Journal of
PERIODONTAL RESEARCH

J Periodont Res 2011; 46: 522–527

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01367.x

Intracytoplasmic enzymes in gingival crevicular fluid of patients with aggressive periodontitis

C. E. Castro[1], M. A. Koss[2], M. E. López[2]

Departments of [1]Periodontology and [2]Biochemistry, Faculty of Dentistry, National University of Tucumán, San Miguel de Tucumán, Argentina

Castro CE, Koss MA, López ME. Intracytoplasmic enzymes in gingival crevicular fluid of patients with aggressive periodontitis. J Periodont Res 2011; 46: 522–527.

Background and Objective: Biochemical parameters of crevicular fluid could provide evidence of periodontal tissue disease. The aim of this study was to analyze enzymes in crevicular fluid in aggressive localized and generalized periodontitis.

Material and Methods: One hundred and twenty-four subjects were classified as having localized ($n = 36$) or generalized aggressive periodontitis ($n = 38$) and subclassified into moderate and severe groups. Controls were 50 periodontitis-free subjects. Activities of the enzymes lactate dehydrogenase, neutrophil elastase, alkaline phosphatase and aspartate aminotransferase were determined. Data were analyzed using one-way ANOVA and Tukey's test.

Results: Among the subjects with localized aggressive periodontitis, values of lactate dehydrogenase and alkaline phosphatase increased notably in moderate and severe periodontitis compared with control subjects. Values for aspartate aminotransferase increased with the severity of the disease, and neutrophil elastase was increased in the moderate and severe states. In generalized aggressive periodontitis, lactate dehydrogenase showed higher values than in control subjects in both periodontal subgroups. Alkaline phosphatase and neutrophil elastase showed higher significant differences between moderate and severe periodontitis compared with the control group. Aspartate aminotransferase showed differences between the severe and moderate periodontitis groups compared with the control group. Of all the enzymes analyzed, only lactate dehydrogenase showed higher values in localized than in generalized aggressive periodontitis.

Conclusion: Lactate dehydrogenase may distinguish localized and generalized aggressive periodontitis. Alkaline phosphatase increases from moderate to severe states in both types of periodontitis. Aspartate aminotransferase and neutrophil elastase only increase with strong evidence of periodontal destruction.

Prof. María Elena López, Cátedra de Química Biológica, Facultad de Odontología, UNT, Avenue Benjamín Aráoz 800, 4000 San Miguel de Tucumán, Argentina
Tel: +54 381 4107317
Fax: +54 381 4227589
E-mail: mariae.lopez@odontologia.unt.edu.ar or mariaelenalopez56@hotmail.com

Key words: enzyme; gingival crevicular fluid; aggressive periodontitis

Accepted for publication February 17, 2011

Recently, aggressive periodontitis was redefined as a complex disease exhibiting microbial alteration and cellular dysfunction, which differs from chronic periodontal disease in the underlying molecular mechanisms of its pathogenesis. Aggressive periodontitis comprises a heterogeneous group of periodontal diseases that affect adolescents and young adults. It is characterized by a very rapid loss of periodontal tissue in otherwise clinically healthy subjects (1); however, aggressive periodontitis is comparatively rare in the general population (0.1–5%; 2). Previous studies have investigated some factors that may increase host susceptibility to tissue

destruction in aggressive periodontitis, including genetic factors, functional defects of polymorphonuclear leukocytes and monocytes, and high levels of inflammatory mediators and cytokines. Aggressive periodontitis is also associated with invasive pathogens, such as *Agregobacter actinomycetemcomitans* and *Porphyromonas gingivalis*, significant reduction in polymorphonuclear neutrophil chemotaxis, family history, rapid progression and low plaque levels (3); destruction of alveolar bone insertion is marked, but episodic. Aggressive periodontitis affects people under 30 years old; however, patients may be older (4). There is considerable interest in studies aimed at understanding the etiology and pathogenesis of this disorder. Aggressive periodontitis can be classified into localized and generalized aggressive periodontitis and, according to the quality of bone loss, into moderate and severe aggressive periodontitis (1). Localized aggressive periodontitis is characterized by interproximal periodontal destruction located on incisors or first molars and with no more than two additional teeth affected. Generalized aggressive periodontitis affects at least three additional teeth. Of the three fluids found within the oral cavity (gingival crevicular fluid, serum and saliva) gingival crevicular fluid has been the focus of most research in recent years. It is an exudate that flows into the oral cavity from periodontal pockets. Its composition resembles that of serum, and the intensity of its flow has been shown to vary as a function of gingival inflammation. Several biochemical parameters of gingival crevicular fluid have been proposed as detectors or predictors of periodontal disease activity (4,5); however, it is difficult to determine which parameter or group of parameters would be the most appropriate for use in clinical practice (6).

For periodontists, the progression of the disease is difficult to predict. However, the evolution of biochemical parameters could help to detect tissue behavior (7).

Sampling of gingival crevicular fluid is relatively easy and noninvasive (8). It can be collected with paper strips or capillary tubes from the sulcus between the tooth and the gingival margin. Gingival crevicular fluid collected from diseased sites has been shown to have high levels of host-derived enzymes, such as neutrophil elastase (NE), alkaline phosphatase (AP) and aspartate aminotransferase (AST; 9,10). Neutrophil elastase, a neutral serine proteinase of primary granules, cleaves elastin and several other components of the gingival tissue (11). A large number of studies have demonstrated a positive relationship between the amount and activity of NE and the amount of inflammation and attachment loss (12,13). Alkaline phosphatase has been measured in gingival crevicular fluid to examine the relationship between periodontal conditions and disease activity (14,15). Intracytoplasmic enzymes, particularly lactate dehydrogenase (LDH) and AST, are very active biochemical markers in gingival crevicular fluid and may contribute to monitoring the progression of periodontal disease. The levels of AST in serum have been used for many years as an aid in the diagnosis of myocardial infarction and other types of necrosis. At the periodontal level, these markers could change as a result of the disease itself or as a result of the therapy used (16,17).

The aim of this study was to compare the values of enzymes present in the gingival crevicular fluid of patients with localized and generalized aggressive periodontitis and their subclassified states, moderate and severe, as a contribution to knowledge regarding early clinical diagnosis of the disease.

Material and methods

Clinical parameters

Clinical considerations for patients with periodontitis have been previously published (18), method of examination (19).

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). The following parameters were included: gingival index (20), plaque index (21), bleeding on probing up to 15 s after gentle testing, probing depth (distance between the gingival margin and the bottom of the sulcus/pocket) and clinical attachment loss (distance between the cemento-enamel junction and the bottom of the sulcus/pocket). Bone resorption in aggressive periodontitis was established on the basis of the clinical and radiographic criteria. Periapical radiographs were taken using a standardized long-cone paralleling technique.

In addition, both forms of aggressive periodontitis were subclassified as exhibiting at least two sites per quadrant in localized aggressive periodontitis and four sites per quadrant in generalized aggressive periodontitis of the following clinical features: moderate group, gingival index > 1, plaque index > 1, bleeding on probing and probing depth and clinical attachment loss between 4.5 and 6 mm; severe group, gingival index > 1, plaque index > 1, bleeding on probing and probing depth and clinical attachment loss > 6 mm; and control subjects, gingival index < 1, plaque index < 1, no bleeding on probing, probing depth and clinical attachment loss < 3 mm.

Study population

The study group consisted of 124 subjects, classified according to their clinical diagnosis (18,19). The localized aggressive periodontitis group (n = 36) consisted of 20 women and 16 men aged 28.5 ± 7.09 years and the generalized aggressive periodontitis group (n = 38) consisted of 22 women and 16 men aged 33.6 ± 0.6 years. The control group (n = 50) was composed of 30 women and 20 men aged 35.9 ± 10.6 years.

Exclusion criteria for all subjects included smoking in the past 6 months (self-reported), systemic diseases, periodontal therapy, and the use of antibiotics, steroidal or nonsteroidal anti-inflammatory agents in the 6 months prior to the study. Inclusion criteria for all subjects were a minimum of 20 natural teeth, excluding third molars, and gingival index > 1,

plaque index > 1, bleeding on probing, probing depth and clinical attachment loss ≥ 4.5 mm for patients; and gingival index < 1, plaque index < 1, no bleeding on probing, probing depth and clinical attachment loss < 3 mm for the control group.

All subjects were of mixed ethnicity (European extraction and Mestizo) and similar socio-economic level according to their higher educational level, employment, housing and living area. They were selected from those who attended the Faculty of Dentistry, National University of Tucumán. Written informed consent was obtained from all patients prior to their participation.

Sampling of gingival crevicular fluid

Gingival crevicular fluid was obtained by a single calibrated investigator from six to eight sites per patient, in order to reach the sample volume for the chemical determinations. Sites with the highest probing depth were selected in periodonitis patients, while upper incisors and first molars were chosen for sampling in the control group. After isolating the tooth with a cotton roll and drying with a gentle stream of air to prevent saliva contamination, supragingival plaque was carefully removed with curettes. Gingival crevicular fluid was collected by placing a 2 mm × 8 mm Whatman no. 1 filter paper strip into the sulcus until mild resistance was felt, and it was left in place for 30 s, following the recommendations of Lamster *et al*. (22). Those with saliva or blood were excluded. Strips were then removed and placed in a preweighed Eppendorf tube. The weight of the fluid of each strip, expressed in migrograms, was converted to volume in microliters by assuming that the density of gingival crevicular fluid is 1.0 mg/mL. Gingival crevicular fluid was eluted from the strips with 100 μL of bidistilled water by a centrifugal method. All samples from each patient were pooled and used for chemical determinations. The final concentration of the chemical components was calculated considering the total eluted volume of each pooled sample and expressed per microliter of gingival crevicular fluid.

Chemical determinations

Laboratory analyses were performed blind to clinical diagnosis. The hydrolysis of a specific peptide, *N*-methoxysuccinyl-ALA-ALA-PRO-VAL-*p*-nitroanilide, was used to measure NE following Nakashima *et al*. (15). Briefly, 25 μL of the sample were incubated for 2 h at 37°C with 6 mg of the substrate in 1 mL of dimethyl sulfoxide and diluted with 10 mL of distilled water. The product of hydrolysis was determined at 405 nm. One unit of NE is the amount of enzyme that releases 1 μM product/min.

For the AP determination, 50 μL of gingival crevicular fluid were added to 50 μL of the sodium phenylphosphate substrate in alkalinity. The amount of phenol released reacts with 4-aminoantipirine and ferricyanide as an oxidant agent. The developed color is directly proportional to the enzymatic activity at 520 nm.

Lactate dehydrogenase was determined by incubating 40 μL of the eluted gingival crevicular fluid with 1.3 mL of 300 mM DL-lactate and 63 mM NaCl in 66 mM glycine–NaOH buffer (pH 10.2). After 15 min incubation at 37°C with 2,4-dinitrophenylhydrazine, color is developed at 505 nm.

The AST activity was evaluated by incubating 100 μL of the gingival crevicular fluid sample with 100 μL of 100 mM aspartate and 2 mM α-ketoglutarate in 100 mM phosphate buffer (pH 7.4). After 30 min incubation at 37°C with 2,4-dinitrophenylhydrazine, color is developed at 505 nm.

Statistical analysis

The data were analyzed by an SPSS system (SPSS Inc., v 13.0, Chicago, IL,USA). Differences among groups were analyzed by one-way ANOVA. When the differences were significant, Tukey's test was used.

Results

The clinical parameters of the selected test sites for both forms of aggressive periodontitis are shown in Table 1 (localized aggressive periodontitis) and Table 2 (generalized aggressive periodontitis). Clinical parameters gradually increased from moderate to severe in both localized and generalized aggressive periodontitis subgroups. Plaque index, gingival index, bleeding on probing, probing depth and clinical attachment loss were statistically higher ($p < 0.001$) in moderate and severe groups than in the control group for both forms of aggressive periodontitis. All parameters were mostly higher in generalized than in localized aggressive periodontitis.

Table 1. Clinical parameters of patients with localized aggressive periodontitis

Clinical parameters	Moderate ($n = 17$)	Severe ($n = 19$)	Control ($n = 50$)
Plaque index	1.6 ± 0.3	1.7 ± 0.5	0.6 ± 0.2
Gingival index	1.9 ± 0.7	1.9 ± 0.5	0.5 ± 0.2
Bleeding on probing (%)	64.7 ± 3.8	76.0 ± 1.5	0
Probing depth (mm)	5.2 ± 0.5	6.6 ± 0.5	2.1 ± 0.5
Clinical attachment loss (mm)	5.3 ± 0.7	6.8 ± 0.8	2.0 ± 0.7

Table 2. Clinical parameters of patients with generalized aggressive periodontitis

Clinical parameters	Moderate ($n = 15$)	Severe ($n = 23$)	Control ($n = 50$)
Plaque index	1.5 ± 0.9	1.8 ± 0.5	0.6 ± 0.2
Gingival index	1.8 ± 0.6	2.3 ± .0.6	0.5 ± 0.2
Bleeding on probing (%)	77.6 ± 6.4	86.7 ± 1.7	0
Probing depth (mm)	5.3 ± 0.5	6.8 ± 0.5	2.1 ± 0.5
Clinical attachment loss (mm)	5.3 ± 0.5	7.0 ± 0.8	2.0 ± 0.7

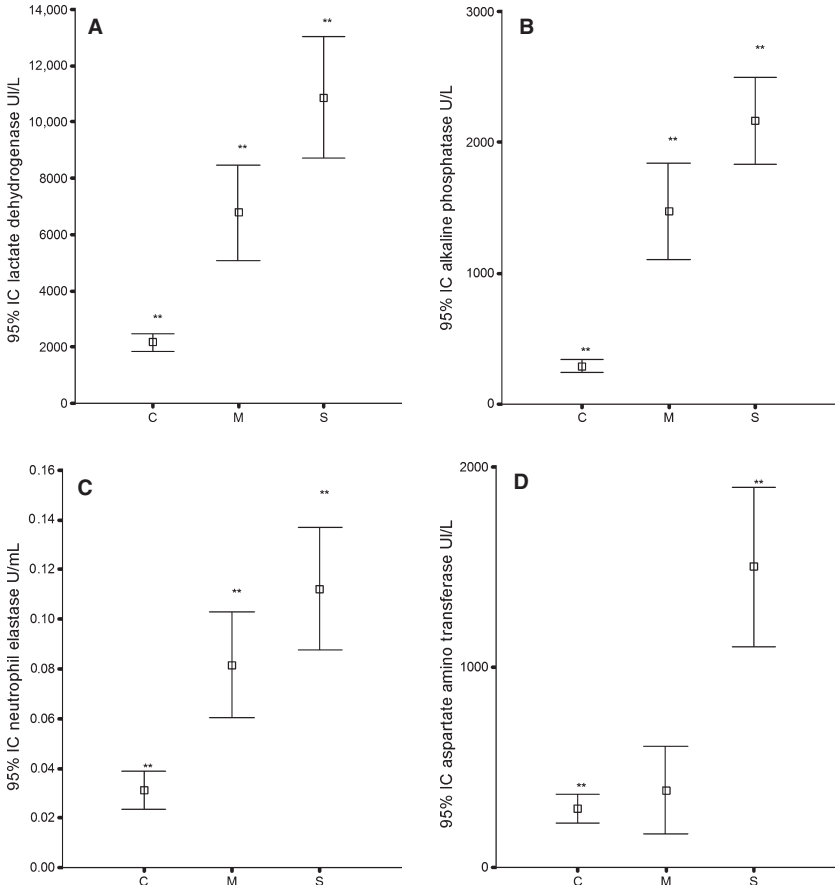

Fig. 1. Lactate dehydrogenase (A), alkaline phosphatase (B), neutrophil elastase (C) and aspartate aminotranferase levels (D) in gingival crevicular fluid of control (C), moderate (M) and severe (S) localized aggressive periodontitis patients. *$p < 0.05$; **$p < 0.001$. Units for NE are: U/mL.

Among patients with localized aggressive periodontitis, values for LDH and AP were notably higher in moderate and severe groups than in the control group ($p < 0.001$), and significant differences ($p < 0.001$) were also found when the moderate group was compared with the severe group (Fig. 1A,B). Values for AST increased significantly ($p < 0.001$) with the severity of the disease when the severe group was compared with the control and moderate groups (Fig. 1C); no significant difference ($p > 0.001$) was detected between control and moderate groups. The level of NE also increased with the severity of the periodontitis (Fig. 1D), and statistically significant differences ($p < 0.001$) were observed between the control group and patients with a diagnosis of moderate or severe periodontitis.

In the generalized aggressive periodontitis subgroups, LDH values were higher ($p < 0.001$) in the moderate and severe groups when compared with the control group (Fig. 2A). Alkaline phosphatase values were higher ($p < 0.001$) in both groups of patients compared with the control group, and significant differences ($p < 0.001$) were also found when the moderate group was compared with the severe group (Fig. 2B). Differences were found in NE ($p < 0.001$) when moderate and severe groups were compared with the control group and when the moderate group was compared with the severe group at $p < 0.05$ (Fig. 2C). Significant differences were found for AST when the moderate and severe groups were compared with the control group ($p < 0.001$; Fig 2D).

Enzyme levels determined in gingival crevicular fluid were similar between localized and generalized aggressive periodontitis, except for LDH, which was higher in the localized form. For AST, the value in the severe subgroup was also higher in localized than in generalized aggressive periodontitis.

Discussion

Although various studies (4,6,10) have demonstrated that a local imbalance of host-derived intracytoplasmic enzyme response may lead to periodontal tissue destruction, none of them has investigated the role of these enzymes in localized and generalized aggressive periodontitis with different degrees of periodontal disease.

The findings of the present study, performed on groups of similar ethnicity and socio-economic characteristics, confirm the association of elevated levels of certain gingival crevicular fluid components with the severity of the periodontitis. Bastos *et al.* (3) found significant differences between plaque index, gingival index, bleeding on probing, probing depth and clinical attachment loss in moderate and severe forms of localized and generalized aggressive periodontitis patients, as in the present study. In a previously published paper, we concluded that the release of high levels of lysosomal enzymes by neutrophils, proteolytic enzymes, such as collagenases, or intracytoplasmic enzymes, such as LDH and AST, can be equally useful to monitor the progression of periodontal disease (23). In the present study, LDH in patients with localized aggressive periodontitis was higher than in patients with generalized aggressive periodontitis, and both forms of aggressive periodontitis had higher values in the severe group than in the moderate and control groups. Our conclusion, based on previous work, is that LDH increases when cell death begins, reaches a maximum after some time, and then begins to decline (24).

Regarding PA activity, our data in localized aggressive periodontitis agree with a previous study that reported higher PA values in inflamed than in healthy gingiva (15). However, Chapple *et al.* (25) reported the development

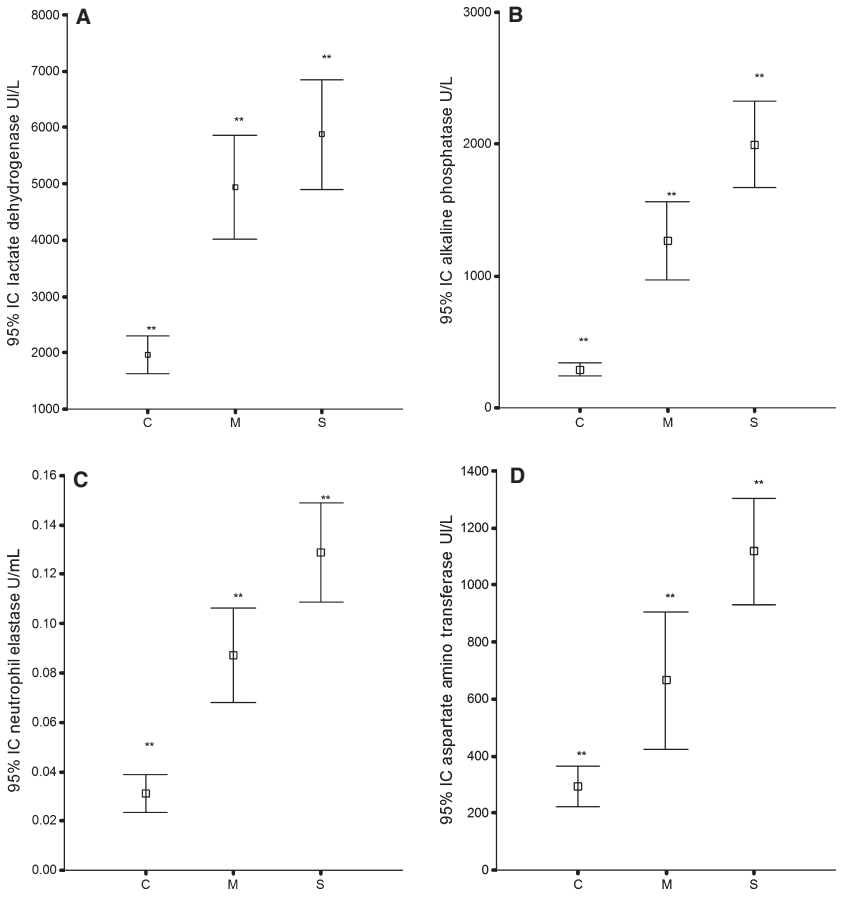

Fig. 2. Lactate dehydrogenase (A), alkaline phosphatase (B), neutrophil elastase (C) and aspartate aminotranferase levels (D) in gingival crevicular fluid of control (C), moderate (M) and severe (S) generalized aggressive periodontitis patients. $*p < 0.05$; $**p < 0.001$. Units for NE are: U/mL.

of a chemiluminescent assay for the determination of AP, which is capable of quantifying the enzyme in submicroliter volumes of gingival crevicular fluid and serum. In this study, PA notably increased in gingival crevicular fluid of patients with moderate and severe degrees of both forms of aggressive periodontitis compared with the control group. We suggested, in agreement with Nakashima *et al.* (6), that the combination of several biochemical parameters in gingival crevicular fluid could provide more information for predicting future clinical attachment loss.

In our previously published study, in patients with chronic periodontitis, the chemical determination of AST in gingival crevicular fluid of periodontally healthy control subjects and patients with different states of periodontal disease showed a substantial relationship between AST levels and the clinical indices, demonstrating that only patients with more severe periodontitis had higher AST levels (24). This enzyme showed significant differences when the moderate and severe groups were compared with the control group, suggesting that it could be used to determine stages in generalized aggressive periodontitis (26–28). Other authors demonstrated that high levels of AST were present at sites that did not subsequently exhibit disease progression. The high prevalence of AST-positive sites due to gingival inflammation diminished the test's ability to discriminate between progressive and stable but inflamed sites. The site specificity AST levels in periodontal disease was further demonstrated.

Regarding NE, major differences were observed between the control group and patients diagnosed with severe periodontitis. Similar results were obtained in other investigations, where high levels of NE were found in patients with chronic periodontitis (29). A longitudinal study demonstrated that this enzyme might serve as a predictor for future clinical attachement loss (30), can reflect the risk and the clinical status of periodontal lesions, and can serve to monitor disease activity (12,13).

Our work shows, through quantitative biochemical methods, differences in the gingival crevicular fluid enzyme levels in patients with localized and generalized aggressive periodontitis, which could contribute to the diagnosis and monitoring of the disease. Lactate dehydrogenase and PA could be used to evaluate different states of aggressive periodontitis, both localized and generalized aggressive, whereas AST and NE only appear when there is greater periodontal destruction. Lactate dehydrogenase levels >7000 UI/mL could also distinguish severe and moderate localized aggressive periodontitis from severe and moderate generalized aggressive periodontitis. Furthermore, prospective studies and an adjusted analysis are required to find out whether these enzymes could be used to monitor the progression of periodontal disease.

Acknowledgements

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

References

1. Armitage GC. Development of a classification system for periodontal disease and conditions. *Ann Periodontol* 1999;**4:**1–6.
2. Albandar JM, Tinoco EM. Global epidemiology of periodontal disease in children and young persons. *Periodontol 2000* 2002;**29:**153–176.
3. Bastos MF, Lima JA, Viieira PM, Mestnik MJ, Faveri M, Duarte PM. a-TNF and IL-4 levels in generalized aggressive periodontitis subjects. *Oral Dis* 2009;**15:** 82–87.
4. Eley BM, Cox SW. Advances in periodontal diagnosis. 2. New clinical methods of diagnosis. *Br Dent J* 1998;**184:**71–74.

5. Embery G, Waddington R. Gingival crevicular fluid: biomarkers of periodontal tissue activity. *Adv Dent Res* 1994;**8:**329–336.
6. Nakashima K, Giannopoulou C, Andersen E *et al*. A longitudinal study of various crevicular components as markers of periodontal disease activity. *J Clin Periodontol* 1996;**23:**832–838.
7. Lamster IB, Grbic JT. Diagnosis of Periodontal disease based on analysis of the host response. *Periodontol 2000* 1995;**7:** 83–99.
8. Kinane DF, Lappin D. Clinical, pathological and immunological aspect of periodontitis disease. *Acta Odontol Scand* 2001;**59:**154–160.
9. Armitage GC. Periodontal disease: diagnosis. *Ann Periodontol* 1996;**1:**21–37.
10. Figueredo CMS, Gustaffson A. Protease activity in gingival crevicular fluid. Presence of free protease. *J Clin Periodontol* 1998;**25:**306–310.
11. Meyle J, Zell S, Brecx M, Heller W. Influence of oral hygiene on elastase concentration of gingival crevicular fluid. *J Periodont Res* 1992;**27:**226–231.
12. Cox SW, Eley BM. Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid. A comparison of levels before and after basis periodontal treatment of chronic periodontitis patients. *J Clin Periodontol* 1992;**16:**1–11.
13. Gustafsson A, Asman B, Begstrom K, Soder PO. Granulocyte elastase in gingival crevicular fluid. A possible discriminator between gingivitis and periodontitis. *J Clin Periodontol* 1992;**19:**535–540.
14. Binder TA, Goodson JM, Socransky SS. Gingival fluid levels of acid and alkaline phosphatase. *J Periodontol* 1987;**22:**14–19.
15. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E_2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. *J Clin Periodontol* 1994;**21:**327–333.
16. Chambers DA, Imrey PB, Cohen RL, Crawford JM, Alves MEAF, Mc Swiggin TA. A longitudinal study of aspartate aminotransferase in humans. *J Clin Periodontol* 1991;**26:**653–657.
17. Kido J, Nakamura T, Kido R. Calprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease. *J Clin Periodontol* 1999;**26:**65–74.
18. Koss MA, Castro CE, Salúm KM, López ME. Changes in saliva protein composition of patients with periodontal disease. *Acta Odontol Latinoam* 2009;**22:**105–112.
19. Lindhe J. Examination of patients with periodontal disease. In: Harring T, Lang NP, eds. *Clinical Periodontology and Implant Dentistry*, 3rd Edition. Munksgaard: Copenhagen, 1998:383–395.
20. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand* 1963;**21:**533–551.
21. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal conditions. *Acta Odontol Scand* 1964;**22:**121–135.
22. Lamster IB, Harper DS, Fiorello LA, Oshrain RL. Lysosomal and cytoplasmatic enzyme activity, crevicular fluid volume, and clinical parameters characterizing gingival sites with shallow to intermediate probing depths. *J Periodontol* 1985;**58:**614–621.
23. Castro CE, Koss MA, López ME. Biochemical markers of the periodontal ligament. *Med Oral* 2003;**5:**322–328.
24. Koss MA, Castro CE, Salúm KM, López ME. Enzymatic profile of gingival crevicular fluid in association to the periodontal status. *Lab Medicine* 2009;**5:**277–280.
25. Chapple IL, Matthews JB, Thorpe GH, Glenwright HD, Smith JM, Saxby MS. A new ultrasensitive chemoluminescent assay for the site-specific quantification of alkaline phosphatase in gingival crevicular fluid. *J Periodont Res* 1993;**28:**266–273.
26. Oringer RJ, Howell TH, Nevins ML *et al*. Relationship between crevicular aspartate aminotransferase levels and periodontal disease progression. *J Periodontol* 2001;**1:** 19–24.
27. Persson GR, Page RC. Effect of sampling time and repetition on gingival crevicular fluid and aspartate aminotransferase activity. *J Periodont Res* 1990;**25:**236–242.
28. Imrey PB, Chambers DA, Cohen RL, Crawford JM, Alwes MEAF, Mc Swiggin TA. A cross-sectional analysis of AST in human gingival crevicular fluid. *J Periodont Res* 1991;**26:**75–84.
29. Yamalik N, Caglayan F, Kilinc K, Kilinc A, Turmer C. The importance of data presentation regarding gingival crevicular fluid myeloperoxidase and elastase-like activity in periodontal disease and health status. *J Periodontol* 2000;**71:**460–467.
30. Palcanis KG, Larjava IK, Wells BR, Suggs KA, Landis JR. Elastase as an indicator of periodontal disease progression. *J Periodontol* 1992;**63:**237–242.