ORIGINAL ARTICLE

EXPRESSION OF MATRIX METALLOPROTEINASE-8 (MMP-8) GENE IN PATIENTS WITH FIXED ORTHODONTIC APPLIANCES

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ABSTRACT

Orthodontic treatment with fixed appliance produces structural and biochemical changes and breaking the balance between the synthesis and the breakdown of the collagen in the periodontium. MMP-8 plays an important role in the remodeling of periodontal ligament during orthodontic movement. The purpose of this study was to observe the expression of MMP-8 gene in the gingival crevicular fluid (GCF) of fixed orthodontic patients. It is expected that the result can be used as a reference to decide the proper time for elastomeric chain to be reactivated.

Orthodontic fixed appliances were placed on 8 patients and elastomeric chains exerting a force of 75 grams were attached to produce canine distalization. GCF samples were collected from the distal side of upper canines before force application, 1-, 2-, 3- and 4 weeks after application consecutively. The samples were analyzed by using RT-PCR. Statistical analyses used were univariate analysis and Mann-Whitney U test.

The expression of MMP-8 in the GCF at t_0 was 31.3% but the force application elevated its expression of 65.6% at t_1, and then decreased continuously at t_2, t_3, and t_4. There was no statistically difference of MMP-8 gene expression between to and t_3.

The highest level of MMP-8 gene expression due to orthodontic forces happened in the first week, but it declined continuously in the following weeks. The proper time to reactivate an elastomeric chain was 3 weeks after application.

Key words: *MMP-8, Fixed orthodontics appliance, RT-PCR*

INTRODUCTION

The main goal of orthodontic treatment is to obtain an optimal function of occlusion and facial aesthetics. Research and clinical observation showed that the treatment will be stable if there is a balance between the teeth and surrounding soft tissue. Number of persons seeking help to improve the irregular position of their teeth have increased. The need for orthodontic treatment has increased not only in Indonesia but also in many other countries.[1,2,3]

The teeth will move if subjected to pressure, followed by changes in the connective tissues. In the past, orthodontic resorption process was related to the pressure side and deposition process on the strain side. Various results of recent research can be used to identify biological and diagnostic tools to monitor orthodontic tooth movement.[4]

The initial phase of orthodontic tooth movement usually involves certain reactions resembling inflammation characterized by vascular changes and migration of leukocytes out of periodontal ligament capillaries. These changes lead to cellular activation and of biologically active substances, such as enzymes and cytokines in the periodontal tissue.[5]

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After the application of pressure, there are structural and biochemical changes that disrupt the molecular balance between synthesis and degradation of collagen in the periodontal tissue. It shows that orthodontic tooth movement in humans, causing an increase in collagenase activity in gingival crevicular fluid (GCF), MMP (matrix metalloproteinase) plays a very important role in periodontal tissue remodeling.[6]

MMP-8 hydrolyzes most effectively collagen type I and III which are the major interstitial collagenases in human gingival inflammation.[7] It has been demonstrated that the expression of MMP-8 and MMP-13 mRNA in rat periodontal ligament was increased during active movement of teeth. Morphological and histo-chemical changes of periodontal ligament cells have been studied, however, there are only few studies on MMP expression on the periodontal tissue due to mechanical pressure.[8]

The purpose of this study was to observe the expression of MMP-8 gene in gingival crevicular fluid during orthodontic tooth movement on fixed appliance wearers. It is expected that the result might be used as a reference to decide the proper time to re-activate elastomeric chain.

METHODOLOGY

This study was conducted at a teaching dental hospital Hj, Halimah Dg. Sikati, Makassar, Indonesia for placing the fixed orthodontic appliances and at the Laboratory of Molecular Biology and Immunology, Faculty of Medicine, Unhas, Indonesia for analyzing samples using Reverse Transcriptase-PCR technique.

The number of subjects in this study was 8 orthodontic patients (6 females and 2 males, aged 19-25 years). All were undergoing fixed orthodontic treatment (Mini Roth bracket, 0.018 inch slot; Ormco, USA). Inclusion criteria for sample selection were; (1) age 18-30 years, (2) patients exhibiting maxillary protrusion and / or anterior crowding, (3). Based on Kesling's space analyses, they required premolar extraction, (4) showed good oral hygiene (5) showed no periapical/ periodontal diseases, (5) had no root anomaly in terms of shape and length, and (6) had never undergone orthodontic treatment. Patients with the following conditions were excluded: (1) suffering from systemic diseases like diabetes mellitus, (2) extreme position of the canines, and (3) gingival crevicular fluid mixed with blood.

GCF samples were collected from distal side of upper canine gingival crevices. The tooth surface was dried gently and kept dry with cotton rolls. Two paper points were inserted into the crevice for one minute and then discarded. The same method was repeated, and the paper points were placed into tubes with the buffered solution (L-6) insides. The samples were then frozen and kept at -20^0 until analyzed.

To move the canines distally, elastomeric chains exerting 75 grams were attached from buccal tube to canine's bracket. GCF samples were taken for 5 times, i.e, before attaching the elastomeric chain (to) one week after the attachment (t_1), (2) weeks (t_2), (3) weeks (t_3), and 4 weeks (t_4) after the attachment consecutively.

The GCF samples were extracted to get a total RNA. The RT-PCR analysis was performed by putting the following reagents into a microfuge tube : 6 µl Reverse Transcription buffer (Primecript, Takara, Japan), 1.5 µl specific primer for MMP-8 i.e. sense primer: TGGACCCAATGGAATCCTTGC and antisense primer: ATAGCCACTCAGAGCCCAGTA which generate 544-bp fragment, 1,5 µl enzyme mixt, 19.5 µl H_2O and 1.5 µl for 15 minutes, to allow the reverse transcription to work. Raise the temperature to 94°C for 2 minutes, 60°C for 2 minutes, 60°C for 2 minutes, and 72°C for 3 minutes. DNA hands were observed after 37 cycles of PCR. GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) was added to each sample served as an internal control/house-keeping gene for the entire process. The PCR product was loaded onto 2% agarose gel for electrophoresis and visualized with UV light after gel incubation in ethidium bromide solution. Laboratory test results with RT-PCR were expressed in a semi-quantitative score of 1-4 as follows;

- Score 1: the light of the DNA band was less bright than the control
- Score 2: the light of the DNA band was the same bright with the control
- Score 3: the light of the DNA band was slightly brighter than the control
- Score 4: the light of the DNA band was much brighter than the control

The data obtained from the study were processed electronically using SPSS software version 15.0, then analyzed by using Mann-Whitney U statistical method and univariate analysis.

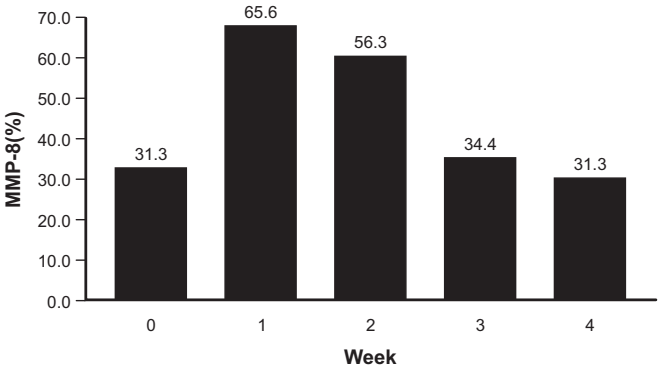

Fig 1: Percentage of MMP-8 gene expression according to the time duration of force

RESULTS

The gene expression of MMP-8 before the attachment of elastomeric chain was 31.3%. After the attachment, it was up-regulated to 65.6% in the first week, but then down-regulated to 56.3% in the second week, decreased again to 34.4% in the third week, and the lowest was 31.3% in the fourth week (Fig 1).

The difference of MMP-8 gene expression between t_0 and t_2 was significant (p=0.003). There was no significant difference between t_0 and t_3 (p=0.602) (Table 1).

DISCUSSION

Matrix metalloproteinase is a member of group of enzyme that can break down protein, such as collagen, that are normally found in the spaces between cells of tissue i.e. extracellular matrix proteins. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as FAS ligand), and chemokine in/activation MMPs also play a major role on cell behavior such as cell proliferation, migration differentiation, angiogenesis, apoptosis and host defense.[10,11,12]

MMP-8 (collagenase-2), is a collagen cleaving enzyme which is present in the connective tissue of most mammals. In human, the MMP-8 is encoded by MMP-8 gene. It is produced primarily by PMNs (polymorphonuclear cells) and released from the specific granules at the sites of inflammation.[13] Examination of the cells found in gingival crevicular fluid (GCF) has consistently shown that neutrophils constitute the largest number (about 92%) of cell.[14] This was one of the reasons why GCF was used as a sample fluid to investigate the MMP-8 gene in the present study.

During orthodontic treatment with fixed appliances, a clinically healthy periodontium with no plaque or food debris accumulation is important. In the present study, all the patients had a clinically healthy periodontium. Some believe that the flow of GCF is induced by microbial accumulation at the dento-gingival junction. This flow increases greatly with inflammatory changes of gingivitis and periodontitis. The expression and activity of MMPs in adult tissues is normally quite low, but increases significantly in various pathological conditions that may lead into unwanted tissue destruction, such as periodontitis.[15]

Orthodontic treatment is mainly at tooth movement by remodeling and adaptive changes in paradental tissue. To affect this outcome, only small amounts of force (20 to 150 g) per tooth might be required. It is assumed that an optimal force moves teeth efficiently into their desired position without causing discomfort or tissue damage to the patient.[16] In the present study, an elastomeric chain exerting 75 g of force was used to move the upper canine distally.

Previous research has revealed that local mediators such as prostaglandin, interleukins, and growth factors play an important role in bone remodeling induced by orthodontic forces. The levels of those mediators in GCF have been well demonstrated to be responsive to orthodontic force in humans. However, only a few studies have been focused on the remodeling caused by MMP in GCF during orthodontic tooth movement.[17] The present in vivo study demonstrated (Fig 1) that the expression of MMP-8 gene at the baseline (t_0) was 31.3%, then the orthodontic force up-regulated sharply the MMP-8 gene expression to 65.6%

TABLE 1: MANN-WHITNEY U TEST RESULT OF THE DIFFERENCE BETWEEN t_0 - t_2 AND t_0 - t_3 TO THE EXPRESSION OF MMP-8 GENE IN FIXED ORTHODONTIC PATIENTS

Variable	Duration	N	Percentage (%)	Significance (p)
MMP-8 gene	0	8	31.3	
expression	2	8	65.6	0.003
	0	8	31.3	
	3	8	34.4	0.602

in the first week. It supported the result of previous study conducted by Apajalahti et al[18] that MMP-8 level in the GCF significantly increased in the initial stage (at 4-8 hours from the application of fixed orthodontic appliance). Unfortunately, it was not reported when the MMP-8 level went down to the same level with that before force application. Ingman et al[19] in their study of MMP-1 and -8 in GCF during 1 month of follow-up after fixed appliance activation using IFMA method showed that the MMP-8 level was 12-fold higher than in control. In contrast with the present study, the level of MMP-8 on the fourth week was 2-fold higher than in the first week. In this study, MMP-8 gene expression was also analyzed after long term (four weeks) tooth movement by using RT-PCR technique. The MMP-8 gene expression down regulated on the second week, more decreased on the third week and the least was on the fourth week. It can be assumed that the force induced by the elastomeric chain decreases with the time. As a consequence, the MMP-8 gene expression will also decrease.

The current study showed no stastically significant difference between the expression of MMP-8 gene before application (t_0) and that in the third (t_3) (Table 1). It means that they had been more or less in the same level. At the time when the MMP-8 gene expression goes down to the same level with its expression before application (to), is assumed to be the proper time to reactivate the elastomeric chain.

CONCLUSION

1 Expression of MMP-8 gene in the GCF was up-regulated by the orthodontic pressure.

2 The highest level of MMP-8 gene expression was evident in the first week, and then decreased gradually in the second, the third and the fourth week.

3 The proper time to reactivate elastomeric chain was three weeks after application.

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