

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

Sun-Yun Lee<sup>1</sup>, Jae-Seung Ko<sup>2</sup>, Yeoun-Ho Jung<sup>2</sup>, Hyun-Man Kim<sup>2</sup>, Kyoung-Hwa Kim<sup>1</sup>,  
Byung-Keun Yang<sup>1</sup>, Soo-Boo Han<sup>1</sup>, Chong-Pyoung Chung<sup>1</sup>,  
Tae-Il Kim<sup>1</sup>, Young Ku<sup>1</sup>, Yong-Moo Lee<sup>1</sup>, In-Chul Rhyu<sup>1</sup>

<sup>1</sup>Department of Periodontology, College of Dentistry, Seoul National University

<sup>2</sup>Department of Oral histology, College of Dentistry, Seoul National University

## 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 periodontitis patients.

\*This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea(00-PJ1-PG1-CH10-0002)  
Corresponding author: In-Chul Rhyu, Department of Periodontology, College of dentistry, Seoul National University, 28, Yongon-Dong, Chongno-Ku, 110-749, Korea

## 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 aggressive 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  $\geq 6$ mm 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, immunosuppressant, 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 gingival crevicular fluid(GCF)

Six GCF samples from healthy( $PD \leq 3$ mm) and 6 GCF samples from periodontitis sites( $PD \geq 6$ mm)

were collected respectively from 8 patients with chronic periodontitis and 4 patients with aggressive periodontitis. Six GCF samples from only healthy sites( $PD \leq 3$ mm) 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  $-70^{\circ}\text{C}$  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  $PD \geq 6$ mm, chronic periodontitis  $PD \leq 3$ mm, aggressive periodontitis  $PD \geq 6$ mm, aggressive periodontitis  $PD \leq 3$ mm). 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 $\geq$ 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 $\leq$ 3mm site) and healthy patients(Table 2-1, 2-2). Elevated level of MMP-9 was found in chronic periodontitis patients(PD $\geq$ 6mm site) compared to healthy

patients. MMP-9 level in chronic periodontitis patients(PD $\geq$ 6mm site) was 5 fold greater than in control group. And lowered level of TIMP-2 was found in chronic periodontitis patients(PD $\leq$ 3mm site) compared to healthy patients.

But there was no statistically significant difference between aggressive periodontitis patients(both PD $\geq$ 6mm site and PD $\leq$ 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 correlation. But MMP-1(r=0,35) and MMP-2(r=0,31) had relatively high correlation 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 $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 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 aggressive periodontitis patients(ng/ml)

	MMP1		MMP2		MMP3		MMP8		MMP9		MMP13		TIMP1		TIMP2	
	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 3mm	PD $\geq$ 6mm	PD $\leq$ 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 $\leq$ 3mm	$\leq$ 3mm	$\leq$ 3mm	$\leq$ 3mm	$\leq$ 3mm	$\leq$ 3mm	$\leq$ 3mm	$\leq$ 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 $\geq$ 6)	CP(PD $\leq$ 3)	AP(PD $\geq$ 6)	AP(PD $\leq$ 3)	CP(PD $\geq$ 6)	CP(PD $\leq$ 3)	AP(PD $\geq$ 6)	AP(PD $\leq$ 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

	CP	Group 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	0.35	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 correlation 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 (Villemann 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 is 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 controversial 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( $\geq 6$ mm) sites of chronic periodontitis patients than control group. The observation that the level of MMP-9 was elevated

in inflamed sites of chronic 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( $\leq 3$ mm) 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 relationship but MMP-1( $r=0.35$ ) and MMP-2( $r=0.31$ ) had relatively high correlations. When data from different groups were separately analyzed, there is no statistically significant relationship. 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 relationship between MMP-1 and GI score( $p$ -value=0.03). MMP-1( $r=0.4$ ) and MMP-2( $r=0.29$ ) had relatively high correlations. But when data from different groups were separately analyzed, there were no statistically significant relationship. 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  $\geq 6$ mm site in chronic periodontitis patients and TIMP-2 could be a marker in pocket depth  $\leq 3$ mm of chronic periodontitis patients.

## V. References

1. Anne-Laure Ejeil,-Sylvie Igondjo-Tchen, Sabah Ghomrasseni, Expression of matrix metalloproteinases(MMPs) and tissue inhibitors of metalloproteinases(TIMPs) in healthy and diseases human gingiva, J Periodontol 2003;73:188-195
2. Birkedal-Hansen, H. Role of matrix metalloproteinases in human periodontal diseases. Journal of periodontology 1993;64:474-484
3. Denis E.KINANE. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. Periodontology 2000. vol.24, 2000:215-225
4. Golub, L. M., Ciano, S., Ramamurthy, N.S., Leung, M & McNamara, T. F. Low-dose doxycycline therapy:effect on gingival and crevicular fluid collagenase activity in humans. Journal of Periodontal Research 1990;26;321-330
5. Golub L,M, Sorsa T, Lee H-M, Ciano S,Sorbi D, Ramamurthy NS, Gruber B, Salo T, Kontinen YT. Doxycycline inhibits neutrophil(PMN)-type matrix metalloproteinases in human adult periodontitis gingivitis. J Clin Periodontol 1995;22:100-109
6. Gulay Tuter, Bulent Kurtis, Muhittin Serdar. Effects of Phase I Periodontal Treatment on gingival crevicular fluid levels of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1. J Periodontol 2002;73:487-493
7. Haerian A, Adonogianaki E, Mooney J, Docherty JP, Kinane DF. Gingival crevicular stromelysin, collagenase and tissue inhibitor of metalloproteinases level in healthy and diseased sites. J Clin Periodontol 1995;22:505-509
8. Hakkarainen, K., Uitto, V-J. & Ainamo, J. Collagenase activity and protein content of sulcular fluid after scaling and occlusal adjustment of teeth with deep periodontal pockets. Journal of periodontal research 23,1998; 204-210
9. Ingman, T., Sorsa, T., Kontinen, Y.Y., Liede, K, Saari, H., Lindy, O.& Suomalainen, K. Salivary collagenase, elastase- and trypsin-like protease as biochemical markers of periodontal tissue destruction in adult and localized juvenile periodontitis. Oral Microbiology and Immunology 1993a;8: 298-305
10. Ingman, T., Sorsa, T., Suomalainen, K., Halinen, S., Lindy, O., Lauhio, A., Saari, H., Kontinen, Y.T.& Goulub, L.M. Tetracycline inhibition and the cellular source of collagenase in gingival crevicular fluid in different periodontal diseases. A review article. 1993b Journal of Periodontology 1993;64;82-88
11. Ingman, T., Sorsa, T., Kangaspunta, P. & Kontinen, Y.T. Elastase and  $\alpha 1$ -Proteinase inhibitor in gingival crevicular fluid and gingival tissue in adult and juvenile periodontitis. Journal of Periodontology 1994;65:702-209
12. Ingman T, Tervahartiala T, Ding Y, Tschesche H, Haerian A, Kinane DF, Kontinen YT, Sorsa T. Matrix metalloproteinases and their inhibitors in gingival crevicular fluids and saliva of periodon-

- titis patients. *J Clin Periodontol* 1996;23:1127-1132
13. John J. Reynolds & Murray C. Meikle. Mechanism of connective tissue matrix destruction in periodontitis. *Periodontology* 2000 vol.14, 1997, 144-157
  13. Jonathan M. Korostoff, Jian Fei Wang, David P. Sarment, Jeffrey C.B. Stewart, Roy S. Feldman. Analysis of In Situ Protease activity in chronic adult periodontitis patients: Expression of activated MMP-2 and a 40 kDa Serine Protease. *J Periodontol* 2000;71:353-360
  14. Larivee, L., Sod<sup>†</sup>, J. & Ferrier, J.M. Collagenase and collagenase inhibitor activities in crevicular fluid of patients receiving treatment for localized juvenile periodontitis. *Journal of Periodontal Research* 1986;21:701-715
  15. Meikle MC, Hembry RM, Holley J, Horton C, McFarlane CG, Reynolds JJ. Immunolocalization of matrix metalloproteinases and TIMP-1 (tissue inhibitor of metalloproteinases) in human gingival tissues from periodontitis patients. *J Periodontal Research* 1994;29:118-126
  16. Nomura, T., Takahashi, T. & Hara, K. Expression of TIMP-1, TIMP-2 and collagenase mRNA in periodontitis-affected human gingival tissue. *J Periodontal Research* 1993;28:354-362
  17. Sorsa, T., Ingman, T., Suomalainen, K., Haapasalo, M., Konttinen, Y. T., Lindy, O., Saari, H. & Uitto, V.-J. Identification of proteases from potent periodontopathogenic bacteria as activators of latent human matrilysin and fibroblast-type interstitial collagenases. *Infection and Immunity* 1992 60;4491-4495
  18. Sorsa, T., Ding, Y., Salo, T., Lauhio, A., Teronen, O., Ingman, T., Ohtani, H., Andoh, N., Takeha, S. & Konttinen, Y. T. Effects of tetracyclines on neutrophil, gingival, and salivary collagenases. A functional and Western blot assessment with special reference to their cellular sources in periodontal diseases. *Annals of the New York Academy of Sciences* 1994 732;112-131
  19. Sorsa, T., Uitto, V.-J., Suomalainen, K., Vauhkonen, M. & Lindy, S. Comparison of interstitial collagenases from human gingiva, sulcular fluid and polymorphonuclear leukocytes. *J periodontal Research* 1998;23:386-393
  20. Veli-Jukka Uitto, Christopher M. Overall & Christopher McCulloch. Proteolytic host cell enzymes in gingival crevice fluid. *Periodontology* 2000, vol.31, 2003, 77-104
  21. Vilella B, Cogen RB, Bartolucci AA and Birkedal-Hansen H. Collagenolytic activity in crevicular fluid from patients with chronic adult periodontitis, localized juvenile periodontitis and gingivitis, and from healthy control subjects. *J Periodontal Research* 1987;22:381-389
  22. Vinay M. Bhide, Lloyd Smith, Christopher M. Overall, Peter Birek, and Christopher A.G. McCulloch. Use of a Fluorogenic Septapeptide Matrix Metalloproteinase Assay to Assess Response to Periodontal Treatment. *J Periodontology* 2000;71:690-700
  23. Woessner, J.F. Matrix metalloproteinases and their inhibitors in connective tissue remodelling. *FASEB Journal* 1991;5:2145-2154.
  22. Howard EW, Bullen EC, Banda MJ. Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. *J Biol Chem* 1991;266:13070-13075
  23. Gangbar S, Overall CM, McCulloch CAG, Sod<sup>†</sup> J. Identification polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: Correlation with periodontal disease activity in adult and juvenile periodontitis. *J Periodont REs* 1990;25:257-267
  24. Teng YT, Sodek J, McCulloch CAG. Gingival



- crevicular fluid gelatinase and its relationship to periodontal disease in human subject. J Periodont Res 1992;27:544-552
25. Murphy G, Reynolds J, Royce PM, Steinmann B, ed. Extracellular matrix degradation in connective tissue and its heritable disorders. New York; Wiley-Liss, 1993;287-316
  26. Makela 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;73:1397-1406
  27. Page RC. The Role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodont Res 1991;26:230-242

## 치주염 환자의 치은열구액에서 MMPs와 TIMPs의 양의 변화

이선윤<sup>1</sup>, 정연호<sup>2</sup>, 김정화<sup>1</sup>, 양병근<sup>1</sup>, 한수부<sup>1</sup>, 정종평<sup>1</sup>, 김태일<sup>1</sup>, 구 영<sup>1</sup>, 이용무<sup>1</sup>, 고재승<sup>2</sup>, 류인철<sup>1</sup>

<sup>1</sup> 서울대학교 치과대학 치주과학교실

<sup>2</sup> 서울대학교 치과대학 구강조직학교실

MMPs(Matrix metalloproteinases)는 치주질환에서 주된 조직파괴단백분해효소인 것은 알려져 있고 TIMPs(Tissue inhibitor of Matrix metalloproteinase)는 MMPs의 작용을 억제한다고 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 만성 치주염을 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