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Effects of phase I periodontal treatment on gingival crevicular fluid levels of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1

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Abstract

Objectives: The aim of this study was to evaluate the effects of phase I periodontal treatment on gingival crevicular fluid (GCF) levels of matrix metalloproteinase (MMP)-3 and tissue inhibitors of metalloproteinase (TIMP)-1.

Methods: Plaque index, gingival index, pocket depth and clinical attachment loss were recorded and GCF samples were collected from 20 chronic periodontitis (CP) patients and 20 periodontally healthy controls (C) before treatment. CP patients received phase I periodontal treatment and all clinical parameters were recorded and GCF samples were collected once more after treatment. Assays were performed by an enzyme-linked immunosorbent assay.

Results: All of the clinical parameters improved significantly after the therapy ($p<0.05$). Baseline GCF levels of MMP-3 were significantly higher than C and that level was reduced significantly by treatment compared with baseline levels ($p<0.05$). Baseline GCF levels of TIMP-1 were lower than post-treatment levels and C ($p<0.05$). GCF levels of TIMP-1 increased significantly by treatment compared with baseline levels ($p<0.05$).

Conclusion: This study shows that the clinical improvements after phase I periodontal therapy are accompanied by reduction in MMP-3 and increasing in TIMP-1 GCF levels.

Key words: gingival crevicular fluid/analysis; matrix metalloproteinases; periodontitis/therapy; tissue inhibitor of metalloproteinase

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Periodontitis is characterized by the loss of connective tissue attachment between the root and the supporting alveolar bone. As degradation of the periodontal connective tissues is a critical component of periodontitis, there appears to be therapeutic value in regulating the activity of enzymes involved in this pathogenic process. One group of enzymes thought to be important in this degradative process is the matrix metalloproteinase (MMP) family. All family members are secreted as inactive proenzymes and are thought to be activated in the tissue by cleavage of the propeptide (Page 1991, Birkedal-Hansen 1993, Nagase & Woessner 1999). MMPs are important enzymes causing the re-modelling and degradation of the extracellular matrix (ECM) in normal and pathologic conditions, e.g., in periodontitis (Ingman et al. 1994). One prominent member of this family, MMP-3 (Stromelysin-1), has been shown to be the product of a variety of cells, including monocytes, endothelial cells, chondrocytes and synoviocytes, as well as gingival fibroblasts (Domeij et al. 2002). MMP-3 is effective in the degradation of numerous ECM substrates including gelatins, proteoglycans, laminin, fibronectin and types IV and IX collagen (Nakaya et al. 1997). In addition to its ability to degrade various connective tissue components, MMP-3 is also known to participate in the proteolytic activation cascades of latent pro-MMP-1, -8 and -9 (Sorsa et al. 1990, Suzuki et al. 1990, Ogata et al. 1992). MMP-3

mediated collagenolysis may be a major pathway in the destruction and re-modelling of connective tissues in periodontitis.

The intrinsic regulation of MMP enzyme activity includes the tissue inhibitors of metalloproteinase (TIMP) family of proteins. TIMPs appear to regulate matrix degradation both by proteinase inhibition and by blockage of autocatalytic MMP activation (Shibata et al. 1999). The matrix is maintained by a careful balance between rates of synthesis, degradation and connective tissue cell division. An imbalance between activated MMPs and their endogenous inhibitors leads to pathologic breakdown of the ECM during periodontitis (Meikle et al. 1994, Kubota et al. 1996). TIMPs are expressed by many cell types including fibroblasts, keratinocytes, monocytes/macrophages, endothelial cells and osteoblasts (Meikle et al. 1994). TIMP-1–4 have been identified and are widely distributed in tissues and body fluids (Stetler-Stevenson et al. 1989). MMPs such as interstitial collagenases (MMP-1 and MMP-8), gelatinase/type IV collagenase (72 and 92 kDa) and stromelysin are all specifically inhibited by TIMP-1, a sialoglycoprotein with a molecular weight of about 28 kDa. TIMP-1 is more effective than TIMP-2 at inhibiting MMP-1 and MMP-3 (Shibata et al. 1999).

In this study, we investigated the level of gingival crevicular fluid (GCF) MMP-3 and TIMP-1 in sites with periodontal breakdown before and after treatment in an attempt to determine: (a) the effect of phase I periodontal therapy on the levels of enzyme and its inhibitor and (b) the relationship between clinical and biochemical parameters.

Material and Methods

Study population and clinical studies

Twenty chronic periodontitis (CP) patients (seven females and 13 males; mean age 44.8 years) and 20 clinically healthy individuals with no history of periodontal disease (11 females and nine males; mean age 29.7 years) were selected from those newly registered with Gazi University, Faculty of Dentistry, Department of Periodontology. The protocol was approved by the Ethical Committee of the Faculty of Dentistry, Gazi University, Ankara, Turkey. Informed consent was obtained from all subjects, and GCF sampling and clinical procedures were fully explained before the study. Periodontal disease status was determined according to clinical and radiographic criteria. CP group comprised patients showing radiographic evidence of bone loss and attachment loss and probing pocket depth more than 4 mm. All subjects were in good general health and none had received periodontal treatment or medication during the past 6 months; no participants had a history of systemic conditions such as heart disease, diabetes and other types of disorders which could influence the course of periodontal disease. They were not on any medication that could affect the manifestations of periodontal disease, such as chronic antibiotic use, phenytoin, cyclosporine, anti-inflammatory drugs, or calcium channel blockers. None of the women were post-menopausal. Smokers were excluded from the study.

The clinical evaluation of patients was based on the following indices: plaque index (PI) (Silness & Löe 1964), gingival index (GI) (Löe 1967), probing depths (PDs) and clinical attachment loss (CAL). All clinical parameters were measured with a Goldman/Fox Williams probe calibrated in millimetres. The most severely affected upper anterior sextant was used as test site for the evaluation of clinical parameters and GCF sampling. Clinical parameters were performed on six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal, mid-palatal and disto-palatal) from upper anterior test site (maxillary incisors and canine teeth). The deepest six pockets were chosen for GCF sampling within the test site. The upper anterior sextant was preferred to others as they were more accessible and these sites were less prone to saliva contamination of the GCF sample. The same sextant was chosen for clinical recording and GCF sampling for group C to provide the standardization. GCF sampling and clinical index scores were recorded pre-treatment and 6 weeks after phase I periodontal therapy. After the baseline clinical recording and GCF sampling, phase I periodontal therapy was initiated. All patients underwent therapy including oral hygiene instruction, scaling, root planing under local anaesthesia. Scaling and root planing were performed once a week for 2 weeks by sharp sickles, gracey and universal curettes and ultrasonic instruments. Mechanical periodontal therapy was not accompanied by any medications like antibiotics or non-steroidal anti-inflammatory drugs.

GCF sampling and processing

GCF samples were collected using periopaper (Oraflow Inc., Plainview, NY, USA) which was commercially available. The sample site was gently air-dried and all supragingival plaque was removed. The area was carefully isolated to prevent samples from being contaminated by saliva. The paper strips were inserted into the crevice until mild resistance was felt or in any event not more than 1 mm and left in place for 30 s. Care was taken to avoid mechanical injury of the gingival tissues. Strips contaminated by bleeding or exudate were discarded. The amount of GCF on the strips was measured by weighing the accumulated fluid. Strips from test and control sites were placed into coded sealed plastic microcentrifuge tubes and weighing was repeated immediately after collection to overcome any evaporation. The microcentrifuge tubes were covered with paraffin and then stored at -70°C until further enzyme processing. The mass of the fluid on each strip was converted to a volume in millilitres by assuming that the density of GCF was one and mass (milligrams) was converted to the volume (millilitres) (Tüter et al. 2002; Kuru et al. 2004).

GCF enzyme-linked immunoabsorbent assay (ELISA) analysis for MMP-3 and TIMP-1

Levels of MMP-3 and TIMP-1 in GCF samples were assayed by sandwich (ELISA) kit (Quantikine R&D Systems Inc., Minneapolis, MN, USA). All assay procedures were carried out according to the manufacturer's instructions. GCF samples were eluted from the strips by a centrifugal method (Griffiths et al. 1988).

Elution was carried out with the addition of 200 μl of sample (test) buffer included in the kit contents. Then the microcentrifuge tubes containing the strips and the buffer were centrifuged for 20 min. at 3000 $\times$ g. After the centrifugation, the strips were removed and the fluid remaining in the tubes were analysed for MMP-3 and TIMP-1 using the above-mentioned commercial ELISA kits. The ELISA plates were then assessed spectrophotometrically at OD 490 nm. The levels of crevicular MMP-3 and TIMP-1 in each sample were determined using the concentration values of

Table 1. Comparison of gingival crevicular fluid (GCF) volumes, clinical parameters, and GCF levels of matrix metalloproteinase (MMP)-3 and tissue inhibitors of metalloproteinase (TIMP)-1

	Median ($n = 20$)			p-Value		
	pre-treatment	post-treatment	control (C) median ($n = 20$)	pre/post-treatment	pre-treatment/C	post-treatment/C
MMP-3 (ng/μl)	9.0 (4.12–33.55)*	5.5 (1.65–10.87)	0.15 (0–4.40)	0.013	0.001	0.021
TIMP-1 (ng/μl)	29.0 (17.20–43.62)	51.3 (25.00–69.22)	93.0 (54.25–122.00)	0.024	<0.001	0.001
GCF volume(μl)	2.3 (1.7–4.9)	1.75 (1.2–2.6)	1.0 (0.5–1.4)	0.002	<0.001	0.001
Pocket depth (mm)	4.18 (4.02–4.91)	3.7 (3.5–4.1)	1.2 (1–1.9)	<0.001	<0.001	<0.001
Plaque index	2.0 (1.83–2.0)	0.95 (0.2–1.6)	0 (0–0)	<0.001	<0.001	<0.001
Gingival index	2.0 (1.70–2.0)	1.55 (1.2–1.6)	0 (0–0)	<0.001	<0.001	<0.001
Clinical attachment loss	4.05 (3.75–4.68)	4.0 (3.7–4.2)	0 (0–0)	0.007	<0.001	<0.001

*The numbers in parentheses are the 25th and 75th percentiles of the raw data.

standards included in the kit contents and a computer-based statistic programme – Microsta – for correlation–regression analysis. The obtained concentration value for each sample was corrected for the original volume of GCF by dividing the volume amount of MMP-3 and TIMP-1 by the volume of sample and the results were expressed as ng/μl MMP-3 and TIMP-1 of GCF. The recovery results were 87–104% for MMP-3 and 94–113% for TIMP-1. The intra-assay precision was 5.7–6.4% and inter-assay precision was 7.9–8.6% for MMP-3 and intra-assay precision was 4.2–5% and inter-assay precision was 3.9–4.9% for TIMP-1. Limit of detection was 0.002 ng/ml for MMP-3 and was 0.08 ng/ml for TIMP-1.

Statistical analysis

Data analysis was performed using the statistical package SPSS (Microsoft Corp., Chicago, IL, USA). All results were analysed by applying Shapiro–Wilk test for determination of the normal and abnormal data distribution. The statistical significance of the differences in MMP-3 and TIMP-1 levels and clinical parameters between pre- and post-treatment were analysed using the Wilcoxon signed-rank test.

Mann–Whitney *U*-tests were used to determine the significance of the differences between baseline and control (C) and between post-treatment and C for all parameters. The correlations among clinical parameters, MMP-3 and TIMP-1 levels were analysed using Spearman's correlation test. The level of power calculation was (1–ß) 0.70. A p-value <0.05 was considered statistically significant.

Results

Measurements of clinical parameters, GCF MMP-3 and TIMP-1 levels and GCF volumes of sampling area for pre- and post-treatment and C groups are given in Table 1. Statistically significant differences were observed both between pre-treatment and C and between post-treatment and C groups for all clinical parameters. All of the clinical parameters showed a reduction at post-therapy visit from baseline, so significant differences were also found between pre- and post-treatment. Although GCF volume decreased after phase I periodontal treatment, GCF volumes were found higher in both pre- and post-treatment groups than C.

Baseline GCF levels of TIMP-1 were significantly higher than C. After therapy, GCF TIMP-1 level increased significantly. There was a significant difference in TIMP-1 levels between pre- and post-treatment and also between post-treatment and C (Fig. 1a).

GCF levels of MMP-3 were significantly lower in C than both pre- and post-treatment. GCF MMP-3 level decreased significantly after phase I periodontal therapy. A statistically significant difference was observed in MMP-3 levels between pre- and post-treatment (Fig. 1b).

A negative significant correlation was found between GCF MMP-3 and TIMP-1 levels. There were significant and positive correlations among all clinical parameters and MMP-3 levels. A positive significant correlation was also noted between GCF volume and MMP-3 levels. There were significant and negative correlations among GCF volume, all clinical parameters and TIMP-1 levels (Table 2).

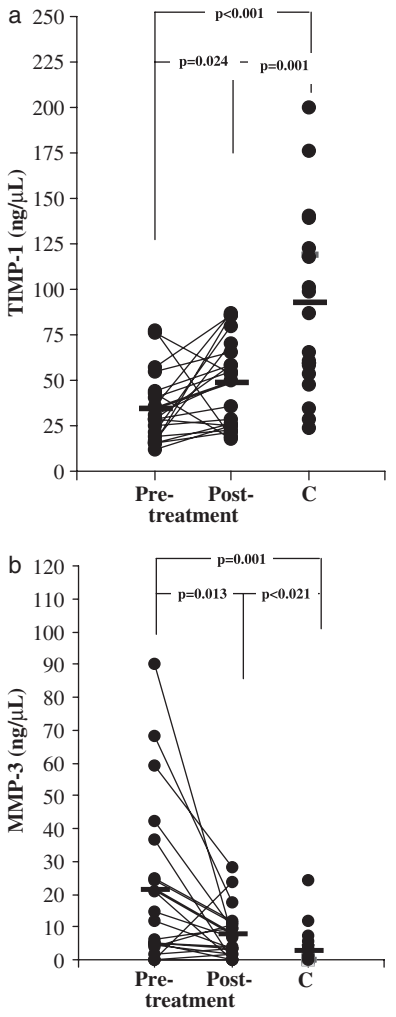

Fig. 1. (a) Gingival crevicular fluid (GCF) levels of tissue inhibitors of metalloproteinase 1 for all groups. (b) GCF levels of matrix metalloproteinase-3 for all groups.

Discussion

Periodontal diseases are characterized by a loss of collagen fibres and other ECM constituents in periodontal tissues. A possible mechanism for the degradation of periodontal ECM is the independent and/or cooperative action of both human and bacterial proteinases (Sorsa et al. 1995). Periodontal tissue destruction is most likely mediated to a significant extent by the host of

Table 2. Correlations among gingival crevicular fluid (GCF) levels of matrix metalloproteinase (MMP)-3, tissue inhibitors of metalloproteinase (TIMP)-1, GCF volume and clinical parameters

	MMP-3 (ng/μl)	GCF volume (μl)	Pocket depth (mm)	Plaque index	Gingival index	Clinical attachment loss
TIMP-1 (ng/μl), *r*	-0.355^{**}	-0.379^{**}	-0.425^{**}	-0.401^{**}	-0.397^{**}	-0.485^{***}
MMP- 3 (ng/μl), *r*		0.321^{*}	0.310^{*}	0.279^{*}	0.362^{**}	0.477^{***}

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

cell-derived MMPs (Birkedal-Hansen 1993). The regulation of the enzymatic action of MMPs relies on conversion from the latent precursor to the active form. MMP-3 is a broad-spectrum MMP and a pivotal activator of latent MMPs (Kubota et al. 1996). It has been shown that MMP-3 could activate procollagenases including pro-MMP-1, -8, -9 (Sorsa et al. 1990, Suzuki et al. 1990, Ogata et al. 1992, Kubota et al. 1996). As the coordinated activity of the MMPs is critical in the collagenetic cascade, the regulatory effects of MMP-3 may be important in the overall regulation of connective tissue degradation in both physiologic and pathologic conditions. Therefore, we have investigated the effect of phase I periodontal treatment on GCF levels of MMP-3 and its inhibitor, TIMP-1.

Previously, MMP-3 expression and increased amount of MMP-3 mRNA in periodontal lesions have been demonstrated (Ingman et al. 1994, Kubota et al. 1996) and it has been suggested that MMP-3 may act as a marker stromal cell in the tissue degradation process (Birkedal-Hansen 1993). A cross-sectional study of GCF analysis has revealed that GCF MMP-3 and TIMP-1 levels can differentiate between healthy and diseased periodontal sites (Haerian et al. 1995). Furthermore, Alpagot et al. (2001) have reported that MMP-3 and TIMP-1 are prognostic factors for the development of attachment loss in established periodontitis sites. In the present study, significantly elevated GCF MMP-3 levels and significantly decreased GCF TIMP-1 levels were detected at baseline compared with C. Haerian et al. (1996) reported a moderate positive and significant correlation between biochemical parameters (GCF stromelysin and TIMP levels) and clinical parameters when data from healthy, gingivitis and periodontitis sites were pooled. However, when data from different groups of sites were separately analysed, there was no correlation between clinical and biochemical parameters. Differently from their study, instead of evaluating GCF stromelysin and TIMP levels as general enzyme groups we worked on specificially MMP-3 and its inhibitor, TIMP-1 in our study. Moreover, there was a significant positive correlation between all clinical parameters including GCF volume and GCF MMP-3 levels and there was a significant negative correlation between all clinical parameters and GCF TIMP-1 levels in the present study. Different methods of GCF sampling and laboratory techniques as well as variations in reporting the results may influence the presence and extent of correlation between clinical and biochemical parameters.

To inhibit disease progression successfully, it is important to reduce the MMP activity in the local inflammatory lesion by periodontal treatment. Haerian et al. (1996) evaluated the effect of scaling and root planing on GCF levels of fibroblast collagenase, stromelysin and TIMP in a group of patients with advanced periodontal disease by a sandwich ELISA. They reported that GCF TIMP levels were increased significantly after the phase I therapy and demonstrated a significant reduction at the follow-up visit performed 3 months later. GCF stromelysin levels were reduced as non-significant after the therapy but the reduction was significant at the follow-up visit. Similar with their findings, GCF MMP-3 levels were reduced significantly after the therapy in our study. Utilizing ELISA, we were able to measure only total enzyme (latent and active) in GCF. It is possible that post-treatment levels of active enzyme was low. According to our results, GCF level of TIMP-1 increased significantly after the treatment. This might be because of a reduction in MMPs which would bind to free TIMP; however, the regulation of TIMP-1 may not solely be dependent on the MMPs (Howard et al. 1991). The increased levels of TIMP-1 may reflect its involvement in the healing process (Haerian et al. 1996). Figueredo et al. (2004) have shown that non-surgical treatment is effective in both improving clinical parameters and reducing protease activity in GCF of CP patients. Differently from their study, we evaluated GCF MMP-3 levels and as parallel to their study, all of the clinical parameters significantly improved and GCF MMP-3 levels reduced after phase I periodontal treatment in our study. Nakaya et al. (2000) have demonstrated an inhibitory effect of bisphosphonate tiludronate on the activity of both MMP-1 and MMP-3 in vitro. These effects appear to occur without altering either mRNA or protein levels for these enzymes. Pourtaghi et al. (1996) have used 25% tetracycline fibre, 2% minocycline gel and 25% metronidazole gel as adjunctive to scaling and root planing and evaluated GCF stromelysin and TIMP levels after the therapy. They have also included a group of periodontitis patients under the treatment of only scaling and root planing into their study. According to their results, while the decrease in the stromelysin level was significant only for tetracycline fibre and minocycline gel adjunctive treatments, the clinical data indicated that a significant attachment gain occurred after all treatments. In fact, it has been shown that MMP-8 and MMP-13 are highly susceptible to inhibition by tetracyclines (Golub et al. 2001). Because of these previous data, an adjunctive therapy was not used in the present study.

In the light of our results, we suggest that GCF MMP-3 levels were reduced and GCF TIMP-1 levels were increased significantly by phase I periodontal treatment without any adjunctive therapy. The imbalance between MMP-3 and TIMP-1 levels was changed by phase I periodontal treatment.

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References

Alpagot, T., Bell, C., Lundergan, W., Chambers, D. W. & Rudin, R. (2001) Longitudinal evaluation of GCF MMP-3 and TIMP-1 levels as prognostic factors for progression of periodontitis. *Journal of Clinical Periodontology* **28**, 353–359.

Birkedal-Hansen, H. (1993) Role of matrix metalloproteinases in human periodontal diseases. *Journal of Periodontology* **64**, 474–484.

Domeij, H., Yucel-Lindberg, T. & Modeer, T. (2002) Signal pathways involved in the production of MMP-1 and MMP-3 in human gingival fibroblasts. *European Journal of Oral Science* **110**, 302–306.

Figueredo, C. M. S., Areas, A., Mirandal, L. A., Fischer, R. G. & Gustafsson, A. (2004) The short-term effectiveness of non-surgical treatment in reducing protease activity in gingival crevicular fluid from chronic periodontitis patients. *Journal of Clinical Periodontology* **31**, 615–619.

Griffiths, G. S., Curtis, M. A. & Wilton, J. M. (1988) Selection of a paper with optimum properties for the collection of gingival crevicular fluid. *Journal of Periodontal Research* **23**, 33–38.

Golub, L. M., Mcnamara, T. F., Ryan, M. E., Kohut, B., Blieden, T., Payonk, G., Sipos, T. & Baron, H. J. (2001) Adjunctive treatment with subantimicrobial doses of doxycycline: effects on gingival fluid collagenase activity and attachment loss in adult periodontitis. *Journal of Clinical Periodontology* **28**, 146–156.

Haerian, A., Adonogianaki, E., Mooney, J., Docchery, J. P. & Kinane, D. F. (1995) Gingival crevicular stromelysin, collagenase and tissue inhibitor of metalloproteinases levels in healthy and diseased sites. *Journal of Clinical Periodontology* **22**, 505–509.

Haerian, A., Adonogianaki, E., Mooney, J., Manos, A. & Kinane, D. F. (1996) Effects of treatment on gingival crevicular collagenase, stromelysin and tissue inhibitor of metalloproteinases and their ability to predict response to treatment. *Journal of Clinical Periodontology* **23**, 83–91.

Howard, E. W., Bullen, E. C. & Banda, M. J. (1991) Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. *Journal of Biological Chemistry* **266**, 13070–13075.

Ingman, T., Sorsa, T., Michaelis, J. & Konttinen, Y. T. (1994) Immunohistochemical study of neutrophil- and fibroblast-type collagenases and stromelysin-1 in adult periodontitis. *Journal of Dental Research* **102**, 342–349.

Kubota, T., Nomura, T., Takahashi, T. & Hara, K. (1996) Expression of mRNA for matrix metalloproteinases and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. *Archives of Oral Biology* **41**, 253–262.

Kuru, L., Griffiths, G. S., Petrie, A. & Olsen, I. (2004) Changes in transforming growth factor-ß1 in gingival crevicular fluid following periodontal surgery. *Journal of Clinical Periodontology* **31**, 527–533.

Löe, H. (1967) The gingival index, plaque index and the retention index systems. *Journal of Periodontology* **38**, 610–616.

Meikle, M. C., Hembry, R. M., Holley, J., Horton, C., Mcfarlane, C. G. & Reynolds, J. J. (1994) Immunolocalization of matrix metalloproteinases and TIMP-1 in human gingival tissue from periodontitis patients. *Journal of Periodontal Research* **29**, 118–126.

Nagase, H. & Woessner, J. F. (1999) Matrix metalloproteinases. *Journal of Biological Chemistry* **274**, 21491–21494.

Nakaya, H., Oates, T. W., Hoang, A. M., Kamoi, K. & Cochran, D. L. (1997) Effects of IL-1 beta on matrix metalloproteinase 3 levels in human periodontal ligament cells. *Journal of Periodontology* **68**, 517–523.

Nakaya, H., Osawa, G., Iwasaki, N., Cochran, D. L., Kamoi, K. & Oates, TW. (2000) Effects of bisphosphonate on matrix metalloproteinase enzymes in human periodontal ligament cells. *Journal of Periodontology* **71**, 1158–1166.

Ogata, Y., Enghild, J. J. & Nagase, H. (1992) Matrix metalloproteinase 3 (stromelysin) activates the precursors for human matrix metalloproteinase 9. *Journal of Biological Chemistry* **267**, 3581–3584.

Page, R. C. (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. *Journal of Periodontal Research* **26**, 230–242.

Pourtaghi, N., Radvar, M., Money, J. & Kinane, D. F. (1996) The effect of subgingival antimicrobial therapy on the levels of stromelysin and tissue inhibitor of metalloproteinases in gingival crevicular fluid. *Journal of Periodontology* **67**, 866–870.

Shibata, Y., Takiguchi, H. & Ağabeyko, Y. (1999) Antisense oligonucleotide of tissue inhibitor of metalloproteinase-1 induces the plasminogen activator activity in periodontal ligament cells. *Journal of Periodontology* **70**, 1158–1165.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and oral condition. *Acta Odontologica Scandinavica* **22**, 121–135.

Sorsa, T., Ding, Y., Ingman, T., Salo, T., Westerlund, V. & Haapasalo, M. (1995) Cellular source, activation and inhibition of dental plaque collagenase. *Journal of Clinical Periodontology* **22**, 709–717.

Sorsa, T., Suomalainen, K. & Uitto, V. J. (1990) The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal diseases. *Archives of Oral Biology* **35**, 193–196.

Stetler-Stevenson, W. G., Krutzsch, H. C. & Liotta, L. A. (1989) Tissue inhibitor of metalloproteinase (TIMP-2). *Journal of Biological Chemistry* **264**, 17374–17378.

Suzuki, K., Enghild, J. J., Morodomi, T., Salvesen, G. & Nagase, H. (1990) Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry* **29**, 10261–10270.

Tüter, G., Kurtiş, B. & Serdar, M. (2002) Effects of phase I periodontal treatment on gingival crevicular fluid levels of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1. *Journal of Periodontology* **73**, 487–493.

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Clinical Relevance

It is known that MMP-3 activates other pro-MMPs like pro-MMP-1, -8 and -9. TIMP-1 is a specific inhibitor for MMP-3. In the present study, we evaluated the effects of phase I periodontal treatment on GCF levels of MMP-3 and TIMP-1 without any adjunctive therapy. Our results showed that GCF levels of MMP-3 decreased and GCF levels of TIMP-1 increased after the therapy. In the various treatment ways of periodontal diseases, the traditional periodontal therapy without any adjunctive treatment could also change the MMP-3 and TIMP-1 profile.