Original Article

Comparison of Tumor Necrosis Factor-α Concentrations in Gingival Crevicular Fluid between Self-Ligating and Preadjusted Edgewise Appliances in the Early Leveling Stage of Orthodontic Treatment

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Abstract

Introduction: Tumor necrosis factor-α (TNF-α) is an important proinflammatory cytokine that regulates the early phase of inflammation reaction during orthodontic tooth movement. The aim of the present study was to compare TNF-α concentrations in the gingival crevicular fluid (GCF) between preadjusted edgewise appliance (PEA) and self-ligating (SL) systems during the early leveling stage of orthodontic treatment. **Materials and Methods:** Eighteen patients (aged 15–35 years) who participated in this study were divided into two experimental groups (PEA and SL) and control group (without orthodontic treatment). The GCF was taken at five sites in the maxilla anterior teeth from each participant just before bracket bonding and at 1, 24, and 168 h after the initiation of tooth movement. Cytokine levels were determined through ELISA. **Results:** The concentration of TNF-α was significantly higher in the experimental groups than in the control group at 24 h after force application. TNF-α levels were significantly decreased at 168 h after force application in the PEA group. Meanwhile, in the SL group, the level of TNF-α at 168 h was still increased, although there was no statistically significant difference. **Conclusion:** TNF-α concentration was increased at 1 h and 24 h after orthodontic force application in both the PEA and SL groups. In the PEA group, TNF-α concentration was significantly decreased at 168 h, meanwhile in the SL group, this value remained increased at this time point. The differences in TNF-α concentration between the PEA and SL groups may be caused by their different types of brackets, wires, and ligation methods.

Keywords: *Gingival crevicular fluid, orthodontic tooth movement, preadjusted edgewise appliance, self-ligating system, tumor necrosis factor-α*

Introduction

Orthodontic tooth movement is based on the application of force at a certain magnitude, frequency, and duration to the periodontal ligament, causing the remodeling of the periodontal ligament and alveolar bone.[1] The early phase of orthodontic tooth movement begins with acute inflammation response, which is marked by vascular dilatation and increased vascular permeability. Thus, these cause the migration of blood cells such as leukocytes from the vascular space to the periodontal ligament space.[2] Orthodontic force application causes vascular and cellular changes in the periodontal ligament, the movement of extracellular ligament fluid, and pressure on and tension in collagen fibers, and the extracellular matrix.[3] These processes engender a response on the part of paradental cells and migrating inflammatory cells from periodontal ligament capillaries through the synthesis and release of various biomolecules similar to cytokines, such as interleukin (IL), tumor necrosis factor-α (TNF-α), macrophage colony-stimulating factor, enzymes, proinflammation mediators such as prostaglandin, growth factor, and vasoactive neurotransmitter. These work simultaneously to regulate biologic responses to orthodontic force application after the initiation of tooth movement and improve alveolar bone resorption by osteoclasts so that the tooth can move.[4,5]

One important discovery regarding bone remodeling in orthodontic tooth movement was the discovery of cytokines, which are bioactive molecules that regulate the inflammation process through autocrine and paracrine signaling system.[6] Cytokine binding with specific receptors on the cell membrane surface leads to various

How to cite this article: Pramustika A, Soedarsono N, Krisnawati, Widayati R. Comparison of tumor necrosis Factor-α concentrations in gingival crevicular fluid between self-ligating and preadjusted edgewise appliances in the early leveling stage of orthodontic treatment. Contemp Clin Dent 2018;9:92-6.

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Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_792_17

Quick Response Code:

biological activities, such as the activation, proliferation, and survival of the cells. Proinflammatory cytokines mainly act at the beginning of orthodontic tooth movement by inducing vascular dilatation and increasing vascular permeability and inflammation response. Some proinflammatory cytokines, such as IL-1B, IL-6, IL-8, and TNF-α, play important roles as regulators of bone remodeling due to mechanical stimulation.[7]

TNF-α is an important proinflammation cytokine involved in osteoclastogenesis. Specifically, this cytokine plays a role in initiating osteogenesis, osteoclast maturation, and maintaining osteoclast activities in alveolar bone areas that undergo resorption so that tooth movement can occur. TNF-α is produced by monocytes, macrophages, and osteoblasts.[6,8] TNF-α is important in the acute inflammation phase of orthodontic tooth movement in that it attracts leukocytes and osteoclast precursor cells to the osteolysis site.[9] TNF-α affects osteoclastogenesis directly through binding with osteoclast precursor cells and indirectly through binding with osteoblasts and enhancing the expression of other important cytokines during the osteoclastogenesis process.[2,10,11] TNF-α stimulates the synthesis of proteolytic enzymes and osteoclastic activities, so TNF-α is an important cytokine for bone resorption.[12] Ren *et al.*, Lowney *et al.*, Uematsu *et al.*, Başaran *et al.*, and Acun Kaya *et al.* demonstrated an increase in TNF-α concentration in the gingival crevicular fluid (GCF) in patients who were treated with a fixed orthodontic appliance as compared to a control group. In a study conducted by Uematsu *et al.*, a significant increase in TNF-α was seen in the first 24 h after receiving an orthodontic force during the early treatment phase, and this value was found to decrease after 7 days.[2-4,6,7]

Orthodontic bracket systems have developed along with the development of the knowledge and technology of orthodontics. Passive self-ligating (SL) systems are the current bracket systems, and they have been reported as having better force distribution toward the periodontal ligament tissues as compared to preadjusted edgewise appliance (PEA) systems. This is because a passive SL bracket has a low-friction force and this force does not decrease as a result of ligation material changes, such as the use of elastomeric materials in the oral cavity. A study conducted by Toygar *et al.* in 2007 found that the use of light, continuous forces, as in SL systems, results in the changes in cytokines that are important in the osteoclastogenesis process, which lasts for a longer duration as compared to cases in which an interrupted force is applied. This supports the notion that bone resorption through osteoclasts is more effective given light and continuous forces, as in a passive SL system.[1,13,14]

TNF-α concentration can be used for studying the inflammation phase and the bone resorption that occurs in early orthodontic treatment with both systems because it can provide a better understanding of the molecular and biological processes involved in orthodontic tooth movement.

Materials and Methods

Eighteen participants (age 15–35 years old) were treated in the orthodontic specialist clinic and given nonextraction orthodontic treatment. The inclusion criteria for the participants were as follows: (1) healthy systemic conditions; (2) diagnosis based on a mild-to-moderate irregularity index for the anterior maxilla; (3) healthy periodontal tissues, no gingivitis or gingiva resection, depth on probing <3 mm, simplified oral hygiene index ≤3, and no significant bone loss upon panoramic radiographic photo; and (4) no use of anti-inflammation medication for the 4 months before orthodontic treatment. Informed consent was obtained from the patients or the parents of patients under 18 years of age. The human participant protocol was reviewed and approved by our Institutional Ethical Committee (Ref No. 70/Ethical Approval/FKGUI/VIII/2017).

Clinical procedures

After the examination, oral hygiene instructions, and informed consent form signing, GCF collection was performed before orthodontic treatment. Thus, GCF sampling was performed at baseline. The GCF was obtained from five points in the distolabial and mesiolabial areas of six anterior maxilla teeth. Orthodontic treatment using passive self-ligating brackets (Damon Q, Ormco, USA) with CuNiTi .014 initial wire (Damon CuNiTi, Ormco, USA) and preadjusted brackets (MBT, Ormco, USA) with NiTi .014 initial wire (DentsplyGAC, USA) was initiated. The next GCF collection was conducted at 1 h, 24 h, and 168 h after the bracket placement samplings were performed, using the same procedures.

Gingival crevicular fluid sampling

GCF sampling was performed using paper points (Gapadent #30 ISO) and the method of Offenbacher *et al.* (1986). The plaque on the tooth surface was carefully cleaned. The teeth were gently washed with water, isolated with cotton rolls to prevent saliva contamination, and then, gently dried with an air syringe. A paper point was inserted carefully 1 mm into the gingival sulcus and remained there for 30 s. The paper points were then taken and inserted into Eppendorf tube that were filled with 500 ml of phosphate-buffered saline and stored at -70°C until the next process began. GCF sampling was performed at the vestibular part of the teeth to prevent saliva contamination. The paper points contaminated with blood or saliva were excluded from the study.

Tumor necrosis factor-α determination

To collect the GCF samples from the paper points, centrifugation was performed for 15 min at 1000 g

and +4°C. The total volume that could be achieved was as much as 400 ml for each Eppendorf tube, and this was stored at −70°C until the next examination. Total protein concentration was determined through a Bradford assay with BSA (bovine serum albumin) standard (Bradford, 1976). Then, the total protein concentration was treated so as to reach 50 μg/mL in each tube.

TNF-α concentrations in the samples were measured using a human TNF-α Quantikine ELISA kit (RandD System, Inc., USA). All samples and standards were examined with Duplo. The number of TNF-α molecules in the sample was compared to a TNF-α standard curve. This curve showed a direct relationship between optical density and cytokine concentration, which was detected in pictograms/mL.

Statistical evaluation

The data were analyzed using Statistical Product and Service Solutions (SPSS) version 21 (IBM SPSS Statistics, Illinois, USA). A one-way ANOVA was used to determine the differences between the SL group, the PEA group, and the control group. A repeated ANOVA was used to determine the differences between time periods (0, 1, 24, and 168 h after bracket placement) for each group.

Results

After orthodontic force application, significant differences in TNF-α values were demonstrated between the experimental (SL and PEA) and control groups [Figure 1]. The concentrations of TNF-α in the groups at each observation time point are shown in Table 1. There was a statistically significant difference in TNF-α concentration at 24 h after orthodontic force application in the PEA group as compared to baseline ($P < 0.05$). Meanwhile, significant differences were observed at 24 h and 168 h after orthodontic force application in the SL system as compared to baseline ($P < 0.05$). The mean TNF-α value was significantly elevated at 24 h after force application in the PEA and SL groups. This result was confirmed by previous reports.

Table 2 compares two adjacent concentrations of TNF-α, which were significantly decreased after 168 h of force application in the PEA group ($P < 0.05$). The TNF-α level increased gradually after 1 h and 24 h of force application in the PEA group; however, there were no statistically significant differences between these values and baseline. On the other hand, there were significantly increased levels of TNF-α between 1 h and 24 h of force application in the SL group. The TNF-α values continuously increased over the 168 h of force application; however, there were no statistically significant differences as compared to baseline. The increased levels of TNF-α seen after 1 h and 24 h of force application were more prominent in the SL group as compared to the PEA group. In contrast to the TNF-α level changes seen in the experimental groups, no statistically significant differences were observed in the TNF-α values of the control group throughout the study period.

Discussion

Orthodontic tooth movement occurs through the remodeling of the alveolar bone as a result of the force exerted by the bracket on the tooth surface. Orthodontic force is transmitted to the periodontal tissue and hence causes cellular and vascular changes. Periodontal ligament cells, such as osteoblasts, osteoclasts, and fibroblasts, produce various cytokines during various phases of orthodontic tooth movement.[6,15] The initial periodontal changes induced by orthodontic force application involve an inflammatory response that is induced and upregulated by proinflammatory cytokines, as demonstrated by the upregulation of several proinflammatory cytokines in the GCF. TNF-α, as one of the proinflammatory cytokines, is one of the earliest cytokines secreted after tissue

Table 1: Tumor necrosis factor-concentrations in preadjusted edgewise appliance, self-ligating, and control groups at 0 h, 1 h, 24 h, and 168 h (pg/mL)

Time (h)	Systems mean (SD)			*P*		
	PEA	SL	Control	PEA versus control	SL versus control	PEA versus SL
0	6.78 (1.45)	7.92 (1.54)	8.25 (1.67)	0.37	1.00	0.68
1	9.98 (1.81)	7.92 (1.54)	7.46 (2.28)	0.20	0.21	1.00
24	13.46 (3.00)	14.23 (2.47)	7.53 (2.15)	0.003*	0.001*	1.00
168	8.08 (0.87)	14.29 (1.82)	7.25 (0.85)	0.82	0.001*	0.001*

*Significant ($P<0.05$). SD: Standard deviation

Table 2: Tumor necrosis factor-concentration changes throughout the experimental period in the preadjusted edgewise appliance, self-ligating, and control groups (pg/mL)

Systems	Time mean (SD)				*P*		
	0	1 h	24 h	168 h	0 versus 1 h	1 versus 24 h	24 h versus 168 h
PEA	6.78 (1.45)	9.98 (1.81)	13.46 (3.00)	8.08 (0.87)	0.296	0.377	0.041*
SL	7.92 (1.54)	9.95 (2.50)	14.23 (2.47)	14.29 (1.82)	0.360	0.006*	1.00
Control	8.25 (1.67)	7.46 (2.28)	7.53 (2.15)	7.25 (0.85)	1.00	1.00	1.00

*Significant ($P<0.05$). SD: Standard deviation

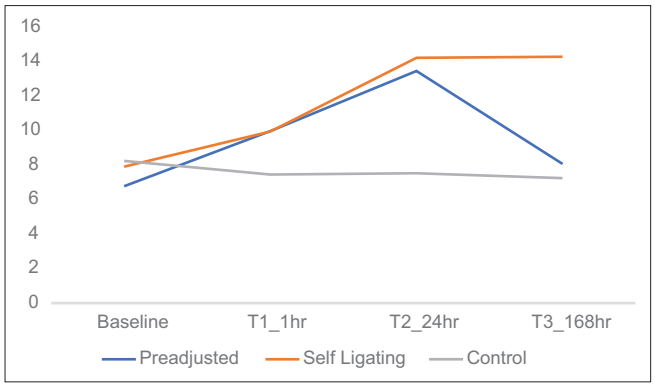

Figure 1: Graphic of tumor necrosis factor- concentration in the gingival crevicular fluid during early orthodontic tooth movement in the preadjusted edgewise appliance, self-ligating, and control groups. Data are presented as mean values (pg/mL)

injury, such as orthodontic tooth movement. Thus, it is upregulated significantly after short-term orthodontic force administration.[7] TNF-α has been shown to be involved in the process of bone resorption. TNF-α plays a prominent role in the mechanism controlling the appearance of osteoclasts at compression sites, and it has been proven to activate osteoclastic bone resorption by stimulating osteoblasts to increase the production of the receptor activator of nuclear factor kappa-B ligand.[6,10]

The gingival sulcus was selected as the testing site because of its continuity with the periodontal tissue. This results in the migration of biomechanical products such as cytokines into the gingival sulcus. During orthodontic tooth movement, cytokines located in the gingival area provide information about bone cell metabolism and reflect bone remodeling during orthodontic treatment.[16] This study was designed to examine the levels of TNF-α in the GCF during early leveling tooth movement due to orthodontic treatment in the PEA and SL groups. The results of this study clearly demonstrated that TNF-α levels were increased at 24 h after force application. This result is in accordance with the previous studies by Kaya *et al.*, Uematsu *et al.,* and Lowney *et al*. This may be caused by the early upregulation of chemotactic activities directly after mechanical force application. This is also in accordance with Krishnan and Davidovitch, who showed an acute inflammatory response during the initial phase of tooth movement.[1,6,17]

In the PEA group, the TNF-α concentration was upregulated at 1 h and 24 h after force application, but TNF-α decreased significantly, reaching nearly to the baseline, after 168 h of force application. This decreased value is assumed to be due to the force decay of the ligation system used in the PEA group. In 1997, Taloumist *et al.* found out that elastomeric which is used as ligation method in preadjusted bracket undergo 53%–68% of force decay in the first 24 h of use because of hydrolysis reaction in a simulated oral environment.[18] The decrease level of TNF-α at 168 h of force application is in accordance with the previous study from Uematsu *et al.* and Acun Kaya *et al*. Meanwhile, the level of TNF-α associated with the Damon system (the SL group) was significantly increased at 1 h and 24 h after force application and remained increased after 168 h of force application, although there was no statistically significant difference from baseline.[2,6]

The increased TNF-α levels seen at 24 h were more significant for the Damon system (the SL group). This result could indicate that the inflammation response caused by orthodontic force was higher for the Damon system (the SL group) as compared to the MBT system (the PEA group). The difference in the results for the two groups may have been caused by differences in the types of the mechanisms used. The Damon system delivered a constant force over the experimental period because of the use of copper NiTi.[19] The Damon system has metal clips. Thus, it will reduce friction, so the force will be transmitted directly to the periodontal ligament without any significant reduction due to the ligature system. The low-friction and low-force mechanics of the Damon system can accelerate cellular activity during tooth movement. Meanwhile, the MBT system, which uses elastomeric ligatures, has a large friction value and undergoes force decay after 24 h.[3,20]

Conclusion

TNF-α concentration was increased at 1 h and 24 h after orthodontic force application in both the PEA and SL groups. In the PEA group, TNF-α concentration was significantly decreased at 168 h, meanwhile in the SL group, it was increased, although this increase was not statistically significantly different. The differences in TNF-α concentration between the PEA and SL groups may have been caused by the different types of brackets, wires, and ligation methods used.

Acknowledgment

Authors would like to thank the Research and Community Service, Directorate of University Indonesia for supporting this research through 2017 PITTA-UI grant.

Financial support and sponsorship

This research was supported PITTA UI 2017 grant awarded by Directorate of Research and Community Service, University of Indonesia.

Conflicts of interest

There are no conflicts of interest.

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