INTRODUCTION

Periodontitis is an inflammatory condition affecting the supporting structures of the tooth namely the periodontal ligament, cementum, and alveolar bone. Bacterial plaque is one of the main etiological factors in the initiation and promotion of periodontal diseases. Traditionally, the diagnosis of periodontal disease is established by clinical and radiographic parameters. These techniques are deficient in identifying and measuring the progression or regression taking place in the previously diseased sites and in the newly developing disease sites. Altered enzymatic action, both at cellular and sub-cellular levels influence the disease process. The measurement of the enzymes can provide valuable diagnostic and prognostic information.

Periodontal disease is considered to progress in periods of disease activity, followed by periods of quiescence. For effective treatment, it is important to know whether the disease is in an active phase or not. Even in apparent health, there are inflammatory changes in gingiva at the molecular level due to the exposure to the oral environment.

The present trend in clinical medicine leans towards the use of non invasive procedures that determine the changes in salivary constituents to diagnose several diseases. The potential diagnostic importance of gingival crevicular fluid (GCF) was...
recognized more than 60 years ago.\[9\] Analysis of the gingival crevicular fluid provides a noninvasive method of studying the host response factors in the periodontium during the initial diagnosis and treatment.\[9,10\] Binder et al.\[11\] demonstrated a strong positive relationship between the levels of the enzyme in GCF and previous disease activity. GCF contains inflammatory products, bacterial products, and products of tissue breakdown. Thus, examination of GCF is an ideal method of evaluating the inflammatory tissue destruction and bacterial activity associated with periodontal disease.

The objective of this study is to compare the gingival crevicular fluid alkaline phosphatase (ALP) levels before and after scaling and root planning in patients with chronic periodontitis. It is postulated that this could serve as a prognostic predictor, as an adjunct to the routine methods used for determination of the disease activity and has a direct influence on the diagnosis, therapy, and prognosis of periodontitis.

**MATERIALS AND METHODS**

**Patient selection**

Twenty patients were randomly selected from the outpatients pool of the department of periodontics, who met the following criteria.

- Adult male/female patient between 20 and 50 years.
- Patients with chronic localized periodontitis having probing pocket depth of 4–6 mm.
- No history of antibiotic, antimicrobial, and/or anti-inflammatory drug usage for last 6 months.
- No history of any systemic diseases, which could influence the development, course and prognosis of periodontal disease and/or periodontal therapy.
- No developmental or anatomic defects detrimental to periodontal treatment prognosis.
- No furcation involvement.
- No history of periodontal treatment for last one year.
- No personal habits that can influence periodontal health and treatment efficiency.
- No restoration or prosthesis on the selected tooth.

**Site selection**

The selection of the site for the collection of gingival crevicular fluid was done one day prior to treatment, to avoid possible contamination of the crevicular fluid with blood after probing. Pocket depth was measured using Williams graduated periodontal probe, held parallel to the long axis of tooth from the free gingival margin to the base of the pocket.

**Plaque assessment and isolation**

Disclosing agent was applied for one minute on the selected site. This was done for the purpose of motivating the patient.

Plaque index (Sillness and Loe) was recorded. The selected site was isolated with sterile cotton and compressed air was used to gently dry the quadrant to prevent contamination.

**Sample collection**

All GCF samples were collected in the forenoon (at same time, of the day) (between 10 and 11 AM) to allow for the circadian variation seen in GCF volume.\[12\] A calibrated volumetric micropipette of 5 μL capacity was introduced into the periodontal pocket of the selected site for collection of gingival crevicular fluid by Brill technique.\[9\] The sample was collected for 20 min. The collected sample was then transferred to a sterilized plastic vial with the help of air-spray. The vial was frozen to −20°C until the sample was transported to the laboratory for analysis.

**Treatment**

A thorough scaling and root planing was then carried out with ultrasonic scalers and Gracey curettes by single operator. Oral hygiene instructions were given to the patient, which included appropriate brushing technique. The patients were recalled on the 7th, 30th, and 60th day after scaling and root planing. The gingival crevicular fluid was collected and clinical parameters were recorded in the similar manner at each recall periods. No periodontal treatment was provided in the recall visits. Only oral hygiene instructions were given to the patients.

**Biochemical analysis**\[13\]

Enzyme (kinetic) analysis of the alkaline phosphatase level in GCF was done using a spectrophotometer. The rate of formation of p-Nitrophenol is measured as an increase in absorbance of 405 nm wavelength light which is proportional to the alkaline phosphatase activity in the sample. The final results were expressed as corrected optical densities or enzyme units, where 1 unit is equal to 1 μmol p-nitro phenylphosphate converted to p-nitro phenol plus inorganic phosphate per minute at the pH and temperature indicated for alkaline phosphate.

The measured values for Total GCF ALP, probing depth and plaque index were tabulated and statistically analyzed. A \( P \leq 0.05 \) was considered to be significant.

**RESULTS AND STATISTICAL ANALYSIS**

Since the GCF ALP levels were showing a uniform distribution, parametric ANOVA repeated measure was utilized for analysis. The statistical test compared all the groups together [Table 1] and also did pairwise comparison [Table 2]. Total alkaline phosphatase levels in gingival crevicular fluid were found to be highest in baseline patients. There was a statistically significant decrease between each of the sampled groups in the duration of the study (baseline, 7th, 30th, and 60th day) at \( P \leq 0.001 \).
Since variables such as probing depth and plaque index were not in uniform distribution nonparametric Spearman’s Rank Correlation test was used to correlate between these variables and GCF total ALP levels. Alkaline phosphatase levels were found to be positively correlated with probing depth. However, there was no correlation found between alkaline phosphatase levels and plaque index P≤0.05 [Table 3].

DISCUSSION

Periodontal diseases alter the biochemical constituents in both tissue and blood through metabolic changes. Tissue destruction is seen as the consequence of bacterial interaction in periodontal disease, which is due to host cells (mainly polymorphonuclear leukocytes) releasing their granular enzymes (lysozyme, β-glucuronidase) that are capable of attaching to all extracellular matrix components that seem to play an important role in the tissue damage.[7,14,15] GCF is an exudate from the microcirculation around the inflamed periodontium and gingiva. It picks up enzymes

Table 1: ANOVA comparing GCF alkaline phosphatase values

| Mean    | Std deviation | N   | Std error | P value |
|---------|---------------|-----|-----------|---------|
| Baseline| 1619.85       | 97.37 | 20        | 21.772  | 0.001   |
| 7 days  | 1317.75       | 115.28 | 20        | 25.776  |
| 30 days | 1136.80       | 106.56 | 20        | 23.828  |
| 60 days | 987.50        | 103.79 | 20        | 23.208  |

Table 2: Pairwise inter group comparison of GCF ALP levels

| Pairwise comparison | Mean difference | Std error | P value |
|---------------------|----------------|-----------|---------|
| Lower bound         | 164.693        |           | 0.001   |
| Upper bound         | 358.120        |           | 0.001   |
| Baseline- 7 days    | 302.100        | 11.764    | 0.001   |
| Baseline- 30 days   | 483.050        | 17.229    | 0.001   |
| Baseline- 60 days   | 632.350        | 14.996    | 0.001   |
| 7 days- Baseline    | −302.100       | 11.764    | 0.001   |
| 7 days- 30 days     | 180.950        | 12.981    | 0.001   |
| 7 days- 60 days     | 330.250        | 13.316    | 0.001   |
| 30 days- baseline   | −483.050       | 17.229    | 0.001   |
| 30 days- 7 days     | −180.950       | 12.981    | 0.001   |
| 30 days- 60 days    | 149.300        | 7.354     | 0.001   |
| 60 days- baseline   | −632.350       | 14.996    | 0.001   |
| 60 days- 7 days     | −330.250       | 13.316    | 0.001   |
| 60 days- 30 days    | −149.300       | 7.354     | 0.001   |

Several other substances have been found in GCF, collected from sites with inflammatory periodontal disease. These constituents include physiologically active substances and enzymes such as prostaglandins,[18,19] collagenase,[20] hydroxyproline,[21] β-glucuronidase,[22] lactate dehydrogenase,[23] glycosaminoglycans,[23] aspartate aminotransferase,[24] and alkaline phosphatase (ALP). [17,19,25] Ishikawa and Cimasoni[26] first recognized the potential of alkaline phosphatase as an important biochemical component of GCF in 1970. The sources of ALP are polymorphonuclear leukocytes (PMNL),[14] bacteria within the dental plaque[27] and osteoblasts and fibroblast cells.[16] Binder et al.[11] demonstrated a strong positive relationship between the levels of alkaline phosphatase in GCF and previous disease activity. The GCF serum ratio for clinically healthy periodontal tissues ranged from 6:1 to 12:1, which implies that the enzyme is locally produced within the periodontium.[28] Thus, gingival crevicular fluid ALP levels from periodontal pockets can be taken as indicative for active periodontal tissue destruction. Chapple et al. noted that total GCF ALP levels increased before increases in the gingival index and appears to be a good marker of gingival inflammation. They stated that majority of ALP in GCF is of PMNL origin[29] Nakashima et al. 1994 found in their study that total amounts of ALP are significantly higher in periodontitis as compared to healthy and gingivitis sites.[19]

There are abundant PMNL in the site of periodontal inflammation and they are a prime source for GCF ALP.[13,29,30] The decrease in the inflammation as a result of the mechanical plaque control
The lack of correlation of ALP levels with the plaque index may be attributed to the fact that even though the plaque bacteria are a source of alkaline phosphatase the percentage contribution to the total GCF ALP levels is not significant.[27] Plaque assays have suggested that bacterial ALP contribute less than 20% to total ALP content of GCF.[29] This suggests that the total ALP level reflects the state of the periodontium more accurately in disease and healing and is not significantly affected by the external bacterial factors in plaque.[17,25,29,30]

CONCLUSION

Total alkaline phosphatase levels in gingival crevicular fluid can be used as a diagnostic biomarker to assess the health and pathology of the periodontium. It can be used in early detection of periodontal changes and can assess the efficacy and prognosis of treatment. However, since there are multiple sources of ALP further studies on evaluation of ALP from single source (isozyme studies) may increase the diagnostic value of alkaline phosphatase level as a disease maker.

REFERENCES

1. Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 1996;11:821-78.
2. Zappa U. Histology of the periodontal lesion: Implications for diagnosis. Periodontol 2000 1995;7:22-38.
3. Waerhaug J. Gingival pocket Anatomy, pathology, deepening and elimination. Odontol Tidskr 1952;60(Suppl 1):1-186.
4. Page RC. Host response tests for diagnosing periodontal diseases. J Periodontol 1992;19:43-8.
5. Fine DH and Mandel ID. Indicators of periodontal disease activity: An evaluation. J Clin Periodontol 1986;13:533-46.
6. Fine DH. Incorporating new technologies in periodontal diagnosis into training programs and patient care: A critical assessment and a plan for the future. J Periodontol 1992;63(4 Suppl):383-93.
7. Van Dyke TE, Lester MA, Shapiro L. The role of the host response in periodontal disease progression. Implications for future strategies. J Periodontol 1993;64(8 Suppl):792-806.
8. Cimasoni G. Crevicular fluid updated. Monogr Oral Sci 1983;12:3-7, 1-152.
9. Brill N, Krass B. The passage of tissue fluid into the clinically healthy gingival pocket. Acta Odontol Scand 1958;16:223-45.
10. Embery G, Waddington R. Gingival crevicular fluid: Biomarkers of periodontal tissue activity. Adv Dent Res 1994;8:329-36.
11. Binder TA, Goodson JM, Socransky SS. Gingival fluid levels of acid and alkaline phosphatase. J Periodontal Res 1987;22:14-9.
12. Bissada NF, Schroff EM, Haus E. Cricardian periodicity of human crevicular fluid flow. J Periodontol 1967;38:36-40.
13. Malhotra R, Grover V, Kapoor A, Kapur R. Alkaline phosphatase as a periodontal disease marker. Indian J Dent Res 2010;21:531-6.
14. Cohn ZA, Hirsch JG. The isolation and properties of specific cytoplasmic granules of rabbit polymorphonuclear lymphocytes. J Exp Med 1960;112:983-1004.
15. Gustafsson GT, Nilsson IM. Fibrinolytic activity in fluid from the gingival crevice. Proc Soc Exp Biol Med 1961;106:277-80.
16. Cabrini RL, Caranza FA. Histochemical study on alkaline phosphatase in normal gingivae varying the pH and substrate. J Dent Res 1951;30:28-32.
17. Chapple IL, Mathews JB, Thorpe GH, Glenwright HD, Smith JM, Saxby MS. A new ultrasensitive chemiluminescent assay for the site-specific quantification of alkaline phosphatase in gingival crevicular fluid. J Periodontal Res 1993;28:266-73.
18. Offenbacher et al. The use of crevicular fluid prostataglandin-E2 levels as a predictor of periodontal attachment loss. J Periodontol Res 1986;21:101-12.
19. Nakashima K, Roehrich N, Cimasoni G, Osteocalcin, prostataglandin E, and alkaline phosphatase in gingival crevicular fluid: Their relations to periodontal status. J Clin Periodontol 1994;21:327-33.
20. Golub LM, Seigal K, Ramamurthy NS, Mandel ID. Some characteristics of collagenase activity in gingival crevicular fluid and its relationship to gingival disease in humans. J Dent Res 1976;55:1049-57.
21. Svaneberg GK. Hydroxyproline determination in serum and gingival crevicular fluid. J Periodontal Res 1987;22:133-8.
22. Lamster IB, Vogel RJ, Hartley LJ, DeGerege CA, Gordon JM. Lactate dehydrogenase, β glucuronidase and aryl sulphatase in gingival crevicular fluid associated with experimental gingivitis in man. J Periodontol 1985;56:139-47.
23. Last KS, Stanberg JB, Embery G. Glycosaminoglycans in human crevicular fluid as indicator of active periodontal disease. Arch Oral Biol 1985;30:275-81.
24. Persson GR, DeRouen TA, Page RC. Relationship between levels of aspartate aminotransferase in gingival crevicular fluid and gingival inflammation. J Periodontal Res 1990;25:17-24.
25. Perinetti G, Paolantonio M, Feminnella B, Serra E, Spoto G. Gingival crevicular fluid alkaline phosphatase activity reflects periodontal healing/recurrent inflammation phases in chronic periodontitis patients. J Periodontol 2008;79:1200-7.
26. Ishikawa I, Cimasoni G. Alkaline phosphatase in human gingival fluid and its relation to periodontitis. Arch Oral Biol 1970;15:1401-4.
27. Bowen WH. Phosphatase in microorganisms cultured from carious dentin and calculus. J Dent Res 1961;40:571-7.
28. Daltanban O, Saygun I, Bal B, Balos K, Serbar M. Gingival crevicular fluid alkaline phosphatase levels in post menopausal women: Effects of phase I periodontal treatment. J Periodontol 2006;77:67-72.
29. Chapple IL, Socransky SS, Dibart S, Glenwright HD, Matthews JB. Chemiluminescent assay of alkaline phosphatase in human gingival crevicular fluid: Investigations with an experimental gingivitis model and studies on the source of the enzyme within crevicular fluid. J Clin Periodontol 1996;23:587-94.
30. Chapple IL, Glenwright HD, Mathew JB, Thorpe GH, Lumley PJ. Site-specific alkaline phosphatase levels in gingival crevicular fluid in health and gingivitis: Cross-sectional studies. J Clin Periodontol 1994;21:409-14.