# Matrix metalloproteinases and Tissue inhibitors of matrix metalloproteinases in gingival crevicular fluids of periodontitis patients

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## I. Introduction

Periodontitis is characterized by the loss of connective tissue attachment between the root and the supporting alveolar bone. One group of enzymes thought to be important in this degradative process is the matrix metalloproteinase family(MMPs).

Matrix metalloproteinases(MMPs) are thought to be the main proteinase of tissue destruction in periodontal diseases and other diseases, such as rheumatoid arthritis, skin diseases and cancer(Birkendal-Hansen 1993).

MMP activity is inhibited by tissue inhibitors of metalloproteinase(TIMPs), which are produced by host cells(Woessner 1991).

The role of tissue inhibitors of metalloproteinases may be to tightly control metalloproteinases activity both at the level of gene expression, activation and subsequent substrate degradation. The tissue degradation is further thought to be induced by an imbalance between MMPs and TIMPs(Nomura et al. 1993).

Metalloproteinases are mainly synthesized by connective tissue cells and also be synthesized by hemopoietic cells, including monocytes, macrophages, keratinocytes, endothelial cells and many types of tumor cells. The family of MMPs has major subgroups, the interstitial collagenases(MMP-1,-8,and-13), the gelatinases(MMP-2 and -9), the stromelysins(MMP-3, -10, -11) and the membrane bound group(MMP-14,-15, -16,-17). Other groups are matrilysin(MMP-7) and metalloelastase(MMP-12).

The purpose of this study is to determine whether specific MMPs(-1,-2,-3,-8,-9,-13) and TIMPs(-1,-2) are present more in GCF of periodontitis patients than in that of healthy patients by using ELISA assays, and whether the results of these assays have relationship with periodontal probing depth and GI scores, which are the most useful methods in evaluating periodontal status and identify the degrees of periodontal disease in periodontititis patients.

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## II. Materials and methods

### 1. Patients selection

Patients for this study were selected from the departments of periodontology , Seoul National University Dental Hospital.

Eight chronic periodontitis patients and 4 aggresiive periodontitis patients with moderate to advanced periodontitis, who have more than 14 remaining teeth except the 3rd molar, 5-6 teeth whose probing pocket depth ≥6mm and definite moderate to advanced periodontitis in radiograph were selected for test group. And 5 patients with clinically healthy periodontal status who have no pocket depth exceeding 5mm were selected for control group. Subjects with these criteria were excluded .

1) Subjects who had systemic antibiotic, immunosupressant, antihistamine therapy
2) Subjects who had diabetes, uncontrolled hypertension, pregnancy, significant risk for infectious disease transmission
3) Subjects who had history of rheumatic fever, congenital heart disease, renal or liver disease, blood dyscrasias or anticoagulant therapy
4) Subjects who had history of antibiotic medication in these 6 months

### 2. Clinical Measurement

Clinical parameters; probing depth(PD), GI score of Loe and Silness were measured at 6 sites of each tooth in all remaining teeth excluding the third molar.

### 3. Collection of gingical crevicular fluid(GCF)

Six GCF samples from healthy(PD≤3mm) and 6 GCF samples from periodontitis sites(PD≥6mm) were collected respectively from 8 patients with chronic periodontitis and 4 patients with aggressive periodontitis. Six GCF samples from only healthy sites(PD≤3mm) were collected respectively from 5 patients with healthy periodontal status. GCF samples were collected by means of sterile paper for 30 seconds. From each patient, 3 samples were collected at 1st visit and 3 samples were collected at 2nd visit.

After isolating the tooth with a cotton roll, the crevicular site was then gently dried with an air syringe. GCF was collected with paper strip(PROFLOW™, New York). Periopaper strips were placed into the pocket/sulcus and left for 30 seconds. And periopaper strips were immediately placed into a sterile tube and stored at -70° C until analysis.

### 4. Measurement of MMPs and TIMPs

Human Biotrak ELISA kit(Amersham pharmacia biotech, UK) were used for quantification of MMPs(-1,-2,-3,-8,-9,-13) and TIMPs(-1,-2).

### 5. Statistical analysis

The different levels of MMPs(-1,-2,-3,-8,-9,-13) and TIMPs(-1,-2) were compared between 5 groups(chronic periodontitis PD≥6mm, chronic periodontitis PD≤3mm, aggressive periodontitis PD≥6mm, aggressive periodontitis PD≤3mm). Because the sample size of each group was not enough to perform ANOVA, Kruskal-wallis test was used. Whether there was difference in MMPs and TIMPs levels between at least 2 groups or not. And then statistically different levels of MMPs and TIMPs were compared between test groups and control groups.

To analyze the relationship between pocket depth and assays of MMPs(-1,-2,-3,-8,-9,-13) and TIMPs(-1,-

2), Pearson correlation analysis was used was used to analyze the relationship between GI score and assays of MMPs and TIMPs.

## III. Results

Mean levels of MMPs and TIMPs in each group were recorded as shown in Table 1-1, 1-2 and 1-3. The results showed that there was a statistically significant difference in MMP-9 level between chronic periodontitis patients(PD≥6mm site) and healthy patients(Table 2-1, Table 2-2). And also there was statistically significant difference in TIMP-2 level between chronic periodontitis patients(PD≤3mm site) and healthy patients(Table 2-1, 2-2). Elevated level of MMP-9 was found in chronic periodontitis patients(PD≥6mm site) compared to healthy patients. MMP-9 level in chronic periodontitis patients(PD≥6mm site) was 5 fold greater than in control group. And lowered level of TIMP-2 was found in chronic periodontitis patients(PD≤3mm site) compared to healthy patients.

But there was no statistically significant difference between aggressive periodontitis patients(both PD≥6mm site and PD≤3mm site) and healthy patients in MMP and TIMP levels.

When data from different groups were pooled, comparing the relationship between amounts of MMPs(-1,-2,-3,-8,-9,-13), TIMPs(-1,-2) and PD showed that there was no statistically significant correlationship. But MMP-1(r=0.35) and MMP-2(r=0.31) had relatively high correlationship with PD(Table 3). When data from different groups were separately analyzed, there was also no statistically significant

Table 1-1. Mean levels of MMPs and TIMPs in chronic periodontitis patients(ng/ml)

| | MMP1 | | MMP2 | | MMP3 | | MMP8 | | MMP9 | | MMP13 | | TIMP1 | | TIMP2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PD | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm |
| mean (ng/ml) | 0.920 | 0.654 | 3.267 | 2.148 | 6.474 | 5.262 | 8.149 | 9.170 | 158.054 | 84.399 | 0.222 | 0.191 | 1.773 | 2.040 | 1.572 | 0.765 |
| SD | 0.815 | 1.004 | 3.508 | 2.306 | 0.615 | 0.647 | 3.626 | 3.044 | 115.666 | 68.474 | 0.188 | 0.155 | 1.132 | 2.170 | 2.457 | 2.163 |

Table 1-2. Mean levels of MMPs and TIMPs in agressive periodontitis patients(ng/ml)

| | MMP1 | | MMP2 | | MMP3 | | MMP8 | | MMP9 | | MMP13 | | TIMP1 | | TIMP2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PD | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm | PD≥ 6mm | PD≤ 3mm |
| mean (ng/ml) | 0.0182 | 0.436 | 0.829 | 0.908 | 12.074 | 12.400 | 3.709 | 5.063 | 24.350 | 16.344 | 6.421 | 0.380 | 4.104 | 11.569 | 5.110 | 12.828 |
| SD | 1.535 | 0.872 | 0.766 | 0.810 | 12.033 | 6.523 | 4.848 | 4.800 | 18.401 | 8.304 | 0.282 | 0.174 | 4.200 | 7.920 | 3.188 | 12.356 |

Table 1-3. Mean levels of MMPs and TIMPs in healthy subjects(control group) (ng/ml)

| | MMP1 | MMP2 | MMP3 | MMP8 | MMP9 | MMP13 | TIMP1 | TIMP2 |
|---|---|---|---|---|---|---|---|---|
| PD | PD≤3mm | ≤3mm | ≤3mm | ≤3mm | ≤3mm | ≤3mm | ≤3mm | ≤3mm |
| mean | 0.142 | 0.542 | 62.157 | 6.559 | 32.780 | 0.337 | 6.844 | 4.040 |
| SD | 0.234 | 0.753 | 56.201 | 3.322 | 28.367 | 0.293 | 9.803 | 3.726 |

Table 2-1. Comparison of MMPs and TIMPs level between 5 groups

| | MMP-1 | MMP-2 | MMP-3 | MMP-8 | MMP-9 | MMP13 | TIMP-1 | TIMP-2 |
|---|---|---|---|---|---|---|---|---|
| p-value | 0.48 | 0.08 | 0.45 | 0.16 | 0.02 | 0.23 | 0.23 | 0.006 |

Testing whether there is difference in MMPs and TIMPs levels between at least 2 groups or not

Table 2-2. Comparison of test groups with control group in MMP9 and TIMP2 level(p-value)

| MMP-9 | | | | TIMP-2 | | | |
|---|---|---|---|---|---|---|---|
| CP(PD≥6) | CP(PD≤3) | AP(PD≥6) | AP(PD≤3) | CP(PD≥6) | CP(PD≤3) | AP(PD≥6) | AP(PD≤3) |
| 0.04(p-value) | 0.11 | 1.00 | 0.62 | 0.45 | 0.049 | 0.54 | 0.22 |

Table 3. Pearson correlation coefficient of MMPs and TIMPs with PD

| | MMP1 | MMP2 | MMP3 | MMP8 | MMP9 | MMP13 | TIMP1 | TIMP2 |
|---|---|---|---|---|---|---|---|---|
| Pearson correlation coefficient | 0.35 | 0.31 | -0.2 | -0.06 | 0.19 | 0.01 | -0.21 | -0.09 |
| p-value | 0.06 | 0.11 | 0.31 | 0.77 | 0.32 | 0.96 | 0.25 | 0.66 |

Table 4. Pearson correlation coefficient of MMPs and TIMPs with PD of each group. ( ) is p-value for no correlation

| | Group | | |
|---|---|---|---|
| | CP | AP | Healthy |
| MMP1 | 0.24(0.37) | 0.51(0.2) | NA |
| MMP2 | 0.27(0.31) | 0.19(0.65) | NA |
| MMP3 | 0.18(0.5) | -0.06(0.89) | NA |
| MMP8 | -0.08(0.78) | 0.07(0.87) | NA |
| MMP9 | 0.26(0.34) | 0.37(0.37) | NA |
| MMP13 | -0.015(0.96) | 0.34(0.42) | NA |
| TIMP1 | 0.04(0.88) | -0.37(0.37) | NA |
| TIMP2 | 0.31(0.24) | -0.45(0.26) | NA |

Table 5. Pearson correlation coefficient of MMPs and TIMPs with GI score

| | MMP1 | MMP2 | MMP3 | MMP8 | MMP9 | MMP13 | TIMP1 | TIMP2 |
|---|---|---|---|---|---|---|---|---|
| Pearson correlation coefficient | 0.4 | 0.29 | -0.1 | 0.009 | 0.26 | 0.04 | -0.179 | -0.126 |
| p-value 0.03 | 0.13 | 0.59 | 0.96 | 0.17 | 0.82 | 0.35 | 0.52 | |

correlationship between amounts of MMPs, TIMPs and PD(Table 4).

When data from different groups were pooled, comparing the relationship between amounts of MMPs(-1,-2,-3,-8,-9,-13), TIMPs(-1,-2) and GI score showed that there was statistically significant positive correlationship between MMP-1 and GI score(p-value=0.03)(see Table 5). And relatively high correlationship was found in MMP-1(r=0.4) and MMP-2(r=0.29) (Table 5). But when data from different

groups were separately analyzed, there was no statistically significant correlationship between MMPs, TIMPs and GI score(Table 6).

## IV. Discussion

Extracellular matrix(ECM) degradation during various periodontal diseases is thought to be mediated by a complex cascade involving both host and microbial-derived proteinases(Sorsa et al. 1992, Page 1991). And the interaction of several types of plaque bacteria with resident gingival cells(fibroblast, epithelial cells) as well as infiltrating inflammatory cells(polymorphonuclear leukocytes of PMNs and monocyte/macrophage) has been found to increase the synthesis and release of tissue-destructive proteinases by such host cells(Birkedal-Hansen et al. 1993, Sorsa et al. 1992).

Assessment of subjects from each of the four groups(control, gingivitis, chronic periodontitis, localized juvenile periodontitis) revealed that more collagenolytic activity was present in GCF from gingivitis patients than from controls and that still more was present than in GCF from periodontitis patients than from those with gingivitis. No difference was observed between the two periodontitis groups (Villela B 1987). Regardless of mechanism, elevated levels of MMPs and other host-derived proteinases (cathepsin, elastase, tryptase/trypsin-like proteinases), able to degrade the various constituents of the ECM, have been detected in inflamed gingiva and in gingival crevicular fluids(GCF) and saliva of human with periodontal disease(Sorsa et al. 1998,Golub et al. 1990, Ingman et al. 1993a, b, 1994). Elevated interstitial collagenase(MMP-1, MMP-8) activities have been detected in GCF of adult periodontitis (AP) and localized juvenile periodontitis(LJP), and these activities have been shown to decrease following periodontal treatment in both groups (Hakkarainen et al. 1988). Ingman(1996) reported that MMP-8 and MMP-9 is the main gelatinase in adult periodontitis GCF, whereas GCF collagenase in localized juvenile periodontitis patients seems to be of the MMP-1 type. Meikle et al (1994) detected immunohistochemically the presence of MMP-1,-2 and- 3 and TIMP-1 in inflamed gingiva of patients with "chronic inflammatory periodontal disease".

Evidence that tissue destruction in disease processes might result from an imbalance of metalloproteinases over tissue inhibitors of metalloproteinases first came from a study showing a reduction in tissue inhibitors of metalloproteinase compared with metalloproteinase in synovial explants from a rabbit model arthritis (Muphy G 1993).

TIMPs share a common two-domain structure in which only the inhibitory N domain in capable of inhibiting MMPs. The amino group of the N-terminal Cys of TIMP co-ordinates with the active site $Zn^{2+}$ of MMPs with the adjacent residues of TIMP occupying the active site cleft of MMPs and contacting the surrounding surface of the catalytic domain of MMPs. Inhibition of MMPs by TIMPs is largely interchangeable, except for the Membrane anchored type-MMPs, which is not inhibited by TIMP-1. TIMP-2 is 2 to 10 fold more effective than TIMP-1 against the gelatinase(MMP-2 and -9), whereas TIMP-1 appears to more effectively inhibit collagenase(MMP-1)(Howard EW, 1991).

And Haerian A(1995) and Nomura(1993) reported that TIMP was higher in diseased sites than healthy sites . Nomura explained that bacterial stimuli initially induce MMP expression by host cells causing an increase in tissue self destruction. Therefore, the host cells recognize the ongoing tissue destruction and attempt to defend the host by producing TIMPs.

However, in contrast to pre-mentioned studies, Larivee et al(1986) concluded that the activity of inhibitors is higher in healthy sites than in diseased

site . And Ingman et al(1994) reported that only low level of TIMP-1 was present in adult periodontitis patients. Moreover, Sorsa found that GCF of adult periodontitis patients contain PMN-derived MMP-8 and -9 but not detectable amounts of TIMP-1. Thus it seems that PMNs do not contain and release TIMP-1 in amounts comparable to MMP-8 and MMP-9(Sorsa 1994). Gulay Tuter(2002) reported that the levels of MMP-1 in GCF decreased and total levels of TIMP-1 in GCF increased after periodontal therapy. The ratio of MMP-1 to TIMP-1 changed after periodontal therapy, becoming close to that of the control.

As reviewed, Whether TIMP level is higher or lower in inflamed site than in healthy site is still controvertial and different GCF sampling methods might result in conflicting results.

Ingman(1996) reported that MMP-8 and MMP-9 is the main gelatinase in adult periodontitis GCF. And also Makela et al.(1994) demonstrated that MMP-9 was the major gelatinase present in gingival crevicular fluid of periodontal disease. Therefore, MMP-9 appeared to be a marker for degradation of the gingival extracellular macromolecules at mild inflammation. Moreover, Anne-Laure(2003) reported that the decrease in collagen fiber was inversely correlated with the significant increase in MMP-1, MMP-9, and MMP-13, with the increase of the active form of MMP-2 and with the active form and proform of MMP-9.

The results of Ingman, Makela and Anne-Laure are coincident with our results in that the level of MMP-9 in inflamed site of chronic periodontitis patients is higher than control group.

The result from this study is that assessment of subjects from 4 groups revealed that higher MMP-9 activity was found in deep pocket($\geq$6mm) sites of chronic periodontitis patients than control group. The observation that the level of MMP-9 was elevated in inflamed sites of chronoic periodontitis patients suggests that MMP-9 could be a marker of inflammation in chronic periodontitis patients.

And there was lowered level of TIMP-2 in shallow pocket($\leq$3mm) sites of chronic periodontitis patients than control group. The fact that the level of TIMP-2 was lowered in non-inflamed sites of chronic periodontitis patients indicates that TIMP-2 could be a marker in non-inflamed site of chronic periodontitis.

And also there were no elevated levels of any MMPs and TIMPs in shallow and deep pockets of aggressive periodontitis patients than control group. From this, we can suggest that any MMPs or TIMPs could not be the marker of aggressive periodontitis.

Villela et al.(1987) reported that positive but weak correlation between PD and GI and collagenase activity while Gangbar et al.(1990) and Teng et al.(1992) failed to find any correlations between collagenase activity and clinical parameters.

In this study, when data from different groups were pooled, comparing the relationship between activities of MMPs(-1,-2,-3,-8,-9,-13), TIMPs(-1,-2) and PD, there is no statistically significant correlationship but MMP-1(r=0.35) and MMP-2(r=0.31) had relatively high correlationships. When data from different groups were separately analyzed, there is no statistically significant correlationship. Thus, pocket depth could not reflect amounts of MMPs and TIMPs in GCF. When data from different groups were pooled, comparing the relationship between activities of MMPs(-1,-2,-3,-8,-9,-13), TIMPs(-1,-2), and GI score showed that there is statistically significant positive correlationship between MMP-1 and GI score(p-value=0.03). MMP-1(r=0.4) and MMP-2(r=0.29) had relatively high correlationships. But when data from different groups were separately analyzed, there were no statistically significant correlationship. So it is hard to clearly say that GI score is

in positive correlation with MMP-1 level.

Different methods of GCF sampling and laboratory techniques as well as different in statistical analysis may have influence on amounts of MMPs, TIMPs and their correlation with clinical parameters.

Further longitudinal study and more sample sizes are required to elucidate whether MMPs and TIMPs can be used as indicator of inflamed sites and non-inflamed sites of chronic periodontitis patients.

In conclusion, only MMP-9 could be a possible marker in pocket depth ≥6mm site in chronic periodontitis patients and TIMP-2 could be a marker in pocket depth ≤3mm of chronic periodontitis patients.

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-Abstract-

# 치주염 환자의 치은열구액에서 MMPs와 TIMPs의 양의 변화

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MMPs(Matrix metalloproteinases)는 치주질환에서 주된 조직파괴단백분해효소인 것은 알려져 있고 TIMPs(Tissue inhibitor of Matrix metalloproteinase)는 MMPs의 작용을 억제한다라고 알려져 있다. 이 둘간의 불균형으로 인해서 조직파괴가 더 가속화될 수 있다. 이 두 연구의 목적은 ELISA kit를 사용하여 특정 MMPs (1,2,3,8,9,13)과 TIMPs(1,2)의 양이 건강한 환자와 비교하여서 치주염 환자에서 달라지는지 알아보고 MMPs(1,2,3,8,9,13), TIMPs(1,2)와 치은열구깊이와 GI score와의 관계를 알아보기로 한다.

8명의 만성 치주염 환자와 4명의 급속진행형 치주염 환자가 실험군으로 참여하였고 5명의 건강한 치주조직을 환자가 대조군으로 참여하였다. 임상적인 측정은 GI score와 치주낭측정을 통하여 이루어졌다. 8명의 만성치주염을 가진 환자와 4명의 급속진행형 치주염을 가진 환자에서 각각 치주낭 깊이가 3mm이하인 부위에서 6개의 치은열구액 표본과 치주낭 깊이가 6mm 이상인 곳에서 6개의 치은열구액 표본을 채취하였다. 건강한 치주조직을 가진 5명의 환자는 단지 치주낭 깊이가 3mm 이하인 건강한 부위에서만 치은열구액 표본을 제공하였다. MMPs(1,2,3,8,9,13)과 TIMPs(1,2)의 측정은 Human Biotrack ELISA kit를 사용하여서 측정하였다. 통계처리는 MMPs, TIMPs의 실험군과 대조군의 차이는 Kruskal-Wallis test를 사용하였고 MMPs, TIMPs와 치주낭 깊이와 GI score의 관계정도는 Pearson' s correlation coefficient를 사용하였다.

실험결과 대조군과 비교하여 만성 치주염 환자에서 치주낭 깊이가 6mm 이상인 부위에서 MMP9의 수치가 통계적으로 유의할만하게 높았으며($p=0.04$) TIMP2의 수치가 대조군과 비교하여 치주낭 깊이가 3mm 이하인 부위를 가진 만성치주염 환자에서 통계적으로 유의할만하게 높았다($p=0.049$). MMPs(1,2,3,8,9,13), TIMPs(1,2)의 활동성과 치주낭 깊이의 관계에서 둘간의 통계적으로 유의할만한 관계는 존재하지 않았으나 MMP1($r=0.35$)과 MMP2($r=0.31$)가 상대적으로 높은 관계를 보였다. 또한 MMPs(1,2,3,8,9,13), TIMPs(1,2)의 활동성과 GI score의 관계에서도 둘간의 통계학적으로 유의할만한 관계는 존재하지 않으나 MMP1($r=0.4$), MMP2($r=0.29$), MMP9($r=0.26$)가 상대적으로 높은 관계를 보였다.

이 실험의 결과로 볼 때 MMP9가 만성치주염 환자의 질환이 있는 부위에서만 염증의 지표가 될 수 있으며 TIMP2가 만성치주염 환자의 염증이 없는 부위에서 높은 농도로 존재하는 것으로 보아서 TIMP2가 MMPs에 대한 억제작용을 하여서 염증의 진행을 방해하는 역할을 한다라고 볼 수 있다.

주요어 : MMPs, TIMPs, ELISA,만성치주염, 급속진행형 치주염