and BUN in saliva spectrophotometrically and compared their values between healthy subjects, gingivitis and chronic periodontitis patients. Clinical parameters like OHI – S (Oral hygiene index - Simplified, SBI (Sulcus Bleeding Index), PPD (Probing Pocket Depth), CAL (Clinical Attachment Level), and PI (Periodontal Index) were recorded.

Results: Obtained results showed statistically significant increases of activity of AST, ALT, ALP, and BUN in saliva from patients with periodontal disease ($p<0.001$) in relation to gingivitis and control groups. There was also an increase in periodontal parameters with an increase in salivary enzymes.

Conclusion: The present study shows that the salivary enzyme activity can be used as biomarkers to determine periodontal tissue damage, which may be useful in diagnosis, prognosis and evaluation of post therapy effects in periodontal disease.

Key Words: Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, periodontal disease, saliva

Introduction

Periodontitis is a chronic infectious condition of the supporting tissues of the teeth that is caused by a complex of anaerobic, Gram-negative bacteria. Various biomarkers like saliva, blood are used for screening and predicting the early changes in the periodontal tissues and also to determine the efficacy of the treatment.[1] Saliva is widely used as a biomarker to determine the periodontal disease activity because it allows rapid screening, provides accurate information, and enables reliable evaluation of periodontal disease condition. The advantage of saliva is the ease of collection and contains various microbial and host response mediators. Estimation of the risk of disease onset and severity, monitoring of disease progression, and evaluation of therapeutic efficacy of the periodontal disease can be performed by analyzing an array of constituents within saliva. Enzymes, proteins, and immunoglobulin's are the most abundant constituents of saliva. Their value as biomarkers has been recognized and extensively explored using proteomic methods.

The correlation between salivary biomarkers and clinical features of periodontal disease has been evaluated for three aspects of periodontitis - inflammation, collagen degradation, and bone turnover. The analysis of enzymes from the salivary secretion helps the clinician to evaluate the pathogenesis and for accurate diagnosis of the periodontal disease.[2] Various biomarkers of chronic inflammation have been detected in the whole saliva of patients with oral disease. The early inflammatory changes of the tissue may promote early diagnosis and aid in monitoring the treatment.[3] Salivary examination reveals the changes of the entire oral cavity rather than the specific areas.[4] Researchers involved in periodontal disease diagnostics are currently investigating the possible use of saliva for disease assessment. Because of its importance in oral biofilm formation and host defense, secreted saliva may have a significant role in the establishment and progression of periodontal disease.[5]

Aspartate aminotransferase (AST) also called as glutamate oxaloacetate transaminase is a member of the transaminase family of enzymes. AST is found in many body tissues including the heart, muscle, kidney, brain, and lung. Alanine

aminotransferase (ALT) also called as glutamate pyruvate transaminase is a member of transaminase family of enzymes. ALT is found in large amounts in the liver and trace amounts are seen in heart, muscle, kidney, lung, and brain. An increased level of alkaline phosphate (ALP) is seen in liver diseases, congestive heart failure, diabetes, infections, and in diseases which impair kidney functions. Decreased blood urea nitrogen (BUN) levels are seen in malnutrition, hepatic failure, and pregnancy.[6,7]

Research objectives in this study were:

1. To estimate the levels of enzymes AST ALT, ALP, and BUN in the saliva of healthy subjects, gingivitis, and chronic periodontitis patients
2. To assess and compare the activity of enzymes between healthy subjects, gingivitis, and chronic periodontitis groups
3. To correlate the level of estimated enzymes with that of clinical parameters in healthy subjects, gingivitis patients, and patients with chronic periodontitis.

Materials and Methods

The study was conducted at Division of Periodontics, Rajah Muthiah Dental College and Hospital, Annamalai University (Chidambaram, India). The study was approved by the Institutional Ethical Board. Patient approval was obtained from each patient via a consent form. A complete medical and personal history was recorded for all of the participants.

Examination included 30 male subjects with periodontal disease, and 10 healthy adult volunteers aged 20-50. Female subjects, patients with systemic diseases, smokers, and patients who underwent previous periodontal therapy were excluded.

At the initial examination, each subject underwent a complete periodontal examination, which included: Oral hygiene index, sulcus bleeding index, Russell's periodontal index, probing depth, and clinical attachment level.

Collection of saliva

Samples of unstimulated, mixed saliva (5 ml) were taken using the "draining method" before treatment, directly from the mouth of the patient and were collected in sterile containers and the saliva samples were centrifuged at 10,000 rpm for 10 min.

Estimation of enzyme activity in saliva

The level of activity of AST, ALT, ALP, BUN in the collected samples was determined spectrometrically using "Enzopak kinetic kit."

Statistical analysis

To compare the enzymatic activity among the subjects without periodontal disease, subjects with gingivitis, and subjects with periodontal disease, one-way Analysis of Variance was used. To determine the significance of differences among groups, the bonferroni method for multiple comparisons was used. Correlation between the activities of the indicated salivary enzymes and the values of clinical indices was determined by Pearson correlations.

Results

The results showed that the salivary enzymes in patients with periodontitis were higher than gingivitis patients and control group, and it is statistically significant with a $P < 0.001$. Table 1 shows the comparison of salivary enzymes between healthy subjects, gingivitis, and chronic periodontitis patients.

The obtained results have shown that the activity of examined enzymes in saliva of the patients with periodontal disease was significantly higher in relation to gingivitis and control group. The established differences showed the statistical significance of a high level ($P < 0.001$).

Correlation between the activities of the indicated salivary enzymes and the values of clinical indexes showed a significant positive correlation between AST, ALT, ALP, and BUN in saliva with clinical parameters studied ($P < 0.001$) for each clinical parameter. Table 2 shows Pearson correlation coefficient to compare salivary biomarkers with clinical parameters.

Discussion

Many enzymes have been used as a biomarker to assess the progression of periodontal diseases. The enzyme AST is one such marker, which has been used as a diagnostic adjunct in human disease conditions such as myocardial infarction, hepatic necrosis, and many other acute, chronic, or necrotic conditions. Levels of the enzyme activity can be correlated with active tissue destruction of periodontal tissues since it is contained in the cytoplasm of cells and released on cell death. Thus, the above correlation indicates that the diagnostic test based on AST levels may be useful in assessing periodontal inflammation.[8,9]

ALT is a cytoplasmic enzyme and its extracellular presence is indicative of tissue cell damage.[10]

Gibert *et al.* (2003) [11] showed an increase in ALP activity in the periodontal ligament due to the constant renewal of the tissue or pathological circumstances.

The aim of the present study was designed to compare the levels of AST, ALT, ALP, and BUN in saliva among healthy controls, gingivitis, and chronic periodontitis patients. The levels of AST, ALT, and ALP were estimated by finding out the concentration of the enzymes in saliva and expressed as IU/L and for BUN it was expressed as mg/dl.

The study excluded female patients in order to avoid the influence of hormones and its effect on periodontium that could be encountered in them. Patients with systemic health conditions such as diabetes, cardiovascular diseases, kidney diseases, cancer,

Table 1: Comparison of salivary enzymes between healthy subjects, gingivitis, and chronic periodontitis patients.

Groups	N	AST		ALT		ALP		BUN		P value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Healthy controls	10	13.62	5.81	19.20	8.20	33.48	15.84	20.62	6.76	0.001 (S)
Gingivitis	15	39.58	15.18	40.26	19.85	76.85	36.77	33.31	9.75	
Chronic periodontitis	15	112.44	45.90	194.53	115.61	164.39	54.45	56.74	20.21	

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, BUN: Blood urea nitrogen

Table 2: Pearson correlation coefficient (test to compare salivary biomarkers with clinical parameters).

Salivary biomarkers	OHI (s)	Russell's index	SBI	PPD	CAL
AST	0.892** 0.000	0.882** 0.000	0.833** 0.000	0.828** 0.000	0.831** 0.000
ALT	0.639** 0.000	0.559** 0.000	0.683** 0.000	0.678** 0.000	0.673** 0.000
ALP	0.826** 0.000	0.827** 0.000	0.804** 0.000	0.818** 0.000	0.831** 0.000
BUN	0.733** 0.000	0.737** 0.000	0.730** 0.000	0.730** 0.000	0.751** 0.000

**P<0.001, *P<0.05, †Non significant, SBI: Sulcus bleeding index, PPD: Probing pocket depth, CAL: Clinical attachment level, OHI: Oral hygiene index

and patients under any medications were also excluded from the study due to the influence created by these on the levels of enzyme activity. Smoking was also an exclusion factor as increasing evidence points to smoking as a major risk factor for periodontitis, affecting the prevalence, extent, and severity of the disease.

The present study selected subjects in the age group between 22 and 50 years, thus minimizing the influence of variables on the levels of AST, ALT, ALP, and BUN. The variability of AST, ALT, ALP, and BUN levels in each group could be due to the difference in severity of periodontal disease at the time of collection of samples.

In the present study, the levels of AST was significantly higher in chronic periodontitis group (112.44) when compared to gingivitis group (39.58) and healthy controls (13.62), which was significant (P < 0.001). The above results were in concurrence with findings of Paknejad*et al.*,[12] Totan *et al.* (2006)[13] and Barbosa *et al.*[14] who examined AST in saliva from patients with periodontal disease and from healthy controls and showed a statistically significant (P < 0.01) increase in AST level in saliva of periodontitis patients in comparison with control group.

Our study was in contradiction to Dewan and Bhatia[15] who studied the relationship between AST levels in saliva and gingival crevicular fluid with periodontal disease progression and their inferences showed that there was no significant difference in the level of AST in between healthy and gingivitis groups. The reason for this outcome could be related either to a low disease activity or the type of tissue affected by necrosis.[9]

In the present study, the levels of ALT were significantly higher in chronic periodontitis group (194.53) when compared to gingivitis group (40.26) and healthy controls (19.20), which was significant (P < 0.001). The results of the current study were in accordance with the study by Rai *et al.*[16] determinedin a pilot study, the association between salivary thiocyanate, ALT, and other enzymes among periodontitis subjects with smoking and without smoking and results showed that higher level of salivary ALT, were observed in periodontitis patients than healthy subjects and level were still higher in smokers as compared to non-smokers. Mohkhedkar and Deshpande[17] concluded that as the inflammatory condition increases there is an increase in ALT level activity due to increased tissue destruction.

In the present study the levels of ALP was significantly higher in chronic periodontitis group (164.39) when compared to gingivitis group (76.85) and healthy controls (33.48), which was significant (P < 0.001). The above results were in concurrence with findings of Desai *et al.*,[18] Malhotra *et al.*,[19] who evaluated the level of ALP in saliva of patients with chronic periodontitis when compared with healthy controls, and showed a statistically significant (P < 0.001) increase in ALP level in saliva of periodontitis patients (136.55 ± 77.08 U/L) in comparison with control group (20.20 ± 6.17 U/L). The results of the current study were also in accordance with the study by Nakamura and Slots[20] who examined ALP activity in chronic periodontitis and healthy subjects, and showed that there was increased activity of ALP in chronic periodontitis subjects when compared to healthy subjects.

In the present study, the levels of BUN was significantly higher in chronic periodontitis group (56.74) when compared to gingivitis group (33.31) and healthy controls (20.62), which was significant (P< 0.001). The above results were in concurrence with findings of Nomura *et al.*[21] who in their study determined the usefulness of salivary biomarkers for screening periodontitis and demonstrated that there was an increase in sensitivity and specificity of BUN (0.60) in patients with chronic periodontitis hence stating that BUN may be a candidate for screening periodontitis.

In this study, it was also noted that the levels of AST, ALT, ALP, and BUN were increased in chronic periodontitis group when compared to Gingivitis and Healthy subjects by applying correlation coefficient test. The levels of these salivary enzymes also increased with a corresponding increase in clinical parameters of periodontitis. This was in accordance with Esfahanian[22] who compared specific activity of AST in saliva of

patients with chronic periodontitis and gingivitis and healthy subjects and results showed that the mean specific activity of AST was quantitatively higher in periodontitis patients than gingivitis patients and also in gingivitis patients than healthy subjects.

Conclusion

The present study provides evidence that activities of AST, ALT, ALP, and BUN were significantly increased in the saliva of patients with periodontal disease in relation to healthy subjects. This may be due to the increased release of intercellular enzymes into the saliva from the diseased periodontal tissues. It was also established the correlation between the enzyme activity and clinical parameters. From the present study, it can be concluded that salivary enzymes can be used as the biochemical markers to accesses the condition of periodontal tissues, thereby providing new opportunities in arriving at a diagnosis and following the efficiency of curing periodontal disease.

References

1. Zhang L, Henson BS, Camargo PM, Wong DT. The clinical value of salivary biomarkers for periodontal disease. Periodontol 2000 2009;51:25-37.
2. Todorovic T, Dozic I, Vicente-Barrero M, Ljuskovic B, Pejovic J, Marjanovic M, *et al.* Salivary enzymes and periodontal disease. Med Oral Patol Oral Cir Bucal 2006;11(2):E115-9.
3. Kathariya R, Pradeep A. Salivary proteomic biomarkers for oral diseases: A review of literature. Arch Oral Sci Res 2010;1(1) 43-9.
4. Chapple IL. Periodontal diagnosis and treatment - Where does the future lie? Periodontol 2000 2009;51:9-24.
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. Periodontol 2000 2009;50:52-64.
6. Lamster IB, Kaufman E, Grbic JT, Winston LJ, Singer RE. Beta-glucuronidase activity in saliva: Relationship to clinical periodontal parameters. J Periodontol 2003;74(3):353-9.
7. Craig RG, Kotanko P, Kamer AR, Levin NW. Periodontal diseases - A modifiable source of systemic inflammation for the end-stage renal disease patient on haemodialysis therapy? Nephrol Dial Transplant 2007;22(2):312-5.
8. Persson GR, DeRouen TA, Page RC. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. J Periodontal Res 1990;25(2):81-7.
9. Imrey PB, Crawford JM, Cohen RL, Alves ME, McSwiggin TA, Chambers DA. A cross-sectional analysis of aspartate aminotransferase in human gingival crevicular fluid. J Periodontal Res 1991;26(2):75-84.
10. Edwards CR, Bauchier IA, Haslett C, Chilver ER. Davidson's Principles and Practice of Medicine, 17th ed. New York: Churchill Livingstone; 1995. p. 489.
11. Gibert P, Tramini P, Sieso V, Piva MT. Alkaline phosphatase isozyme activity in serum from patients with chronic periodontitis. J Periodontal Res 2003;38(4):362-5.
12. Paknejad M, Emtiaz S, Khoobyari MM, Gharb MT, Yazdi MT. Analysis of aspartate aminotransferase and alkaline phosphatase in crevicular fluid from implants with and without peri-implantitis. Implant Dent 2006;15(1):62-9.
13. Totan A, Greabu M, Totan C, Spinu T. Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase: Possible markers in periodontal diseases? Clin Chem Lab Med 2006;44(5):612-5.
14. Barbosa e Silva E, Salvador SL, Fogo JC, Marcantonio RA. Use of aspartate aminotransferase in diagnosing periodontal disease: A comparative study of clinical and microbiological parameters. J Oral Sci 2003;45(1):33-8.
15. Dewan A, Bhatia P. Evaluation of AST enzyme levels in saliva and GCF fluid with periodontal disease progression - A pilot study. J Int Oral Health 2011;3(3):19-24.
16. Rai B, Kharb S, Anand SC. Salivary enzyme and thiocynate: Salivary markers among smokers and non-smokers: A pilot study. Adv Med Dent Sci 2007;1(1):1-4.
17. Mohkhedkar AS, Deshpande BM. Comparative study of alkaline phosphatase, aspartate transaminase and alanine transaminase in serum and gingival crevicular fluid in gingivitis and periodontitis. JIDA 2010;4(11):393-5.
18. Desai S, Shinde H, Mudda J, Patil V. Levels of ALP in saliva of patients with chronic periodontitis; a clinical and biochemical study. Internet J Dent Sci 2009;8(1):1-5. (DOI: 10.5580/17d4).
19. Malhotra R, Grover V, Kapoor A, Kapur R. Alkaline phosphatase as a periodontal disease marker. Indian J Dent Res 2010;21(4):531-6.
20. Nakamura M, Slots J. Salivary enzymes. Origin and relationship to periodontal disease. J Periodontal Res 1983;18(6):559-69.
21. Nomura Y, Tamaki Y, Tanaka T, Arakawa H, Tsurumoto A, Kirimura K, *et al.* Screening of periodontitis with salivary enzyme tests. J Oral Sci 2006;48(4):177-83.
22. Esfahanian V. Relation of saliva AST & ALT titre and periodontal disease. Periodontal Research (Poster Session III); 2006.