Original Article

Comparative evaluation of salivary, serum, and GCF alkaline phosphatase levels in chronic periodontitis patients before and after nonsurgical periodontal therapy: A clinico-biochemical study

ABSTRACT

Background: Chronic periodontitis is a multifactorial disease that causes the supporting tissues around the teeth to become inflamed and destroyed, which further causes tooth mobility and eventual tooth loss. The enzyme alkaline phosphatase (ALP), which is involved in bone resorption and gingival inflammation, is an important biomarker. The current study's objective is to compare the serum, gingival crevicular fluid (GCF), and salivary levels of ALP in individuals with chronic periodontitis before and after nonsurgical periodontal therapy.

Materials and Methods: On the basis of clinical and radiographic examinations, 72 participants were split into two groups: Group I (healthy individuals) and Group II (chronic periodontitis patients). All patients who were in an aseptic condition had their serum, GCF, and unstimulated saliva taken, and samples were then tested for ALP levels using ALP kit.

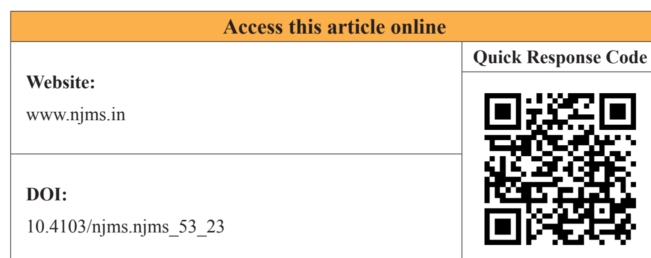
Results: The difference in salivary, serum, and GCF ALP levels between the control group (23.44 ± 4.76, 58.88 ± 8.29, and 776.76 ± 121.91) and the study group (105.66 ± 16.33, 102.38 ± 4.43, and 1,825.77 ± 275.12) was found to be statistically significant with $P < 0.001$. The difference in salivary, serum, and GCF ALP levels from baseline (105.66 ± 16.33, 102.38 ± 4.43, and 1,825.77 ± 275.12) to postoperative (49.54 ± 5.69, 83.46 ± 4.22, and 1,148.38 ± 129.01) was found to be statistically significant with $P < 0.001$. The results demonstrated that patients with chronic periodontitis have considerably higher levels of serum, GCF, and salivary ALP than healthy individuals.

Conclusion: Salivary and GCF ALP can thus be used as a key inflammatory diagnostic biomarker in periodontal diseases.

Keywords: Alkaline phosphatase, biomarker, chronic periodontitis, nonsurgical periodontal therapy

INTRODUCTION

One of the major causes of tooth loss is the destruction of supporting periodontal apparatus by the interaction between the microbes present in the dysbiotic biofilm and immune responses from the hosts leading to a second-rate inflammation. Periodontal disorder is caused by a variety of etiologies, including complex subgingival biofilms, social and behavioral modulations, and host genetic and epigenetic features, all of which are regulated by the hosts' immunological and inflammatory responses.[1] Chemical mediators of inflammation are responsible for the destruction of periodontal tissues, despite the fact that microbes are the principal causative agents.[2]



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Traditional periodontal disease diagnostic methods depend on clinical indicators of inflammation, such as CAL, BOP, and PD, to measure tissue loss and radiographic criteria, to assess bone degeneration. These criteria do not accurately reflect the current state of the disease. This could cause a delay in the diagnosis of the disease, prompting the search for better options. One of the newly developed ways is the use of biomarkers to detect a hidden deadly threat to the periodontium before the disease progresses.[3,4]

Biochemical indicators can identify inflammatory changes expeditiously, but radiographs take a longer period to discover meaningful changes in bone density. These biomarkers might help to predict, present, and future disease activity. They could also be able to determine the present activity level of histologically sick areas.

These markers can be obtained from whole saliva, GCF, plaque, or serum. Enzymes released by host cells are easily obtained within the oral cavity via GCF or whole saliva.[5]

ALP is a biomarker of bone metabolism and is a hydrolase enzyme that removes phosphate groups from a variety of biomolecules. It is a glycoprotein that is located on cell membranes. During inflammation, it is secreted by polymorphonuclear leukocyte, osteoblast during bone production, and periodontal ligament fibroblast during periodontal regeneration. ALP is an essential enzyme in the periodontium because it is involved in proper periodontal ligament turnover and bone homeostasis.[6]

ALP activity in serum has been studied for systemic diseases in recent years and it has been proposed that it allows bone mineralization by releasing an organic phosphate and assisting in the deposition of calcium-phosphate complexes into the osteoid matrix; elevated levels have been linked to chronic kidney disease.[7] Its activity has also been found to be higher in postmenopausal women with periodontitis as compared to nonperiodontitis individuals.[8]

ALP levels in saliva and GCF may be identified using ultraviolet spectrometry in the same way that serum ALP levels can be estimated and the analysis is cost-effective. The levels of salivary and GCF ALP have been studied in relation to gingivitis, chronic periodontitis, and their correlation with clinical indicators. However, there is a lack of information on the effects of ALP in serum, saliva, and GCF after periodontal treatment.

This study aims to compare the levels of ALP in serum, saliva, and GCF in chronic periodontitis patients before and after nonsurgical periodontal therapy to test the hypothesis that saliva or GCF can be used as an alternative to serum for evaluating ALP as a biomarker in periodontal disease progression. A chair-side test for ALP as a biomarker may therefore prove helpful in the future for both the initial diagnosis of chronic periodontitis and the evaluation of subsequent cases.

MATERIAL AND METHODS

A clinical investigation was carried out on a total of 72 willing participants, aged 25–55 years. The control and study groups each had 36 patients who were periodontally healthy and had chronic generalized periodontitis, respectively. The study received ethical clearance from the Institutional Review Board (Ethical Clearance was obtained from Banaras Hindu university, institute of medical sciences Ethical Committee with Ref no Dean/2021/EC/2722 date 23.06.2021). All subjects who accepted to participate in the study received a thorough explanation of its objectives and signed consent forms.

The control group participants were required to have at least 20 natural teeth, a probing pocket depth of 2–3 mm, no attachment loss, and no bleeding during probing with a 20% site rate. Additionally, study group participants had to have five qualifying sites in two quadrants with at least two affected teeth in each quadrant, a probing depth of 5 mm, attachment loss of 3 mm, and bleeding on probing at each site [Figure 1]. The study excluded individuals with systemic disorders, smokers, pregnant women, and those who did not maintain good dental hygiene.

All participants underwent baseline clinical parameter evaluations for plaque index, gingival index, probing depth, and CAL. Both groups then had saliva, GCF, and blood samples collected, which were analyzed for ALP estimate. On the first day after sample collection, thorough ultrasonic scaling was done on patients with chronic periodontitis. Within 15 days

Figure 1: Probing depth >5 mm at baseline

of the initial visit, complete root planning was finished in two consecutive sessions. Patients were evaluated 30 days after finishing nonsurgical periodontal therapy; blood, GCF, and saliva samples were taken and tested for ALP activity [Figure 2].

Before performing any procedure in the patient's oral cavity, saliva should be collected. As a result, saliva samples were usually taken before GCF samples. All of the trial participants were instructed to rinse with regular water to remove exfoliated cells and then wait 5 min.

It was advised that 5 cm^3 of unstimulated saliva be obtained from each patient between 9:00 to 11:00 a.m.[9] The patients were requested not to swallow the passively accumulated saliva in their mouths while they were comfortably seated in an upright position. They were told to spit into a sterile sample collecting container marked with the patient's name and the collection date once there was enough whole, unstimulated saliva in their mouth.

All GCF samples were collected at the forenoon of the same day in order to take the circadian variation in GCF volume into account (between 10 and 11 a.m.).[10] The supragingival plaque was scored with the PI prior to collecting crevicular fluid and the patient's oral cavity was appropriately isolated using a cheek retractor and cotton rolls. To avoid saliva from contaminating the GCF, any debris at the location of GCF collection was cleaned with a curette while being careful not to bleed. The area was then gently dried for 5 s. In our investigation, we collected GCF extra-secularly using volumetric micropipettes with a 5-µl capacity. The benefit of using micropipettes over periopapers or threads is that they are precalibrated on a 1-µl scale, eliminating the need for a periotron to measure the quantity of GCF collected. This approach does not require the use of specific buffers to remove the fluid from the absorbent papers or threads during analysis, is easier to collect, less technique-sensitive, and gathers specified volumes.[11] The Brill technique was used to collect GCF from the periodontal pocket of the selected site (tooth with the deepest pocket) using a calibrated volumetric micropipette with a capacity of 5 µL.[12] The sample collection process endured for a span of twenty minutes, during which any specimens tainted with blood or extraneous debris were promptly rejected. Subsequently, a renewed endeavor was made to procure an uncontaminated sample. GCF was injected with a jet of air pressure into an Eppendorf tube that contained 250 µl of normal saline and was kept at −80°C.

The antecubital vein was punctured to obtain 2 ml of blood from each patient, which was then sent to the biochemistry laboratory for analysis of serum biomarkers. The samples of saliva, GCF, and serum were sent right away to the lab, where they were centrifuged at 3,000 rpm for 5 min in accordance with the International Federation of Clinical Chemistry and Laboratory Medicine's recommendations. The ALP enzyme activity in the saliva, GCF, and serum was then determined spectrophotometrically with the aid of a semi-auto-analyzer using an ALP enzyme kit [Figure 3]. The results were expressed in international units [Figure 4].

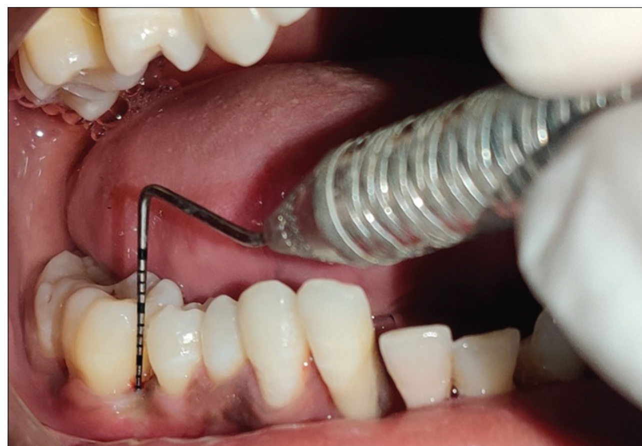

Figure 2: Probing depth after 1 month of phase 1 periodontal therapy

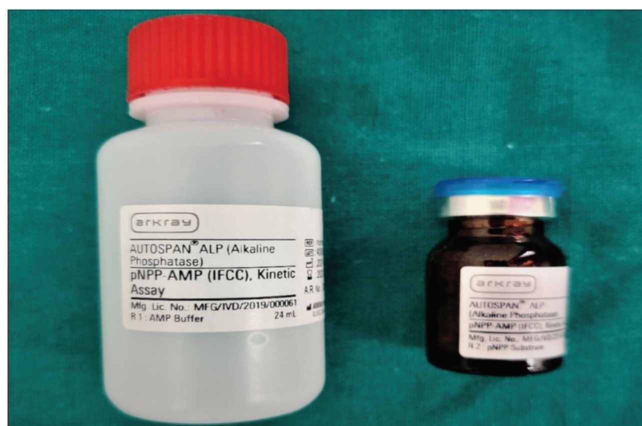

Figure 3: ALP Kit (AUTOSPAN)

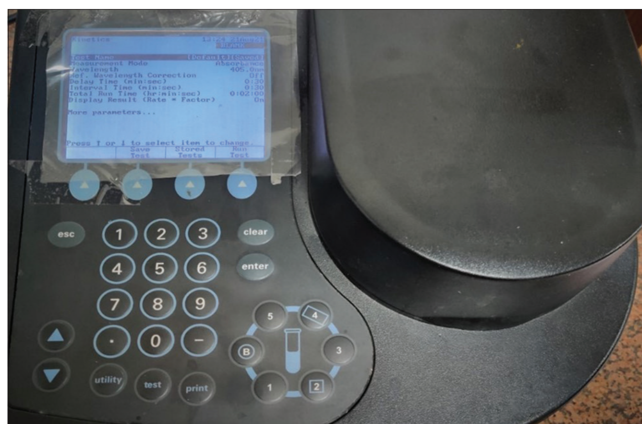

Figure 4: Kinetic estimation of ALP in spectrophotometer

Following the collection of samples, all patients in the study group underwent comprehensive ultrasonic scaling. Patients were instructed to brush their teeth twice daily with a modified bass method and to use chlorhexidine mouthwash twice a day. Root planning was performed after 15 days from baseline in two following visits, as needed. On the 30th day after completing nonsurgical periodontal therapy, all patients were summoned back for a review and postoperative sample collection.

RESULTS

All clinical parameters were measured; samples were collected at baseline and 30 days after scaling and root planning. The ALP levels in the collected samples were determined using spectrophotometric analysis. The obtained results were tabulated and the data gathered were subjected to statistical analysis using SPSS 21 software.

Clinical parameters and biomarkers like serum, salivary, and GCF ALP levels were compared at baseline and postoperatively using the paired *t*-test. Enzyme levels in saliva, serum, and GCF were compared between the control group and the study group at baseline and postoperatively using the independent *t*-test. The link between clinical variables and biomarker concentrations was established using the Pearson correlation coefficient.

When the clinical parameters plaque index, gingival index, probing depth, and CAL [Table 1] in the study group were compared, the mean PI and GI score at baseline in the study group was 1.89 ± 0.51 and 2.07 ± 0.33, respectively, and the postoperative score was found to be 0.56 ± 0.28 and 0.52 ± 0.31, respectively. There was an improvement in plaque and gingival index scores as well as there was a statistically significant decrease in probing depth and an increase in CAL postoperatively. The mean probing depth and CAL in the study group at baseline were 4.33 ± 0.85 mm and 4.30 ± 0.66 mm, respectively, and postoperatively 2.47 ± 0.63 mm and 3.10 ± 0.65 mm.

When the mean salivary, serum, and GCF ALP levels of the control group were compared to the baseline values of the study group, the difference in salivary, serum, and GCF ALP levels between the control group (23.44 ± 4.76, 58.88 ± 8.29, and 776.76 ± 121.91) and the study group (105.66 ± 16.33, 102.38 ± 4.43, and 1,825.77 ± 275.12) was found to be statistically significant with $P < 0.001$ [Table 2]. When the mean baseline salivary, serum, and GCF AL*P* values in the study group were compared to the postoperative values, the difference in salivary, serum, and GCF ALP levels from baseline (105.66 ± 16.33, 102.38 ± 4.43, and 1,825.77 ± 275.12) to postoperative (49.54 ± 5.69, 83.46 ± 4.22, and 1,148.38 ± 129.01) was found to be statistically significant with $P < 0.001$ [Table 3].

DISCUSSION

Periodontitis is an inflammatory condition caused by the hosts' reaction to microorganisms in the subgingival plaque.[13] As a result, the periodontal natural histological architecture is disrupted, leading to persistent inflammation and permanent loss of mineralized and nonmineralized tissues. Destructive cellular enzymes, cytokines, chemokines, and other pro-inflammatory mediators of tissue destruction are released at the tooth and periodontal pocket interfaces following the activation of the patients' host response. Multiple markers found in GCF, saliva, and serum have been tested as a periodontal disease diagnostic tool with high specificity and sensitivity. Due to the complex mechanism of periodontitis, the availability of a single independent biomarker is unlikely. As a result, the search for a combination of biomarkers is relentless.[14]

Many cells in the periodontium and gingival sulcus produce alkaline phosphatase (ALP), a membrane-bound glycoprotein. The distribution of ALP in gingival tissues was investigated and it was discovered that ALP can be found in endothelial cells, capillary walls, and possibly connective tissue fibers,[15] demonstrating a strong link between periodontitis and higher ALP levels in serum, saliva, and GCF.[16,17]

Table 1: Clinical parameters at baseline and 1 month following phase I periodontal therapy in patients with chronic periodontitis

Clinical parameters	Baseline	After 1 month	*P*
PI	1.89±0.51	0.56±0.28	<0.001
GI	2.07±0.33	0.52±0.31	<0.001
PD (mm)	4.33±0.85	2.47±0.63	<0.001
CAL (mm)	4.30±0.66	3.10±0.65	<0.001

Table 2: Comparison of baseline salivary, serum, and GCF ALP values of the control group with baseline ALP values of the study group

	Control group	Study group	*P*
Salivary ALP	23.44±4.76	105.66±16.33	<0.001
Serum ALP	58.88±8.29	102.38±4.43	<0.001
GCF ALP	776.76±121.91	1,825.77±275.12	<0.001

Table 3: Comparison of baseline salivary, serum, and GCF ALP values with 1 month postoperative ALP values of the study group

	Baseline	Postoperative	*P*
Salivary ALP	105.66±16.33	49.54±5.69	<0.001
Serum ALP	102.38±4.43	83.46±4.22	<0.001
GCF ALP	1,825.77±275.12	1,148.38±129.01	<0.001

The gingival crevicular fluid is a pool of inflammatory and immunological indicators, tissue breakdown products, bacterial enzymes, host-derived enzymes, and inhibitors of these enzymes. It has proven to be a useful tool for analyzing biomarkers since they directly reflect biochemical changes in the PDL and alveolar bone.[18]

Saliva is another oral fluid that can be easily and inexpensively collected. There are numerous advantages to using saliva as a diagnostic tool, including its readily available nature and the ability to collect it in a comfortable manner, as opposed to blood collection via venepuncture and the associated fear of the needle, whereas saliva can be collected in a noninvasive manner, making patient compliance a reliable factor.[19]

Some research in recent years has demonstrated the possibility to detect and evaluate a variety of biomarkers in saliva and GCF for the diagnosis and monitoring of periodontal disease progression and health.[20,21] This study shows the relationship between periodontal disease and ALP levels in saliva with clinical parameters in healthy controls, gingivitis patients and was the first in the Indian population to correlate periodontal disease status with salivary ALP levels.[22-26]

So far, all studies have attempted to rationalize the use of ALP from saliva, serum, or even GCF solely in patients with chronic periodontitis with comparisons to periodontally healthy individuals. None of the investigations, however, have evaluated serum, salivary, and GCF ALP levels in chronic periodontitis patients before and after nonsurgical periodontal therapy or compared them to periodontally healthy people.

The current study found that ALP levels were elevated in saliva, GCF, and serum in patients with chronic generalized periodontitis.[27] The study also revealed that after phase I periodontal therapy, there was a significant decrease [Table 3] in salivary, serum, and GCF ALP levels in patients with chronic generalized periodontitis, as well as an improvement in clinical parameters [Table 1] on salivary ALP levels at baseline and postoperatively.[28]

The results showed that the mean PI and GI scores in the chronic periodontitis group decreased significantly from baseline to 30 days [Figure 5] after therapy. This implies that the patient maintains good oral hygiene following periodontal phase 1 therapy, which is a critical factor in the treatment outcome prognosis.[3,8,29]

Mean probing pocket depth and mean CAL readings are significantly lower from baseline to 30 days after scaling and root planning [Figure 6], which results in the resolution of the inflammatory response and the cessation of periodontal disease progression.[30,31] Scaling and root planning also aid in the elimination of bacteria at the site, reducing the colonization of periodontal pathogens and creating a favorable environment for the patient's oral hygiene maintenance.

CONCLUSION

Salivary and GCF ALP can be considered as a marker of chronic periodontitis as its level is significantly elevated above that of periodontally healthy people. In the majority of cases, the elevation of level of salivary ALP is directly proportional to the level of periodontal parameters. Further longitudinal studies with larger samples are warranted to confirm our results. If access to a portable analyzer was available, the chair-side diagnosis could have been performed. Cases with an undiagnosed systemic disease can be misleading due to the raised level of ALP due to other than periodontal sources like liver, gut, and renal pathologies.

An expected benefit of oral fluid-based periodontal diagnostics would be the identification of highly susceptible

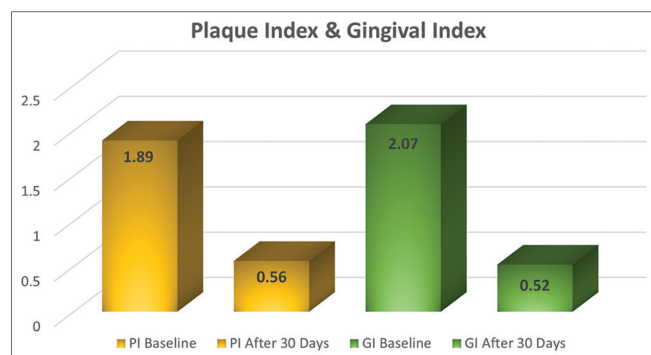

Figure 5: Change in PI and GI score from baseline to 1 month postoperative in the study group

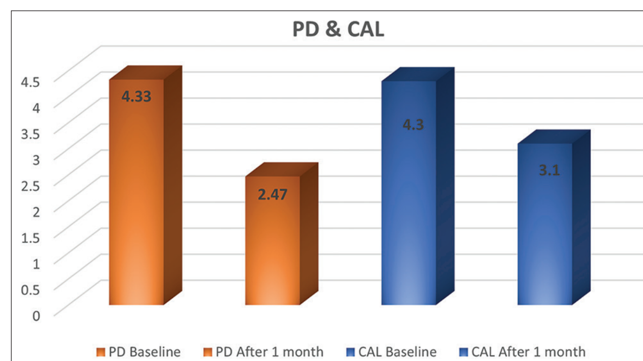

Figure 6: Change in probing depth and CAL from baseline to 1 month postoperative in the study group

individuals prior to aggressive disease. Timely detection and diagnosis of disease offer earlier, less invasive, and more cost-effective treatment.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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