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# MATRIX METALLOPROTEINASE-8 GENE EXPRESSION IN GINGIVAL CREVICULAR FLUID OF PATIENTS TREATED WITH REMOVABLE ORTHODONTIC APPLIANCES

## (EKSPRESI GEN MATRIKS METALOPROTEINASE-8 DALAM CAIRAN KREVIKULER GINGIVA PASIEN YANG DIRAWAT DENGAN PIRANTI ORTODONTI LEPASAN)

**Susilowati*, Imam Mudjari****

***** Department of Orthodontics,
Faculty of Dentistry, University of Hasanuddin
****** Department of Dental Puplic Health,
Faculty of Dentistry, University of Hasanuddin
Jl. Perintis Kemerdekaan KM 10, Tamalanrea
Makassar 90245, Sulawesi Selatan
Email: susmudjari@yahoo.co.id

**Abstract**

Orthodontic treatment with removable appliance produces structural and biochemical changes and breaking the balance between the synthesis and breakdown of the periodontium collagen. MMP-8 plays an important role in the remodeling of periodontal ligament during orthodontic movement. The purpose of this study was to observe the MMP-8 gene in the gingival crevicular fluid (GCF) of patients with removable orthodontic appliance. It is expexted that the result can be used as a reference to decide the proper time for finger spring to be reactivated**.** A sample of 8 patients wearing removable orthodontic appliances was obtained. The finger springs were activated with 75 grams of force 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 sample was analyzed by using RT-PCR. Statistical analyses used were univariate analysis and Mann-Whitney U test**.** The result showed that the expression of MMP-8 in the GCF at $t_0$ was 28.1% but the force application elevated its expression to 62.5 % at $t_1$, and then decreased continously at $t_2$ (37.5%), $t_3$ (34.4%), and to $t_4$ (31,3%). There was no statistical significant difference of MMP-8 gene expression between and to $t_2$. In conclusion, the highest level of MMP-8 gene expression due to orthodontic forces of removable appliance was happenend in the first week, but it declined continously in the following weeks. The proper time to reactivate the finger spring was 2 weeks after application.

**Key words:** matrix metalloproteinase-8, removable orthodontic appliance, gingival crevicular fluid

## 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. Along with the growing science of orthodontics, people who seek help to improve their irregular position of their teeth are increased. The need for orthodontic treatment increases not only in Indonesia but also in many other countries.[1-3]

In the past, orthodontic resorption process is related to the pressure side and the apposition process on the strain side. Along with the development of science and technology, new discoveries have shown that bone tissue remodeling is seen from various basic sciences and clinical disciplines, which are all useful to human beings. From the various results of recent research, a perspective on orthodontic tooth movement based on molecular biology and immunology focused on the metabolic function of the extracellular matrix of periodontal tissues and bone, can be used to identify biological and diagnostic tools to monitor orthodontic tooth movement.[4]

The initial phase of orthodontic tooth movement usually involves many reactions resembling inflamemation characterized by vascular changes and migration of leukocytes out of periodontal ligament

capillaries. These changes lead to cellular activation and release of biologically active substances, such as enzymes and cytokines in the periodontal tissue.[5]

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

MMP-8 hydrolyzes most effectively to collagen type I and III which are the major interstitial collagenases in human gingival inflamemation.[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 histochemichal changes of periodontal ligament cells have been studied, but only few studies on MMP expression on the periodontal tissue due to mechanical pressure.[8]

The earlier studies mostly conducted on orthodontic patients with fixed appliances. However, removable appliance is still frequenttly worn in developing countries, such as Indonesia. The purpose of this study was to observe the expression of MMP-8 gene in gingival crevicular fluid during orthodontic tooth movement on patients treated with removable appliance. It is expected that the result can be used as a reference to determine the proper time to reactivate finger spring of removable appliance.

## MATERIALS AND METHODS

This study was conducted at RSGMP-FKG Unhas Makassar for placing the removable orthodontic appliances and at Laboratory of Molecular Biology and Immunology, Faculty of Medicine Unhas 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 removable orthodontic treatment. Inclusion criteria for sample selection are as follows: aged 18-30 years, patients were suffering from maxillary protrusion and/or anterior crowding, based on Kesling's space analyses, they required premolar extraction, had good oral hygiene, no periapical/periodontal diseases, no root anomaly in terms of shape and length, and never undergone orthodontic treatment. Patients with the following conditions were excluded: suffering from systemic diseases (Diabetes mellitus), extreme position of the canines, and 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 but then discarded. The same method was repeated, then the paper points were placed into tubes with the buffered solution (L-6) insides. The samples were then frozen and kept at -20$^{o}$ until analyzed.

To move the canines distally, the finger spring was activated with amount of 75 grams of force by using gauge meter and during the research, it was performed by one person. GCF samples were taken for 5 times, i.e. before the finger spring being activated (baseline) to followed by one week ($t_1$), two weeks ($t_2$), three weeks ($t_3$), and four weeks ($t_4$) after the finger spring being activated 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 anti sense primer:ATAGCCACTCAGAGCCCAGTA which generate 544 bp fragment, 1,5 µl enzyme mixt, 19.5 µl $H_2O$ and 1.5 µl mRNA. sample. Then, the tube was incubated at 37$^{o}$ C for 15 minutes, it allows the reverse transcription to work. Raise the temperature to 94$^{o}$ C for 2 minutes, 60$^{o}$ C for 2 minutes, and 72$^{o}$ C for 3 minutes. DNA bands 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 semiquantitative score of 1-4 as follows: score 1 if the light of the band was less than the control, score 2 if the light of the band was the same with the control, score 3 if the light of the band was slightly lighter than the control, and score 4 if the light of the band was much brighter than the control. Data obtained from the study were processed electronically using SPSS software version 15.0, and then analyzed using Mann-Whitney U statistical method.

## RESULTS

The gene expression of MMP-8 before the finger spring activation was 28.1%. After the activation, it

was up-regulated to 62.5% in the first week, but then down-regulated to 37.5% in the second week, decreased again to 34,4% in the third week, and the lowest was 31,3% in the fourth week (Figure 1).

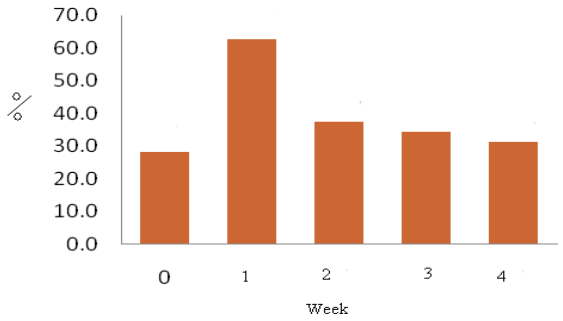


Figure1. Percentage of MMP-8 gene expression according to the duration of force

There was a significant difference of MMP-8 gene expression between $t_0$ and $t_1$ ($p = 0.001$), but there was no significant difference between $t_0$ and $t_2$ ($p = 0.117$) (Table 1).

Table 1. Mann-Whitney U test result of the difference between $t_0 - t_1$ and $t_0 - t_2$ to the expression of MMP-8 gene in orthodontic patients with removable appliance

| Duration of force (week) | N | MMP-8 gene expression Percentage | P |
|---|---|---|---|
| 0 | 8 | 28.1 | |
| 1 | 8 | 65.6 | 0.001 |
| 0 | 8 | 28.1 | |
| 2 | 8 | 37.5 | 0.117 |

## DISCUSSION

Matrix metalloproteinase is a member of group of enzyme that can break down protein, such as collagen, that is 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 are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense.[10-12]

MMP-8 (collagenase-2), is a collagen cleaving enzyme which 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 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 cells. 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 orthodontic appliances, a clinically healthy periodontium with no plaque or food debris accumulation is important. In the present study, all patients had a clinically healthy periodontium. Some believe that the flow of GCF is induced by microbial accumulation at the dentogingival 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 undesired tissue destruction, such as periodontitis.[14]

Orthodontic treatment is mainly aimed at tooth movement by remodeling and adaptive changes in paradental tissue. To affect this outcome, only small amounts of force (20 to 150 grams) 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 patient.[15] In the present study, the force used to move the upper canine distally was 75 grams.

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.[16] The present *in vivo* study demonstrated (Figure1) that the expression of MMP-8 gene at the baseline ($t_0$) was 28.1%, and then the orthodontic force up-regulated the MMP-8 gene expression to 62.5% in the first week. There was statistical significant difference between the gene expression at $t_0$ and $t_1$ (Table 1). It supported the result of previous study conducted by Apajalahti et al.[17] that MMP-8 level in the GCF significantly increased in the initial stage (at 4-8 hours from the application of orthodontic appliance). Unfortunately, they did not report when the MMP-8 level went down to the same level with its level before force application. Ingman et al.[18] 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 that in control. In contrast with our present study, the level of MMP-8 in the fourth week was 2-fold higher than that in the first week. In this present study, MMP-8 gene expression was also analyzed

after long term (four weeks) tooth movement but it was used removable appliance and the MMP-8 was analyzed by using RT-PCR technique. The MMP-8 gene expression down regulated in the second week, more decreased in the third week and the least was in the fourth week. It can be assumed that the force induced by the finger spring decreases with the time. It might be caused by the longer the time, the lesser of pressure from the finger spring. Consequently, the MMP-8 gene expression will decrease too. In this study result, there was no difference significantly between the expression before application ($t_0$) and that in the second week (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, is assumed as the proper time to reactivate the finger spring.

It can be concluded that expression of MMP-8 gene in the GCF was up-regulated by orthodontic pressure caused by removable appliance. The highest level of MMP-8 gene expression was happened in the first week, and then decreased gradually in the second, the third and the fourth week. The proper time to reactivate finger spring is two weeks after force application.

**References**


1. Erbay EF, Caniklioglu CM, Erbay SK. Soft tissue profile in Anatolian Turkish adults. Part I: Evaluation of horizontal lip position using different soft tissue analysis. Am J Orthod Dento-fac Orthop 2002; 121(1): 57-64.
2. Alkhatib MN, Bedi R, Foster C. Ethnic variation in orthodontic treatment need in London school-children.<http://www.biomedcentral.com/14726831/5/8 > (January 2009).
3. Artun J, Kerosuo H, Behbehani F, Al-Jame B. Early orthodontic treatment need and experience among adolescents in Kuwait <http//iadr.confec.com /afrmde05/preliminary program /indext.html> (October 2009).
4. Bildt MM, Bloemen M, Kuijpers-Jagtman AM, Von den Hoff JW. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid during orthodontic tooth movement. Eur J Orthod 2009; 39: 529-35.
5. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofac Orthop 2006; 129: 432-54.
6. Surlin P, Rauten AM, Manolea H. The involvement of metalloproteinase and their tissular inhibitors in the processes of periodontal orthodontic remodelling. Am J of Morphology and Embryology 2009; 50(2): 181-4.
7. Sasano Y, Jing-Xu Zhi, Tsubota M. Gene expression of MMP-8 and MMP-13 during embryonic development of bone and cartilage in the rat mandible and hind limb. J Histochem & Cytochem 2002; 50(3): 325-332.
8. Takahashi I, Nishimura M, Mitani H. Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats. J Dent Res 2003; 82(8): 646-651.
9. Casaccia GR, Gomes JC, Alviano DS. Micro-biological evaluation of elatomeric chains. J Angle Orthod 2007; 77(5): 890-893.
10. Henneman S, Maltha JC, Kujper-Jagtman AM, Hoff JW. CMT-3 inhibits orthodontic tooth displacement in the rat <http://hdl.hand-le.net/2066/52939> (December 2009).
11. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinase and TIMP. Cardiovascular Res 2006; 69(3): 562-7.
12. Dozier S, Escobar GP, Lindsey ML. Matrix metalloproteinase-7 activates MMP-8 but not MMP-13. Medical Chemistry 2006; 2(5): 523-6.
13. Toft-Hansen H, Nutall KR, Edward DR, Owens T. Key metalloproteinases are expressed by specific cell type in experimental autoimmune encephalomyelitis. J Immun 2004; 173: 5200-18.
14. Sorsa T, Tjaderhane L, Salo T. Matrix Metall-oproteinase (MMPs) in oral diseases. Oral Diseases 2004; 10(6): 311-8.
15. Proffit WR. Contemporary orthodontics. St Louis: CV.Mosby Co., 2000; 150-2.
16. Tae-Yeon Lee, Kee-Yeon Lee, Hyoung-Seon Baik. Expression of IL-1β, MMP-9 and TIMP-1 on the pressure side of gingival under orthodontic loading. The Angle Orthodontist 2008. 79(4): 733-9.
17. Apajalahti S, Sorsa T, Railavo S, Ingman T. The in vivo levels of matrix metalloproteinase-1 and -8 in gingival crevicular fluid during initial orthodontic tooth movement. J Dent Res 2003; 82: 1018-22.
18. Ingman T, Apajalahti S, Mantyla P, Sorsa T. Matrix metalloproteinase-1 and -8 in gingival crevicular fluid during orthodontic tooth movement: a pilot study during 1 month of follow-up after fixed appliance activation. European J Orthod 2005; 27: 202-7.