

Levels of Alkaline Phosphatase (ALP) In Saliva of Patients with Chronic Periodontitis; a Clinical and Biochemical Study

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Citation

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Abstract

Objectives: The purpose of our study was to evaluate ALP levels in saliva of patients with chronic periodontitis. **Materials and methods:** Forty patients were assigned to each one of three groups C0, C3 and C4, based on their largest Community Periodontal Index of Treatment Needs CPITN code, totaling 120 participants. Unstimulated saliva was collected and analyzed with autoanalyzer (RA-XT Tecnicon® Auto Analyzer, USA) by using International Federation of Clinical Chemistry IFCC method in order to quantify their ALP concentration. **Results:** There were significant differences between levels of ALP from groups C0, C3 and C4. There was a significant positive correlation between clinical parameters and ALP concentration in saliva in each group. **Conclusion:** ALP levels in saliva increased with increase in the CPITN score. Group C0 had the least while group C4 had the highest ALP levels. Salivary ALP levels may be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease.

INTRODUCTION

Periodontal disease is one of the common inflammatory diseases with complex etiology and multifactorial in origin. Traditional periodontal diagnosis involves measures of probing depth, gingival recession, probing attachment level using graduated periodontal probe. These are indirect measures of bone loss. ¹

Various factors like probing force, presence of inflammation or long junctional epithelium can affect periodontal probing, leading to inaccuracies in recording the true pocket depth. ² The height of the alveolar bone margin and the shape and form of its outline are also examined with radiographs. Unfortunately, the sensitivity of radiographs in detecting an early osseous lesion is poor. ³ Biochemical markers can detect inflammatory changes in short period of time where as longer period is required to detect measurable changes in bone density using radiographs. ¹

Saliva is an oral fluid, and interest in it as a diagnostic medium has advanced exponentially in the last 10 years. ⁴ Saliva has been used as a diagnostic fluid in medicine and dentistry. ⁵ Salivary components for periodontal diagnosis include enzymes and immunoglobulins, hormones of host

origin, bacteria and bacterial products, ions, and volatile compounds. ⁶⁷

Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva. Several enzymes that are evaluated for the early diagnosis of periodontal disease are aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP), and gamma glutamyl transferase (GGT). ⁶⁸⁹¹⁰

The enzyme ALP plays a role in bone metabolism. It is a membrane-bound glycoprotein produced by many cells, such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice. ¹¹ Gao J et al. (1999) ¹² found that ALP activity was highest in osteoblasts, moderate in periodontal ligament PDL fibroblasts, and lowest in gingival fibroblasts. No activity was detected in cementoblasts. In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. Some forms of enzyme are also produced by plaque bacteria. ¹³

Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, and after the periodontal therapy, the activity of these enzymes restored to the value as found with the healthy persons.¹⁴ ALP level has often been measured in GCF to examine the relationship between periodontal conditions and disease activity.¹¹¹⁵¹⁶ Plagnat et al. (2002)¹⁷ studied ALP in GCF from implants with and without periimplantitis and suggested that ALP could be a promising marker of bone loss around dental implants. Gilbert et al. (2003)¹⁸ studied ALP activity in serum from patients with chronic periodontitis and showed a relationship between loss of attachment in periodontal disease and ALP activity in serum. Perinetti et al. (2002)¹⁹ suggested that ALP activity in GCF reflects the biologic activity in the periodontium during orthodontic movement. Todorovic T et al. (2006)²⁰ examined the activity of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva from patients with periodontal disease before and after periodontal treatment. They concluded that the activities of CK, LDH, AST, ALT, GGT, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease when compared to healthy subjects. Daltaban O et al. (2006)²¹ found no significant differences in the concentrations of ALP between periodontitis and control groups. Most of the studies are related to levels of ALP in GCF of patients with periodontal disease¹¹¹⁵¹⁶¹⁷¹⁸¹⁹²⁰²¹, very few studies have evaluated salivary ALP levels in patients with periodontitis.⁴²⁰²²

The purpose of this study was to evaluate ALP levels in saliva of patients with periodontitis as compared to healthy individuals.

MATERIALS AND METHODS

120 patients visiting out patient department of Periodontics, H.K.E Society's S. Nijalingappa institute of dental sciences and research, Gulbarga, Karnataka were screened and classified according to their CPITN scores, by a single professional, using a WHO 621 periodontal probe.²³²⁴ This index was primarily designed for rapid and practical assessments²⁵ of periodontal treatment needs rather than periodontal status, but it is based on various disease indicators such as gingival bleeding after probing, pocket depth, which are related to tissue destruction.

Patients of both genders between 20 to 65 years of age were included in the study. Individuals, who participated in the study, were assigned to one of the following three groups based on their highest CPITN score found among all the

examined sites:

- Group C0: No periodontal disease (Healthy periodontium).
- Group C3: Pathological pocket 4 to 5 mm in depth. Gingival margin situated on black band of the probe.
- Group C4: Pathological pocket 6mm or more in depth. Black band of the probe not visible.

Other clinical parameters recorded were Gingival index (Loe and Silness 1963) and Turesky - Gilmore - Glickman modification of Quigley - Hein plaque index (1970). Their medical and dental histories were obtained by interview. Patients with systemic diseases associated with an increase in ALP, patients with lesions of oral mucosa other than gingivitis and periodontitis, pregnant, lactating, post menopausal females, patients with removable prosthesis and/or caries, smokers, patients with disturbances in salivary flow related with systemic processes or therapeutic procedures, patients with history of any periodontal treatment in previous 6 months, patients with history of antibiotic therapy in previous 3 months were excluded from the study.

1 ml of whole saliva sample was collected by unstimulated passive drool in a sterile disposable plastic container. Patients were instructed not to brush or eat 8 hours before collection of the samples. No dental examination was carried out 48 hours prior to saliva collection. Samples were stored at 4°C and sent to the laboratory of Biochemistry. ALP activity remains stable at room temperature for four hours; frozen samples should be kept at room temperature for 12 hours to assure complete enzyme reactivation.²⁶ The level of ALP was estimated with an auto analyzer (RA-XT Technicon®) by using International Federation of Clinical Chemistry (IFCC) method.

For analysis, each saliva sample was centrifuged at 5000 rpm for 10 minutes. Reagents were added to about 10 µl of supernatant sample by the auto analyzer and the value of ALT estimated in U/L. The reagents used in estimation of saliva ALP are listed in Table (1).

Figure 1

Table-1: Reagents

ALP Reagent	
p-Nitrophenyl phosphate	16 mmol/L
Mg ²⁺	4 mmol
Tris/Carbonate buffer (pH 10.2 ± 0.2 at 25°C)	-
Aqua 4	
Double deionised 0.2 micron filtered particle free reagent grade water	

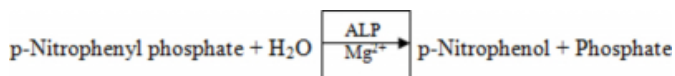
Aqua 4 is added to reagent bottle and swirled to dissolve. Vigorous shaking is avoided.

Methodology:

Adaptation by Wilkinson et al. ²⁷ of the Bessy, Lowrey et al. method. ²⁸

Principle:

Figure 2



At pH 10.3 alkaline phosphatase catalyses the hydrolysis of p-Nitrophenyl phosphate to yellow colored p-Nitrophenol and phosphate. The change in absorbance measured by autoanalyzer at 405 nm wavelength is proportional to ALP activity in the sample.

Data collected were compared using unpaired t test.

RESULTS

All patients (56 men and 64 women) selected for the study were 20–65 years old (mean 33 years). Mean and standard deviation of ALP concentration in saliva are reported in Table (2).

Figure 3

Table-2: ALP levels (U/L) in saliva from 120 patients

Group	Mean	Standard deviation
C0	20.20	± 6.17
C3	78.95	± 25.77
C4	136.55	± 77.08

The mean concentration of ALP in saliva for group C0 was 20.20 ± 6.17 U/L; for C3 was 78.95 ± 25.77 U/L; and for C4 was 136.55 ± 77.08 U/L. There was statistically significant difference in ALP concentrations in saliva for the groups C0, C3 and C4. Comparing the salivary ALP levels of group C0 and group C3, the results were statistically highly significant (t = 9.95, P < 0.005). Comparing the ALP levels of group C0 and group C4, the results were statistically highly significant (t = 8.25, P < 0.001). ALP levels of group C4 were significantly greater than ALP levels of group C3 (t = 3.19, P < 0.01).

There was a significant positive correlation between clinical parameters and ALP concentration in saliva in each group. (Table-3. r = 0.14 to 0.36)

Figure 4

Table-3: Co-efficient correlation (r) between clinical parameters and ALP concentration

Group	Gingival Index	Plaque Index
C0	r = 0.24	r = 0.32
C3	r = 0.20	r = 0.16
C4	r = 0.36	r = 0.16

DISCUSSION

Periodontitis is a chronic destructive category of periodontal disease that progresses to the resorption of alveolar bone, which leads to progressive bone destruction and tooth loss. As a consequence of resorption, breakdown of products are released into periodontal tissues, migrating toward the gingival sulcus and gathering from the surrounding site in whole saliva, where several of them have been identified. ^{29,30}

Among the several host enzymes proposed as diagnostic

indicators of periodontal status, ALP was one of the first to be identified.³¹ ALP is released from polymorphonuclear cells (PMNs) during inflammation¹⁰ and from osteoblasts³² and periodontal ligament fibroblasts³³ during bone formation and periodontal regeneration respectively.

ALP activity in serum has been extensively studied, and it was suggested that ALP allows bone mineralization by releasing an organic phosphate that contributes to the deposition of calcium phosphate complexes into the osteoid matrix.^{34,35} ALP might also promote mineralization by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution, within the extracellular calcifying matrix vesicles.³⁵

CPITN has shown to be a rapid and practical method of periodontal status assessment and determining treatment needs.³⁶ The proposed method allowed establishing a quantitative relationship between AST levels in saliva and periodontal status assessed through CPITN recordings. This method simplified the clinical procedures involved in sample collection compared to the current diagnosis systems; only a 1.0 ml sample of the saliva must be deposited in a sterile disposable plastic container by the patient by unstimulated passive drool. Stimulated saliva collected by asking the patient to chew a gum base may get contaminated with blood as inflamed periodontal tissue may get damaged during chewing. This can cause alteration in the salivary enzyme levels.³⁷

Previous studies¹¹¹⁵¹⁶¹⁷¹⁸¹⁹²⁰²¹ mainly investigated the activities of this enzyme in gingival crevicular fluid, which is in a much closer contact with periodontal tissues and, due to this, it surely much better reflects the occurrences in them. However, the problem with the gingival crevicular fluid is in that the technique of collecting is rather complicated and that in a routine procedure, which possibly might be established, it would be hardly feasible in practice. Contrary to the gingival crevicular fluid, there is plenty of saliva, the procedure of its sampling is much easier and more bearable for the patient and, however, the same enzymes as those in the gingival crevicular fluid can be detected.²⁰ Because of the simple and non-invasive method of collection saliva samples were preferred in our study instead of GCF samples.

More sophisticated techniques to detect bone loss such as subtraction radiography and computer-assisted image analysis have been used as research tools, but at present, these have not found a place in routine clinical practice.³⁸

Changes in ALP in GCF, saliva and serum have been used as an inflammatory marker of periodontium as well as a marker of bone metabolism.¹¹¹⁸²¹³⁹ Various enzymes in serum have been routinely examined for the diagnosis of systemic diseases. Therefore, no specific laboratory devices are necessary.³⁷ We have used these conventional laboratory tests to estimate ALP levels in saliva sample.

Changes in the bone architecture take time to manifest, changes in the bone turnover can be ascertained relatively early by measuring increased levels of serum and urine biochemical markers of bone turnover,²⁹⁴⁰ such as collagen cross-links, ALP, and Osteocalcin.³⁴⁴¹ As ALP is a marker of bone remodeling, CPITN Code 3 (Pathological pocket 4 to 5 mm in depth) and Code 4 (Pathological pocket 6mm or more in depth) were included and Code 1 (Bleeding observed during or after probing) and Code 2 (Calculus or other plaque retentive factors either seen or felt during probing) were excluded from the study.

In our study, differences in ALP concentrations in saliva for the groups C0, C3 and C4 were statistically significant. Probing pocket depth greater than 6 mm (Group C4) was related to the highest ALP levels. The results showed statistically significant relationship between salivary ALP levels and pocket depth. Our results are in agreement with previous studies of ALP done in saliva.⁴²⁰²² The high levels of ALP in saliva in our study may be due to an increase in the inflammation and bone turnover rate as ALP is produced by PMNs, osteoblasts, macrophages, fibroblasts¹¹ and plaque bacteria¹³ within periodontal tissues or periodontal pocket.¹¹³¹ The increased activity of ALP is probably a consequence of destructive process in the alveolar bone in advanced stages of development of periodontal disease.

In our study, GI was positively correlated with increase in pocket depth, although not statistically significant. (Data is shown in table-3) Our results are in agreement with study done by Yoshie H et al. (2007)⁴² who reported statistically significant increase in GI with increase in pocket depth. The increase in salivary ALP levels might have come from PMNs during inflammation within the periodontal pocket. On the basis of results of this study it can be suggested that the activity of ALP enzyme was significantly increased in the saliva of patients with periodontal disease when compared to healthy subjects. It also established the correlation between the enzyme activity and the value of the gingival index. This is probably a consequence of pathological processes in periodontal tissues from where this

intracellular enzyme is increasingly released into saliva.

CONCLUSION

Salivary ALP levels may be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease. Diagnosis of periodontal disease by measuring salivary levels of ALP is economical, feasible, simple and convenient approach that does not require expert examiners. Further studies are warranted to confirm the reliability of saliva ALP levels for screening of periodontal disease.

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