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Research Article

Determination of Salivary Alkaline Phosphatase and β Glucuronidase in Treated Periodontal Disease Patients

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Received: April 29, 2019; **Published:** May 21, 2019

Abstract

Background: Periodontitis can be considered among the prevalent and important global health problems in terms of quality of life and at the present. It is diagnosed entirely on the basis of an array of clinical measurements. A one of the major challenges in the field of periodontology is to discover a method of predicting the future of periodontal disease. Saliva can be used for screening and predicting the early changes in the periodontal tissues, so this study was designed to evaluate alkaline phosphatase and β-glucuronidase as salivary biochemical markers to monitoring patients after nonsurgical periodontal therapy.

Material and Methods: The study group consisted of 31 adults. They were classified according to their clinical diagnoses as having periodontitis A and B degree, stage I to III. The control group consisted of 28 periodontitis-free adult subjects. Estimation of salivary β glucuronidase and ALP was determined before and after periodontal nonsurgical treatment by a colorimetric and a kinetic method respectively. Data were analyzed using one-way ANOVA and Tukey tests.

Results: Alkaline phosphatase and β glucuronidase activities showed statistically significant difference between healthy and periodontitis groups. After a month of nonsurgical periodontal treatment, β glucuronidase activity showed statistical difference, in between the pre and post treatment patients belonging to stage I, II and III and Alkaline phosphatase only between the pre and post treatment in patients belonging to stage III.

Conclusion: Salivary β glucuronidase activity could be used to evaluate the nonsurgical treatment in the A and B degree periodontal patients corresponding to stage I to III.

Keywords: *Alkaline Phosphatase; β-Glucuronidase; Saliva; Periodontitis*

Introduction

Periodontal disease is a common inflammatory disease caused by the interaction between certain Gram-negative bacterial species and components of the host immune response [1] that affect the connective tissue attachment and supporting bone around the teeth. It can be considered among the prevalent and important global health problems in terms of quality of life [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 [3]. The periodontal inflammatory disorder leads to the accumulation of different cells types in the site of infection including the polymorphonuclear leukocytes (PMNs) which exercise an important role in protecting action against the body

infection. In their lysosomes those cells contain enzymes capable of destroying periodontal tissues when they are released [4]. Between all of them, β-glucuronidase is considered a marker for PMNs primary granules and a potential biomarker of tissue destruction [5]. It contributed to the non- collagenous matrix degradation in periodontal disease [6].

As well as β-glucuronidase, alkaline phosphatase (ALP) it is also produced by PMNs, osteoblasts, macrophages and fibroblasts. It is a membrane-bound glycoprotein that exert its action in the gingival area [7] and may be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease [8]. Their activities can be used to detect the early changes in the periodontal tissues and also to evaluate the efficacy of the treatment [9]. The advantage of using saliva is that it is easy to collect, multiple samples can be obtained without causing inconvenience for the patient, it does not cause pain and it can also be used to evaluate analytes in a large population [10-12].

At present, periodontitis is diagnosed by a summation of clinical index and radiographic criteria including clinical attachment level (CAL), bleeding on probing (BOP), probing depth (PD). An important achievement for the diagnosis of periodontal disease is linked to the development of a co-adjuvant biochemical methodology for its prediction and monitoring [2]. Therefore, this study aimed to attempt to establish ALP and β-glucuronidase as salivary biochemical markers for periodontitis and its evaluation after nonsurgical periodontal therapy.

Materials and Methods

Clinical parameters

The clinical considerations for patients with periodontitis were made taking into account the new classification of 2018 [13]. On each tooth clinical indexes were assessed at six sites (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual) by using a manual periodontal probe (Hu-Friedy, NC, USA). Others parameters: gingival index (GI) [14], plaque index (PI) [15], bleeding on probing (BOP) up to 15 s 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) were included. Bone resorption was established using clinical and radiographic criteria. Periapical radiographs were taken using a standardized long-cone paralleling technique. Periodontal patients considered in the present study were classified in stage I: GI > 1, PI > 20%, BOP, and PD and CAL between 1 and 2 mm; stage II: GI > 1, PI > 20%, BOP, and PD and CAL between 3 and 4 mm and stage III: GI > 1, PI > 20%, BOP and PD and CAL ≥ 5 mm forms of the disease and exhibited at least one site with the clinical features. Healthy periodontal subjects: GI < 1, PI < 20%, no BOP, PD ≤ 3 mm and no CAL.

Population

The study group included 31 adults aged 37.9 ± 4.3 yrs. In considering their clinical diagnosis they were classified as having periodontitis A and B degrees stage I to III. 28 periodontal healthy adult subjects aged 32.8 ± 2.9 yrs constituted the control group. All periodontal patients attended the School of Dentistry (National University of Tucumán) for consultation. Each of the individuals who participated in the study signed a consent to participate. 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 < 1 mm for the C group. Exclusion criteria for all periodontal patients included periodontal treatment prior to saliva sample collection, systemic affections and/or intake of medicines that might impact directly on periodontal status, use of antibiotics, anti-inflammatory steroidal or non-steroidal agents in the 6 months prior to the study.

All patients received basic periodontal therapy after the diagnosis that consisted of oral hygiene instructions, biofilm control and scaling and root planning. No chemical plaque control agents were used.

Saliva collection

It was requested that patients do not drink or eat for 2h prior to the sample collection. Unstimulated whole saliva was collected for 10 min at the initial visit, before periodontal treatment, and after 30 days of initial therapy, between 8 and 10 a.m. in a sitting position all the subjects who participated in the study collected the saliva produced into an sterile plastic tube on ice. Whole saliva was centrifuged at 10,000 rpm for 10 min at 4°C and immediately frozen at -20°C until chemical determinations were performed.

After 30 days of initial therapy, patients were recalled for assessment of saliva sample for analysis following the same considerations as previously enunciated.

Chemical determinations

Estimation of β glucuronidase was performed by the method of Szasz (1967) [16] using p-nitrophenyl glucuronide obtained from Santa Cruz Biotechnology (Dallas, Texas USA. The system of the assay consisted of 0.2 ml de acetate buffer (0.2M pH = 5) 1.0 ml of glycine (0.6M pH = 11.7), 100 µl saliva and 0.1 ml of p-nitrophenyl glucuronide (50 mM) as substrate. After incubation at 37º for 2h absorbance at 546 nm was read in a T60 UV- Visible Spectrophotometer against water blank and the activity was expressed in milliunits (mU).

ALP activity was determined at 37ºC using 10 **µ**l of saliva by a kinetic method (ALP 405 AA, Wiener Lab Arg.) based in the hydrolysis of p-nitro phenylphosphate 10 mM in diethanolamine buffer 1M in presence of Mg^{+2} 0.5 mM. The velocity of apparition of p-nitrophenol at 405 nm was proportional to the enzyme activity. The unities of ALP (U/L) were calculated by the following form: ALP (U/L) = **Δ**A / min x 5.460.

Statistical analysis

Data were analyzed using the using ANOVA one-way (SPSS system) and when differences were significant, was applied the Tukey test.

Results

A statistically significant difference were found between healthy and periodontitis groups respect to ALP and β glucuronidase ($p < 0.001$). ALP activity in saliva of the periodontal disease patients was 147.27 ± 12.96 U/L and 67.6 ± 12.6 U/L in the healthy patients group. β glucuronidase showed values in between 1.03 ± 0.21 mU and 0.41 ± 0.27 mU in the periodontitis and healthy groups respectively. After a month of nonsurgical periodontal treatment, ALP activity registered only a slight decrease ($p > 0.05$). However, β glucuronidase showed a significate statistic decrease ($p < 0.05$) compared to the levels in the periodontal patients at the initial state (Table 1). Both enzymes in this period do not reach the levels of the healthy periodontal free patients.

	Control patients (n = 28)	**Periodontal patients (n = 31)**	**Periodontal treatment patients (n = 27)**
ALP (U/L)	67.6 ± 12.6	147.27 ± 12.96*[a]	133.98 ± 13.87
β Glucuronidase (mU)	0.41 ± 0.27	1.03 ± 0.21*[a]	0.72 ± 0.32*[b]

Table 1: *Mean and standard error of alkaline phosphatase and* **β glucuronidase** *levels in periodontal patients before and after periodontal nonsurgical treatment.*

[a]: Significant differences between control and periodontal patients ($p < 0.001$).

[b]: Significant differences between periodontal and periodontal treatment patients ($p < 0.05$).

When we assessment the enzymes activity in considering the periodontal diagnosis, ALP showed statistical difference between the pre and post treatment states in patients belonging to stage III ($p < 0.05$) (Figure 1). β glucuronidase activity, in between the pre and post treatment patients belonging to stage I, II and III ($p < 0.001$) (Figure 2).

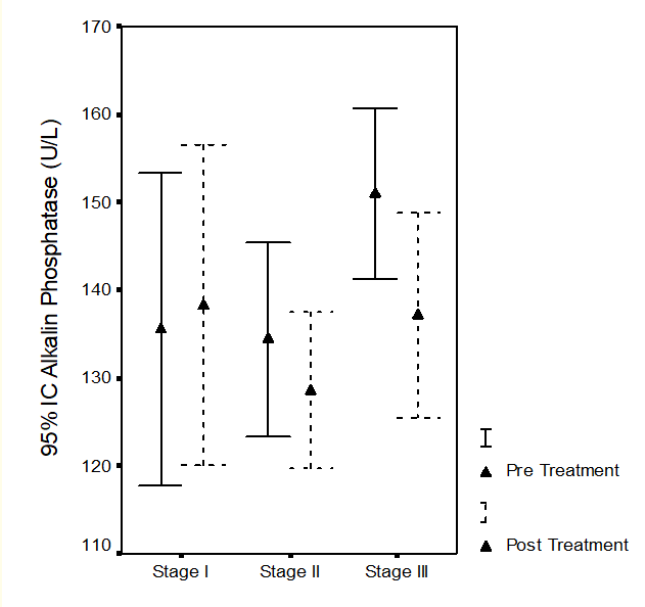

Figure 1: *Mean and standard error of alkaline phosphatase (U/L) in periodontitis patients stage I, II and III before and after the nonsurgical periodontal treatment.*

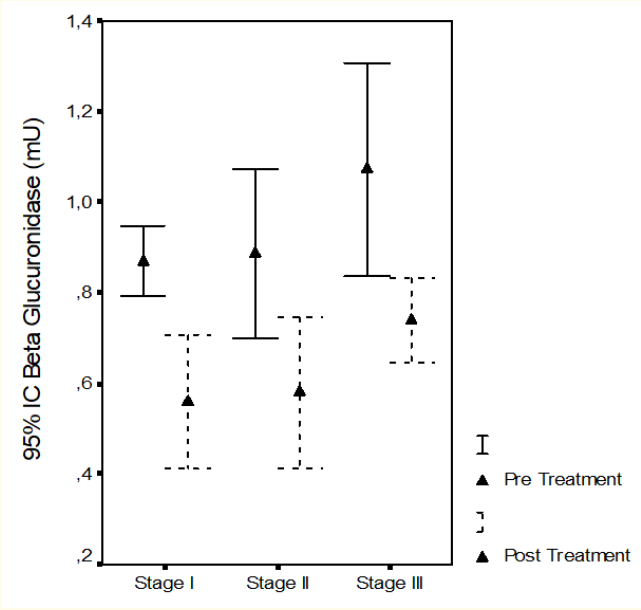

Figure 2: *Mean and standard error of β glucuronidase (mU) in periodontitis patients stage I, II and III before and after the nonsurgical periodontal treatment.*

Discussion

Periodontitis is an inflammatory disorders that result from the host response to sub-gingival plaque microorganisms [17]. As a result, the normal histological architecture of the periodontium is disturbed, with persistent inflammation associated with the irreversible loss of mineralized and non-mineralized tissues [1].

Diagnosis, stratification, prognosis, and therapy for inflammation include the measurement of biological markers in biological specimens [18]. Saliva provides noninvasiveness and stress-free sample collection, easy and multiple sampling opportunities, reduced need for sample pre-processing, and minimal risk of contracting infectious organisms [19]. This study compare the salivary ALP and β-glucuronidase levels between periodontally healthy and diseased subjects and also evaluate its activity after the nonsurgical periodontal therapy using a simple and accessible biochemical method. The present study showed high levels of salivary ALP and β-glucuronidase activities in periodontal patients respect to the control group. Our findings are similar to those found by others authors [5,20-22]. A previous study carried out by our group [23] showed significant correlation between ALP activity and clinical diagnosis in patients with mild, moderate and advanced periodontitis. Other study conducted by Ohshima., *et al.* [11] found that in a 78% of diagnostic accuracy β-glucuronidase followed by ALP in 77%, are the most promising biomarkers to predict the future activity of the disease.

One month after the nonsurgical periodontal treatment, a significant decrease in β glucuronidase activity was found in all periodontitis forms, however those results were only valid for the severe periodontitis (III degree) respect to ALP activity. A diagnostic tool should provide pertinent information to aid differential diagnosis, screening, presence, location, severity or staging and prognosis of a disease [24]. We could infer from the present study, that β glucuronidase is a better biomarker than ALP to monitor the periodontal disease treatment since it evidences periodontal tissue metabolism changes in a shorter period of time.

The most studies conducted so far, have aimed to evaluate the use of saliva biomarkers, especially comparing them between patients with periodontitis and periodontally healthy patients; however, the present study compared the behavior of both biomarkers after periodontal treatment in patients with different severity of the disease. The results of present study showed that β glucuronidase is a good candidate to monitor the response to periodontal treatment in the three different considered degrees of periodontitis while ALP only for the severe form (III degree). These findings should be correlated with the traditional clinical indexes used to diagnose and monitor the periodontal disease.

Conclusion

Salivary β glucuronidase activity could be used to evaluate the nonsurgical periodontal treatment in periodontitis degree A and B patients corresponding to stage I to III, and ALP only for the stage III in those patients.

Acknowledgements

This study was partially supported by a grant from CIUNT (Consejo Nacional de Investigaciones Científicas y Técnicas de la Universidad Nacional de Tucumán) and Facultad de Odontología de la Universidad Nacional de Tucumán.

Conflict of Interest

The authors do not have any financial interest or any conflict of interest.

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Volume 18 Issue 6 June 2019