INTRODUCTION

Orthodontic treatment need and demand at the present time is increasing in many countries around the world. The use of fixed orthodontic appliances is booming, but removable orthodontic appliances are still widely used, particularly in developing countries.¹

Research on tooth movement began about 100 years ago, viewed from the aspects of cellular, histological, radiological, and more recently molecular biology and genetics. Profiles of various cytokines, growth factors, genes, and enzymes associated with orthodontic tooth movement have been widely studied, and the samples might be taken from gingival crevicular fluid (GCF) in the gingival sulcus.

¹ Associate Professor at Department of Orthodontics, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia, Kompleks Dosen Unhas, Jl. Sunu CX-6, Makassar 90213, Sulawesi Selatan, Indonesia. Email: susmudjari@yahoo.co.id

² Teaching at Department of Dental Public Health, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia
The teeth will move when subjected to pressure. Active orthodontic appliances, can provide the desired pressure in order to correct malocclusion. Pressure on the teeth will cause changes in periodontal tissue and alveolar bone, called remodeling. In the remodeling process, it needs activity of osteoclast and osteoblast in the extracellular matrix. To facilitate the mobility of these cells, an enzyme needed to break down collagen, which is the largest component of the extracellular matrix, called collagenase (matrix metalloproteinases/MMP), such as MMP-1, -8, and -13.

The action of MMP is regulated by specific inhibitors called Tissue Inhibitor of metalloproteinase (TIMP). There are four TIMPs, namely: TIMP-1, -2, -3, and -4. The most widely studied is TIMP-1, a glycoprotein synthesized by most connective tissue cells. TIMP inhibits the action of MMPs by binding to the active sites. ²

Morphological and biochemical changes of periodontal ligament cells have been studied, but still a bit of research on gene expression of MMP-8 and TIMP-1 in gingival crevicular fluid (GCF) as a result of mechanical stresses originating from orthodontic appliances. ³,⁴ Regarding the expression of genes, the studies conducted in western countries mostly used fixed orthodontic appliances, not the removable one. The data obtained also examined mostly the levels, not the expression of genes.

Therefore it needs to be investigated how the dynamics of gene expression of MMP-8 and TIMP-1 in the GCF during tooth movement of removable orthodontic appliance wearers works. The results of this study is expected to increase the understanding of paradental tissue response to orthodontic pressure in general and the role of MMP-8 and TIMP-1 in the biomechanics of tooth movement due to pressure from removable appliances in particular.

**METHODOLOGY**

The type of this research is clinical trial with time series design. The insertion of orthodontic appliances were carried out at a dental hospital, while the examination of gene expression (TIMP-1 and MMP-8) using RT-PCR tests were performed at the Laboratory of Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

The study subjects were 8 (6 women and 2 men, aged 19-25 years old) wearing removable appliances who met the inclusion criteria as follows: complete teeth from upper left to right first molar, patient suffered from anterior crowded teeth or protrusion, based on the Kesling’s space analysis required removal of the upper teeth, good oral hygiene, there are no periapical/periodontal abnormalities, there is no root extreme anomaly in terms of shape and size (length), never undergone orthodontic treatment, and willing to participate in the research process to completion.

Exclusion criteria were as follows: suffering from systemic disease (Diabetes Mellitus), the position of the canine was extreme distoversion, mesioversion, or ectopic, the canine and/or the first molar was mobile, and gingival crevicular fluid was mixed with blood.

The orthodontic removable appliances were inserted into patients a week after the first upper premolar extractions. Gingival crevicular fluid collection was performed on the distal side of the upper canine teeth at the following times: prior to activation of the finger spring (t₀), a week after activation (t₁), two weeks after activation (t₂), three weeks after activation (t₃), and four weeks after activation (t₄). The initial force given to activate the finger spring was 75 grams.

The gingival crevicular fluid collection was done as follows: the patient was asked to rinse his/her mouth and the tooth surface was cleaned and then dried with air and kept dry with cotton roll. Two paper points were inserted into the gingival sulcus, kept there for a minute and then discarded. The same activity was repeated, but the paper points were placed in the sulcus for one minute, then they were kept in a tube with transport buffer inside. Samples were stored in the refrigerator at a temperature of -20°C until analysis.

The gingival crevicular fluid samples were extracted to obtain total RNA. Analysis of RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) was done by putting the following reagents into a microtube: 6 ml Reverse Transcription buffer (Primecript, Takara, Japan), 1.5 ml specific primers for MMP-8, i.e. sense primer:

TGGACCCAATGGAATCCTTGC and antisense primer: ATAGCCACTCAGAGCCCAGTA that produce fragments of 544 bp, 1.5 ml enzyme mixtures, 19.5 ml H2O, and 1.5 ml sample mRNA. Then the tubes were
incubated at temperatures 37°C for 15 minutes, which allows reverse transcription work. The temperature was raised to 94°C for 2 minutes, 60°C for 2 minutes, and 72°C for 3 minutes. Bands of DNA were seen after 37 cycles of PCR. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) was added to each sample which serves as an internal control. PCR products were passed through 2% agarose gel for electrophoresis and viewed under UV light.

All of the above procedures were repeated for TIMP-1 by using: sense primer: 5'-GGGGACACCAGAAGTCAACCAGA-3', and antisense primer 5'-CTTTTCAGAGCCTTGGAGGAGCT-3'. Capturing images of DNA in agarose gel made with a digital camera. Laboratory test results with RT-PCR is expressed in a semi-quantitative score of 1-4 as follows:

- score 1 if the light from the light band is less than the control
- score 2 if the light of the band is the same as the control
- score 3 if the light from the band a little brighter than the control
- score 4 if the light of the band is much brighter than the control.

The data obtained from the study processed electronically using SPSS version 15.0, then statistically analyzed by using univariate analysis. Data were presented in graphic form of percentage to overview the distribution of gene expression of MMP-8 and TIMP-1 based on the length of time and pressure.

RESULTS

The research was conducted on 8 patients wearing removable orthodontic appliances, with a finger spring activated initially at 75 grams. Further observations were carried out over 4 weeks of gene expression of MMP-8 and TIMP-1 in the gingival crevicular fluid.

Fig 1 shows that the gene expression of MMP-8 before the given pressure was 28.1%. The pressure given in the first week up-regulated to 62.5%. Furthermore it declined in the second week to 37.5%. In the third week, it decreased to 34.4%, then decreased again during the fourth week to 31.3%.

Fig 2 provides information that before the given pressure, the percentage of TIMP-1 was 40.6%. By administering pressure for one week, increasing the percentage of TMP-1 to 59.4%. In the second week was dropped to 43.8%. In the third week, it decreased to 40.6%. Furthermore decreased again to 37.5% in the fourth week.

DISCUSSION

In this study, all patients had good oral hygiene and did not suffer from gingivitis or periodontitis. The presence of food debris on the surface of the tooth can lead to gingivitis and periodontitis. Periodontitis is an inflammatory disease that may cause progressive damage of the supporting tissue attachment and alveolar bone. One possible cause is a bacterial infection. Bac-
Bacteria in the plaque, including lipopolysaccharide (LPS) and lipoteichoic acid, interacts with toll-like receptor in epithelial cells, leukocytes and fibroblasts, stimulates the production of cytokines such as IL-1 beta, TNF-alpha, IL-6, IL-8, and prostaglandin E2 (PGE2). To facilitate the infiltration of leukocytes, fibroblasts are stimulated by IL-1 beta and TNF-alpha secreting MMPs that degrade ECM molecules including collagen. It has been observed that periodontitis may increase MMP-8 level in GCF.

Tooth movement induced by orthodontic force is characterized by changes in dental and parodontal tissue remodeling, including pulp, periodontal ligament, alveolar bone, and gingiva. The tissue when subjected to a certain amount, frequency, and duration of pressure, shows the changes macroscopically and microscopically. Orthodontic pressure will change vascularization, resulting in local synthesis of important molecules such as neurotransmitters, cytokines, growth factors, colony stimulating factor, and arachidonic acid metabolites. These molecules can trigger cellular responses by various types of cells in and around the teeth, creating conditions suitable for tissue microresorption and deposition.

The pressure on the teeth due to orthodontic appliances resulting in increased levels of MMP-8 in gingival crevicular fluid. A study on orthodontic appliances wearers, the GCF was analyzed by IFMA (Immunofluorometric Assay) technique, proved that the concentration of MMP-8 in GCF increased 2-5 times compared to the baseline after 4 hours and increased 3-5 times after 6 hours.

Ingman et al examining levels and activity of MMP-1 and -8 in GCF of orthodontic fixed appliance wearers observed every day for a month also showed an increase in the average levels of MMP-8 by 12 times compared with controls. The techniques used were IFMA and Western Blot. Although the average levels of MMP-8 for a month has increased, but the highest concentration of it was at the fourth week. Mantyla et al in his research proved that the presence of increased levels of MMP-8 derived from PMN and fibroblasts, showing a periodontal remodeling process.

In this study we examined the expression, not the levels of MMP-8 gene. Theoretically, the increased gene expression would increase the level of the encoded enzyme, and vice versa. The MMP-8 gene expression was increased sharply at week 1 (Figure 1) likely due to the recently activated finger spring. While the decline of expression due to the possibility that the force of the finger spring to move the canines distally, derived from the coil as energy deposits which is routed through the arm of the finger spring. When the finger spring being activated, it will gradually return to its original position.

TIMP-1 is a protein encoded by TIMP gene families, as a natural inhibitor of matrix metalloproteinase (MMP). It induces the proliferation of various cells, and has anti-apoptotic properties. TIMP-1 gene transcription is influenced by various cytokines and hormones.

The activity of MMP is controlled by TIMP, plays a very important role in the physiological remodeling of the periodontium as well as to respond to mechanical forces during orthodontic treatment. Inhibition of synthetic MMP has been shown reducing the orthodontic tooth movement. Orthodontic tooth movement tested in animals (usually mice), suggesting an increased expression of MMP-1, -2, -8, -9, and -13 and TIMP-1 and -3 in the PDL and alveolar bone. An increase in MMP and TIMP according to Bildt et al occurs in the resorption and apposition sides, but according to Garlet et al the increase in TIMP-1 only occurs in apposition side.

On the removable appliance wearers, TIMP-1 gene expression before the given pressure (baseline) was 40.6% (Graph 2). With the pressure given during the first week, raising the value of TIMP-1 to 59.4%. Then decreased at weeks 2, 3, and 4. This is in line with the increased expression of MMP-8. The increase in TIMP-1 gene expression could be as a compensation to suppress the increasing expression of MMP-8.

The proper balance between TIMP and MMP activity is important for the integrity of ECM components such as collagen, vibronectin, laminin, elastin, and proteoglycans. TIMP-1 inhibits all types of MMP except MMP-14. It has been believed by some researchers that TIMP useful to limit bone resorption/remodeling process so that it can restrict translationally orthodontic tooth movement. If the levels of TIMP-1 in the resorption side decreases, bone resorption will increase. In the apposition side, TIMP-1 levels increased so that resorption will decrease.
In a healthy periodontal tissue, TIMP levels are usually higher than in the inflamed periodontal tissue, and the MMP level exceeds the TIMP level. However, the MMP and TIMP levels in the apposition and resorption sides are higher than control.

CONCLUSIONS

1. The dynamics of gene expression of MMP-8 and TIMP-1 in GCF of patients wearing removable orthodontic appliances have the same pattern.

2. Before the appliances being activated, the expressions of MMP-8 and TIMP-1 were low.

3. Activation of the appliances increased the expression and reached the highest peak at the first weekend, then declined contiously in week 2, 3, and 4. Gene expression levels began to return to normal during the second week.

REFERENCES


