The comparison of inflammatory mediator expression in gingival tissues from human chronic periodontitis patients with and without type 2 diabetes mellitus

Sang-Don Joo, Jae-Mok Lee*

Department of Periodontology, School of Dentistry, Kyungpook National University

I. INTRODUCTION

Chronic periodontitis is an inflammatory disease that results in the destruction of the tooth-supporting tissues, that is, the gingiva, periodontal ligament, root cementum, and alveolar bone\(^9\). Chronic periodontitis is strongly associated with the presence of cytokines, such as IL-1\(\alpha\) and \(\beta\). Indeed many cell types in the periodontium have the capacity to produce these compounds. The cytokine IL-1 is now fully characterized polypeptide with multiple overlapping biological activities and act as early mediator of inflammation and immunities\(^2\). IL-1 has been shown to have a dual function in collagen digestion. It inhibits the intracellular phagocytic pathway, but at the same time, it strongly promotes extracellular digestion by inducing the release of collagenolytic enzymes like collagenase.

Matrix metalloproteinase (MMP)\(^4,5\) and matrix-degrading serine proteinases were shown to participate in the breakdown of extracellular matrix in disease processes as diverse as rheumatoid arthritis, atherosclerotic plaques and cancer metastasis. In the oral environment, MMPs take part in developmental events involving teeth and salivary glands and in collagen turn over, besides playing an important role in pathologic processes such as periodontal tissue destruction, oral lichen planus, dysplasia, squamous cell carcinoma, bone and cartilage degradation and root caries. It is now recognized that during active periodontitis, degradation of gingival tissue (mainly collagen) is due in part to matrix metalloproteinases (MMPs)
expressed in situ by inflammatory cells (monocytes, macrophages, lymphocytes, polymorphonuclear leukocytes) and resident cells (fibroblasts, epithelial cells and endothelial cells). Collagenase-3, also known as MMP-13 is expressed by the basal cells of the gingival pocket epithelium that rest on the underlying connective tissue. Collagen is the major extracellular matrix component of gingiva and thus is implicated in pathological states such as periodontitis. It is now recognized that during active periodontitis, degradation of gingival tissue (mainly collagen) is due in part to matrix metalloproteinases (MMPs) expressed in situ by inflammatory cells (monocytes, macrophages, lymphocytes, PMNLs) and resident cells (fibroblasts, epithelial cells, and endothelial cells).

Matrix metalloproteinases (MMPs) are a family of metal dependent proteolytic enzymes that mediate the degradation of extracellular matrix and basement membranes. MMPs are composed of at least 23 related zinc or calcium dependent endopeptidases that are able to degrade extracellular matrix proteins at a pH close to neutral. MMPs are secreted as inactive form (proenzyme) and are activated in the extracellular compartment or in the vicinity of the cell membranes by other MMPs or serine proteinases through peptide cleavage. Three major types of MMPs have been identified: the collagensases degrade interstitial collagen types I, II and III; the gelatinases (also called type IV collagenases) degrade type IV collagen and denatured interstitial collagens; and the stromelysins, which demonstrate wider substrate specificity, degrade proteoglycan, laminin, fibronectin, and the non-helical regions of collagens. Recently MT-MMPs (Membrane type matrix metalloproteinases) were also found. They can degrade gelatin after cleavage of collagen molecules to 1/4 and 3/4 segments by interstitial collagenases (MMP-1, MMP-13).

The actions of collagenolytic enzymes are controlled at least at three distinct levels involving production, activation and inhibition. First, the enzymes are synthesized and secreted in an inactive proform. Second, the enzymes are activated by autoactivation, by stromelysin, or plasmin. Third, once activated, the enzymes perform their catalytic activities and are subsequently inhibited by specific tissue inhibitors of metalloproteinases (TIMPs). Tissue inhibitors of matrix metalloproteinases (TIMPs) are modulating factors of MMPs activity, and four members of TIMPs have been reported. Among these, TIMP-1 and TIMP-2, which have inhibitory effects on all MMPs, are found in periodontal lesions. TIMP-2 shows strong inhibition against PMN-derived MMPs, whereas TIMP-1 shows greater inhibition against fibroblast-derived MMPs. 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 co-ordinates with the active site Zn$^{2+}$, with the adjacent residues occupying the active site cleft and containing the surrounding surface of the catalytic domain.
Diabetes mellitus is an important risk factor of periodontal diseases. It is one of the main contributing factors for periodontal disease and also limiting factor for periodontal treatments such as implant therapy. Although diabetes itself does not cause periodontitis, periodontal disease progresses more rapidly and leads to more tooth losses in patients with poorly controlled blood glucose. Severe periodontitis has been associated with an increased risk of poor glycemic control and, in turn untreated advanced periodontal disease can deteriorate the metabolic control of diabetes. Various pathogenic factors have been suggested to explain the increased prevalence and severity of periodontitis in diabetes. Numerous studies of PMNLs function in patient with diabetes have shown impaired chemotaxis, adherence, phagocytosis, and suggest these defects could lead to impaired host resistance to infection. The severity of periodontitis has been correlated with defective chemotaxis, which may be linked to extensive periodontal tissue destruction; diabetic patients with severe periodontitis had depressed PMN chemotaxis compared to those with mild periodontitis or non-diabetic subjects with severe or mild periodontitis.

The production of collagenolytic enzyme, its activators and inhibitors are mediated by a variety of compounds among which cytokines like IL-1, BGP, PGP, PDGF, TGF-β, TGF-β. Therefore, the modulation by cytokines of the cascade of proenzyme-activator -enzyme inhibitor are likely to have a high relevance in processes like wound healing, chronic inflammatory disease like periodontitis, rheumatoid arthritis, tumor invasion.

In inflammatory response, the roles and interactions of IL-1β, MMP-13 and TIMP-1 are not clear. The relative contribution of IL-1β, MMP-13 and TIMP-1 in the pathogenesis of periodontitis is still not entirely established. Moreover none of the in vivo studies simultaneously analysed each IL-1β, MMP-13 and TIMP-1 and it’s interrelationship for the diabetic and nondiabetic patients with chronic periodontitis. The purposes of this study were to compare and quantify the expression of IL-1β, MMP-13 and TIMP-1 in the gingival tissues of patients with type 2 diabetes mellitus and healthy adults with chronic periodontitis.

II. MATERIALS AND METHODS

1. Study population and Tissue sampling

Study population consisted of 8 patients with type 2 diabetes and chronic periodontitis, 8 patients with chronic periodontitis, and 8 healthy individuals. Marginal gingival tissue samples were obtained by internal bevel incision at the time of periodontal surgery (including surgical crown lengthening) or tooth extraction and informed consent was obtained from all of the participants before the surgery.

According to the patient’s systemic condition (age, sex, blood glucose level, obesity,
smoking) and clinical criteria of gingiva (Sulcus bleeding index value, probing depths) and radiographic evidences of bone resorption, each gingival sample was divided into the three groups. Group 1 (normal, n=8) is clinically healthy gingiva without bleeding and no evidence of bone resorption or periodontal pockets, obtained from systemically healthy 8 patients. Group 2 (chronic periodontitis, n=8) is inflamed gingiva from patients with chronic periodontitis. The diagnosis of chronic periodontitis was established on the basis of clinical and radiographic criteria (bone resorption) according to the classification system for periodontal disease and condition. All patients of group 2 were systemically healthy and had more than one periodontal pockets ≥ 5 mm and at least one pocket with ≥4mm loss of attachment. All gingival samples were obtained from the teeth with probing depth ≥ 5 mm, swelling of the marginal gingiva, and bleeding corresponding to Gingival sulcus bleeding index ≥ 3 according to Mithuleman and Sön. Group 3 (chronic periodontitis & type 2 DM, n=8) is inflamed gingiva from patients with chronic periodontitis associated with type 2 diabetes. Patients in group 3 were diagnosed type 2 diabetes mellitus since 6 months and showed above 200 mg/dl blood glucose level in 2PP. Patients in group 2 & 3 have similar periodontal condition, but systemically patients in group 2 were healthy and patients in group 3 had type 2 diabetes under medication. Gingival sample were obtained by similar way described above. Following surgery, excised tissue specimens were immediately placed on liquid nitrogen and subsequently frozen (-70°C).

2. Protein Isolation and Western blotting

For Western blotting, as previously described technique by Kang[3] frozen tissues were homogenized in RIPA lysis buffer (10 mM EDTA, 0.15M NaCl) with 1:30 diluted protease inhibitor cocktail (Roche, Germany)[30]. The lysates were sonicated 3 times for 10 seconds and centrifuge at 12,000g for 15 minutes. Protein concentrations of supernatant were routinely determined by a Bradford protein assay (Quick Start, BIO-RAD, USA) using BSA as standard.

Lysates were boiled in SDS samples buffer (1M Tris-Cl(pH6.8), 40% glycerol, 8% SDS, 2% mercapto-ethanol, 0.002% Bromophenol blue).

Prepared samples were separated by 15% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane.

The membranes were subsequently blocked in Tris-buffered saline (TBS) containing 5% powdered milk and 1% BSA for 1 hour, and then incubated with polyclonal anti-IL-1β, anti-MMP-13, and anti-TIMP-1(Santa Cruz Biotechnology, Inc USA) antibody for 1.5 hours at room temperature.

The membranes were washed (five times for 5 minutes with Tween 20) and incubated with a horseradish peroxidase(HRP)- con-
jugated goat anti-rabbit secondary antibody for anti-IL-1β antibody and donkey anti-goat secondary antibody for anti-MMP-13, anti-TIMP-1 antibody (diluted 1: 2000 in TBS) for 1 hour at room temperature. After additional washing (five times for 5 minutes with Tween 20) the Western blot procedure was completed with an ECL Plus development kit (Amsterdam, Beckinghamshire, U.K.)

The quantification analysis of IL-1β, MMP-13, TIMP-1 expression was performed using a densitometer (Image Gauge V 3.46, Kashiin Graphic Systems, FUJI PHOTO FILM CO, Japan). After normalization to β-actin (Abcam® U.K.) in each sample, level of IL-1β, MMP-13, TIMP-1 were expressed as a ratio of IL-1β or MMP-13 or TIMP-1/β-actin and the differences of density between 3 groups were determined.

3. Statistical Analysis of the Western blot results

All data were presented as means ± standard deviation and results were statistically analyzed. The IL-1β, MMP-13, TIMP-1 levels among each 3 groups were compared using one way ANOVA followed by Tukey test. P value <0.05 was considered to statistically significant.

III. RESULTS

Both chronic periodontitis group & chronic periodontitis with type 2 DM group showed the expression of IL-1β, MMP-13 and TIMP-1 in all samples. To compare IL-1β expression levels in human gingiva with chronic periodontitis with or without associated to Type 2 diabetes mellitus, IL-1β specific antibodies was used to detect the cytokine in the tissues (Figure 1A, B). In order to quantify the level of IL-1β expression in the groups, the expression levels of IL-1β in each sample were measured by densitometer. The expression levels of β-actin were also measured by anti-β-actin specific western blot analysis. Then IL-1β expression levels were normalized by β-actin (ratio of IL-1β/β-actin). The levels of gingival IL-1β expression are given in Table 1.

The mean amount of IL-1β expression (ratio of II-1β/β-actin) were 0.694 ± 0.274 in group 1, 0.685 ± 0.105 in group 2, 0.998 ± 0.255 in group 3. The expression levels of IL-1β in the group 3 were significantly higher than group 1 and group 2.

The comparison of MMP-13 expression levels were also studied by western blot analysis using MMP-13 specific antibody which detected about 60 kDa molecular weight of MMP-13 in all three groups (Figure 2A). The levels of MMP-13 expression were also quantified with β-actin normalization (Figure 2B) and normalized levels of individual gingival MMP-13 expression are given in Table 2. The mean amounts of MMP-13 expression (ratio of MMP-13/β-actin) were 0.645 ± 0.119 in group 1, 0.894 ± 0.230 in group 2 and 1.044
Figure 1A. IL-1β Western analysis of 4 representative samples in each group. IL-1β corresponding to molecular weight 35 kDa was shown to be expressed in all samples including healthy gingiva, and the expression levels of IL-1β were increased in patients with type 2 diabetes mellitus than in control healthy subjects. In order to quantify the IL-1β levels, β-actin levels were also performed.

Group 1: healthy gingiva from systemically healthy person
Group 2: Inflamed gingiva from patient with chronic periodontitis
Group 3: Inflamed gingiva from patient with chronic periodontitis and type 2 DM

Table 1. IL-1β intensity was normalized by β-actin intensity to quantify the expression levels of IL-1β expression and the value were presented as a ratio of IL-1β/β-actin.

<table>
<thead>
<tr>
<th>sample</th>
<th>group 1</th>
<th>group 2</th>
<th>group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.567</td>
<td>0.648</td>
<td>0.856</td>
</tr>
<tr>
<td>2</td>
<td>0.446</td>
<td>0.707</td>
<td>1.050</td>
</tr>
<tr>
<td>3</td>
<td>0.403</td>
<td>0.610</td>
<td>0.935</td>
</tr>
<tr>
<td>4</td>
<td>0.559</td>
<td>0.619</td>
<td>0.869</td>
</tr>
<tr>
<td>5</td>
<td>1.101</td>
<td>0.813</td>
<td>0.643</td>
</tr>
<tr>
<td>6</td>
<td>0.574</td>
<td>0.840</td>
<td>1.277</td>
</tr>
<tr>
<td>7</td>
<td>1.079</td>
<td>0.712</td>
<td>1.443</td>
</tr>
<tr>
<td>8</td>
<td>0.822</td>
<td>0.531</td>
<td>0.910</td>
</tr>
</tbody>
</table>

Mean±SD 0.694±0.274 0.685±0.105 0.998±0.255* +

* significant difference between group 1 and group 3 (P<0.05)
+ significant difference between group 2 and group 3 (P<0.05)

Figure 1B. The average amounts (Ratio of IL-1β/β-actin) and standard deviation of gelatinase IL-1β in group 1, 2 and 3. In the inflamed gingiva with diabetes (group 3), IL-1β seemed to be increased compared to group 1 and group 2.

* significant difference between group 1 and group 3 (P<0.05)
+ significant difference between group 2 and group 3 (P<0.05)

Group 1: healthy gingiva from systemically healthy person
Group 2: Inflamed gingiva from patient with chronic periodontitis
Group 3: Inflamed gingiva from patient with chronic periodontitis and type 2 DM

358
Table 2. MMP-13 intensity was normalized by β-actin intensity to quantify the expression levels of MMP-13 expression and the value were presented as a ratio of MMP-13/β-actin.

<table>
<thead>
<tr>
<th>sample</th>
<th>group 1</th>
<th>group 2</th>
<th>group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.698</td>
<td>0.689</td>
<td>1.345</td>
</tr>
<tr>
<td>2</td>
<td>0.842</td>
<td>0.526</td>
<td>0.890</td>
</tr>
<tr>
<td>3</td>
<td>0.693</td>
<td>1.141</td>
<td>0.865</td>
</tr>
<tr>
<td>4</td>
<td>0.623</td>
<td>0.936</td>
<td>0.979</td>
</tr>
<tr>
<td>5</td>
<td>0.555</td>
<td>0.860</td>
<td>1.030</td>
</tr>
<tr>
<td>6</td>
<td>0.671</td>
<td>1.190</td>
<td>0.859</td>
</tr>
<tr>
<td>7</td>
<td>0.431</td>
<td>0.765</td>
<td>1.247</td>
</tr>
<tr>
<td>8</td>
<td>0.651</td>
<td>1.050</td>
<td>1.137</td>
</tr>
</tbody>
</table>

Mean±SD 0.645±0.119 0.894±0.230* 1.044±0.183 +

* significant difference between group 1 and group 2 (P<0.05)
+ significant difference between group 1 and group 3 (P<0.05)
Figure 3A. TIMP-1 Western analysis of 4 representative samples in each group. TIMP-1 corresponding to molecular weight 26.5 kDa was expressed in all samples including healthy gingiva and the expression levels of TIMP-1 were increased significantly in group 2, group 3 than in control subjects. In order to quantify the TIMP-1 levels, β-actin levels were also measured.

Group 1 : healthy gingiva from systematically healthy person
Group 2 : inflamed gingiva from patient with chronic periodontitis
Group 3 : inflamed gingiva from patient with chronic periodontitis and type 2 DM

Table 3. TIMP-1 intensity was normalized by β-actin intensity to quantify the expression levels of TIMP-1 expression and the value were presented as a ratio of TIMP-1/β-actin.

<table>
<thead>
<tr>
<th>sample</th>
<th>group 1</th>
<th>group 2</th>
<th>group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.554</td>
<td>0.751</td>
<td>0.708</td>
</tr>
<tr>
<td>2</td>
<td>0.684</td>
<td>0.987</td>
<td>0.808</td>
</tr>
<tr>
<td>3</td>
<td>0.703</td>
<td>1.056</td>
<td>0.695</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>0.628</td>
<td>0.98</td>
</tr>
<tr>
<td>5</td>
<td>0.574</td>
<td>0.926</td>
<td>0.672</td>
</tr>
<tr>
<td>6</td>
<td>0.589</td>
<td>0.61</td>
<td>0.824</td>
</tr>
<tr>
<td>7</td>
<td>0.747</td>
<td>0.71</td>
<td>0.893</td>
</tr>
<tr>
<td>8</td>
<td>0.719</td>
<td>0.509</td>
<td>0.866</td>
</tr>
</tbody>
</table>

Mean±SD 0.642±0.0789 0.797±0.185* 0.806±0.108+

* significant difference between group 1 and group 2 (P<0.05)
+ significant difference between group 1 and group 3 (P<0.05)

Figure 3B. The average amounts (Ratio of TIMP-1/β-actin) and standard deviation of TIMP-1 in group 1, 2 and 3. In the inflamed gingiva (with or without diabetes, group 2 & 3), TIMP-1 seemed to be not increased compared to healthy gingiva. And the difference was not statistically significant (P>0.05).

* significant difference between group 1 and group 2 (P<0.05)
+ significant difference between group 1 and group 3 (P<0.05)

Group 1 : healthy gingiva from systematically healthy person
Group 2 : inflamed gingiva from patient with chronic periodontitis
Group 3 : inflamed gingiva from patient with chronic periodontitis and type 2 DM
± 0.183 in group 3. The MMP-13 expression levels in group 3 and 2 were higher than in group 1 (P<0.05).

In the case of MMP-13, the significant difference was observed between the control group and the chronic periodontitis group, but the difference between the chronic periodontitis groups and the chronic periodontitis with type 2 DM group was not statistically significant. In the study of TIMP-1 expression levels using Western blot analysis, molecular weight of TIMP-1 was identified as 28.5kDa size(Figure 3A). The normalized quantification data were presented in Table 3. The mean amounts of TIMP-1 expression (ratio of TIMP-1/β-actin) were 0.642 ± 0.0789 in group 1, 0.797 ± 0.185 in group 2 and 0.806 ± 0.108 in group 3.

The significant difference was observed between the control group and the chronic periodontitis with type 2 DM group. In the interrelationship of IL-1β, MMP-13 and TIMP-1 expression was analyzed, as expression of IL-1β, MMP-13 were increased, TIMP-1 expression showed increasing tendency in chronic periodontitis associated to type 2 DM, although there were no proportional relationship between the interrelationship of IL-1β, MMP-13 and TIMP-1 expression.

IV. DISCUSSION

Multiple studies have demonstrated the link between diabetes and periodontal disease in human subjects. Although diabetes itself does not cause periodontitis, periodontal disease progresses more rapidly and leads to more tooth loss in patients whose diabetes is poorly controlled. Various pathogenetic factors have been suggested to explain the increased prevalence and severity of periodontitis in diabetes. Makela and colleagues reported that during active phase of periodontitis, IL-1β and MMP-13 levels in gingival crevicular fluid are pathologically elevated and Collin et al. found that MMP-13 in saliva tissue is elevated and they suggested that MMPs may have a key role of periodontal destruction. The gene expression of MMPs is regulated by inflammatory mediators, such as cytokines and prostaglandins.

It was known that the major cause of tissue resorption in periodontal diseases is the interaction between bacterial antigens and inflammatory cells leading to the production of IL-1β.

Naturally occurring MMP inhibitors, such as TIMP(Tissue Inhibitors of Metalloproteinase), are important controlling factors in the actions of MMPs, and tissue destruction in disease processes often correlates with an imbalance of MMPs over TIMP. One of the major MMP inhibitor is TIMP-1 (or TIMP) glycoprotein that is synthesised by most cells. A second unglycosylated inhibitor, TIMP-2, which is less abundant, has the interesting property of binding to the proform of gelatinase A and is involved in controlling its activation.

The expression of MMPs and TIMPs by
cells is regulated by many cytokines (particularly, IL-1), growth factors and hormones, some of which are specific to cell type and others are ubiquitous (e.g., transforming growth factor β, TGF-β). Many of these factors are products of monocytes/macrophages and their production in inflammatory situations is therefore part of the chain of events leading to tissue degradation. From many recent studies it seems that tissue destruction, both physiologic and pathologic, is correlated with an imbalance of inhibitors over proteases.

The MMP-13 is thought to play an important role in the degradation of denatured collagens (gelatin), basement membrane (type IV collagen) and other matrix. The degenerative process causes the loss of attachment apparatus between tooth and epithelium, connective tissue which accelerates inflammatory process resulting in deepening of periodontal pocket. Several studies on the expression and activities of these MMPs were executed in saliva and crevicular fluid.

But direct study on the expression of IL-1β, MMP-13 and TIMP-1 in diabetic gingival tissue related to periodontitis is limited. The purpose of this study was to quantify and compare the expression of IL-1β, MMP-13 and TIMP-1 in the gingival tissues of the patients with chronic periodontitis associated to type 2 DM, in order to understand the contribution of these proteins to periodontal destruction in type 2 diabetic patients, especially MMP-mediated host response in type 2 diabetic patients.

The amount of IL-1β expression was higher in inflamed gingiva with chronic periodontitis associated to type 2 DM than in healthy gingiva from systemically healthy person and inflamed gingiva from patients with chronic periodontitis. The differences among three groups were statistically significant between group 1, 2 and group 3 (P<0.05). Table 1, Figure 1B) There is little difference between healthy gingiva from systemically healthy person and inflamed gingiva from patients with chronic periodontitis. These results were different comparing to Honig et al’s report that increasing pattern in inflamed tissue. It is assumed that IL-1β was activated in early stage of inflammation, but our samples were in the later stage of inflammation and the degree of inflammation severity and turn over rate of IL-1β in each sample were considered.

When the expression levels of IL-1β between non diabetic patients with chronic periodontitis and chronic periodontitis with type 2 DM were compared, chronic periodontitis with type 2 DM showed higher level than chronic periodontitis without DM. These findings seen in human diabetes are strongly supported by the observation in rat models of insulin-deficient diabetes. These animal model studies have shown reduced collagen solubility and turnover resulting evidently from the formation and action of elevated levels of advanced glycation end-products (AGEs). AGEs can induce the expression of MMPs through the enhanced
production of IL-1β, vascular hardening and basement membrane disintegration, reduced solubility and decreased turnover rate. Reactive oxygen species are produced to a significant extent by triggered degranulating neutrophils, to a lesser extent by other cells such as monocytes and macrophages. Collin et al. reported that the role of oxidative stress in diabetes can exert multifactorial action regarding the development of periodontitis and it can be potent non-prototype pro-MMP activators.

The amounts of MMP-13 expression were higher in chronic periodontitis group of systemic health person and chronic periodontitis with type 2 DM compared to healthy gingiva from systemically healthy person. The levels in inflamed gingiva with type 2 DM were a little higher than chronic periodontitis in systemic healthy person. It assumed that IL-1β increased MMP-13 and other cytokines was involved. The MMP-13 expression was showed a little increasing pattern in DM than systemic healthy group. This might be due to the fact that MMP-13 is secreted from PMNLs, inflammatory cells that can react immediately to any stimulus, and can be secreted easily by other various cytokines. It also might be due to that AGBs produced from diabetic environments enhances production of MMPs. MMP-13 is a significant enzyme because it appears to be released from fibroblasts and pocket epithelial cells during tissue destruction.

The intensity of MMP activity correlated with the activity of extracellular matrix breakdown and remodelling of the inflammatory sites. These differences in cell lines could influence on inflammation resulting in higher expression level of MMP-13. In diabetes, the same matrix metalloproteinases involved in the development of periodontitis as the sixth long-term complication of diabetes have also been associated with other well known complications of diabetes such as nephropathy, angiopathy, retinopathy and wound-healing problems. It was reported impaired PMN function in diabetes regarding to chemotaxis, chemokinesis and degranulation. This might explain the increased susceptibility of diabetic patients to various infectious diseases including periodontitis. These results make it possible to consider that PMN dysfunction may be reflected in the gingival neutrophil degranulation product which is released from neutrophils recruited to inflammatory gingiva. In addition, it couldn't be excluded that age, blood glucose and other unknown factors influence on the results although sex difference was not found among groups.

A major group of inhibitors of the MMPs, TIMPs, has been studied in this study. The levels of TIMP were significantly different between control group and inflamed gingiva either with or without type 2 DM. But the levels were similar expression between inflamed gingiva with systemic healthy gingiva and inflamed gingiva with type 2 DM. In inflammatory response, as the level of MMP-13 increased, TIMP-1 level was increased, but in case of inflamed gingiva.
with type 2 DM, MMP-13 value was not proportional to TIMP-1. In inflamed gingiva from patient with chronic periodontitis and type 2 DM, there was no significant difference between group 2 and group 3. The TIMP-1 is a glycoprotein and is synthesized and secreted by most connective tissue cells as well as by macrophages. It also resides in platelet α granules and could be identified in most body fluids. The amino acid sequences of human TIMP-1 were largely conserved across species that is it forms high affinity, essentially irreversible, non-covalent complexes with the active forms of MMPs. Drouin et al. detected the presence of TIMP-1 and interstitial collagenase in saliva of healthy controls and detected TIMP-1, but not collagenase, in parotid and submandibular saliva. The expression of MMPs and TIMPs by connective tissue cells in culture is regulated by a number of cytokines (particularly IL-1), growth factors and hormones. It assumed in this result that decreasing ability of TIMP-1 production in tissue and other inflammation mechanism existed in DM compared to general inflammation tissue in healthy person.

In interrelationship between IL-1β, MMP-13 and TIMP-1, as IL-1β and MMP-13 expressions showed increasing tendency in chronic periodontitis associated to type 2 DM, expression of TIMP-1 were increased in Group 3 than Group 2, but, there were no significant interrelationship among IL-1 β, MMP-13 and TIMP-1 (P>0.05). In inflamed gingiva from patient with chronic periodontitis and type 2 DM, despite of increasing IL-1β and MMP-13, TIMP-1 values were not different from the inflamed gingiva from patient with chronic periodontitis. It assumed that there was higher inflammatory responses in chronic periodontitis associated to type 2 DM. Thus several possible reasons for the results are suggested. First, according to the effect of antimicrobial periodontal treatment on circulating TIMP-1 and glycated Hb level in patient with type 2 DM and other studies by Hayakawa et al., the circulating TIMP-1 itself was very low. So it can be suggested that the difference on TIMP-1 between the groups was not significant enough to influence solely on the expression of IL-1β and MMP-13. Second, the changes in MMPs synthesis and activity might be time dependent. After TIMP-1 produced by monocytes or adipocytes acted on the target cells that were activated at the early stages of inflammation, the immune cells that arrived in the later stage or other resident gingival cells (fibroblast or epithelial cells) activated by TIMP-1 could start to produce MMPs. Bjeil et al. reported that MMP-13 showed significant increase in the later stage of inflammation. Third, though the concentration of TIMP-1 expressed was not enough to show the difference of MMP expression between the groups, the target cells to TIMP-1 in the inflammatory areas are increased in periodontitis group compared to control group which might enhance the chance of activation. Fourth, the activity of MMPs is tightly regulated at sev-
eral levels including gene expression, secretion of pro-enzymes that require activation and inhibition by TIMPs. Death et al. found that high glucose may regulate MMP gene expression via its effects on gene transcription or growth factors through the increased activation of its own response elements.

In conclusion, this study demonstrated that the expression levels of MMP-13 and TIMP-1 had an increasing tendency in inflamed tissue. It can be assumed that IL-1β and MMP-13 may be partly involved in the progression of periodontal inflammation associated to type 2 DM.

Finally, it seemed that more studies are needed to investigate the effect and inter-relationship between MMPs and other cytokines that affect the progression of periodontal disease at a higher level.

V. SUMMARY

Gingival tissue samples were obtained during periodontal surgery or tooth extraction. According to the patient's systemic condition & clinical criteria of gingiva, each gingival sample was divided into three groups. Group 1 (n=8) is clinically healthy gingiva without bleeding and no evidence of bone resorption or periodontal pockets, obtained from systematically healthy 8 patients. Group 2 (n=8) is inflamed gingiva from patients with chronic periodontitis. Group 3 (n=8) is inflamed gingiva from patients with chronic periodontitis associated with type 2 diabetes. Tissue samples were prepared and analyzed by Western blotting. The quantification of IL-1β, MMP-13 and TIMP-1 were performed using a densitometer and statistically analyzed by one-way ANOVA followed by Tukey test.

1. The expressions of MMP-13 and TIMP-1 showed increasing tendency in group 2 & 3 compared to group 1.
2. The expressions of IL-1β & MMP-13 were showed increasing tendency in group 3 compared to group 2.
3. As IL-1β levels were increasing, MMP-13 showed increasing tendency in group 3, and although IL-1β, MMP-13 levels were increasing, TIMP-1 levels were similar expressed comparing to group 2.

In conclusion, this study demonstrated that the expression levels of MMP-13 and TIMP-1 had an increasing tendency in inflamed tissue. It can be assumed that IL-1β and MMP-13 may be partly involved in the progression of periodontal inflammation associated to type 2 DM.

VI. REFERENCES

3. Melik MC, Atkinson SI, Ward RV, Murphy G and Reynolds JI. Gingival fibroblasts degrade type I collagen fibres when stimulated with tumor necrotic factor and interleukin 1 : Evidence that break


21. Yalda B, Offerbacher S and Collins JG.
22. Bissada NP, Manoucher-Pour M, Haddock M and Spagnuolo PJ. Neutrophil functional
23. Armitage GC. Development of a classification system for periodontal disease and
activity during diabetes: Preliminary observations and a proposed new mechanism
32. Lalla E, Lamster IB, Felt M et al. Blockade of RAGE suppresses periodontitis
33. Ide M, Jegadev D, Coward PY et al. The short-term effects of treatment of chronic
periodontitis on circulating levels of endotoxin, C-reactive protein, tumor necrosis
35. Tuter G, Kurtis B and Serdar M. Effects of Phase I Periodontal Treatment on
Gingival Crevicular Fluid Levels of Matrix Metalloproteinase-1 and Tissue inhibitor of
36. Drozin L, Overall CM and Sodee J. Identification of matrix metalloproteinase
39. Hayakawa H, Yamauchi K and Ohwaki K. Collagenase activity and tissue inhibitor of
metalloproteinases-1(TIMP-1)content in human whole saliva from clinically healthy


단순 만성 치주염 환자 및 2형 당뇨병환자의 만성 치주염 치은조직에서 염증성 매개인자의 발현 양상 비교

주상돈, 이재목 *
경북대학교 치과대학 치주과학교실

치주질환의 병원균은 세포벽의 항원에 의하여 조직내 존재하는 mononuclear phagocytes가 활성화되어 cytokine들이 생성됨으로써 치주 결체조직의 파괴를 진행시킨다. 이런 판련된 cytokine들은 순차적으로 상주하는 치온세포 및 대식세포가 Matrix metalloproteinase 합성을 하도록 유도하여 조직파괴를 시작한다. 당뇨병은 치주질환의 위험요소 중 하나로 당뇨 환자에서는 치주질환의 유병율이 일반인에 비해 높고 치주질환의 중증도도 더 심하게 진행한다고 알려져 있다.

그 병리 기전 중 하나로 당뇨 환자에서는 혈관 염증 내 중성구 유래의 Matrix metalloproteinase의 활성 증가 및 TIMP-1 발현의 변화가 추정되고 있으나 아직 치은조직에서 이에 대한 연구는 미흡한 실정으로 이에, 본 실험에서는 제2형 당뇨병 환자와 비당뇨 환자들에서 만성 치주염 부위의 치은 및 건강한 치온에서 염증 매개체 중 하나인 IL-1β, MMP-13 및 TIMP-1의 발현에 대해 상호 비교 분석함으로써 염증, 혈당이 미치는 영향을 밝히고 제2형 당뇨병 환자에서 심한 치주조직 파괴의 기전을 연구하고자 하였다.

경북대학교병원 치과 내원환자 중 제2형 당뇨병 환자와 비당뇨 환자들 및 치주질환이 없는 건강인 대조군을 대상으로 여러 가지 치주상태를 기록하고, 전신적으로 건강한 환자의 건강한 부위(n=8, Group 1), 전신적으로 건강한 환자의 만성 치주염 부위(n=8, Group 2), 제2형 당뇨병 환자의 만성 치주염 부위(n=8, Group 3)에서 각각 변연치은을 채득하고 액화질소에 급속 동결하였다. Western blotting을 이용하여 각 조직 내 IL-1β, MMP-13 및 TIMP-1의 발현을 관찰, densitometer를 이용하여 상대적 발현을 정량, 각 조직의 β-actin을 이용하여 표준화하여 실험군과 대조군들의 평균치를 비교하였다.

비당뇨 환자들의 만성 치주염 부위 및 제2형 당뇨병 환자들의 만성 치주염 부위에서 모두 건강대조군에 비해, MMP-13과 TIMP-1의 발현이 증가되었으며, 또한 IL-1β와 MMP-13는 2형 당뇨 환자의 만성 치주염 부위가 비당뇨 환자의 만성 치주염 부위보다 증가된 발현량상을 보았다. 2형 당뇨 환자의 만성 치주염 부위에서 IL-1β가 증가함에 따라 MMP-13이 증가하였으며, IL-1β 및 MMP-13이 증가하였지만 TIMP-1 수치는 비당뇨 환자의 만성 치주염환자와 유사한 양상을 보였다. 결론적으로 MMP-13과 TIMP-1이 염증조직에서 증가하는 양상을 보였으며 IL-1β, MMP-13이 2형 당뇨환자의 만성 치주염 진행과정에 부분적인 기여 인자로써 역할을 하는 것으로 생각된다.

Key words: MMP-13, TIMP-1, chronic periodontitis, diabetes mellitus

369