

# Detection of MMP-2 and MMP-9 salivary levels in patients with chronic periodontitis before and after periodontal treatment

## Detecção de MMP-2 e MMP-9 antes e após o tratamento periodontal de pacientes com periodontite crônica

### Abstract

**Purpose:** Patients suffering from periodontal disease have high salivary levels of gelatinases, especially metalloproteinases (MMPs). The aim of this study was to evaluate MMP-2 and MMP-9 levels in whole saliva of patients with chronic periodontitis before and after non-surgical treatment, using a zymographic assay.

**Methods:** The test group comprised 8 patients with chronic periodontitis before (G1) and after (G2) periodontal treatment. The control group (GC) consisted of 8 subjects with no evidence of periodontal disease. The whole stimulated saliva was collected and analyzed by zymography. Gels were assessed with the AphaDigiDoc RT<sup>®</sup> software program. Data were analyzed by paired t test, ANOVA, and t-Student tests.

**Results:** The MMP-9 mean values ( $\pm$ SD) in arbitrary units (AU) for G1 were 74.17 ( $\pm$ 16.28) AU, for G2 65.58 ( $\pm$ 20.18) AU, and for GC 74.75 ( $\pm$ 23.16) AU. MMP-2 mean values were 100.33 ( $\pm$ 25.24) AU for G1, 93.62 ( $\pm$ 30.93) AU for G2, and 97.50 ( $\pm$ 24.82) AU for GC. No statistically significant differences were found between groups for MMP-2 and MMP-9 activity ( $P > 0.05$ ).

**Conclusion:** The results suggest that the analysis of gelatinases in saliva using zymography may not be a precise indicator of periodontal disease activity.

**Key words:** Periodontitis; metalloproteinase-2; metalloproteinase-9; periodontal treatment; saliva

### Resumo

**Objetivo:** Pacientes com doença periodontal possuem altos níveis de gelatinases, especialmente metaloproteínases (MMPs) na saliva. O objetivo deste estudo foi avaliar os níveis de gelatinases (MMP-2 e MMP-9) na saliva total de pacientes portadores de periodontite crônica antes e após tratamento periodontal, utilizando a zimografia.

**Metodologia:** O grupo teste foi constituído por 8 portadores de periodontite crônica generalizada antes (G1) e (G2) depois do tratamento periodontal. O grupo controle (GC) constituído por 8 indivíduos sem sinais de doença periodontal. A saliva total estimulada foi coletada e analisada por zimografia. Os géis foram avaliados pelo programa AlphaDigiDoc<sup>®</sup> RT 2. Os dados foram analisados por teste-t pareado, ANOVA e teste-t de Student.

**Resultados:** Para MMP-9 os valores médios ( $\pm$ DP) em unidades arbitrárias (UA) foram 74,17 ( $\pm$ 16,28) UA para G1, 65,58 ( $\pm$ 20,18) UA para G2, e 74,75 ( $\pm$ 23,16) UA para GC. Para MMP-2, os valores foram 100,33 ( $\pm$ 25,24) UA para G1, 93,62 ( $\pm$ 30,93) UA para G2 e 97,50 ( $\pm$ 24,82) UA para GC. Não houve diferença estatisticamente significativa entre os grupos para os níveis de MMP-2 e MMP-9 ( $P > 0,05$ ).

**Conclusão:** Os resultados sugerem que a análise da gelatinase na saliva utilizando a zimografia não é um indicador preciso da atividade da doença periodontal.

**Palavras-chave:** Metaloproteínase 2 de matriz; metaloproteínase 9 de matriz; periodontite crônica; saliva

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## Introduction

The diagnosis of active phases of periodontal disease and the identification of patients at risk of periodontal disease are still a challenge for periodontal research. A variety of criteria employed to establish the periodontal disease clinical diagnosis reflects the difficulty to select a classification of the disease (1). Clinical parameters including probing depth, clinical attachment level, bleeding on probing, plaque index, and radiographic loss of alveolar bone are used to assess disease severity (1). Clinical and radiographic parameters are the most used and reliable methods to identify the presence, extension, and severity of periodontal diseases, but these measurements are not useful parameters of disease activity (2).

One objective of periodontal research is to develop diagnostic tests to detect metabolic alterations that occur in the initial phase of periodontal disease. The use of saliva as sampling material has the advantages of being an easy and non-invasive collection. Furthermore, saliva contains locally-derived and systemically-derived markers of periodontal disease, and hence may offer the basis for a patient specific diagnostic test for periodontitis (3).

Salivary components such as proteins of host origin and enzymes have been evaluated as an adjunct periodontal diagnostic test. Host-derived enzymes in saliva appear to hold the greatest promise as indicators of periodontal disease (3), and these enzymes are potentially useful in the diagnosis and assessment of periodontal disease (4).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral proteinases involved in the physiological degradation of extracellular matrix proteins and basement membranes. Gelatinases form a subgroup of enzymes within the MMP family and include MMP-2 (72 kDa type IV collagenase, gelatinase A) and MMP-9 (92 kDa type IV collagenase, gelatinase B), which especially degrade type IV collagen and regulate basement membrane remodeling (5). Even though these two enzymes are very similar in their structure and substrate specificity, their cellular origins are different. MMP-2 is derived from fibroblasts, endothelial cells, and osteoblasts, while MMP-9 is expressed by polymorphonuclear leukocytes, macrophages, and epithelial cells (6). The expression of different MMPs may be upregulated in pathological conditions such as inflammation and tumor invasion. Matrix metalloproteinase-9 (MMP-9), also known as gelatinase B, is extremely evident during periodontitis (7). The balance between activated MMPs and tissue inhibitors of metalloproteinases (TIMPs) controls the extent of extracellular matrix remodeling.

Since gelatinases may be reflected in periodontal status, the aim of this study was to detect gelatinase levels (MMP-2/MMP-9) in whole saliva from patients with adult periodontitis before and after treatment using zymographic analysis. Zymography is an electrophoretic technique, based on *SDS-PAGE*, which includes a *substrate* copolymerized with the *polyacrylamide* gel for the detection of *enzyme* activity. It

has been used extensively in the qualitative evaluation of proteases present in tumors, cell culture conditioned media, gingival crevicular fluid, and saliva.

## Methods

This study was approved by the *Human Research Ethics Committee* of the University of São Paulo, São Paulo, Brazil. All patients received detailed information about the objective of the study and signed an informed consent form before the procedures.

The experimental groups comprised 8 patients (5 women and 3 men) with a mean age of 40 ( $\pm 4.5$ ) year-old, all diagnosed with chronic periodontitis. The control group included 8 students (4 men and 4 women), aged 23 to 34 year-old, whose periodontium did not show any clinical signs of inflammation and no detected gingival pockets (all probing depths  $\leq 3$  mm). All subjects were systemically healthy and none had received periodontal treatment, antibiotics, or anti-inflammatory medication during the previous 6 months; no patients had a history of systemic conditions such as heart disease, diabetes, and other types of disorders which could influence the course of periodontal disease. Smokers were excluded from the study.

The clinical evaluation of periodontal disease patients was based on the following indices: plaque index, gingival index, probing depths (6 sites/tooth), and clinical attachment level (6 sites/tooth). Saliva samples and clinical index scores were recorded at baseline, before treatment, and 4-6 weeks after basic periodontal therapy. All patients received therapy including oral hygiene instruction, scaling, and root planning. Reminders of oral hygiene procedures and ultrasonic scaling were applied once a week during the experimental period. Periodontal disease status was determined from clinical (probing depth  $> 4$  mm) and radiographic bone loss records. Groups were divided into G1 – before treatment, G2 – after treatment, and GC – control group.

A stimulated whole saliva sample was collected from the subjects between 9 a.m and 2 p.m., after they had carefully rinsed their mouths with water, and chewed on a paraffin block for 5 minutes. After collection, the saliva was transported on ice, centrifuged, and the supernatant was transferred to micro tubes and stored at  $-80$  °C.

### Gelatin zymography

MMP-2 and MMP-9 activity from collected samples was assayed using 0.05% gelatin zymography. Briefly, 10  $\mu$ L of each sample was mixed with equal amount of BioRad Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue) and separated in 10% SDS-PAGE gel copolymerized with 0.05% gelatin. Enzyme activity was regained by removing SDS, gels were thrice washed in 2.5% Triton-X-100 for 1.5 h at room temperature (RT), after electrophoresis. The gels were then bathed in proteolysis buffer (50 mM  $\text{CaCl}_2$ , 0.5 M NaCl, 50 mM Tris, pH 7.8) and incubated at 37 °C for 24 hours. Gels were rinsed in 2.5% Triton-X-100 solution and stained

temperature with coomassie blue (45% methanol, 44.75% H<sub>2</sub>O, 10% acetic acid, 0.25% coomassie blue R250) at room temperature for 1 hour on a rotator. Gels were destained (40% methanol, 7.5% acetic acid, 52.5% H<sub>2</sub>O) until white bands appeared clearly from the blue background.

The band of proteolysis was estimated by densitometric analysis using the AlphaDigiDoc® RT 2 (Alpha Innotech Corporation 2401 Merced St. San Leandro, CA, USA). The results were expressed in arbitrary units (AU, pixels/mg protein).

**Statistical Analysis**

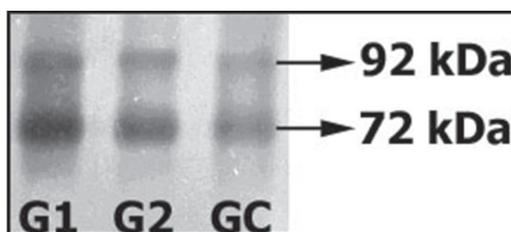
Data from groups were normally distributed, so the paired samples t-test was employed to analyze the difference between pre- and post-treatment groups. Differences among pre-treatment, post-treatment, and control groups were evaluated by one-way analysis of variance (ANOVA), complemented by t-Student tests. A level of significance of 0.05 was adopted for all tests.

**Results**

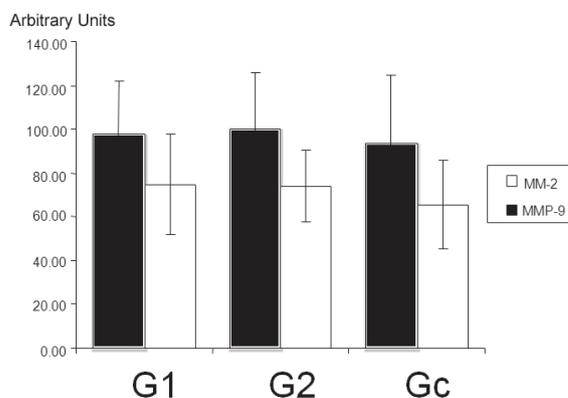
The clinical parameters of the patients with chronic periodontitis before and after scaling and root planning are presented in Table 1. All of the clinical parameters evaluated improved significantly after treatment. The mean number of teeth with a probing depth greater than 4mm in at least one site significantly decreased from 21 teeth before treatment to 17 teeth after treatment. Periodontal treatment significantly reduced the mean probing depth of 3.61 before treatment to 2.89 after treatment, and the mean clinical attachment level statistically improved after periodontal treatment (a mean CAL of 4.54 before treatment compared with 4.27 after treatment). The mean number of sites that bled on probing was also significantly reduced after periodontal treatment (from 83.5% to 37.8%).

Bands with a molecular weight around 90 kDa and 70 kDa corresponded to MMP-9 and MMP-2, respectively. Figure 1 shows two stronger bands in all experimental groups.

The MMP-9 mean values (±SD) for G1 were 74.17 (±16.28) AU, for G2 65.58 (±20.18) AU and for GC 74.75 (±23.16) AU (Fig. 2). Comparisons between groups showed that the three groups were similar (P=0.60), and the comparison between G1 and G2 also did not show any difference (P=0.18). Data for MMP-2 was: G1 – 100.33 (±25.24) AU, G2 – 93.62 (±30.93) AU and GC – 97.50 (±24.82) AU. No difference was found among groups (P=0.88). Comparing G1 and G2, no difference was detected (P=0.25), i.e., the values were similar for the three groups (Fig. 2).



**Fig. 1.** Zymographies showing gelatinolytic activities for MMP-9 (92kDa) and MMP-2 (72kDa) in saliva of one patient before (G1) and after (G2) periodontal treatment and in saliva of a subject of the control group (GC).



**Fig. 2.** Levels of MMP-2 and MMP-9 in saliva of patients before (G1) and after periodontal treatment (G2) and in subjects of the control group (GC).

**Table 1.** Clinical parameters of patients in groups G1 (baseline) and G2 (post-treatment).

Patient	Age (years)	N. of teeth with PD≥4mm baseline	N. of teeth with PD≥4mm post-treatment	PD baseline (±DP)	PD post-treatment (±DP)	CAL baseline (±DP)	CAL post-treatment (±DP)	BOP baseline (%)	BOP post-treatment (%)	Control Group	Control Group Age (Years)	Control Group n. teeth
1	39	22	18	3.67 (1.78)	3.33 (1.70)	3.70 (1.74)	4.19 (.94)	90.38	52.56	1	29	28
2	41	25	15	3.58 (1.81)	2.67 (1.29)	4.63 (2.33)	4.34 (1.97)	94.64	44.04	2	34	28
3	50	22	15	3.92 (1.98)	2.99 (1.38)	4.28 (2.32)	3.76 (2.04)	96.29	37.89	3	27	28
4	42	12	10	3.85 (2.24)	3.24 (2.28)	4.58 (2.71)	4.29 (2.59)	94.11	66.66	4	26	28
5	30	17	4	2.95 (1.31)	2.26 (0.75)	4.09 (1.96)	3.52 (1.65)	68.00	11.80	5	26	28
6	39	9	3	2.83 (1.36)	2.39 (0.99)	3.68 (1.84)	3.35 (1.42)	89.70	20.80	6	27	28
7	41	13	11	4.53 (1.86)	3.64 (1.74)	6.15 (2.28)	6.00 (2.30)	80.00	51.00	7	23	28
8	41	16	8	3.57 (1.85)	2.60 (1.24)	5.17 (2.79)	4.68 (2.57)	55.00	18.00	8	26	28
		P=0.004		P=0.013		P=0.003		P=0.001				

PD - probing depth; CAL - Clinical attachment level; BOP - bleeding on probing

Although visual differences in band intensity of each group (Fig. 1), and a slight decrease in levels of MMP-9 and MMP-2 (Fig. 2) after periodontal treatment can be observed, no statistically significant differences were found among groups ( $P > 0.05$ ).

## Discussion

This clinical case-control study evaluated the levels of MMP-2 and MMP-9 in saliva of patients with chronic periodontitis before and after periodontal treatment, in comparison with periodontally healthy patients. No statistically significant differences were found among groups for both MMP-2 and MMP-9 activity as recorded by the zymographic assay.

Inflammation-mediated periodontal tissue destruction is thought to occur in cycles of exacerbation and quiescence (8), but the clinical parameters currently used to diagnose periodontal disease are not sufficiently accurate to predict or detect active destruction of the tissue. Since the major structural protein of periodontium is collagen, the assessment of collagen degradation or of the proteinases thought to be responsible for collagenolysis during inflammation provides a rational approach towards finding a biochemical marker of disease activity (5).

In this study, periodontal treatment consisted only of non-surgical procedures. The patients received oral hygiene instruction and scaling and root planning, and these procedures significantly improved the clinical parameters evaluated. However, even after treatment, a mean number of 17 teeth had a probing depth greater than 4 mm in at least one site, and, although the number of sites that bled on probing after treatment decreased from the baseline values, 37.84% of the sites still bled on probing, which means that gingival inflammation occurred in the G2 group. Possibly, if the patients had been evaluated after surgical procedures that reduced probing depth and gingival inflammation, then different values of gelatinase levels (MMP-2/MMP-9) in whole saliva would have been obtained. Some studies that have evaluated periodontitis and enzymes from saliva have reinforced the potential utility of assessing saliva for components of tissue destruction that might be associated with periodontal diseases (9). Ingman et al. (10) demonstrated that multiple molecular forms of gelatinases occurred in GCF and saliva in adult periodontitis and localized juvenile periodontitis, but molecular weight profiles do not differentiate between the different forms of periodontitis.

The results of this study clearly show that collagenolytic enzymes can be detected using stimulated whole saliva. Saliva is a complex biological fluid and may contain non host-derived bacterial enzymes (11,12). The main host of these source of this proteins is the gingival crevicular fluid and, to a lesser extent, the tonsillar tissue. This was confirmed by Drouin et al. (13), who observed that there was no collagenolytic activity in fresh saliva collected directly from salivary ducts, and by Gangbar et al. (5), who observed that edentulous patients did not show any active or latent forms of gelatinase.

Although no statistically significant differences were found between groups, including the control group, MMP-2 bands were more intense than MMP-9 bands in the three studied groups. This finding disagrees with Ingman et al. (10), who detected lower levels of MMP-2 than MMP-9 in the saliva of patients with two distinct periodontal diseases (adult periodontitis and localized juvenile periodontitis). They suggested that multiple forms of gelatinases present in saliva may be involved in tissue destruction, but the molecular weight profile did not allow differentiation between the different forms of periodontitis.

In the present study, Figure 2 shows a decrease in MMP-9 levels after periodontal treatment. This reduction, although not significant, may be related to the disease status since extravasated degranulating PMNs are the major source of MMP-9 in gingival tissues, crevicular fluid, and saliva of patients with periodontitis (14).

Mäkela et al. (7) described periodontitis patients with higher amounts of gelatinases than healthy subjects. Furthermore, periodontal treatment significantly reduced MMP-9 in the oral samples while MMP-2 was not detected in the samples after treatment. These results are in contrast with the present findings, since the control group had values similar to those obtained from patients with the disease. Slightly lower concentrations of MMP-2 and MMP-9 were observed when comparing pre- and post-treatment results, but with no statistic significance. In agreement with the results presented here, Gangbar et al. (5) found that salivary enzymes were decreased after periodontal treatment, but the relationship between level of enzymes and clinical parameters, such as the gingival index, plaque index, and pocket depth, was not significant. Maeso et al. (15) compared the levels of MMP-2 e MMP-9 in gingival crevicular fluid in patients with gingivitis and periodontitis and in healthy control subjects and found the same slightly higher concentrations of MMP-9 in patients with periodontitis, without statistical significance. In contrast, MMP-2 was found to be slightly diminished in these patients. They also observed that there was a significant increase in the tissue inhibitor of matrix metalloproteinase-1 (TIMP1) after periodontal treatment, suggesting that in disease scenarios there is a breakdown of balance between the amount of MMPs and their inhibitor.

The fact that the differences between the healthy and the diseased group were not statistically significant in the present study may be due to a higher variability in the gelatinase assay that could possibly be reduced by refining the technique. The results presented were not related to the case study (eight control patients and eight patients with the disease) because the MMP-2 and MMP-9 levels of the control patients were proven to be similar to patients with periodontitis.

The gelatinase quantified in this assay represents the total gelatinase activity using SDS-PAGE, and only a single substrate concentration was used (5). In the analysis of possible changes in the proportion of the latent and active forms of gelatinases, it is important to evaluate the degree

of proteolytic environment related to the tissue destruction. The zymographic analysis of gelatinase activity is capable of detecting both active and latent forms of this enzyme (16). In the present study we were not able to detect active and latent forms of gelatinase due to the high levels of protein present in the samples.

Zymography is a simple, sensitive, quantifiable, and functional approach for the analysis of proteolytic activity in cell and tissue extracts, which was introduced more than 20 years ago (17). It is widely used to study extracellular matrix (ECM)-degrading enzymes, in particular the MMPs. Zymography offers advantages over other methods, such as ELISA. Expensive materials are not required (*e.g.*, antibodies), and proteases with different molecular weights showing activity towards the same substrate can be detected on a single gel. But in this study the differences between values for gelatinases were not statistically significant in any of the groups, consequently they do not indicate a definite potential for this assay in the clinical assessment of patients with periodontal disease. Moreover, densitometric analysis did not allow the association of this parameter to the severity of the disease. It would be necessary to complement the analysis of enzymes in whole saliva with quantitative tests, such as Western blotting or fluorometric assay and Elisa. Although studies based on analyses of gingival crevicular fluid are more complex, they could give more reliable results since crevicular fluid is the major source of collagenolytic enzymes to periodontally diseased sites.

A single nucleotide polymorphism in the promoter region of human MMP-9 gene is associated with the risk of some inflammatory diseases. Recent studies have evaluated the prevalence of polymorphism of the MMP-9 gene in patients with chronic periodontitis. According to Kelles et al. (18), MMP-9 promoter gene polymorphism seems to be associated with severe generalized chronic periodontitis,

but two studies by Holla et al. (19,20) evaluated a possible association between susceptibility to chronic periodontitis and polymorphisms in the MMP-2 and MMP-9 gene, and the results were not significant for mild and moderate forms of the disease.

Considerable variations exist in the amount of salivary components among individuals and populations, and few well-accepted normal values have been established for any specific marker. Although the identification of some host-derived salivary enzymes such as gelatinase may be significantly correlated with existing periodontitis, their ability to predict future disease remains unclear. Those factors may yet represent markers with potential diagnostic value, but may not necessarily provide information regarding a particular individual's susceptibility to periodontitis prior to the onset of periodontal breakdown (21). Additional controlled, longitudinal studies will be necessary to more fully elucidate the utility of salivary components as potential diagnostic or predictive markers.

## Conclusions

The results suggest that although the analysis of gelatinases in saliva is a simple and non-invasive method of analyzing periodontal status, it is not a precise indicator of periodontal disease using zymographic assay. More refined quantitative techniques would be necessary to achieve the desired results.

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## References

- Gomes Filho IS, Macedo TC, Cruz SS, Soledade KR, Trindade SC, Sarmento VA. Comparison of clinical diagnosis criteria to establish periodontal disease. *Rev Odonto Ciênc* 2006;21:77-81.
- Polson AM, Goodson JM. Periodontal diagnosis. Current status and future need. *J Periodontol* 1985;56:25-34.
- Griffiths GS, Wilton JM, Curtis MA, Maiden MF, Gillett IR, Wilson DT et al. Detection of high-risk groups and individuals for periodontal diseases. Clinical assessment of the periodontium. *J Clin Periodontol* 1988;15:403-10.
- Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis – a review. *J Clin Periodontol* 2000;27:453-65.
- Gangbar S, Overall CM, McCulloch CA, Sodek J. Identification of polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: correlation with periodontal disease activity in adult and juvenile periodontitis. *J Periodontol Res* 1990;25:257-67.
- Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. *Acta Odontol Scand* 2007;65:1-13.
- Mäkela M, Salo T, Uitto V-J, Larjava H. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. *J Dent Res* 1994;73:1397-406.
- Ingman T, Tervahartiala T, Ding Y, Tschesche H, Haerian A, Kiname DF et al. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* 1996;23:1127-32.
- Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. *J Clin Periodontol* 1984;11:21-32.
- Ingman T, Sorsa T, Lindy O, Koski H, Kontinen YT. Multiple forms of gelatinases/Type IV collagenases in saliva and gingival crevicular fluid of periodontitis patients. *J Clin Periodontol* 1994;21:26-31.
- Uitto VJ, Suomalainen K, Sorsa T. Salivary collagenase. Origin, characteristics and relationship to periodontal health. *J Periodontol Res* 1990;25:35-42.
- Sorsa T, Suolomanien K, Uitto VJ. The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal tissues. *Arch Oral Biol* 1990;35(Suppl):193S-6S.

13. Drouin L, Overall CM, Sodek J. Identification of matrix metalloendoproteinase inhibitor (TIMP) in human parotid and submandibular saliva: partial purification and characterization. *J Periodontol Res* 1988;23:370-7.
14. Westerlund U, Ingman T, Lukinmaa PL, Salo T, Kjeldsen L, Borregaard N et al. Human neutrophil gelatinase and associated lipocalin in adult and localized juvenile periodontitis. *J Dent Res* 1996;75:1553-63.
15. Maeso G, Bravo M, Bascones A. Levels of metalloproteinase-2 and -9 and tissue inhibitor of matrix metalloproteinase-1 in gingival crevicular fluid of patients with periodontitis, gingivitis, and healthy gingiva. *Quintessence Int* 2007;38:247-52.
16. Pozo P, Valenzuela MA, Melej C, Zaldívar M, Puente J, Martínez B et al. Longitudinal analysis of metalloproteinases, tissue inhibitors of metalloproteinases and clinical parameters in gingival crevicular fluid from periodontitis-affected patients. *J Periodontol Res* 2005;40:199-207.
17. Heussen C, Dowdle EB. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. *Anal Biochem*. 1980;102:196-202.
18. Keles GC, Gunes S, Sumer AP, Sumer M, Kara N, Bagci H et al. Association of matrix metalloproteinase-9 promoter gene polymorphism with chronic periodontitis. *J Periodontol* 2006;77:1510-4.
19. Holla LI, Fassman A, Muzík J, Vanek J, Vasku A. Functional polymorphisms in the matrix metalloproteinase-9 gene in relation to severity of chronic periodontitis. *J Periodontol* 2006;77:1850-5.
20. Holla LI, Fassman A, Vasku A, Goldbergova M, Beranek M, Znojil V, Vanek J et al. Genetic variations in the human gelatinase A (matrix metalloproteinase-2) promoter are not associated with susceptibility to, and severity of, chronic periodontitis. *J Periodontol* 2005;76:1056-60.
21. Sahingur SE, Cohen RE. Analysis of host responses and risk for disease progression. *Periodontol* 2000 2004;34:57-83.