

## The level's changing of transforming growth factor $\beta$ 2 during canine retraction in non-growing age patient

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### ABSTRACT

**Background:** Orthodontic tooth movement occurred as a result of alveolar bone remodeling and collagen due to mechanical load. This mechanical load applied to the tooth will exert a number of cytokine and growth factors. One of the growth factors that are often associated with orthodontic tooth movement is transforming growth factor- $\beta$  (TGF- $\beta$ ). It has 3 isoforms, TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. It has been known that in adult patient, tooth movement rate was slower. **Purpose:** The aim of this study was to investigate the changing level of TGF- $\beta$ 2 in non-growing patient due to mechanical load in canine retraction. **Method:** Gingival crevicular fluid from 6 subjects who undergo canine retraction was taken to investigate changing level of TGF- $\beta$ 2. Distal site of each upper canine served as an experimental tooth. The gingival crevicular fluid from experimental tooth was taken just prior to mechanical load, at 24h and 72h after mechanical load. **Result:** ELISA reader showed that level of TGF- $\beta$ 2 was decreasing during experiment time. **Conclusion:** It can be concluded that in non-growing patient, TGF- $\beta$ 2 has less role in alveolar bone resorption in orthodontic tooth movement.

**Keywords:** Bone resorption; non-growing; orthodontic tooth movement; TGF- $\beta$ 2

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### INTRODUCTION

Malocclusion has a high impact in individual life quality. The prevalence of malocclusion in Indonesia is around 80% and in third place after caries and periodontal disease. Along with the improvement of the knowledge society and the desire to improve the quality of life, the demand for orthodontic needs in the community was also increased. The aims of orthodontic therapy were to corrected dental irregularities and disharmony in jaw relations. It utilizes the potential of the periodontal ligament and the alveolar bone to adapt to changing mechanical circumstances by tissue remodeling. By these adaptations teeth can be moved through the alveolar bone and also distant skeletal locations can be affected. By these two mechanisms a stable occlusion and a proper jaw relation can be established.<sup>1</sup>

There was an increase in the demand for adult orthodontic therapy in the past decades. However, our

knowledge on efficiency of adult tooth movement is still incomplete. There is a common believe among the orthodontists that orthodontic procedure will be more time-consuming in adult patient or non-growing age patient than in growing age patient. Younger patient had a greater tooth movement than adults.<sup>2</sup> Another study also showed there was a faster initial tooth movement in juvenile than in adult animals.<sup>3</sup> This finding corroborate another study by Ren,<sup>4</sup> who found that cellular response in crevicular fluid are less responsive to orthodontic force in old rat than in juvenile.

Orthodontic tooth movement is achieved by the remodeling of periodontal ligament and alveolar bone in response to mechanical loading. This remodeling is mediated by the activation of several bones remodeling marker. Many studies were done in order to give us additional information about this molecular signalization, for example TIMP-1, Col-1, RANKL, IL-6, sgp-130, etc.<sup>3-7</sup> One of the growth factor that still has an unclear role in orthodontic tooth movement is transforming growth factor

$\beta$  (TGF- $\beta$ ). Some author found it as mediator for suppressed osteoclast activity<sup>8,9</sup>, but in conversely, others found it may contribute to the induction of bone resorption.<sup>10,11</sup> TGF- $\beta$  has 3 isoforms, they are TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. Overexpression of TGF- $\beta$ 2 was found to give a result on an osteoporosis-like phenotype.<sup>12</sup>

Consider there is a role of TGF- $\beta$ 2 in bone remodeling, the aim of this study was to investigate the changing level of TGF- $\beta$ 2 due to mechanical loading in orthodontic tooth movement. This changing level of TGF- $\beta$ 2 was investigated at just prior to mechanical load application, 24 hours after mechanical load and 72 hours after mechanical load.

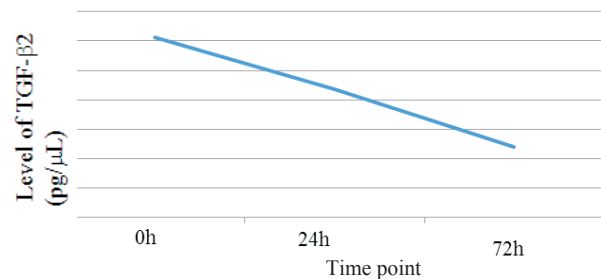
## MATERIALS AND METHODS

Subjects of this study were 6 patient age 30-35 years old who undergo canine retraction in private clinic. Changing level of TGF- $\beta$ 2 was investigated in gingival crevicular fluid (GCF). Ethical approval was obtained from Ethic Committee and Research Faculty of Dentistry Universitas Indonesia. Subjects of this research were selected according to these following criteria: (1) no history of bone metabolism disease; (2) pocket probing less than 4 mm; and (3) no clinical and radiographic sign of gingivitis and periodontitis. Subjects were excluded if they were smoker, and use of antibiotics or non-steroid anti-inflammatory agents in the 6 months prior to the study. Informed consent in written form was obtained from the subject before the beginning of the study.

GCF samples were collected using filter paper strips (Whatman, No. 1) (Figure 1 and 2). Samples were collected at distal side of upper right and left canine at just prior to mechanical load (0 hour), 24 hours after mechanical load, and 72 hours after mechanical load. Teeth were gently washed with water, the site under study were isolated with cotton rolls, and dried gently with air-syringe before paper strips were applied. Paper strips were inserted 1 mm



**Figure 1.** GCF collection with filter paper strip. GCF was collected at distal site of canine represented resorption area.



**Figure 2.** Graphic showed mean value of TGF- $\beta$ 2 at just prior to mechanical loading, 24 h after mechanical loading, and 72 h after mechanical loading.

**Table 1.** The Changing level of TGF- $\beta$ 2 at just prior to application of mechanical load, 24 h and 72 h after mechanical load

Time	$\bar{x} \pm SD$ (pg/ $\mu$ L)	p
0h – 24h	0.87864 $\pm$ 0.96999	0.077
0h – 72h	1.86711 $\pm$ 0.77038	0.002*
24h – 72h	0.98847 $\pm$ 1.28459	0.118

\*p<0.05

subgingivally for 30 seconds. Mechanical load applied to teeth was 100 g of force with elastomeric chain. Force magnitude was measured with dontrix gauge. Paper strip contained TGF- $\beta$ 2 was then diluted with phosphate buffer saline pH 7,7 (Gibco) in 1,5 ml Eppendorf tube and stored in -80°C in Oral Biology Laboratory Faculty of Dentistry University of Indonesia.

ELISA assay was done to determinate the level of TGF- $\beta$ 2 in GCF. Eppendorf tube contained filter paper strip and phosphate buffer saline were centrifuged at 12000 x 9 rpm for 10 minutes to separate supernatant form. ELISA assay was done under the standard procedure.

## RESULTS

The changing level of TGF- $\beta$ 2 due to mechanical loading in canine retraction was shown in Figure 2. This study showed that there is a changing level of TGF- $\beta$ 2 at different time point. Mean value of TGF- $\beta$ 2 level before application of mechanical load is 24.06  $\pm$  0.62 pg/ $\mu$ l. At 24 hour after mechanical load, level of TGF- $\beta$ 2 was slightly decreased (23.28  $\pm$  1.05 pg/ $\mu$ l). 72 hours after mechanical load, it continued to decrease (22.19  $\pm$  1.03 pg/ $\mu$ l).

T-test dependent was used to evaluated the statistical significance (p<0.05) between 0 hour compare to 24 hours, 0 hours compare to 72 hours, and 24 hours compare to 72 hours. The results showed at Table 1. There was a significance difference at 0 hour compare to 72 hours

( $p=0.02$ ;  $p<0.05$ ). However, it was found there were no significances different at 0 hour compare to 24 hours and 24 hours compare to 72 hours.

## DISCUSSION

Orthodontic tooth movement is a good example to easily understand bone remodeling due to mechanical load. In orthodontic treatment, tooth was moved as a result of applied force. This force, according to pressure and tension theory will cause bone resorption in compressed area, and bone formation in stretched area. This movement can be controlled by the magnitude of the applied force and the biological responses from the periodontal ligament (PDL). The force applied on the teeth will cause changes in the microenvironment around the PDL due to alterations of blood flow, leading to the secretion of different inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating factors and arachidonic acid metabolites. As a result of these secretions, remodeling of the bone occurs.<sup>13</sup>

Monitoring the biological system in clinical situations would diminish the gap between basic research and clinical implications. It would be advantageous for the clinician and the patient if the reactions of the biological system could be monitored during treatment. This would allow the adaptation of the treatment to the biological condition of the patient.

TGF- $\beta$  is a multifunction growth factor that played role in bone remodeling. TGF- $\beta$  is a part of TGF- $\beta$  super family along with activin, nodal, bone morphogenetic proteins (BMP) and so on. TGF- $\beta$  have 3 isoforms, they are TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. How its role in orthodontic tooth movement was remain unclear. This study was done as a pilot study to confirm the present of TGF- $\beta$ 2 due to mechanical load in orthodontic tooth movement. Some authors found that level of TGF- $\beta$  is higher in compression site than tension site.<sup>10,11</sup> Others found that it has the same level in both compression and tension site.<sup>5</sup> During development, TGF- $\beta$ 2 expressed later than TGF- $\beta$ 1 and TGF- $\beta$ 3.<sup>14</sup> However, 2 days after application of mechanical load, tooth movement begins as a result of osteoclast and osteoblast remodels the bony socket.<sup>13</sup> This is the main reason for time interval choosing in this study.

Subjects of this study were 30-35 years old patient, because we need to be sure these subjects has no potential adolescent growth to be expected. So there will be no growth hormone affected the changing level of TGF- $\beta$ 2. Distal site was choose as an experimental site because we believe this site will be more represent the bone resorptive response than in mesial site in this kind of experimental design. GCF sampling was taken from compression site because according to Erlebacher *et al.*,<sup>12</sup> overexpression

of TGF- $\beta$ 2 will cause an osteoporosis-like phenotype. Thus, it can be concluded that TGF- $\beta$ 2 may play role in bone resorption.

We have detected a level of TGF- $\beta$ 2 before application of mechanical load, this might be because there still remain a force used for leveling and aligning. At 24 hours after mechanical load, there was a slight decrease level of TGF- $\beta$ 2. Its decrease was continued until 72 hours after mechanical load. We found a statistical significance in comparison of 0 hour and 72 hours after mechanical load ( $p=0.02$ ). This happen because level of TGF- $\beta$ 2 continued to decrease at 72 hours after mechanical load. This result was different from Uematsu *et al* because they found that in 24 hours after mechanical loading, TGF- $\beta$ 1 was reached its peak level before continued to decrease at 7 days after mechanical loading.<sup>10</sup> However, it remains unknown whether TGF- $\beta$ 2 exhibits activity similar to that of TGF- $\beta$ 1.<sup>15</sup>

It seemed like in non-growing patient, TGF- $\beta$ 2 had no or less effect on bone remodeling in orthodontic tooth movement. This might be an explanation why some treatment in orthodontic seems more time-consuming in adult than in younger patient, as our previous study showed that in juvenile, level of TGF- $\beta$ 2 were increased at 72 hours after mechanical loading. Ren<sup>3</sup> found that in early state of orthodontic tooth movement, mediator response in adult was slower than in juvenile. This is why there was a delay on initial tooth movement in adult rat.<sup>4</sup> On the other hand, in a study experimental on rats, TGF- $\beta$  showed a lack responsiveness in old than in young rats.<sup>16</sup> This result might explain our finding, why in non-growing patient, TGF- $\beta$ 2 continued to decrease after mechanical loading.

The result of this study might give the orthodontist additional information of how bone remodeling marker were reacted in adults. However, further study with prolonged time is needed to give information about changing level pattern of TGF- $\beta$ 2 due to mechanical load in orthodontic tooth movement in adult patient. There was a changing of level of TGF- $\beta$ 2 during application of mechanical load in orthodontic tooth movement. TGF- $\beta$ 2 was decreased at 24 h and 72 h after mechanical load. It can be concluded that in non-growing patient, TGF- $\beta$ 2 are less responsive to mechanical load in orthodontic tooth movement.

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