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MMP-2, MMP-8 Expression in gingival tissue of Chronic Periodontitis associated to Type 2 Diabetes Mellitus

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Ⅰ. Introduction

Diabetes mellitus(DM) in itself does not cause periodontitis. However, there are epidemiologic evidences that diabetes predisposes and accelerates the irreversible periodontal destruction. 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[1-4].

Accumulation of plaque is not related directly to the early onset and rapid progression of periodontal tissue destruction in diabetes. Various pathogenic factors have been suggested to explain the increased prevalence and severity of periodontitis in diabetes. An exaggerated inflammatory response to potential periodontopathogenic bacterial virulence factors, such as lipopolysaccharide(LPS), may influence the periodontitis in diabetes. Moreover, an impaired recruitment and cellular function of polymorphonuclear leukocytes(PMNLs) has been linked to extensive periodontal tissue destruction[1-3].

Structual studies on diabetic patients have revealed vascular changes in gingiva and other tissues, a thickening of subendotherial basement membrane and, paradoxically, an increased permeability of basement membrane to virulent pathogenic microflora and to its potential vilurent factors[5-7]. Futhermore, a prolonged exposure to hyperglycemia can be primary factor responsible for the formation of non-enzymatic advanced glycosylation end-products(AGEs), demonstrated recently in periodontal tissues of diabetic patients. AGEs are further responsible for

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diabetic collagen cross-links, which again lead to the above-mentioned microvascular complications such as vascular hardening and basement membrane disintegration[2,8,9]. AGEs also bind to macrophage receptors and induce a cycle of cytokine(Interleukin-1:IL-1, Tumor Necrosis Factor-α:TNF-α) upregulation. These can, in turn, induce an elevated expression of matrix metalloproteinases(MMPs) in diabetic periodontal tissue[10-12].

MMPs are a family of at least 16 secreted and membrane-bound zinc- and calcium- dependent protease which function in the degradation and remodeling of extracellular matrix proteins during different developmental processes such as organ morphogenesis, bone formation, angiogenesis, and remodeling during reproductive processes, as well as in pathological processes such as inflammation, chronic degenerative disease and tumor invasion[13].

In summary, they are responsible for degradation of extracellular matrix components such as collagen and proteoglycans. MMPs, produced by both infiltrating and resident cells of the periodontium, play a role in physiologic and pathologic events. It is recognized that an imbalance between activated MMPs and their endogenous tissue inhibitors of metalloproteinases(TIMPs) leads to pathologic breakdown of the extracellular matrix during chronic periodontitis[14-17].

Of all MMPs, matrix metalloproteinase-2 (MMP-2, 92 kDa gelatinase A) is the most widely distributed. MMP-2 has been identified in fibroblast, keratinocytes, endothelial cells, monocytes/macrophages, osteoblast, odontoblast and chondrocytes. And expression of MMP-2 in periodontium is localized mainly in fibroblast and granulation tissue of periodontal connective tissue(CT). In human periodontium, MMP-2 affects integrity of basement membrane and attachment of junctional epithelium and elevated MMP-2 expression level in chronic periodontitis is already reported in several investigations[15-20]. These results suggest the involvement of MMP-2 in periodontal destruction.

The major collagenase species detected in inflamed human periodontium is inductive collagenase-2 or MMP-8 rather than constitutively expressed fibroblast-type MMP-1, which is more associated with normal tissue remodeling.[8,13] MMP-8 is mainly a polymorphonuclear leukocyte-(PMN-) specific matrix metalloproteinase stored in specific granules of PMNs.[13,17] When neutrophils are recruited to a site of inflammation, they release large quantities of MMP-8 stored in specific granules, and the inflammatory response is sustained by recruitment of new cells rather than by the local synthesis of MMP-8. MMP-8 activity and release are regulated by factors such as cytokines(TNF-α or IL-1β) and various bacterial virulence factors that affect MMP-8 release by PMN degraulation following PMN infiltration to the site of inflammation.[2] During active phase of peridontitis, MMP-8 levels in GCF & inflammed gingival tissue are pathologically elevated, and may have a key role of periodontal destruction.[13,14] On the other hands, several studies show evidences that type 2 diabetes mellitus induces dysregulation of the MMP and

Tissue Inhibitors of Metalloproteinase (TIMP) system in human gingival tissue [3,8,21]. The effect of type 2 diabetes on MMP and TIMP expression effectively amplifies the degradation arm of the MMP/TIMP system that could lead to net activation of matrix breakdown.

Because investigations to examine MMP-2, MMP-8 expression in diabetic gingival tissue related to periodontitis are limited, we investigated the effect of type 2 diabetes mellitus on MMP-2, MMP-8 production in human gingival tissue with chronic periodontitis. The purpose of this study is to quantify and to compare relation of the expression of MMP-2, MMP-8 in the gingival tissue of patients with type 2 DM and systemically healthy adults with chronic periodontitis.

Ⅱ. Materials and Methods

1. Study population and Tissue sampling

Study population comprised 8 patients with type 2 diabetes mellitus aged 36-68 years and 16 non-diabetic control subjects of the similar age group. Before surgery, patient's systemic condition(age, sex, blood glucose level) and clinical criteria of gingiva(Sulcus Bleeding Index according to Muhlemann and Son[22]:SBI, pocket depth) were recorded. Avoiding local anesthetic infiltration into biopsy site, marginal gingival tissue samples were obtained during periodontal surgery or tooth extraction following informed consent.

Each gingival samples were divided into three groups based on the patient's systemic condition & clinical status of gingiva (gingival color, gingival bleeding, probing depths, and radiographic evidence of bone resorption).

Group 1(normal, n=8, mean age 41 [30 to 60], 5 males and 3 females) is clinically healthy gingiva obtained from 8 systemically healthy adults. Gingival samples were obtained during crown lengthening procedure from tooth with sulcus depth ≤3 mm, no loss of attachment and no inflammatory signs. Mean glucose level was 110.87 mg/dl. Group 2(chronic periodontitis, n=8, mean age 40 [33 to 48], 5 males and 3 females) is inflammed 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 diseases and conditions[23]. All patients in group 2 were systemically healthy and had more than one periodontal pocket of ≥5 mm depth and at least one pocket with ≥4 mm loss of attachment. All gingival samples were obtained during periodontal flap surgery, gingivectomy or extraction from teeth with probing depth ≥5 mm, swelling of the marginal gingiva and bleeding corresponding to Sulcus Bleeding Index 3. Mean glucose level was 103.25 mg/dl and this value was slightly lower than that of group 1. Group 3 (chronic periodontitis associated to type 2 DM, n=8, mean age 55 [36 to 71], 6 males and 2 females) is inflammed gingiva from type 2 diabetic patients with chronic periodontitis. Patients in group 2 and 3 had

similar periodontal conditions, but systemically, patients in group 2 were healthy and patients in group 3 were suffering from type 2 diabetes mellitus. Mean glucose level was 163.57 mg/ dl in FBS and 228.57 mg/dl in 2PP. Diabetic control was performed by insulin medication in all 8 patients and, additionally, diet control was also performed in 3 of them. Gingival samples were obtained by similar way described above.

The sample cohort consisted of 8 clinically healthy, 8 inflammed and 8 diabetic patients' inflammed samples from total of 24 subjects. Following surgery, excised tissue specimens were immediately placed on liquid nitrogen and subsequently frozen(-70℃).

2. Tissue preparation, Protein Isolation and Western blotting

For Western blotting, as previously described technique by Cho,[24] frozen tissues were homogenized in RIPA lysis buffer(10 mM phosphate buffer, pH 7.4, 10% glycerol, 1% NP-40, 0.1% SDS, 4 mM EDTA, 0.15 M NaCl) with 1:30 diluted protease inhibitor cocktail(Roche). The lysates were sonicated 3 times for 10 seconds and centrifuged at 12,000 g for 15 minutes. Protein concentration of supernatant were routinely determined by a Bradford protein assay using Bio-Rad protein assay reagent with BSA as a standard.

Lysates were boiled in SDS sample buffer (1M Tris-Cl(pH 6.8), 40% glycerol, 8% SDS, 2%-mercaptoethanol, 0.002% Bromophenole blue). Prepared samples were separated by 10% sodium dodecyl sulfate(SDS)-polyacrylamide gels and transferred to a polyvinylidene difluride membrane. The membranes were subsequently blocked in Tris-buffered saline(TBS) containing 5% powdered milk and 1% BSA for 1 hours, and then incubated with polyclonal anti-MMP-2 antibody(Sigma-Aldrich Corporation, ST. Luis, U.S.A, prepared in rabbits, diluted 1: 1000 in TBS/1% BSA) for 1.5 hours at room temperature. The membrane was washed (five times for 5 minutes with Tween 20) and incubated with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody(diluted 1: 1000 in TBS) for 1 hours at room temperature. After additional washing(five times for 5 minutes with Tween 20) the Western blot procedure was completed with an ECL Advance development kit(Amersham, Beckinghamshire, U.K.)

The quantitative analysis(ratio of MMP-2, MMP-8/β-actin) of MMP-2 MMP-8 expression was performed using a densitometer (Image Gauge V 3.46, Koshin Graphic Systems, FUJI PHOTO FILM CO, Japan). After normalization to β-actin(Abcam Ltd., Cambridge Science Park, UK) in each sample, level of MMP-2 was expressed as a ratio(ratio of MMP-2, MMP-8/β-actin) and the differences of densities among 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 relative MMP-2, MMP-8 levels among each 3 groups were compared

using one way ANOVA followed by Scheffe test. The criterion for statistical significance was defined as $P<0.05$.

Ⅲ. Results

Antibodies to MMP-2, MMP-8 cross-reacted with 72, 60kDa molecular weight of MMP-2, MMP-8 in 3 groups. In order to quantify detected MMP-2, MMP-8 normalization to β-actin was performed. The quantification(ratio of MMP-2, MMP-8/β-actin) of MMP-2, MMP-8 in each sample was performed using a densitometer. The average amounts of MMP-2, MMP-8 among 3 groups were compared in Table 1, 2 and Figure 1, 2, 3. Although age, glucose level and SBI value were similar within the same group, various amount of MMP-2, MMP-8 was detected in each samples.

As to mean MMP-2 levels in each 3 groups, the amount of MMP-2(ratio of MMP-2/β-actin) was 2.108, 2.560 and 3.266 in group 1, 2 and 3. There were some tendencies that mean amount of MMP-2 was rather increased in inflamed gingiva compared to healthy gingiva and was more increased in type 2 diabetic gingival tissue. That is, the amount of MMP-2 was increased in group 2 compared to group 1. Also, increased amount of MMP-2 was seen in group 3 compared to group 2. But the differences among 3 groups were not statistically significant ($P>0.05$).

As to mean MMP-8 in each 3 group, the amount of MMP-8(ratio of MMP-2/β-actin) was 2.380, 2.783, 3.682 in group 1, 2, 3. The amount of MMP-8 was increased in group 2 compared to group 1. Also, increased amount of MMP-8 was observed in group 3 compared to group 2. But the difference among 3 groups was not statistically significant($P>0.05$).

That is, the amount of MMP-2, MMP-8 was increased in group 2, 3 compared to group 1. Also, increased amount of MMP-2, MMP-8 was observed in group 3 compared to group 2. But there were no statistically significant difference in among 3 groups ($P>0.05$)

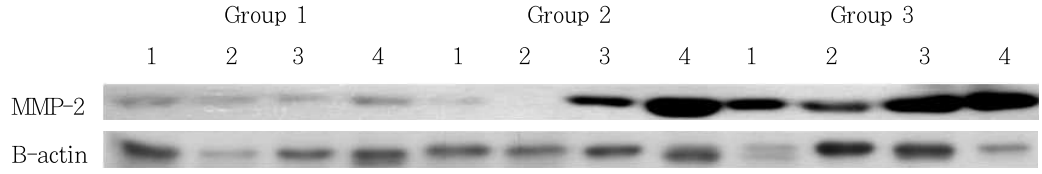

Figure 1. Western analysis of samples for MMP-2. MMP-2 corresponding to molecular weight 72 kDa was expressed in all samples including healthy gingiva, and expression of MMP-2 was increased in group 3 than in group 1 subjects. In order to quantify detected MMP-2, normalization to β-actin was performed.

Group 1 : healthy gingiva from systemically healthy patient
Group 2 : inflammed gingiva from systemically healthy patient with chronic periodontitis
Group 3 : inflammed gingiva from type 2 diabetic patient with chronic periodontitis

Table 1. The quantitative analysis(ratio of MMP-2/β-actin) of MMP-2 of each sample was performed using a densitometer and average amount of MMP-2 in 3 groups were identified. The differences between 3 groups were not statistically significant(P>0.05).

sample \ Group	1	2	3
1	2.210	1.422	3.619
2	2.676	0.690	2.019
3	2.413	3.821	2.958
4	1.829	4.867	6.071
5	2.116	2.290	2.713
6	1.332	2.730	3.047
7	1.984	2.368	3.046
8	2.304	2.296	2.655
Mean ±SD	2.108±0.407	2.560±1.304	3.266±1.219

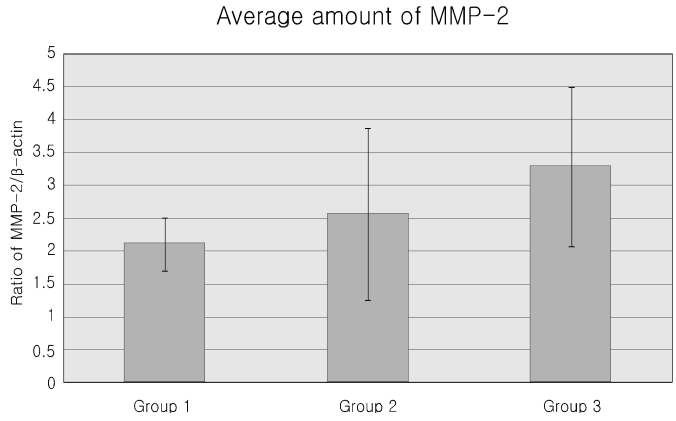

Figure 2. Mean amounts(ratio of MMP-2/β-actin) and standard deviation of MMP-2 in group 1, 2 and 3. In the inflamed gingival(with or without diabetes, group 2 and 3), MMP-2 was increased compared to healthy gingiva. But the differences were not statistically significant(P>0.05).

Table 2. The quantification(ratio of MMP-2/β-actin) of MMP-8 of each sample was performed using a densitometer and average amount of MMP-8 in 3 groups were identified.

sample \ Group	1	2	3
1	2.754	5.334	1.785
2	2.692	2.133	4.948
3	3.629	2.429	5.029
4	0.724	2.334	3.659
5	2.253	2.150	3.270
6	2.614	2.446	4.706
7	2.173	2.189	3.423
8	2.202	3.249	2.638
Mean ±SD	2.380±0.818	2.783±1.092	3.682±1.158

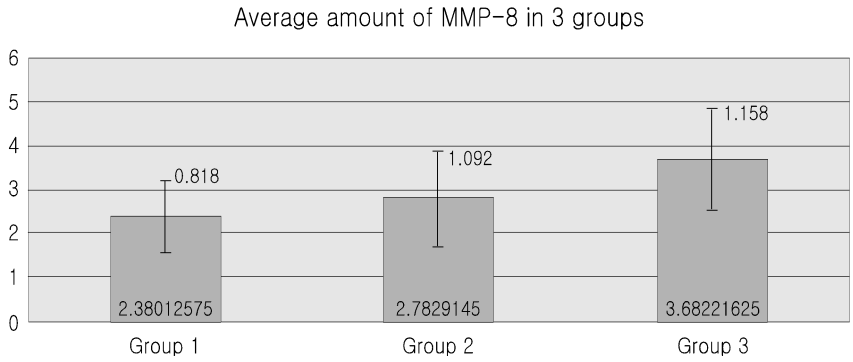

Figure 3. Mean amounts(ratio of MMP-8/β-actin) and standard deviation of MMP-8 in group 1, 2 and 3. In the inflamed gingiva(with or without diabetes, group 2 and 3), MMP-8 was increased compared to healthy gingiva. In group 3, the increase of MMP-8 was statistically significant compared to group 1(P>0.05).

Ⅳ. Discussion

The purpose of this study was to investigate the effect of type 2 diabetes mellitus on MMP-2, MMP-8 production in human gingival tissue with chronic periodontitis.

MMP-2, MMP-8 corresponding to molecular weight 72, 60kDa was expressed in most samples. The quantitative analysis(ratio of MMP-2,-8/β-actin) of MMP-2, MMP-8 level in each sample was performed using a densitometer and we could find some tendencies that mean amount of MMP-2, MMP-8 was rather increased in inflamed gingiva compared to healthy gingiva and was more increased in type 2 diabetic gingival tissue.

Enhanced expression of MMP-2 in chronic periodontitis patient and its activation mechanisms were investigated in several studies[15,17,20]. For instance, Anne-Laure Ejeil[15] et al studied the relation between degree of gingival inflammation and expression of MMPs, and showed that active forms of MMP-2 are highly increased in inflamed gingiva. About its overexpression mechanism, Chang et al[25] studied the regulation of MMP-2 synthesis and secretion in human periodontal ligament fibroblast(PDLF) and found that periopathogens, such as P. gingivalis and A actionomycetemcomitans, and IL-1α can elevate MMP-2 secretion in human PDLF. In periodontitis, PDL cells are a major group of cells highly affected by the P. gingivalis product. Pattamapon et al[26] observed MMP-2 activation in human periodontal ligament fibroblast by adding P. gingivalis supernants in vitro study and Grayson et al[27] observed elevated MMP-2 levels in gingival crevicular fluid of chronic periodontitis patient and concluded that P. gingivalis produces typsin-like proteases and these activated latent pro MMP-2 to active form in concentration- and time- dependent manner.

Therefore, it was suggested that enhanced expression of MMP-2 in periodontal disease is mainly related to increase of P. gingivalis, A. actinomycetemcomitans and IL-1 etc. However investigations in chronic periodontitis associated to type 2 DM are limited and, to our knowledge, no regulatory mechanisms of MMP-2 in type 2 diabetic gingival tissue were identified or suggested.

On the other hands, several studies demonstrate that MMP-2 & MMP-9 are major MMPs highly affected by type 2 diabetes in other human tissue.[5-7,28,29] Alison et al[7] studied the regulation of MMP-2 synthesis and secretion in 2 key vascular cells, endotherial cells and macrophages, and found that hyperglycemia can increase MMP-2 gene expression in these cell types thereby accelerating atherogenesis in diabetes. Wall et al[28] incubated diabetic dermal fibroblast, assessed MMP-2 production by gelatin zymography and western blotting and concluded that human diabetic dermal fibroblasts isolated from unwounded skin show significantly increased production of pro-MMP-2 when compared with their nondiabetic counterparts. Therefore type 2 DM-associated overexpression of MMP-2 in periodontium can be also expected and this is supposed by the fact that type 2 DM can upregulate AGEs-induced IL-1 and can alter fibroblast in human periodontal connective tissue. In addition, increased susceptability to periopathogens, especially A. actionomycetemcomitans and P. gingivalis, in diabetes can be additional factor. In human periodontium, expression of MMP-2 is localized mainly in fibroblast of CT cells. Fibroblast in PDLs and CT are a major group of cells highly affected by P. gingivalis products and can elevate MMP-2 expression if affected.

MMP-8 is the major collagenase species detected in inflamed human periodontium, and MMP-8 present in gingival tissue in all subjects are predominantly derived from degranulating triggered neutrophils.[13,14,17,] To a minor extent they can be derived from the non-PMN lineage cellular sources such as

resident fibroblasts, endothelial and epithelial cells.[30,31] During periodontitis, MMP-8 released from PMNs, in a latent, inactive proform becomes activated by the independent and/or combined actions of host- and microbial-derived proteases and reactive oxygen species(ROS) produced by triggered PMNs. MMP-8 synthesis by neutrophils is completed during granulocyte precursor cell differentiation in the bone marrow. When neutrophils are recruited to a site of inflammation, they release large quantities of MMP-8 stored in specific granules, and the inflammatory response is sustained by recruitment of new cells rather than by the local synthesis of MMP-8.[32]

In diabetes, neutrophils have been shown to exert various degrees of dysfunction regarding chemotaxis, chemokinesis and degranulation.[33] This is suggested to explain the increased susceptibility of diabetic patients to various infectious diseases including periodontitis. In our present study, although it is not statistically significant, the mean MMP-8 level was slightly higher in patients with type 2 diabetes mellitus than in control subjects. The results suggest that PMN dysfunction is not reflected in the gingival neutrophil degranulation product(i.e. MMP-8), which released from neutrophils recruited to inflammatory gingiva.

Elevated degree of activation of MMP-8 has been observed in GCF, gingival tissue and mouthrinse samples during active phases of periodontitis,[33] and suggested that may have a key role of periodontal destruction.[8,14] Also, previous studies have shown that diabetic patients with periodontitis have elevated MMP-8 levels in GCF,[14,30,31] and it seemed to be related to accelerated periodontal destruction in diabetes. The findings seen in human diabetes are strongly supported by observation in rat models of insulin-deficient diabetes.[32,33] These animal model studies have shown reduced collagen soubility and turnover resulting evidently from the formation and action of elevated levels of advanced glycation end-products (AGEs). These AGEs act on the target cells through the cell surface receptors, that can induce the expression of MMPs, adhesion molecules, cytokines and prostaglandins.[33] Elevated collagenase level in gingiva of rats with streptozocin induced diabetes strongly associate with non-enzymatic glycation of proteins, alveolar bone loss and other tissue destructive diabetic complications.

In these findings, elevated level of MMP-8 may related to MMP-2 expression each other and inflammation were in turn accelerated. Although statistically not significant, the mean MMP-8 level was slightly higher in inflammed gingiva than in healthy gingiva, and higher in patients with type 2 diabetes mellitus than in control subjects. Theses results have shown type 2 diabetes may more induce MMP-2, MMP-8 expression.

Because MMP-2, MMP-8 affects integrity of basement membrane and attachment of junctional epithelium, increased MMP-2, MMP-8 production induced by type 2 diabetes may have important consequences for periodontal destruction(eg. formation of periodontal pocket, disruption of extracellular matrix, alteration in periodontal wound healing etc).

These can be one of the reasons why chronic periodontitis associated to type 2 diabetes shows more periodontal breakdown compared to that in systemically healthy patients. Exacerbated periodontal inflammation further upregulates other MMPs & inflammatory cytokines and these, in turn, worsen metabolic control of diabetes. Thus, some reports have suggested that the inhibition of MMP activities can be a therapeutic strategy for periodontitis and type 2 diabetes.

In conclusion, this study has shown that expression of MMP-2, MMP-8 which increased by periodontal disease was rather upregulated by type 2 diabetes. expression levels of MMP-2, MMP-8 may affect to MMP expression each other and inflammation. However, this study is limited to small sample size and insufficient to establish definitive differences between chronic periodontitis groups with or without type 2 diabetes. To make definitive conclusion of the effect of type 2 DM on gingival MMP-2, MMP-8 levels, further studies including healthy gingiva from type 2 diabetes patients and large sample size are needed.

V. Summary

The purpose of this study was to quantify and compare the level of MMP-2, MMP-8 in the healthy, inflammed gingival tissue and inflammed gingival tissue associated with type 2 DM. We investigate whether expression of MMP-2, MMP-8 is increased by chronic periodontitis associated with type 2 DM.

Gingival tissue samples were obtained during periodontal surgery or tooth extraction. Based on patient's systemic condition & clinical criteria of gingiva, each gingival samples were 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 8 systemically healthy patients. Group 2(n=8) is inflammed gingiva from patients with chronic periodontitis. Group 3(n=8) is inflammed gingiva from type 2 diabetic patients with chronic periodontitis. Tissue samples were prepared and analyzed by Western blotting. The quantification of MMP-2, MMP-8 was performed using a densitometer and statistically analyzed by ANOVA.

MMP-2, MMP-8 was expressed in all samples including healthy gingiva and increased in group 3 compared to group 1 and 2. and showed that significant variation was observed between group 1 & 3 in MMP-8 results.

In conclusion, this study demonstrated that human gingival tissue with chronic periodontitis associated to type 2 diabetes showed slightly elevated MMP-2, MMP-8 levels compared to healthy gingiva and non-diabetic inflamed gingiva.

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-Abstract-

2형 당뇨병을 동반한 만성 치주염 환자의 치은조직에서 MMP-2, MMP-8의 발현 양상 비교

강민구 · 차현정 · 송선희 · 박진우 · 서조영 · 이재목

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치주질환은 치주지지 조직의 상실로 특징 지워지는 감염성 질환으로, 치주조직의 세포외 기질(특히 교원질)이 주요 degradation target이 된다. 현재로서는 MMP family가 주역을 담당하고 있음이 밝혀졌는데, 구강조직에서 가장 광범위하게 분포하는 것이 MMP-2, MMP-8이다. 치주조직에서 MMP-2는 주로 결합조직내 섬유아세포와 육아조직에서, 발현되며 , basement membrane의 integrity와 접합상피 부착에 영향을 줄 수 있고, MMP-8은 neutrophils에서 발현되어 다양한 염증세포와 염증진행에 영향을 주는 것으로 알려져 있다. 치주질환에서 MMP-2, MMP-9의 발현 증가는 이미 치은 열구액을 분석한 여러 연구에서 보고 되어 그 발현 수준이 치주질환 진단의 좋은 indicator가 될 수 있음이 시사되었다.

치주질환에서 MMP-2, MMp-8의 발현이 증가하는 것은 주로 P. gingivalis, IL-1과 연계되어 그 기전이 추정되어 왔는데, 이는 2형 당뇨병이 치주 질환의 심도에 영향을 미치는 기전과 많은 부분을 공유한다. 따라서 본 실험에서는 2형 당뇨병 환자와 비당뇨자에서 만성 치주염 부위의 치은, 건강한 치은에서 염증 매개체 중 하나인 MMP-2, MMP-8의 발현에 대해 비교 분석함으로써 염증, 혈당이 미치는 영향을 밝히고 2형 당뇨환자의 만성치주염 치은조직에서 치주조직 파괴의 기전을 연구하고자 하였다.

경북대 병원 치주과 내원 환자중 2형 당뇨병 환자와 비당뇨 환자를 대상으로 여러 가지 환자요소, 임상 치주상태를 기록하고, 비당뇨 환자의 만성 치주염 부위(n=8, group 1), 비당뇨 환자의 건강한 부위(n=8, group 2), 2형 당뇨병 환자의 만성 치주염 부위(n=8, group 3)에서 각각 변연 치은을 채득하고, 액화 질소에 급속 동결하였다. Western blotting을 이용하여 각 조직 내 MMP-2, MMP-8의 발현을 관찰, densitometer를 이용하여, 상대적 발현을 정량, 각 조직의 β-actin을 이용하여 표준화하여 실험군들과 대조군들의 평균치를 비교하였다.

그 결과 정상군에 비해 치주 환자군에서 채득된 시편에서 MMP-2, MMP-8의 발현이 증가하였으며, 염증군의 당뇨군과 비당뇨군 사이에서는 2형 당뇨병을 동반한 치주 환자군에서 가장 높게 나타났지만, 통계학적으로 유의한 차이를 나타내지는 않았다($P<0.05$).

결론적으로 본 연구에서 2형 당뇨병을 동반한 치주 환자군의 치은 조직에서 정상군과 비당뇨 치주 환자군보다 MMP-2, MMP-8의 발현이 다소 증가하는 양상을 보였다.

주요어 : 치주염, 당뇨병, Matrix metalloproteinase, MMP-2, MMP-8, 치은조직