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ORIGINAL ARTICLE

# Gingival crevicular fluid matrix metalloproteinase-13 levels and molecular forms in various types of periodontal diseases

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**BACKGROUND: The purpose of this study was to evaluate the levels, molecular forms and activation degree of matrix metalloproteinase-13 (MMP-13) in the gingival crevicular fluid (GCF) of patients with periodontal diseases and to correlate these findings with periodontal clinical parameters.**
**METHODS: Sixty one subjects participated in this study as healthy ($n = 18$), gingivitis ($n = 17$), aggressive periodontitis (AgP; $n = 15$) and chronic periodontitis (CP; $n = 11$) groups. Clinical measurements and GCF samples were obtained from each subject. The molecular forms of MMP-13 in GCF samples were analyzed by Western immunoblotting method. Differences among the groups were assessed using non-parametric statistical analysis.**
**RESULTS: In the CP group, levels of 29–30 kDa fragment of MMP-13, total MMP-13, and activated form of MMP-13 were significantly higher than in the healthy, gingivitis and AgP groups. GCF levels of all molecular forms of MMP-13 in AgP group were similar to those of healthy and gingivitis groups. Total and activated MMP-13 levels were positively correlated with all clinical parameters. 29–30 kDa fragment levels of MMP-13 were also positively correlated with papillary bleeding index and plaque index.**
**CONCLUSION: These results indicate that elevated GCF MMP-13 levels may play an important role in the pathogenesis of CP. These data demonstrate, for the first time, pathologically activated and elevated MMP-13 in GCF.**
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**Keywords:** periodontal disease; pathogenesis; chronic periodontitis; aggressive periodontitis; collagenases; gingival crevicular fluid; activation; matrix metalloproteinase-13

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## Introduction

Inflammatory periodontal diseases are characterized by the irreversible destruction of collagen fibers and other matrix constituents of the gingiva, periodontal ligament, and alveolar bone (Birkedal-Hansen, 1993). Extracellular matrix degradation in periodontal disease is mediated by complex cascades involving both host- and microbial-derived proteinases (Uitto *et al*, 1989; Sorsa *et al*, 1992; Reynolds, 1996). In fact, the interaction of dental plaque bacteria with resident gingival cells and infiltrating inflammatory cells results in synthesis and release of tissue-destructive proteinases (Uitto *et al*, 1989; Sorsa *et al*, 1990; Birkedal-Hansen *et al*, 1993). The host-derived matrix metalloproteinases (MMPs) are known to be the main endogenous proteinases of physiologic tissue remodeling and pathologic extracellular matrix degradation in periodontitis (Birkedal-Hansen, 1993; Birkedal-Hansen *et al*, 1993).

Matrix metalloproteinases can be classified as interstitial collagenases, gelatinases, stromelysins, membrane-type MMPs, minimal domain, and others (Palosaari *et al*, 2003). Interstitial collagenases are categorized as collagenase −1, −2, and −3 (Freije *et al*, 1994; Ryan and Golub, 2000). Resident fibroblasts, epithelial cells, endothelial cells, chondrocytes, osteoblasts and macrophages synthesize MMP-1 (collagenase-1), while MMP-8 (collagenase-2) is mainly released by infiltrating neutrophils (Birkedal-Hansen, 1993; Birkedal-Hansen *et al*, 1993). MMP-13 (collagenase-3) expression was first discovered in breast cancer (Freije *et al*, 1994). It has since been shown that gingival sulcular epithelium, gingival fibroblasts, macrophage-like cells and plasma cells express MMP-13 mRNA and protein in chronic periodontitis (CP) (Uitto *et al*, 1998; Tervahartiala *et al*, 2000; Wahlgren *et al*, 2001; Kiili *et al*, 2002). MMP-13 is also expressed by osteoblastic cells adjacent to the osteoclasts at sites of active bone resorption (Fuller and Chambers, 1995). Proinflammatory cytokines, such as interleukin-1, interleukin-6,

tumor necrosis factor-alpha, transforming growth factor-beta and keratinocyte growth factor, induce pro-MMP-13 expression (Kusano *et al*, 1998; Uitto *et al*, 1998).

Matrix metalloproteinase-13 is considered as an enzyme responsible for bone resorption and cartilage destruction in rheumatoid arthritis and osteoarthritis (Martel-Pelletier and Pelletier, 1996). Elevated MMP-13 levels in gingival crevicular fluid (GCF) of CP patients have shown that MMP-13 could also contribute to alveolar bone destruction in periodontitis (Golub *et al*, 1997, 1998). It has been shown that MMP-13 is expressed in periodontal pocket epithelium affected by periodontitis (Uitto *et al*, 1998) and reaches high levels in GCF of CP patients compared with healthy subjects (Tervahartiala *et al*, 2000). However, there are very limited data regarding GCF levels and molecular forms of MMP-13 in periodontal diseases such as gingivitis, CP, and aggressive periodontitis (AgP). Therefore, the purposes of the present study were to: (i) investigate the levels, molecular forms and degree of activation of MMP-13 in the GCF obtained from patients with gingivitis, CP, and AgP, and (ii) correlate MMP-13 levels with periodontal clinical parameters.

## Subjects and methods

*Study population*

A total of 61 subjects (25 male, 36 female) were enrolled from the Department of Periodontology, School of Dentistry, Ege University, Izmir. Prior to participation, the purpose and procedures were fully explained to all subjects, and written informed consent was obtained from each subject in line with the Declaration of Helsinki. Complete medical and dental histories were obtained from all subjects. Only non-smokers were included in this study to avoid the effects of smoking on GCF MMP-13 levels. Subjects were excluded if they had a history of systemic diseases and had used antibiotics and/or anti-inflammatory drugs within the last 3 months or had received periodontal treatment within the last 6 months. Radiographic examination was performed to assess alveolar bone destruction. The subjects were selected according to the clinical and radiographic criteria proposed by the 1999 International World Workshop (Armitage, 1999).

*Healthy control group*

Eighteen periodontally healthy subjects (10 female and eight male; age 23–62 years, median 36.5 years) had no clinical evidence of gingival inflammation [no bleeding on probing and papillary bleeding index (PBI) scores = 0 (Saxer and Mühlemann, 1975)], no radiographic evidence of bone loss and no probing depths (PD) $>3$ mm.

*Gingivitis group*

This group included nine female and eight male (age 15–54 years, median 35 years). Clinical evidence of gingivitis in these patients was based on the presence of bleeding on probing at any site, as determined by PBI. No radiographic evidence of bone loss was observed in these patients.

*Aggressive periodontitis group*

Fifteen patients with AgP (eight female and seven male; age 18–39 years) participated in the present study. These patients showed severe periodontal tissue destruction and loss of periodontal support inconsistent with age and plaque levels. Localized aggressive periodontitis (L-AgP) patients (four female, two male; age 19–27 years, median 24 years) exhibited characteristic bone loss localized to the first molars and/or incisors, clinical attachment loss (CAL) ≥4 mm in at least two permanent molars or incisors (at least one first molar must have been affected) and up to two additional teeth, and minimal inflammation and plaque in areas other than those sites that demonstrated disease. Patients with generalized aggressive periodontitis (G-AgP) (four female, five male; age 18–39 years, median 30 years) showed generalized CAL of ≥4 mm on eight or more teeth; at least three of those were other than central incisors or first molars.

*Chronic periodontitis group*

Eleven patients (three female, eight male; age 34–62 years, median 46.7 years) had at least four sites with a PD of ≥6 mm and attachment loss of ≥4 mm at the same site. Diagnosis of CP was made if the CAL was commensurate with the amount of local factors of the patients.

*Clinical periodontal parameters*

Clinical periodontal measurements including PD, CAL, plaque index (PI) (Silness and Löe, 1964) and PBI (Saxer and Mühlemann, 1975) were recorded at sampling sites. All clinical parameters were measured by the same clinician (SV-S).

*Gingival crevicular fluid sampling*

Gingival crevicular fluid samples were collected from a total of 122 sites from 61 subjects in four groups. In all study groups, mesial approximal surfaces of two non-adjacent teeth were selected for GCF sampling sites. In the CP and AgP groups, GCF samples were collected from two approximal sites with ≥6 mm PD and ≥4 mm CAL. GCF samples in the gingivitis group were collected from two sites with bleeding upon probing and ≥2 mm PD. In the healthy group, GCF samples were collected from two sites with ≤3 mm PD and without bleeding upon probing. Prior to GCF sampling, supragingival plaque was removed from the interproximal surfaces by a sterile curette. These surfaces were air-dried gently and were isolated by cotton rolls. GCF samples were collected by inserting filter paper strips (Periopaper; ProFlow, Inc., Amityville, NY, USA) for 30 s. During this process, care was taken to avoid mechanical injury and strips contaminated with blood were discarded. GCF volume was estimated (Periotron 8000; ProFlow, Inc.) and strips were placed into a sterile polypropylene tube prior to freezing at −40°C.

*Western immunoblot analysis*

The molecular forms of MMP-13 in the GCF samples were performed by using Western immunoblotting method as described previously Prikk *et al*, 2001; Kiili *et al*, 2002). The GCF samples were treated with Laemmli's buffer, pH 6.8, and heated for 5 min at +100°C. High- and low-range prestained SDS-PAGE standards (Bio-Rad, Laboratories, Hercules, CA, USA) served as molecular weight markers. Samples were separated on 8–10% SDS-polyacrylamide cross-linked gel at 200 V for 45 min and electrophoretically transferred to a nitrocellulose membrane (Bio-Rad Laboratories). Non-specific binding was blocked by incubation with 3% gelatin in 10 mM Tris-HCl, pH 8.0, containing 22 mM NaCl and 0.05% Tween. This membrane was incubated with polyclonal rabbit anti-human MMP-13 IgG (Lindy *et al*, 1997) overnight at +20°C. The MMP-13 antibody was used at a concentration of 0.5 $\mu$g ml$^{-1}$. After repeated washings, the membrane was incubated with alkaline phosphatase-labeled anti-rabbit immunoglobulins (Sigma, St Louis, MO, USA) (1:100) for 1 h at +20°C. After a 15 min washing with 10 mM Tris-HCl, pH 8.2, containing 22 mM NaCl and 0.05% Tween washing buffer, the color was developed by addition of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate diluted with *N*-dimethyl-formide (Sigma) in 100 mM Tris-HCl, pH 9.5, containing 5 mM MgCl2 and 100 mM NaCl. The secondary antibody alone did not recognize the band detected with specific antibodies in immunoblotting. The Western blots were scanned using an image densitometer (model GS-700; Bio-Rad Laboratories) using the Molecular Analyst Program (Image analysis system version 1.4) to determine the relative amounts of MMP-13. Results are expressed as arbitrary densitometric units/immunoblot absorbances (Prikk *et al*, 2001). Human rheumatoid synovial fibroblast-derived MMP-13 was used as positive control (Lindy *et al*, 1997).

*Statistical analysis*

Data were analyzed for normality and thereafter non-parametric tests were performed. The unit of analysis in this study was the patient. As the L-AgP group was relatively small (six patients), L-AgP and G-AgP patients were grouped into a single AgP category for the purpose of statistical analysis. Comparisons between the study groups were assessed using Kruskal–Wallis test. In case of significant difference among the study groups ($P < 0.05$), *post hoc* two-group comparisons were analyzed by Bonferroni-corrected Mann–Whitney *U*-test, and *P*-values < 0.0083 were considered to be statistically significant. The correlations of MMP-13 levels with clinical parameters and age were determined by the Spearman rank correlation analysis and *P*-values < 0.05 were considered as significant. Statistical analyses were performed using a statistical package (ver. 13.0; SPSS Inc., Chicago, IL, USA).

## Results

*Analysis of clinical periodontal parameters*

Clinical parameters of the sampling sites in study groups (mean ± SD) are outlined in Table 1. Both PBI and PI scores of gingivitis group were significantly higher than those of the healthy control group ($P = 0.000$). All clinical parameters of CP and AgP groups were significantly higher than those of the healthy control group ($P = 0.000$). All clinical parameters in the CP group were similar to those in the AgP group ($P > 0.05$).

*Western immunoblot analysis*

The staining for MMP-13 on immunoblots of GCF was present in 60, 48, and 29–30 kDa bands corresponding, respectively, to proMMP-13, active MMP-13, and processed low-molecular-weight MMP-13 fragments similar to pure human MMP-13 and to recombinant human MMP-13 (Figure 1) (Knäuper *et al*, 1996; Lindy *et al*, 1997). Elevated levels of immunoreactivities for high-molecular-weight (≥100 kDa) MMP-13 complexes presumably represent MMP-13 species combined with their endogenous inhibitors or dimers. In addition, elevated levels of low-molecular-weight fragments (29–30 kDa) represent degraded or truncated enzyme fragments (Figure 1). The levels of high- and low-molecular-weight fragments of MMP-13 together with active MMP-13 represent the level of activated MMP-13 levels. Total MMP-13 levels represent all forms (dimers, high molecular size, pro- and active forms, all small

**Table 1** Clinical characteristics of the sampling sites in the study groups

| | *Healthy control (n = 18)* | | *Gingivitis (n = 17)* | | *AgP (n = 15)* | | *CP (n = 11)* | |
|---|---|---|---|---|---|---|---|---|
| | *Median* | *Range* | *Median* | *Range* | *Median* | *Range* | *Median* | *Range* |
| PD (mm) | 2 | 1–2 | 2.5 | 2–3 | 7.5** | 6–9 | 7** | 6–8 |
| CAL (mm) | 0 | 0 | 0 | 0 | 8** | 6–9.5 | 7.5** | 6.5–11 |
| PI | 0* | 0 | 1.5 | 1–2 | 1 | 0.5–1.5 | 2 | 1–3 |
| PBI | 0* | 0 | 2*** | 1–3 | 3 | 2–4 | 4 | 3–4 |
| GCF ($\mu$l) | 0.15* | 0.075–0.24 | 0.25*** | 0.12–054 | 0.59 | 0.44–0.84 | 0.65 | 0.33–0.86 |

AgP, aggressive periodontitis; CP, chronic periodontitis; PD, probing depth; CAL, clinical attachment loss; PI, plaque index; PBI, papillary bleeding index; GCF, gingival crevicular fluid.
*Significant difference from the gingivitis, AgP and CP groups ($P < 0.05$).
**Significant difference from the healthy control and gingivitis groups ($P < 0.05$).
***Significant difference from CP and AgP groups ($P < 0.05$).

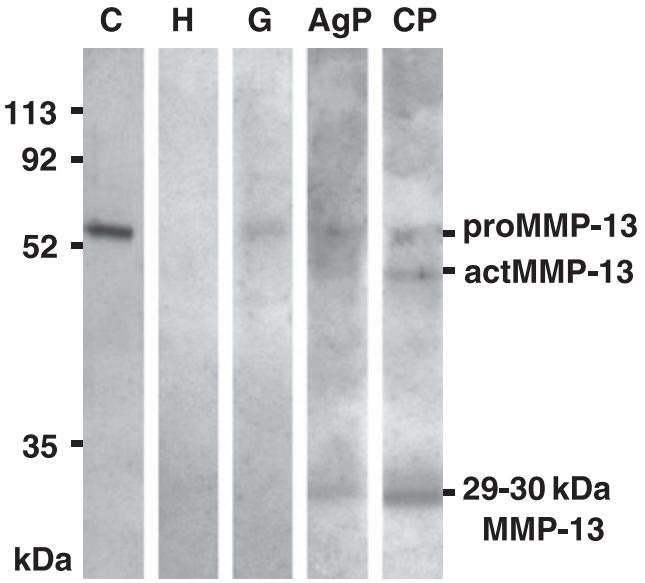


**Figure 1** Molecular forms and expression of MMP-13 (collagenase-3) in gingival crevicular fluid (GCF) detected by the Western immunoblot method. Lane C (control): purified human proMMP-13 from rheumatoid synovium; lane H (healthy): representative GCF sample from healthy group; lane G (gingivitis): representative GCF sample from gingivitis group; lane AgP: representative GCF sample from aggressive periodontitis group; lane CP (chronic periodontitis): representative GCF sample from chronic periodontitis. Molecular weight markers are indicated on the left

molecular weight fragments) of MMP-13 detected by Western immunoblots.

The total and activated MMP-13 GCF levels in the gingivitis group were significantly higher compared with the healthy control group ($P = 0.006$ and 0.007, respectively). The levels of total, activated, and 29–30 kDa fragment of GCF MMP-13 in the CP group were significantly higher than those of the healthy control group ($P = 0.000$). GCF levels of all molecular forms of MMP-13 (total MMP-13, activated MMP-13, and 29–30 kDa fragment of MMP-13) in the AgP group were similar to those of the healthy control group ($P = 0.012$, 0.015, and 0.848, respectively). Total, activated, and 29–30 kDa fragment levels of MMP-13 in the CP group were significantly higher compared with those in the gingivitis group ($P = 0.001$, 0.001, and 0.002, respectively), while these levels in the AgP group were similar to those of the gingivitis group ($P = 0.806$, 0.748, and 0.262, respectively). GCF total, activated, and 29–30 kDa fragment levels of MMP-13 in the CP group were also significantly higher than those of the AgP group ($P = 0.001$, 0.002, and 0.001, respectively) (Table 2).

Statistical analysis revealed a significantly positive correlation between the levels of total MMP-13 and the levels of activated/and 29–30 kDa MMP-13 fragments ($P = 0.000$). Total MMP-13 levels were positively correlated with PD, CAL, PBI, and PI values ($P = 0.000$, 0.004, 0.000, and 0.000, respectively). The activated MMP-13 levels were also positively correlated with PD, CAL, PBI, and PI values ($P = 0.000$, 0.006, 0.000, and 0.000, respectively). In addition, 29–30 kDa fragment levels were correlated with PBI and PI scores ($P = 0.010$ and 0.004, respectively). However, the levels of total, activated, and 29–30 kDa fragment of MMP-13 showed no significant correlation with age ($P = 0.241$, 0.278, and 0.086, respectively) (Table 3).

## Discussion

In the present study, the molecular forms, activation degree and levels of MMP-13 were addressed in GCF of CP and AgP patients. As it was not possible to find healthy and gingivitis sites in all periodontitis patients,

**Table 2** GCF MMP-13 levels in the study groups

| *MMP-13 (immunoblot absorbances)* | *Healthy control (n = 18)* | | | *Gingivitis (n = 17)* | | | *AgP (n = 15)* | | | *CP (n = 11)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Mean* | *Median* | *Range* | *Mean* | *Median* | *Range* | *Mean* | *Median* | *Range* | *Mean* | *Median* | *Range* |
| Total | 0.25 | 0 | 0–0.99 | 0.85 | 0.51 | 0–3.69** | 0.98 | 0.46 | 0–3.64 | 4.69 | 5.03 | 0.26–10.11* |
| Activated | 0.22 | 0 | 0–0.87 | 0.80 | 0.46 | 0–3.69** | 0.78 | 0.46 | 0–3.06 | 3.88 | 3.61 | 0.26–8.86* |
| 29–30 kDa fragment | 0.01 | 0 | 0–0.15 | 0.07 | 0 | 0–0.40 | 0.03 | 0 | 0–0.26 | 0.73 | 0.71 | 0–1.93* |

AgP, aggressive periodontitis; CP, chronic periodontitis.
*Significant difference from the healthy control, gingivitis and AgP groups ($P < 0.05$).
**Significant difference from the healthy control group ($P < 0.05$).

**Table 3** Correlation between clinical parameters and total, activated, and 29–30 kDa fragment measurements

| | *Age* | *PD* | *CAL* | *PBI* | *PI* | *MMP-13 total* | *MMP-13 activated* |
|---|---|---|---|---|---|---|---|
| MMP-13 total | 0.152 | 0.468* | 0.363* | 0.511* | 0.493* | | |
| MMP-13 activated | 0.141 | 0.456* | 0.346* | 0.504* | 0.489* | 0.994* | |
| MMP-13 29–30 kDa fragment | 0.222 | 0.240 | 0.186 | 0.328* | 0.367* | 0.620* | 0.598* |

PD, probing depth; CAL, clinical attachment loss; PI, plaque index; PBI, papillary bleeding index.
*Significant differences according to Spearman's rho test ($P < 0.05$).

periodontally healthy individuals and gingivitis patients were included in this study as the control groups. To our knowledge, this is the first study evaluating the molecular forms, levels and degree of activation of MMP-13 in patients with various types of periodontal diseases.

Similar to collagenase-2 (MMP-8), which is the major type of collagenase in GCF (Sorsa *et al*, 1988, 1990; Kiili *et al*, 2002), MMP-13 (collagenase-3) is synthesized and released by cells into the extracellular milieu as latent 60 kDa proform (Knäuper *et al*, 1996). Upon *in vitro* activation 60 kDa proMMP-13 has been shown to be converted to a 48-kDa active form, and 29–30 kDa fragments processed to low-molecular-weight fragments (Knäuper *et al*, 1996; Moilanen *et al*, 2003). Our unpublished findings indicated that hypochlorous acid, one of the reactive oxygen species that is relevant to proinflammatory mediators in periodontitis (Ding *et al*, 1997) can activate proMMP-13 producing 48 kDa and 29–30 kDa forms. The present study is the first study demonstrating *in vivo* MMP-13 activation in human inflammatory fluid exudates, e.g., GCF of periodontitis patients. MMP-13 was observed as elevated levels of 48 kDa active MMP-13 species together with 29–30 kDa MMP-13 fragments, especially in the CP group. These MMP-13 species (48 and 29–30 kDa) cannot be separately analyzed from 60 kDa proMMP-13 in GCF samples by currently available enzyme-linked immunosorbent assays. Therefore, in the present study, we used Western immunoblotting with a specific anti-MMP-13 antibody (Lindy *et al*, 1997) together with computerized densitometry to separately analyze different immunoreactive MMP-13 species in GCF.

It has been stated that MMP-13 and MMP-8 are the major collagenolytic enzymes in gingival tissues of patients with CP and L-AgP (Uitto *et al*, 1998; Tervahartiala *et al*, 2000). MMP-13 levels in GCF were also found to be significantly higher in the CP group than in the healthy control group (Tervahartiala *et al*, 2000). Our findings regarding high GCF MMP-13 levels in CP and the positive correlation between GCF MMP-13 levels and clinical parameters are in agreement with the data of Tervahartiala *et al* (2000). In previous studies (Ingman *et al*, 1996; Tervahartiala *et al*, 2000), the activities and levels of certain MMPs in GCF of CP patients were found to be significantly different compared with AgP patients. Tervahartiala *et al* (2000) reported that GCF MMP-13 levels in L-AgP group were lower than those in the CP group. However, the presence of total and active MMP-13 levels in GCF has been demonstrated in only two of seven L-AgP patients. Ingman *et al* (1996) stated that GCF MMP-8 and -9 levels of the CP group were significantly higher than those of the L-AgP and healthy control groups. On the other hand, GCF MMP-1 level of the L-AgP group was found to be significantly higher than those of the CP group (Ingman *et al*, 1996). The GCF MMP-13 levels of the CP group in the present study are in parallel with GCF MMP-8 and -9 levels in the CP group reported by Ingman *et al* (1996). Taken together, MMP-13 may contribute to tissue degradation mediated by MMP-8 and -9 in CP. Moreover, it has been shown that adjunctive subantimicrobial dose of doxycycline inhibits GCF MMP-13 levels in CP (Golub *et al*, 1997). All these findings likely emphasize the destructive role of MMP-13 in CP.

Matrix metalloproteinase-13 can degrade not only fibrillar type collagens, but also basement membrane type IV collagen, gelatin, fibronectin, proteoglycan, and tenascin (Knäuper *et al*, 1996; Mitchell *et al*, 1996; Johansson *et al*, 2000). As a significant correlation has been demonstrated between the amounts of MMP-13 and collagen loss during gingival inflammation and periodontitis, MMP-13 is considered to play a substantial role in collagen degradation (Uitto *et al*, 1998; Ejeil *et al*, 2003). However, the mechanism by which MMP-13 takes part in gingival extracellular matrix degradation has not yet been clarified. Uitto *et al* (1998) demonstrated that MMP-13 is the primary collagenase in human periodontal pocket epithelium, and might play a role in the proliferation of the epithelium into the connective tissue during gingival inflammation. As MMP-13 is expressed by the epithelium and connective tissue cells of the inflamed periodontium, it is suggested to play an important role in connective tissue destruction in periodontal inflammation (Uitto *et al*, 1998). It has been shown that *Fusobacterium nucleatum* can increase MMP-13 expression and migration of human epithelial cells (Uitto *et al*, 2005). Moreover, all collagenases, especially MMP-13, are able to cleave laminin-5 $\gamma$ 2-chain of the gingival basement membrane (Pirilä *et al*, 2003). Emingil *et al* (2004) demonstrated that the presence of the elevated laminin-5 $\gamma$ 2-chain fragments in GCF of CP patients could reflect basement membrane destruction caused by inflammation. In the present study, elevated levels of GCF MMP-13, especially in the CP group, is supported by the hypothesis that this enzyme can play a crucial role in gingival extracellular matrix and basement membrane destruction in CP (Golub *et al*, 1997; Uitto *et al*, 1998, 2005; Tervahartiala *et al*, 2000; Kiili *et al*, 2002; Ejeil *et al*, 2003).

The proteolytic enzymes responsible for the degradation of organic matrix in bone consist of MMPs (MMP-1, -2, -9, -12, -13, and -14), which are produced by osteoblasts and osteoclasts (Mansell *et al*, 1997; Greenwald *et al*, 1998). Tezuka *et al* (1994) demonstrated the selective expression of MMP-9 in osteoclasts. MMP-13 is expressed in osteoblastic cells adjacent to osteoclasts at the sites of active bone resorption (Fuller and Chambers, 1995). Therefore, MMP-13 in osteoblasts and MMP-9 in osteoclasts may act in concert to promote bone matrix degradation (Kusano *et al*, 1998). It is well documented that MMP-13 produced by osteoblasts is responsible for removing the non-mineralized osteoid layer covering the bone surface, which protects bone minerals from osteoclastic bone resorption (Chambers *et al*, 1985). Ingman *et al* (1996) and Ma *et al* (2003) reported that GCF MMP-9 levels were significantly higher in sites with CP and periimplantitis compared with healthy sites. In the present study, GCF MMP-13 levels were higher in the CP group than in the healthy control group. Taken together, one may expect to find significantly elevated MMP-13 levels in GCF of

patients with AgP compared with healthy subjects, considering the role of MMP-13 in bone destruction. However, although clinically severe destruction has been detected in AgP patients, GCF MMP-13 levels in AgP groups were not different from those in the healthy control group. This finding suggests that different mechanisms may be involved in bone destruction in AgP. Likewise, it has been previously documented that AgP differs from CP in host-response characteristics (Van Dyke *et al*, 1980; Kantarcı *et al*, 2003).

In conclusion, the findings of the present study suggest that elevated GCF MMP-13 in the active form may be one of the host-response components in CP. Further studies are required to evaluate the exact role of MMP-13 in various types of periodontal diseases.

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