

Gingival Crevicular Fluid Levels of Prolidase and Alkaline Phosphatase in Periodontitis

SUMMARY

Background/Aim: The purpose of this study was to investigate gingival crevicular fluid (GCF) alkaline phosphatase (ALP) and prolidase levels in subjects with different periodontal status. **Material and Methods:** Fifteen periodontitis, fifteen gingivitis and fifteen healthy subject were included. GCF samples were collected from participants. Probing depth, clinical attachment level, gingival index was recorded. ALP and prolidase levels were determined in GCF by spectrophotometrically. **Results:** Higher values of ALP were found in periodontitis compared with gingivitis and healthy control ($p < 0.001$). The values of prolidase were lower in periodontitis than healthy control ($p < 0.05$). A statistically significant positive correlation was found between clinical parameters and ALP levels ($p < 0.001$). There is no significant correlation between clinical parameters and prolidase levels ($p > 0.05$). Additionally, no significant correlation was detected between ALP and prolidase ($r = -0.309$, $p > 0.05$). **Conclusion:** Our preliminary data suggest that low prolidase level in periodontitis was not associated with ALP and clinical parameters, which represent periodontal destruction and inflammation.

Keywords: Prolidase, ALP, Periodontitis, Gingivitis

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Introduction

Periodontitis is an inflammatory condition affecting the tooth-supporting tissues. It is characterized by destruction of periodontal tissues. Markers of bone formation are proteins revealing osteoblast activity like products of collagen synthesis, matrix proteins or osteoblastic enzymes^{1,2}. Alkaline phosphatase (ALP) is a glycoprotein, which functions as a phospho-hydrolytic enzyme, is a calcium- and phosphate-binding protein. It is released from many cells including neutrophils, fibroblasts and osteoblasts during bone formation. ALP is detected in gingival crevicular fluid (GCF) collected from inflamed periodontium as well as from osteoblasts during bone formation³.

Prolidase (EC 3.4.13.9) is an enzymes that hydrolyzes dipeptides containing proline or 4-hydroxyproline at the C-terminal. The dipeptidase has a major role in collagen turnover and rate-limiting enzyme for collagen resynthesis⁴. Prolidase deficiency is a rare autosomal recessive disease

characterized by skin lesions and recurrent infections⁵. On the other hand, increase in prolidase activity may be accompanied by increase in tissue collagen deposition^{6,7}.

The aim of our study was to evaluate ALP and prolidase activities in gingival crevicular fluid in patients with gingivitis and periodontitis.

Material and Methods

Clinical parameters

This study consisted of 45 individuals (23 males and 22 females) admitted to the Periodontology Department, Faculty of Dentistry of Bulent Ecevit University. The ethical committee of Bulent Ecevit University approved this study, and a written informed consent was obtained from each patient.

All individuals underwent radiography and a full-mouth periodontal examination including probing pocket depth

(PPD), clinical attachment level (CAL) and gingival index (GI)⁸. Clinical parameters were assessed at six sites on each tooth (mesiobuccal, mediobuccal, distobuccal, mesiolingual, mediolingual and distolingual) using a manual periodontal probe (Hu-Friedy, Chicago, IL, USA). Inclusion criteria for all subjects were a minimum of 20 natural teeth, exclusion criteria included a history of periodontal treatment in the last 6 months, use of antibiotics, anti-inflammatory drugs within the previous 3 months, pregnancy, or lactation, systemic diseases and smokers.

Patients were categorized into three groups based on PPD, CAL and GI scores and radiographic evidence of bone loss⁹.

Healthy control: 15 subjects (7 males and 8 females) with clinically healthy periodontium (GI = 0, PPD and CAL ≤ 3 mm).

Gingivitis: 15 subjects (7 males and 8 females) with gingival inflammation (GI ≥ 1, PPD and CAL ≤ 3 mm).

Periodontitis: 15 subjects (8 males and 7 females) who showed chronic periodontitis clinical signs of inflammation GI > 1, PPD and CAL ≥ 5 mm with alveolar bone loss affecting >30% of the existing teeth on clinical and radiographic examination.

Collection of Samples

In all groups, two sites per individual were selected for sampling. GCF samples were obtained from the mesio-buccal or disto-buccal surfaces of single rooted teeth. To prevent the contamination of GCF with blood associated with the probing of inflamed sites, all clinical examinations and sampling site selections were performed 2 days before GCF samples were collected.

Each crevicular site included in the study was isolated with cotton rolls. Before the GCF collection, any supragingival plaque was removed with cotton pellets and a gentle air stream was directed toward the tooth surface for 5 seconds to dry the area. GCF was sampled with filter paper (PerioPaper, ProFlow, Amityville, NY) using the intracrevicular method¹⁰. Paper strips were placed into the crevice until mild resistance was felt and were left in position for 30 seconds. Strips with visible saliva or blood contamination were discarded. Immediately after collection, the paper strips were transferred to plastic vials and stored at -40 °C until analysis.

Biochemistry Assay

On the day of the assay, 400 µL phosphate-buffered saline (pH 7.4) was added to each of the tubes containing the sample strips. The tubes were vortexed and homogenized for 1 minute and then centrifuged at 3,000g for 15 minutes at 4°C. The supernatants were collected the GCF samples were measured for ALP levels by using an Alkaline Phosphatase Detection kit (Beckman Coulter, CA, USA) according to the manufacturer's instructions.

Prolidase activity was determined by a photometric method based on the measurement of proline levels

produced by prolidase. Fluid was diluted 40-fold with 25 mmol/L Mn₂⁺, 40 mmol/L Trizma HCl buffer (pH 8.0) and preincubated at 37°C for 2 h. The reaction mixture containing 30 mmol/L Gly-Pro, 40 mmol/L Trizma HCl buffer (pH 8.0), and 100 µL of preincubation fluid in 1 mL was incubated at 37°C for 30 min. The reaction was stopped by adding 0.5 mL 20% trichloroacetic acid solution. The supernatant was used for measurement of proline by the method proposed by Myara et al., which is a modification of Chinard's method¹¹.

Statistical Analyses

All data were analyzed using statistical software (SPSS version 10.5, SPSS, Chicago, IL, USA). Statistical analysis was performed using nonparametrical techniques. Comparisons between the study groups were performed using the Kruskal-Wallis test. The correlations between biochemical and clinical parameters were determined by Spearman's correlation coefficient. The results were considered statistically significant when p-values were less than 0.05.

Results

Mean ages of control, gingivitis and periodontitis groups were 34.1 ± 3.2, 34.5 ± 2.7 and 36.1 ± 3.3 years, respectively. No significant difference was observed between the mean age of groups. The clinical parameters are shown in Table 1. GI, PPD and CAL were statistically higher in gingivitis and periodontitis than in control. All parameters were mostly higher in periodontitis than in gingivitis.

Table 1. Comparison of clinical parameters between the groups

Clinical parameters	Control (n = 15)	Gingivitis (n = 15)	Periodontitis (n = 15)
Gingival index	0.3±0.25	1.67±0.30a	2.26±0.16a,b
Probing pocket depth (mm)	1.75±0.5	2.33±0.45a	4.88±0.53a,b
Clinical attachment level (mm)	1.75±0.5	2.33±0.45a	5.59±0.54a,b

^a Significant difference from healthy group (p<0.05)

^b Significant difference from gingivitis group (p<0.05)

Higher values of ALP were found in periodontitis compared with gingivitis (p<0.001) and healthy control (p<0.001). There was no significant difference between gingivitis and healthy control groups with regard to ALP levels (p>0.05) (Figure 1). The values of prolidase were lower in periodontitis than healthy control (p<0.05). With respect to prolidase levels, no significant differences between gingivitis group and control group were observed (p>0.05) (Figure 2).

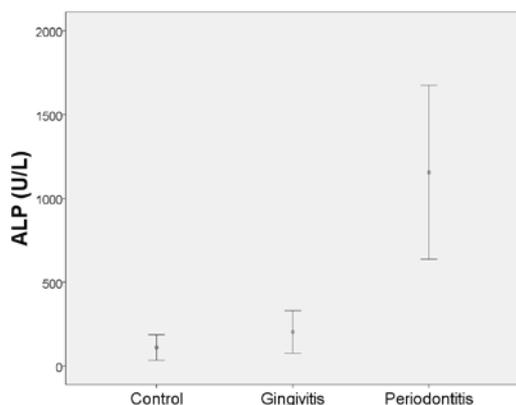


Figure 1. GCF ALP levels in relation to periodontal status

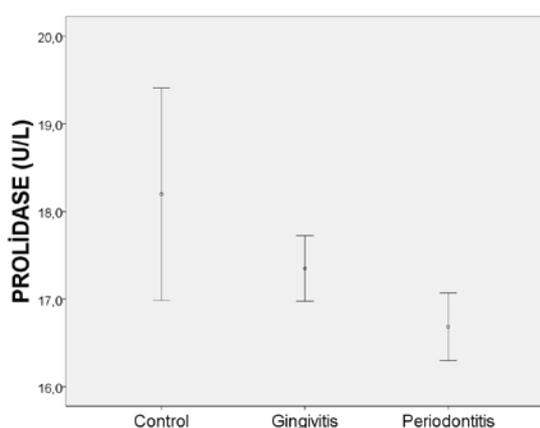


Figure 2. GCF prolidase levels in relation to periodontal status

The correlation coefficients are shown in Table 2. A statistically significant positive correlation was found between clinical parameters and ALP levels ($p < 0.001$). There is no significant correlation between clinical parameters and prolidase levels ($p > 0.05$). Additionally, no significant correlation was detected between ALP and prolidase ($r = -0.309$, $p > 0.05$).

Table 2. Correlations between biochemical and clinical parameters

Clinical parameters	ALP	Prolidase
Gingival index	0.728*	-0.267
Probing depth	0.619*	-0.261
Clinical attachment level	0.620*	-0.257

Spearman correlation (* $p < 0.05$).

Discussion

Alkaline phosphatase is a very important enzyme, that contributes to the normal turnover of periodontal ligament and bone homeostasis¹². It is produced by many cells, especially it is considered to be a marker of osteoblastic activity^{13,14}, but the main source of ALP in GCF is

neutrophils¹². In this study, ALP notably increased in GCF of patients with periodontitis compared with gingivitis and the control group. Furthermore, positive correlations of ALP levels with clinical parameters, including PD, GI and CAL were found. These findings support previous data that raised ALP levels in GCF are associated with severity of periodontal destruction and inflammation¹⁵. Also, ALP is thought to play a role in superoxide generation in neutrophils¹⁶. Oxidative stress, an excess of reactive oxygen species (ROS) and depletion of anti-oxidant levels in tissues is importance in periodontal pathogenesis¹⁷. Previous studies showed that oxidative stress can stimulate prolidase activity^{18,19}. Prolidase is a cytosolic enzyme capable of degrading dipeptides in which a proline or hydroxyproline residue is located at the C-terminal position. Collagen biosynthesis may require prolidase activity to play an important role capable of degrading proline-containing dipeptides to produce free proline as a product²⁰.

Collagen is the main component of extracellular matrix in the periodontal tissues, which is considered as an important component determining the pathophysiology of periodontal disease²¹. Collagen degradation is initiated by activation of matrix metalloproteinases (MMPs) resulting in smaller proteins and peptides which are degraded into tripeptides and dipeptides by endopeptidases and exopeptidases²⁰. Some researchers suggest that MMPs levels in GCF could be used as a diagnostic marker for periodontitis progression²². Previous studies also showed that elevated concentrations of collagen cross-link residues were found in gingiva and GCF with periodontitis^{23,24}. We hypothesized that prolidase may possess higher capacity to reflect the collagen turnover increased in the periodontitis. Interestingly, in the present study, we found that prolidase activity in GCF was lower in periodontitis. Also, we did not observe significant correlations between prolidase activity and ALP activity as well as between prolidase activity and clinical parameters. It is possible that collagen degradation products, when in excess, can inhibit prolidase activity. This study suggest that a more detailed study is needed to determine the mechanism of prolidase regulation in periodontitis.

There is still a limitations in the present study due to its cross-sectional design, thus, the findings should be confirmed by longitudinal studies investigating the levels of these biomarkers after periodontal treatment.

Conclusion

In summary, we observed that prolidase activity was significantly decreased in GCF of patients with periodontitis. However, it was not correlated with ALP and clinical parameters, which represent periodontal destruction and inflammation. We think that more studies with larger groups are needed to investigate prolidase activity and its relation with periodontal tissue in the future.

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