Section: Research Paper

EVALUATION OF SERUM AND SALIVARY ALKALINE PHOSPHATASE LEVELS IN CHRONIC PERIODONTITIS PATIENTS

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Abstract

Background: The customary way of diagnosing periodontitis involves evaluating the destruction of periodontal tissue using clinical measurements and radiographic tools. However, saliva has the potential to be a useful diagnostic fluid for oral diseases. The objective of this research was to compare the levels of alkaline phosphatase (ALP) in both saliva and serum, both before and after scaling and root planing, in patients who have chronic generalized periodontitis. **Materials and Methods**: This study involved a total of 100 participants, of which 90 had chronic generalized periodontitis and 10 were periodontally healthy volunteers. The age range of the participants was between 30 to 50 years. The researchers measured various clinical parameters, including the simplified oral hygiene index (OHI-S), gingival index, probing depth, and clinical attachment loss (CAL). Afterward, samples of saliva and blood were collected from each participant and analyzed for their ALP levels using spectrometry. Following Phase I periodontal therapy, the clinical parameters, saliva, and serum ALP levels were measured again after 30 days. The data were then statistically analyzed using the paired t-test and one-way ANOVA. **Results**: The study found that subjects with chronic generalized periodontitis had significantly higher levels of ALP in both saliva and serum compared to periodontally healthy individuals. These higher levels of ALP were also associated with increased clinical parameters such as OHI-S, gingival index, probing depth, and CAL. However, after Phase I periodontal therapy, there was a significant decrease in both saliva and serum ALP levels, along with an improvement in clinical parameters. **Conclusion**: Despite the limitations of this study, it can be concluded that ALP levels in saliva have the potential to be used for the diagnosis of the active phase of periodontal disease, as well as for evaluating treatment outcomes following Phase I periodontal therapy.

Keywords: Alkaline phosphatase, biomarkers, chronic periodontitis, saliva, serum

Introduction

Of the various illnesses that affect teeth, periodontitis is a common disease results in the destruction of supporting structures of the teeth, ultimately which cause tooth loss. Although periodontitis is an infectious disease of gingival tissue origin, changes that occur in the bone are crucial as the alveolar bone destruction is responsible for tooth loss.[1,2] The most common cause of alveolar bone destruction in periodontitis is the extension of inflammation from the marginal gingiva to the underlying periodontal tissues.

Salivary constituents for diagnosing periodontal disease include enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, and volatile compounds.[3]

Intracellular enzymes are released progressively into the gingival crevicular fluid (GCF) and saliva from the damaged cells of periodontal tissues. Several enzymes that are evaluated for the early diagnosis of periodontal disease include lactate dehydrogenase, aspartate and alanine aminotransferase, creatine kinase, alkaline and acid phosphatase (ALP), and gamma-glutamyltransferase.[4]

ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules and is a marker of bone metabolism.[5] It is a membrane-bound glycoprotein produced by a various number of cells, such as polymorphonuclear leukocytes, macrophages, fibroblasts, and osteoblasts, within the area of the periodontium and gingival crevice.[6]

Various studies have assessed the levels of salivary ALP with respect to gingivitis, chronic periodontitis, and correlation of the same with clinical parameters. However, there was lagging evidence regarding the

comparative effects of ALP in serum and saliva following periodontal treatment.[7] ALP levels in saliva can be detected similarly to serum ALP estimation by using ultraviolet spectrometry.[8] So far, ALP has not been supported by research findings as a predictive indicator for future periodontal tissue breakdown.

The purpose of this study was to compare the serum and salivary ALP levels in chronic periodontitis patients before and after Phase I periodontal therapy which served to hold to the hypothesis that saliva can be used as an alternative to serum for evaluating ALP as a biomarker in periodontal disease progression.

Material and methods

This clinical study was conducted at the Department of Periodontology and involved a total of 100 participants between the ages of 30 and 50. The control group consisted of 10 periodontally healthy individuals, while the study group consisted of 90 patients with chronic generalized periodontitis. The participants were selected from the outpatient division of periodontics, and all were informed about the nature of the study and signed an informed consent form.

To be included in the control group, participants had to have at least 20 natural teeth with probing pocket depths of 2-3 mm, no attachment loss, and less than 20% sites with bleeding on probing. For the study group, participants had to have at least five qualifying sites in two quadrants, with a minimum of two affected teeth in each quadrant. Each site had to have a probing depth of at least 5 mm, clinical attachment loss (CAL) of at least 3 mm, and bleeding on probing. Patients with systemic diseases, smokers, pregnant women, and those who were not maintaining their oral hygiene were excluded from the study.

All individuals who participated in the study provided informed consent. Clinical indices, including the simplified oral hygiene index (OHI-S) and gingival index, as well as clinical parameters, such as probing depth and clinical attachment loss (CAL), were measured at baseline for the study group. Saliva and blood samples were collected from both the study and control groups and analyzed for ALP levels. For patients with chronic periodontitis, complete ultrasonic scaling was performed on day 1, followed by complete root planing in two subsequent visits within 15 days from baseline. On the 30th day, after the completion of Phase I periodontal therapy, patients were reviewed, and saliva and blood samples were collected and analyzed again for ALP activity.

All the patients in the study were asked to rinse their mouth with normal water (to wash out exfoliated cells). After 5 min, 5 ml of unstimulated saliva and 5 ml of intravenous blood were collected from each patients from 9.00 to 11.00 am. Unstimulated saliva was collected by the spit method in a sterile sample collection container. The saliva and serum samples were sent to the laboratory immediately where it was centrifuged at 3000 rpm for 5 min, and then, the ALP enzyme activity in saliva and serum was determined spectrophotometrically with the help of a semi-autoanalyzer (BTS 350, BIOSYS®) using ALP enzyme kit (Diasys®) with the International Federation of Clinical Chemistry and Laboratory Medicine recommendations and the results were expressed in international units. Following the sample collections, complete ultrasonic scaling was performed to all the patients in the study group. All the patients were instructed to maintain their oral hygiene with modified bass brushing technique and to use chlorhexidine mouthwash twice daily. Root planing, wherever required, was done after 15 days from baseline within two subsequent visits. All the patients were recalled on the 30th day following completion of Phase I periodontal therapy for review and postoperative sample collection.

Results

This clinical study aimed to evaluate the levels of serum and salivary ALP in patients with generalized chronic periodontitis before and after nonsurgical periodontal therapy and compare the outcomes with healthy participants. At baseline, all clinical parameters were measured, and saliva and blood samples were collected on the same day, and then 30 days after Phase I periodontal therapy. The collected samples were analyzed for ALP levels using spectrometry.

The obtained results were tabulated and analyzed using statistical software SPSS. The paired-t test was used to assess the baseline and postoperative values of clinical parameters, and one-way ANOVA was used to compare the enzyme levels in saliva and serum between the study group at baseline and postoperatively along with control group.

In the study group, there was improvement in OHI-S and gingival index scores along with a reduction in probing depth and a gain in CAL postoperatively. These changes were found to be statistically significant.

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

Clinical parameters	**Baseline**	**1 month postoperatively**
OHI-S	3.00±0.03	0.99±0.04
GI	1.98±0.05	0.61±0.02
PD (mm)	3.63±0.17	1.95±0.09
CAL (mm)	4.14±0.18	2.21±0.07
OHI-S: Oral hygiene index-simplified**, GI:** Gingival index**, PD:** Probing depth**, CAL:** Clinical attachment level**, ±:** Standard deviation.		

On comparing the mean values of salivary and serum ALP levels of control group with baseline values of the study group, the difference in salivary and serum ALP levels between control group (23.01 ± 6.66 and 72.71 ± 2.20) and study group (79.57 ± 6.41 and 97.61 ± 4.18) was found to be statistically significant with P = 0.000** and 0.009** (**indicates statistically highly significant) for saliva and serum, respectively. On comparing the mean baseline salivary and serum ALP values with postoperative values in study group, the difference in salivary and serum ALP levels from baseline (79.57 ± 6.41 and 97.61 ± 4.18) to postoperative (49.48 ± 5.11 and 85.40 ± 4.11) was found to be statistically significant with P = 0.000 and 0.009 for saliva and serum, respectively.

Discussion

The term biomarker refers to biologic substances that can be measured and evaluated to serve as indicators of biological health, pathogenic processes, environmental exposure, and pharmacologic responses to a therapeutic intervention.[9]

Among several biomarkers of periodontal disease activity, ALP, being a phenotype marker of bone turnover rate has been found to be elevated in a variety of bone disorders with the highest elevations occur in Paget's disease (osteitis deformans). Other bone disorders including osteomalacia, rickets, hyperparathyroidism, and osteogenic sarcoma have also shown elevated levels of ALP. In addition, increased levels were also seen in the case of healing bone fractures and during periods of physiologic bone growth.[10,11]

In the past few years, various cross-sectional clinical studies in humans have been conducted and proved the robust relationship between the periodontitis and elevated ALP levels in serum and in GCF.[12]

Although predictable, the sampling of blood by intravenous method is invasive and causes discomfort to the patients; its use for periodontal disease is of less patient compliance. Although reliable, sampling from GCF is technique sensitive and takes longer time compared with the sample collection time for saliva.

Various studies in the past few years have revealed the potential to identify and measure numerous biomarkers in saliva for the diagnosis of periodontal diseases and monitoring its progression and health.[13]

The study conducted by Miglani et al.[14], in 1974 revealed the relationship between periodontal disease and ALP levels in saliva was the first study in the Indian population, correlating the periodontal disease status with salivary ALP levels. Later, various studies that include Todorovic et al.[15], in 2006, Desai et al.[16], in 2008, Dabra and Singh[17] in 2012, Trivedi and Trivedi[18] in 2012, Ramesh et al.[19], in 2013, and Luke et al.[20], in 2015 have correlated the relationship between the enzyme ALP levels in saliva with that of clinical parameters in healthy controls, gingivitis patients, and patients with chronic periodontitis and the significant outcomes of the ALP levels after Phase I periodontal therapy.

All the studies conducted so far has aimed to rationalize the use of ALP from either saliva or serum or even GCF solely in patients with chronic periodontitis and even comparison of the same with periodontally healthy individuals.[21-23] However, none of the studies have compared the serum and salivary ALP levels in chronic periodontitis patients before and after periodontal treatment and correlating the same with periodontally healthy individuals.

This was the first study to compare and evaluate the ALP levels in saliva and serum in patients with chronic generalized periodontitis and to correlate the ALP levels with that of the healthy individuals.

The results of present study showed that ALP levels were increased in both saliva and serum in patients with chronic generalized periodontitis which was in accordance with the study conducted by Malhotra et al.[24], in 2010. The study also showed that the following Phase I periodontal treatment, there was a significant decrease in the salivary and serum ALP levels in patients with chronic generalized periodontitis in accordance with the results obtained from the study conducted by Dabra and Singh[17] in 2012 along with an added improvement in the clinical parameters following Phase I periodontal therapy.

Conclusion

Based on the constraints of the current research, it can be inferred that the measurement of ALP levels in saliva may serve as a means of diagnosing the active stage of periodontal disease, as well as a predictor of treatment success following Phase I periodontal therapy. However, to reinforce the findings of this study, further research with a larger sample size and varying durations of ALP evaluation in saliva is necessary.

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