ECRONICON OPEN ACCESS

EC DENTAL SCIENCE
Research Article

Comparison between Two Methods Used in the Determination of Salivary Alkaline Phosphatase in Periodontal Disease Patients

Myriam A Koss[1]*, Cecilia E Castro[2], Santiago Martínez Guarnieri[1] and María E López[1]

[1]*Department of Biochemistry, School of Dentistry, National University of Tucumán, Argentina*

[2]*Department of Periodontology, School of Dentistry, National University of Tucumán, Argentina*

***Corresponding Author:** Myriam A Koss, Department of Biochemistry, School of Dentistry, National University of Tucumán, Argentina.

Received: April 04, 2018; **Published:** May 14, 2018

Abstract

Background: Salivary alkaline phosphatase may be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontitis, an inflammatory disease that affects the connective tissue attachment around the teeth. Clinical diagnosis provides limited information about patients at risk for future periodontal breakdown. The aim of this study was to compare the alkaline phosphatase levels determined in total saliva through kinetic and colorimetric methods in patients with different diagnoses of periodontitis.

Material and Methods: The study group consisted of 27 adults classified according to their clinical diagnoses as having mild, moderate and severe chronic periodontitis and a control group consisted of 28 periodontitis-free adult subjects. Alkaline phosphatase was quantified in whole saliva using two commercial serum kits. Data were analyzed using one-way ANOVA and Tukey tests.

Results: Salivary alkaline phosphatase was detected by the two methods in all subjects. Levels in patients with periodontal disease were significantly higher than those in healthy subjects using both methods. However, a significant correlation between the determination of alkaline phosphatase and the clinical diagnoses was only found using the kinetic method.

Conclusion: Salivary alkaline phosphatase determination by the kinetic kit could represent a useful test for the diagnosis of the periodontal disease due to its marked correlation with the clinical diagnosis.

Keywords: *Alkaline Phosphatase; Saliva; Periodontitis*

Introduction

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. It is widely accepted that the initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms capable of causing disease [1].

After its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the junctional epithelium, formation of deepened periodontal pockets, and resorption of alveolar bone [2]. If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss.

Traditional periodontal diagnostic parameters used clinically include probing depths, bleeding on probing, clinical attachment levels, plaque index and radiographs assessing alveolar bone level [3], however provide limited information about patients at risk for future periodontal breakdown [4].

Several intracellular enzymes are released increasingly from the damaged cells of periodontal tissues into the gingival crevicular fluid and saliva [5], their estimation can be used for screening and predicting the early changes in the periodontal tissues and also to determine the efficacy of the treatment [6]. The advantage of saliva is the ease of collection, furthermore may offer a cost-effective approach to assess periodontal disease incidence in a large population [7-9]. Alkaline phosphatase (ALP) is a membrane-bound glycoprotein produced by many cells such as polymorphonuclear leukocytes (PMNLs), osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice [10]. Some studies have shown a remarkably increase in the activity of these enzymes in periodontitis and a reduction after periodontal therapy [11,12]. Also, salivary alkaline phosphatase levels may be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease [13].

Aim of the Study

The aimed of the study was to test two existing serum ALP kits to determine its useful in total saliva of chronic periodontitis patients and its relations with the clinical diagnosis.

Material and Methods

Clinical parameters

Clinical considerations for patients with periodontitis have been published previously [14] taking into consideration an adaptation of Lindhe [15]. Clinical parameters were assessed at six sites on each tooth (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual) using a manual periodontal probe (Hu-Friedy, NC, USA). The following parameters were included: gingival index (GI) [16], plaque index (PI) [17], bleeding on probing (BOP) up to 15s after gentle testing, probing depth (PD) (distance between the gingival margin and the bottom of the sulcus/pocket) and clinical attachment level (CAL) (distance between cement-enamel junction and the bottom of the sulcus/pocket). Bone resorption in periodontal chronic patients (CP) was established on the basis of clinical and radiographic criteria. Periapical radiographs were taken using a standardized long-cone paralleling technique. Patients with CP included in this study had mild (1): GI > 1, PI > 20%, BOP, and PD and CAL between 3 and 5 mm; moderate (2): GI > 1, PI > 20%, BOP, and PD and CAL between 5 and 6 mm and severe (3):GI> 1, PI > 20%, BOP, and PD and CAL ≥ 7 mm forms of the disease and exhibited at least one site with the clinical features. Free periodontal subjects (C): GI < 1, PI < 20%, no BOP, PD ≤ 3 mm and no CAL. Clinical and radiographic characteristics are show in table 1 [14].

Diagnoses	Clinical and Radiographic Characteristics
Control	Gingival index =0. Plaque Index 20%. Probing depth 3 mm. No attachment loss on the basis of clinical criteria. Characteristics compatible with health: light pink gums with a rough appearance. Absence of bleeding on probing, furcation, mobility and bony defects.
Mild Periodontitis	Gingival index >1. Plaque Index >20%. Probing depth 4mm. Pocket depth and attachment level (measurement and radiographic analysis) not exceeding one third of the length of the root. Bleeding on probing. Absence of furcation, mobility and bony defects.
Moderate Periodontitis	Gingival index >1. Plaque Index >20%. Probing depth > 5 mm. Pocket depth and attachment level (measurement and radiographic analysis) between one third and half the length of the root. Bleeding on probing. First degree furcation. Eventual first degree mobility.
Severe Periodontitis	Gingival index > 1. Plaque Index >20%. Probing depth > 7 mm. Pocket depth and attachment level (measurement and radiographic analysis) over half the length of the root. Bleeding on probing. Second or third degree furcation. Eventual second or thirddegree mobility. Angular bony defects.

Table 1: *Characteristics associated to control subjects and to the clinical and radiographic diagnoses of mild, moderate and severe chronic periodontitis groups.*

Study population

The study group consisted of 27 adults. They were classified according to their clinical diagnoses as having mild, moderate and severe chronic periodontitis (CP) aged 37.9 ± 4.3 yrs. The control group (C) consisted of 28 periodontitis-free adult subjects aged 32.8 ± 2.9 yrs. All patients attended the School of Dentistry at the National University of Tucumán for periodontal consultation. Written informed consent was obtained from all patients prior to participation. Inclusion criteria for all subjects were a minimum of 20 natural teeth excluding third molars GI > 1, PI > 1, BOP, PD and CAL ≥ 4.5 mm for patients and GI < 1, PI < 1, no BOP, PD and CAL < 3 mm for the C group. Exclusion criteria for all subjects included periodontal therapy prior to saliva collection, systemic medical affections and/or medicines that might impact directly on periodontal status or salivary constituents and salivary flow, use of antibiotics, steroidal or non-steroidal anti-inflammatory agents in the 6 months prior to the study.

Saliva collection

Patients were instructed not to eat or drink for 2h prior to sample collection. Unstimulated whole saliva was collected at the initial visit, before periodontal treatment, between 8 and 10 a.m. for 10 minutes. The volunteers were asked not to eat and drink for 1h prior to the experiment. While in a sitting position, the participants were asked to swallow saliva, then remain motionless and allow the saliva to drain passively for 10 minutes over the lower lip into a sterile plastic vial on ice. Saliva accumulated in the antero-vestibular and sublingual regions of the mouth was collected with a saliva ejector, avoiding contact with the mucosa, and placed in a test tube on ice. Whole saliva was centrifuged at 10,000 rpm for 10 minutes at 4°C and immediately frozen at -20°C until chemical determinations were performed.

Chemical determinations

Two diagnostic kits were used for the estimation of ALP activity. The kinetic method (ALP 405 AA, Wiener Lab Arg.) is based in the hydrolysis of p-nitro phenylphosphate (p-NFF) 10 mM in diethanolamine buffer (DEA) 1M in presence of Mg^{+2} 0.5 mM at 37°C using 10 ul of saliva. The velocity of apparition of p-nitrophenol at 405 nm is proportional to the enzyme activity. The unities of ALP (U/L) were calculated by the following form:

$$ALP\ (U/L) = \Delta A / min \times 5.460$$

The colorimetric method (optimized ALP, Wiener Lab Arg.), is based in the degradation of sodium phenylphosphate with amino methyl propanol (AMP) 3M pH = 10 at 37ºC. A color at 520 nm proportional to the ALP activity was determined by the phenol liberated whit 4-amino antipyrina 29mM and ferrycyanure 10 mM as oxidant agent. A modification in the sample volume was necessary to make, so 100 ul of saliva was used instead the 10 ul indicated in the protocol form. A phenol standard solution was (200 UI/l)included in the kit. The ALP unities (UI) were calculated with the following form taking into account the adaptation made:

$$ALP\ (UI) = 200\ UI/L \times DO_m/DO_{St}$$

Statistical analysis

Data were analyzed by the SPSS system (11.0). Differences among groups were studied using one-way ANOVA. When differences were significant, the Tukey test was applied. The clinical diagnosis and the salivary alkaline phosphatase levels were analyzed by means Spearman rank correlation.

Results

Salivary ALP activity was detected by the two serum kits. The mean and the standard deviation for both of them are show in figure 1. ALP activities showed statistically significant difference ($p < 0.001$) between healthy and periodontitis groups with the two methods used. The average activity of ALP in saliva of the periodontal disease patients was 136.4 ± 23.4 U/L with the kinetic method and 129.8 ± 60.9 UI/L with the colorimetric method and in the healthy patients groups 67.6 ± 12.6 U/L and 33.4 ± 17.3 UI/L respectably. In the healthy group ALP levels showed significant differences in between two methods ($p < 0.001$), however this difference is not showed in periodontitis patients. A gentle and sustained increase of ALP activity was registered from mild, moderate to severe periodontitis using the kinetic method (Table 2), so a significance correlation (Figure 2), was found between kinetic methods with the clinical diagnosis $r = 0.543$ ($p < 0.001$)however, no correlation with the colorimetric methods was found (data no show).

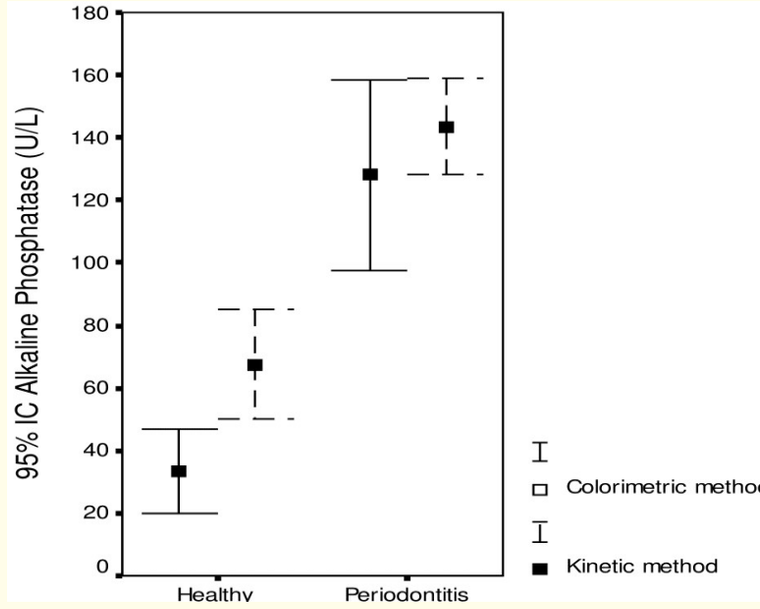

Figure 1: *Mean and standard error of alkaline phosphatase (U/L) in healthy and chronic periodontitis patients.*

	ALP			
	Mild periodontitis	Moderate periodontitis	Severe periodontitis	P value
Kinetic method	120.7 ± 5.7 U/L	127.9 ± 14.3 U/L	148.8 ± 30.0 U/L	p > 0.05
Colorimetric method	115.6 ± 81.5 UI/L	105.4 ± 71.0 UI/L	138.4 ± 56.5 UI/L	P > 0.05

Table 2: *Mean and standard error of alkaline phosphatase (U/L) levels with kinetic and colorimetric methods in mild, moderate and severe periodontal patients.*

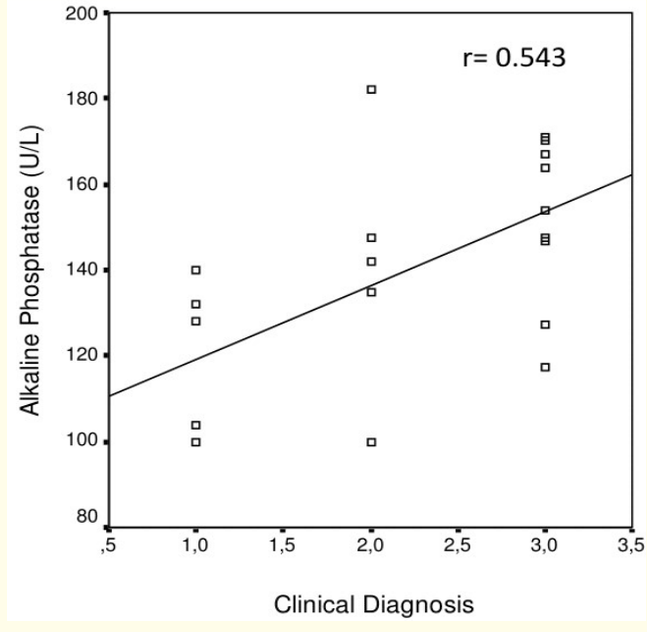

Figure 2: *Correlation between alkaline phosphatase (U/L) with the clinical diagnosis (Mild periodontitis: 1, Moderate periodontitis: 2 and Severe periodontitis 3).*

Discussion

Diagnostic test in serum and/or urine are commonly used in evaluation of many systemic diseases. Salivary diagnostics is a dynamic and emerging field in the diagnosis of oral and systemic diseases [18]. ALP is detected in the parotid, submandibular, and minor salivary glands, as well as in desquamated epithelial cells, leukocytes, and bacteria from dental plaque. ALP is considered to be an important indicator of bone formation and is a phenotypic marker for osteoblast cells [5]. It has been shown that its concentration in saliva, are elevated in patients with periodontal disease [10]. ALP in a 77% of diagnostics accuracy is one of the three most promising biomarkers for predicting future disease activity together with β glucuronidase(78%) and cathepsin B (99%) [9].

The present study was designed to evaluate the useful of two serum kits to determine ALP salivary activity in periodontal disease patients. The study selects a healthy subject group to take a referential salivary ALP level in periodontal free patients and a mild, moderate to advanced periodontal patients group.

As show our results, both kits could detect the enzymes in saliva of healthy and disease patients, however with the colorimetric method was necessary to increase the sample volume to detect the enzyme in saliva. We found statistical difference in ALP activity in periodontal patients with the two methods respect to the control group. Salivary ALP activity in saliva is usually indicative of inflammation and/or destruction of the periodontal tissues [5]. As well as the study done by Bezerra., *et al*. in 2010 [19], significant correlation was found between ALP level and clinical diagnosis in periodontal mild, moderate and advanced patients with the kinetic method. Increased ALP activity has been shown to have a predictive value in terms of attachment loss that is significantly more accurate than the use of clinical parameters [20,21], while other researchers showed that a combination of ALP salivary levels and the *P. gingivalis* count in saliva in periodontal disease patients may be useful biomarkers for predicting the progression of periodontal disease during supportive periodontal therapy [22].

Saliva as a mirror of the oral and systemic health is a valuable source for clinically relevant information, because it contains biomarkers specific for the unique physiological aspects of periodontal disease [23,24]. A biomarker or biologic marker is a substance that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. In considering that changes in ALP levels in saliva and/or serum has been used as an inflammatory marker of periodontium, we can conclude that salivary ALP testing by the kinetic kit could therefore represent a non-invasive, sensitive and useful test for quantify the biomarker for application to diagnose periodontal disease and to prognosticate treatment. Recently due to the combination of biotechnological development a large number of medically valuable analytes in saliva are unveiled and some of them represent biomarkers for different disease including cancer, auto immune disease, viral disease, bacterial diseases [18]. Thus, ALP determination in saliva using this test can represent an easy and accessible form to measure the biomarker. In according of our results we can conclude that salivary ALP levels in a range between 54.8 - 80.2 UI/L can associated to healthy periodontal disease subjects, while an increase in ALP activity with an inflammatory tissue state as characterized the periodontal disease. Changes of the clinical parameters from the moderate form to advanced periodontal disease show correlation in this study with ALP levels. These findings however should be interpreted taking into account some limitation of the study, in particular the sample size.

Conclusion

Statistical differences in salivary alkaline phosphatase activity in periodontal patients respect to the control group were found by the two serum ALP kits. Salivary ALP determination by the kinetic kit could represent a useful test for the diagnosis of periodontal disease. It does not require any adaptation or modification of the technique for its application in saliva samples and show sensitivity and a marked correlation with the clinical diagnosis.

Bibliography

1. Taba M., *et al*. "Diagnostic Biomarkers for Oral and Periodontal Diseases". *Dental Clinics of North America* 49.3 (2005): 551-571.

2. Offenbacher S. "Periodontal diseases: pathogenesis". *Annals of Periodontology* 1.1 (1996): 821-878.

3. Armitage GC. "The complete periodontal examination". *Periodontology 2000* 34 (2004): 22-33.

4. Lamster IB. "Evaluation of components of gingival crevicular fluid as diagnostic tests". *Annals of Periodontology* 2.1 (1997): 123-137.

5. Sanikop S., *et al*. "Gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal disease". *Journal of Indian Society of Periodontology* 16.4 (2012): 513-518.

6. Zhang L., *et al*. "The clinical value of salivary biomarkers for periodontal disease". *Periodontology 2000* 51 (2009): 25-37.

7. Luke R., *et al*. "Estimation of Specific Salivary Enzymatic Biomarkers in Individuals with Gingivitis and Chronic Periodontitis: A Clinical and Biochemical Study". *Journal of International Oral Health* 7.9 (2015): 54-57.

8. Ohshima M., *et al*. "Hepatocyte growth factor in saliva: a possible marker for periodontal disease status". *Journal of Oral Science* 44.1 (2002): 35-39.

9. Kaufman E and Lamster IB. "Analysis of saliva for periodontal diagnosis-a review". *Journal of Clinical Periodontology* 27.7 (2000): 453-465.

10. Daltaban O., *et al*. "Gingival crevicular fluid alkaline phosphatase levels in postmenopausal women: Effects of phase I periodontal treatment". *Journal of Periodontology* 77.1 (2006): 67-72.

11. Dabra S and Singh P. "Evaluating the levels of salivary alkaline and acid phosphatase activities as biochemical markers for periodontal disease: A case series". *Dental Research Journal* 9.1 (2012): 41-45.

12. Perinetti G., *et al*. "Gingival crevicular fluid alkaline phosphatase activity reflects periodontal healing/recurrent inflammation phases in chronic periodontitis patients". *Journal of Periodontology* 79.7 (2008): 1200-1207.

13. Gibert P., *et al*. "Alkaline phosphatase isozyme activity in serum from patients with chronic periodontitis". *Journal of Periodontal Research* 38.4 (2003): 362-365.

14. Koss MA., *et al*. "Changes in saliva protein composition of patients with periodontal disease". *Acta Odontologica* L*atinoamericana* 22.2 (2009): 105-115.

15. Lindhe J. "Examination of patients with periodontal disease". In: Harring, Lang (Eds.), Clinical Periodontology and Implant Dentistry, Munksgaard, Copenhagen (1998): 383-395.

16. Löe H and Silness J. "Periodontal disease in pregnancy. I. Prevalence and severity". *Acta Odontologica Scandinavica* 21 (1963): 533-551.

17. Silness J and Löe H. "Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal conditions". *Acta Odontologica Scandinavica* 22 (1964): 121-135.

18. Koneru S and Tanikonda R. "Salivaomic- A promising future in early diagnosis of dental diseases". *Dental Research Journal* 11.1 (2014): 11-15.

19. Bezerra AA., *et al*. "Evaluation of organic and inorganic compounds in the saliva of patients with chronic periodontal disease". *Revista Odonto Ciência* 25.3 (2010): 234-238.

20. Chapple ILC., *et al*. "Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels". *Journal of Clinical Periodontology* 26.3 (1999): 190-198.

21. Binder TA., *et al*. "Gingival fluid levels of acid and alkaline phosphatase". *Journal of Periodontal Research* 22.1 (1987): 14-19.

22. Nomura Y., *et al*. "Salivary biomarkers for predicting the progression of chronic Periodontitis". *Archives of Oral Biology* 57.4 (2012): 413-420.

23. Miller CS and King CP. "Salivary biomarkers of existing periodontal disease-A cross-sectional study". *Journal of American Dental Association* 137.3 (2006): 322-329.

24. Marcotte H and Lavoie MC. "Oral microbial ecology and the role of salivary immunoglobulin A". *Microbiology and Molecular Biology Reviews* 62.1 (1998): 71-109.

Volume 17 Issue 6 June 2018