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Estimation of Salivary Level of Alkaline Phosphatase In Chronic Periodontitis Patients: A Biochemical Study

Kumar Vivek[1*] Amrita[2] Verma Anshul[3] Pratap Mahender[4] Kumar Gaurav[5] Rawat Gargee[6]

[1]Assistant Professor, Department of Periodontology, Hazaribagh College of Dental Sciences and Hospital, Hazaribagh, Jharkhand, India.
[2]Assistant Professor, Department of Periodontology, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, India.
[3]Assistant Professor, Department of Prosthodontics, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, India.
[4]Assistant Professor, Department of Orthodontics, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, India.
[5]Assistant Professor, Department of Orthodontics, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, India.
[6]Assistant Professor, Department of Endodontics, Uttaranchal Dental and Medical Research Institute, Dehradun, Uttarakhand, India.

ABSTRACT

Background: Alkaline phosphatase (ALP) is a hydrolase intracellular destruction enzyme in the periodontium. ALP has often been measured as possible indicators of gingival inflammation and bone metabolism. Hence, the aim of the present study was to estimate and compare salivary alkaline phosphatase levels in healthy controls, generalized gingivitis, and chronic periodontitis patients.

Materials and Methods: A total of 60 patients were divided into 3 groups. Group 1 (healthy) consisted of 20 individuals with healthy gingiva of probing depth ≤3mm, GI =<1, PI=<1 and CAL=0, Group 2 (chronic gingivitis) consisted of 20 individuals who had signs of gingival inflammation with probing depth ≤3mm, GI >1, PI>1 and CAL=0 and Group 3 (chronic periodontitis) consisted of 20 individuals who had signs of clinical inflammation, presence of at least 12 teeth, excluding third molars; and a diagnosis of CP with PPD ≥ 5 mm, GI >1, PI> 1and CAL≥ 3mm. Whole saliva samples were collected and subjected to estimation of salivary alkaline phosphatase levels. The results were analyzed by SPSS and Mann–Whitney analysis.

Results: The level of salivary ALP increased as the disease progressed from health to periodontitis. The highest ALP levels from the Saliva were detected in group 3 while the lowest were detected in group 1. The result showed a statistically significant increase in the levels of salivary ALP in periodontitis patients in comparison to healthy group.

Conclusion: The level of alkaline phosphatase was higher in the saliva of chronic periodontitis patients. Salivary ALP may be used as an inflammatory marker for detection of periodontal disease.

Keywords: alkaline phosphatase, enzyme, periodontitis, saliva.

INTRODUCTION

Periodontitis is a chronic inflammatory disease of periodontium, which destroys the connective tissue and bone that supports teeth.[1] Periodontal disease is generally occurs due to an imbalance between pathogenic microbes and the local and systemic host responses. Periodontitis is an infectious condition caused by periodontal pathogens, which affect the composition and integrity of periodontal structure and cause destruction of cells and connective tissue matrix, clinical attachment loss (CAL), alveolar bone resorption, periodontal pocket formation, and gingival inflammation.[2,3]

Saliva is one of the most complex, versatile, and important body fluids supplying large range of physiological needs. It is used as a diagnostic tool to evaluate various biomarkers associated with periodontal disease.[4]

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*Correspondence Dr. Vivek Kumar.
Department of Periodontology, Hazaribag College of Dental Sciences and Hospital, Hazaribag, Jharkhand, India.
Email: drvivek1909@gmail.com

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ALP which is produced by various cells such as polymorphonuclear leucocytes, osteoblasts and fibroblasts present within the area of periodontium and gingival crevice is a membrane bound glycoprotein. ALP is released during inflammation from polymorphonuclear neutrophils, by osteoblasts during bone formation and by periodontal ligament during fibroblasts periodontal regeneration, presenting dual involvement in the process of periodontal inflammation and healing/regeneration.[5] ALP is stored in specific granules and secretory vesicles of neutrophils and is mainly released during their migration to the site of infection. ALP allows bone mineralization by releasing an organic phosphate and by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxy apatite crystal formation and dissolution. [6]

ALP level has often been measured in GCF to examine the relationship between periodontal conditions and disease activity. There is a need to estimate the salivary alkaline phosphatase level and compare them between the healthy controls and patients with generalized gingivitis and chronic periodontitis to determine whether alkaline phosphatase levels in saliva can be used as an early diagnostic marker and help in the prevention of periodontal diseases. The objective of the study is to estimate and compare salivary alkaline phosphatase levels in healthy controls, generalized gingivitis, and chronic periodontitis patients.

MATERIALS AND METHODS

This case–control, cross-sectional study was performed from January 2017 to July 2017 in the Department of Periodontology after obtaining ethical approval from the institutional review board of the college. A total of 40 male and female subjects aged between 25 and 50 years participated in this study. Written informed consent was taken from all the participants before the start of the study.

Participants were categorized into two groups based on the Gingival index(GI), Plaque index (PI), pocket probing depths (PPD) and clinical attachment level (CAL).

Group 1 (healthy) consisted of 20 individuals with absence of gingival inflammation and bleeding on probing

Group 2 (chronic gingivitis) consisted of 20 individuals who had signs of gingival inflammation and bleeding on probing without clinical attachment loss (CAL)

Group 3 (chronic periodontitis) consisted of 20 individuals who had signs of clinical inflammation and a diagnosis of CP with PPD ≥ 5 mm, GI >1, PI> 1and CAL≥ 3mm.

Exclusion criteria

(1) cigarette smoking or tobacco use and alcoholism; (2) systemic diseases such as diabetes mellitus, hypertension, and rheumatoid arthritis; (3) pregnancy; (4) systemic bacterial, viral, or fungal infection; (5) history of antibiotic therapy or use of anti-inflammatory medications during the past 6 months; (6) periodontal therapy during the past 2 years; and (7) patients with aggressive periodontitis.

Sample collection

Subjects were requested to refrain from eating and drinking for at least 2 h before saliva collection. Using the spitting method, unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes and the samples were centrifuged at 3,000 rpm for 10 minutes and the supernatant was analyzed. The sample was stored at −70°C until the experiment. The estimation of salivary alkaline phosphatase was carried out by using semiautomated analyzer and the sample was assayed according to the manufacturer's instructions. This semiautomated analyzer works on the principle of absorbance transmittance photometry. The rate of formation of p-Nitrophenol is measured as an increase in absorbance of 540 nm wavelength light which is proportional to the alkaline phosphatase activity in the sample.

Data analysis

Statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The salivary level of alkaline phosphatase was compared between the two groups using the Mann–Whitney test and t-test. $P < 0.05$ was considered statistically significant. Data were presented as mean ± standard deviation.

Table 1: Values(mean ± SD) of alkaline phosphatase, PPD, CAL, GI, PI in both Groups.

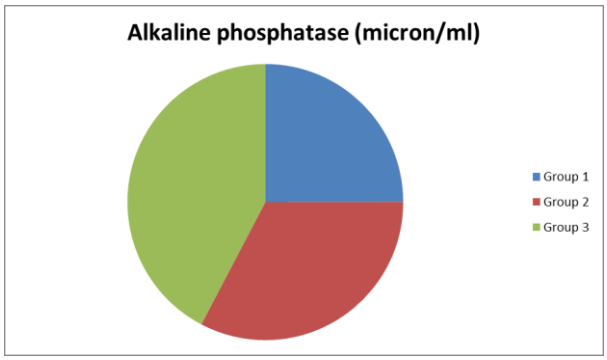
Variable	Group 1	Group 2	Group 3
ALP (micron/ml)	99.47±4.52	130.32±5.47	168.53 ± 7.89
PPD	1.47±0.57	1.75±0.84	6.80±1.21
CAL	0	0	4.13±1.31
GI	0.28±0.25	2.03±0.57	2.32±0.49
PI	0.22±0.19	2.38±0.84	2.13±0.41

ǂ PPD – Pocket probing depth; CAL– clinical attachment level; GI – Gingival index; PI – Plaque index; SD - Standard deviation.

Table 2: Consolidated Pairwise Comparison (p value) Among the Groups (p<0.05).

Variable	**Groups**	**Mean difference (95% CI)**	**t**	**df**	**p**
ALP	Group1 vs Group2	-30.850(−34.06-−27.62)	-19.443	38	< 0.0001*
	Group1 vs Group3	-69.060(−73.17-−64.93)	-33.965	38	< 0.0001*
	Group2 vs Group3	-38.210(−42.54-−33.86)	-17.779	38	< 0.0001*
PPD	Group1 vs Group2	-0.280 (−0.73-−0.17)	-1.234	38	0.2250
	Group1 vs Group3	-5.330 (−5.93-−4.71)	-17.821	38	< 0.0001*
	Group2 vs Group3	-5.05 (−5.71-−4.37)	-15.332	38	< 0.0001*
CAL	Group1 vs Group2	--	--	--	--
	Group1 vs Group3	--	--	--	--
	Group2 vs Group3	--	--	--	--
GI	Group1 vs Group2	-1.750 (−2.03-−1.46)	-12.574	38	< 0.0001*
	Group1 vs Group3	-2.040 (−2.28-−1.78)	-16.585	38	< 0.0001*
	Group2 vs Group3	-0.290 (−0.63-−0.05)	-1.725	38	0.0926
PI	Group1 vs Group2	-2.160 (−2.54-−1.76)	-11.216	38	< 0.0001*
	Group1 vs Group3	-1.910 (−2.11-−1.70)	-18.903	38	< 0.0001*
	Group2 vs Group3	0.250 (0.67-0.17)	1.196	38	0.2391

*Statistically significant at P<0.05; p – Probability; Independent sample t-test; CI - Confidence interval, PPD – Pocket probing depth; CAL– clinical attachment level; GI – Gingival index; PI – Plaque index.



Graph 1: Bar diagram shows the comparison of the salivary alkaline phosphatase level in both groups.

RESULTS

The mean values of the clinical and biochemical parameters were expressed as mean ± SD (Table 1). The highest alkaline phosphatase levels from the Saliva were detected in group 3 (168.53 ± 7.89 micron/mL), while the lowest were detected in group 1 (99.47±4.52 micron/mL) (Graph 1). Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in alkaline phosphatase levels in the saliva was found when group 1 was compared with group 3 (P<0.0001) and group 2 was compared with group 3(P<0.0001). Statistically significant differences were observed when gingival index and plaque index of group 1 was compared with group 2 and group 3. A significant difference in pocket probing depth was found when group 1 and group 2was compared with group 3.

DISCUSSION

The potential role of saliva in the diagnosis of oral and systemic health is evident in researches.

Salivary biomarkers could be used to screen periodontal health status and disease progression.[7,8] Salivary Ca may be important with regard to dental and gingival health by way of its effect on mineralization of plaque. In the present study unstimulated whole saliva was used for the study as it predominantly bathes the oral cavity most of the time, and is a representative of pooled subgingival plaque samples.[9]

The results of the present study revealed that the subjects in the periodontitis group had significantly higher levels of salivary alkaline phosphatase than healthy group. The finding in this study are in accordance with the studies conducted by Nakamura and Slots,[10] Chapple,[11] Binder et al,[12] Beck,[13] Dabra et al[14] who found that there was a higher alkaline phosphatase concentration in the saliva in the periodontitis subjects as compared to the healthy subjects. Hence, salivary ALP concentrations were positively correlated with the periodontal disease progression. Nakashima et al.[15] found in their study that total amounts of ALP are significantly higher in periodontitis as compared to healthy and gingivitis sites which is similar to our study.

The increase in salivary alkaline phosphatase level in periodontitis patients could be attributed to the fact that the diseased periodontal tissue produces large amount of ALP enzyme which is considered as a potential marker of alveolar bone resorption. ALP enzyme is considered as a potential marker of periodontal disease.[16,17]

Statistically significant difference in PPD was observed when group 1 and group 2 were compared with group 3. A mean probing depth of 6.80±1.21mm, GI score of 2.32±0.49 mm, and PI score of 2.13±0.41 mm a in group 3 established the presence of an active inflammatory component along with a destructive component to the prevalent periodontal disease.

CONCLUSION

At present there are several biomarkers, studied in relation to periodontitis. In the current study, level of ALP was higher in the saliva of chronic periodontitis patients. Salivary ALP may be used as an inflammatory marker for detection of periodontal disease. The limitation of this study was that we did not have information about the level of ALP after treatment of periodontitis. Further long-term and interventional studies with larger sample sizes are required to assess the efficacy of this biomarker for early detection of periodontal disease and prevention of its progression. Finally, for considering salivary ALP as biomarkers of inflammation in periodontitis, further studies are needed with more sample size in different populations.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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