Original Research

Levels of Tumor Necrosis Factor-α (TNF-α) and Transforming Growth Factor-β1 (TGF-β1) in Gingival Crevicular Fluid During Canine Retraction Using Elastic Chain and Closed Coil Spring

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Abstract

Introduction: To investigate the levels of human tumor necrosis factor-α (TNF-α) and transforming growth factor β1 (TGF-β1) in gingival crevicular fluid (GCF) during canine retraction movement using elastic chains and closed coil springs. **Materials and Methods:** One hundred patients (49 men and 51 women; mean age, 23.6 years) entering the space closure phase of fixed orthodontic treatment participated in this research. An upper canine of each patient was retracted using an elastic chain, and the contralateral canine was retracted using a closed coil spring. GCF samples were collected from the canine before and 7 days after the force was applied. human TNF-α and TGF-β1 were determined by enzyme-linked immunosorbent assay. **Results:** There were significant increase in the concentrations of both TNF-α and TGF-β1 at 7 days in both groups ($P < 0.05$). In the evaluation of between-group differences, significantly higher values were determined in the closed coil spring group. **Conclusions:** The results suggest that closed coil springs give higher TNF-α and TGF-β1 concentrations as compared to elastic chains, which make more bone remodeling to occur; therefore, closed coil springs can be considered as the treatment of choice for orthodontic canine retraction.

Keywords: Closed coil spring, elastic chain, gingival crevicular fluid, TNF-α, TGF-β1

Introduction

The main objectives of orthodontic treatment are to improve malocclusion and achieve correct occlusion and dentofacial function.[1] Orthodontic treatment often involves the extraction of premolars followed by space closure. Canine retraction is one of the most time-consuming stages of premolar extraction-based orthodontic treatment. Any procedure which reduces the time required to perform this stage will also serve to shorten overall treatment time.[2] When space closure is performed via sliding mechanics, the most commonly used methods of applying the force are elastic chains and closed coil springs. Elastic chains have the potential disadvantage of a significant force decrease over the time.[3,4] Closed coil springs are relatively more expensive to use, but there is almost no decrease in force during the movement of teeth.[5]

Orthodontic forces cause an inflammatory reaction within the periodontal tissue, which in turn may trigger the biological processes associated with bone remodeling.[6] Molecules such as chemokines, cytokines, and growth factors maintain the vascular and cellular changes by stimulating or inhibiting cellular activity.[7] Some of these cytokines, including tumor necrosis factor-α (TNF-α) and transforming growth factor beta 1 (TGF-β1), stimulate osteoclast differentiation, function, and survival, contributing to the bone remodeling mechanism and tooth movement.[8-10]

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Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate present in the gingival sulcus. During inflammation, the region tends to increase in volume and exhibits increased capillary permeability.[11] Various studies have attempted to clarify the molecular mechanisms affected by canine retraction by performing the biochemical analyses of GCF. The results were inconclusive with either significant[12,13] or insignificant[14] changes. Therefore, the aim of this study was to evaluate the changes of human TNF-α and TGF-β1 levels in GCF during canine retraction movement and to compare the expression of these markers between the use of elastic chains and closed coil springs.

Materials and Methods

Study samples

The samples were obtained from patients attending for orthodontic treatment at the Prof. Soedomo Dental Hospital, Universitas Gadjah Mada, Indonesia. A total of 100 patients (49 men and 51 women; mean age, 23.6 ± 4.9 years) were enrolled and selected to participate in this study, based on the following inclusion criteria: had upper premolar extraction in each quadrant (no other extraction), were undergoing orthodontic treatment using a Roth prescription preadjusted edgewise appliance (0.022, Forestadent, German), and had an 0.018 stainless steel working archwire in place for at least 4 weeks. Participants were excluded if they were smokers, had gingivitis, had probing pocket depths ≥4 mm, had loss of clinical attachment ≥2 mm in the selected or adjacent teeth, or had taken anti-inflammatory medications within the previous 6 months. Before the beginning of this study, the selected volunteers had been informed of the objectives of the study, and they indicated their agreement to participate by signing an informed consent form that had been approved by the Ethical Committee of UGM (No. 001134/KKEP/FKG-UGM/EC/2017). The volunteers were then subjected to a supragingival prophylaxis and were given oral hygiene instructions to follow at home as a procedure to eliminate inflammation.

Canine retraction

For each volunteer, one randomly selected upper canine was retracted using a short clear colored polyurethane elastic chain (American Orthodontic, USA), and the contralateral canine was retracted using a 9 mm Nickel-titanium closed coil spring (American Orthodontic, USA) [Figures 1 and 2]. The amount of force applied at the beginning was 150 g for both elastic chain and closed coil spring and was measured using a tension gauge.

Gingival crevicular fluid sampling

GCF samples were collected using #25 paper point (Sendoline, Sweden). The samples were collected at the buccal sites of the Maxillary canine gingival sulcus before the force was applied and at 7 days after. Day 7 was chosen because day 7 is the turnover time for enzymes.[14] Teeth were isolated with cotton rolls, cleaned of any plaque deposits, and gently drained using air jets to exclude the remaining saliva. A paper point was inserted 1 mm subgingivally for 30 s. The collected samples then were stored in sterile tubes at −80°C until biochemical analyses were assayed.

Biochemical analyses

A blinded biochemist at Clinical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada performed the GCF cytokine measurement. After thawing at room temperature, stored GCF samples were assayed in duplicate for human TNF-α and TGF-β1 by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Finetest®, China). Optical density (OD) was measured on a plate reader using 450 nm wavelength. A standard curve generated from the OD values of standards provided by the manufacturer was used to determine cytokine concentration. The amount of each biomarker was determined in picograms/millimeter (pg/ml).

Statistical analyses

TNF-α and TGF-β1 levels data were subjected to a test of normality and homogeneity. Paired *t*-tests were used to compare cytokine levels at the different time points in which samples were taken from the patients. The differences between the groups at a time interval were compared by independent *t*-test. A *P*-value of <0.05 was considered statistically significant. Statistical analyses were processed with the Statistical Package for the Social Sciences version 20.0 software (SPSS Inc., Chicago, Illinois, USA).

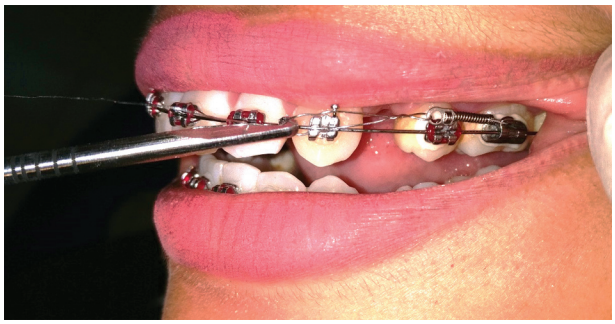

Figure 1: Canine retraction using closed coil spring

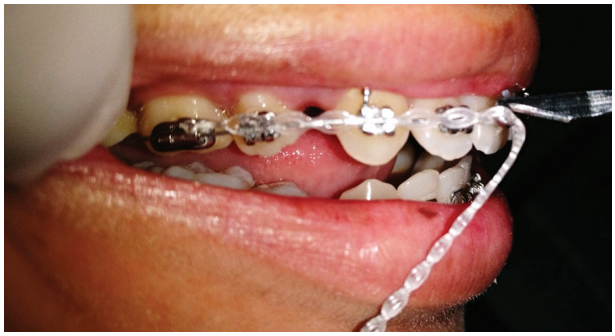

Figure 2: Canine retraction using elastic chain

Table 1: Descriptive statistics of the measured concentrations of TNF-α and TGF-β1 (pg/ml)

	Statistic	Before retraction		7 days after retraction	
		Elastic chains	Closed coil springs	Elastic chains	Closed coil springs
TNF-α	Mean ± SD	111.75 ± 16.15	120.68 ± 25.68	142.47 ± 18.59	162.92 ± 23.31
	Minimum	75.99	79.87	122.31	121.04
	Maximum	135.93	155.52	189.24	205.86
TGF-β1	Mean ± SD	50.51 ± 6.23	56.16 ± 8.49	58.75 ± 5.67	99.32 ± 35.73
	Minimum	43.69	42.82	49.97	62.96
	Maximum	61.46	71.73	66.01	182.26

Results

All the participants maintained good oral hygiene throughout the study. There were no inflammatory clinical signs of gingival and periodontal status. The descriptive statistics that include mean, standard deviations, minimum, and maximum value of TNF-α and TGF-β1 levels of this study are listed in Table 1.

The independent *t*-test showed no statistically significant difference was detected for TNF-α ($P = 0.365$) and TGF-β1 ($P = 0.109$) levels in GCF before the canine retraction between the elastic chain group and the closed coil spring group. However, at 7 days, significantly higher values of TNF-α ($P = 0.036$) and TGF-β1 ($P = 0.002$) were determined in the closed coil spring group. The paired *t*-test showed that there were significant increase in the levels of TNF-α and TGF-β1 at 7 days in both retraction groups compared to before canine retraction [Table 2].

Table 2: Paired *t*-test to compare the levels of TNF-α and TGF-β1 on canine retraction using elastic chains and closed coil springs

	Retraction types	*P*
TNF-α	Elastic chains	0.000*
	Closed coil springs	0.001*
TGF-β1	Elastic chains	0.001*
	Closed coil springs	0.003*

*Significant difference ($P < 0.05$).

Discussion

This study intended to observe the levels of TNF-α and TGF-β1 in GCF during canine retraction movement and to compare the efficiency of orthodontic force generated by elastic chains and closed coil springs by measuring the cytokines changes. TNF-α is a proinflammatory cytokine that is involved in bone resorption and acute as well as chronic inflammations. Even though TNF-α is produced primarily by activated monocytes and macrophages, it can also be produced by osteoblasts, epithelial cells, and endothelial cells. *In-vitro* studies have demonstrated that in bones, TNF-α can directly and indirectly induce osteoclastogenesis by binding to its p55 receptor on osteoclast precursors and by upregulating the expression of receptor activator of nuclear factor-kB ligand (RANKL), Macrophage-colony stimulating factor (M-CSF), and other chemokines on osteoblasts.[15] TNF-α is also an apoptotic factor for osteocytes, which could be the signal for osteoclast recruitment to resorb bone in the PDL pressure side, at the same time inhibiting osteoblasts.[7,16] TGF-β1 is a multifunctional cytokine produced by a variety of cells, including osteoblasts and mechanically stimulated fibroblasts and has highly osteogenic properties, and increases osteoblast activities. Further studies have showed TGF-β1 effects on pressure sites after an orthodontic force application and it is an essential factor for RANKL-induced osteoclastogenesis and, consequently orthodontic tooth movement.[17] Neither TNF-α nor TGF-β1 is the sole factor that explains the process of bone remodeling; however, these cytokines are available in GCF and considered useful tools for studying cellular response to mechanical stress, *in vivo*.

The results of this study demonstrated that in both elastic chain group and closed coil spring group, there were significant increase in the levels of TNF-α and TGF-β1 at 7 days compared to before canine retraction. The finding was similar to previous studies.[13,18,19] The results implied that the force applied to a tooth induced an acute inflammatory response of the cell within the periodontal tissue which was characterized by the release of inflammatory cytokines. The released cytokines may interact either directly with bone cells or indirectly with neighboring cells such as fibroblasts, monocytes, and lymphocytes, and trigger the bone remodeling process.[20] However, Basaran *et al.*[14] reported that the increased mean concentration of TNF-α was not statistically significant compared to the baseline. Furthermore, a decrease was found between the last stage of the leveling and the starting stage of the distalization period which also differs from our study. The finding might be due to the large variation of individuals, the amount of load applied, and the type of material used to generate force in the study.[15]

The levels of TNF-α and TGF-β1 were found to be significantly higher in the closed coil spring group than the elastic chain group at 7 days after the force applied. It could be explained that the closed coil springs still exerted force throughout the study period whereas the elastic chains had large force decay. Kishorekumar found that NiTi closed coil springs lost less than 10% capability of force delivery after 7 days activation.[21] Cox *et al.* also showed that the force decay of NiTi closed coil springs was approximately

12% after 4 weeks of clinical usage.[22] On the other hand, *in-vitro* study by Mirhashemi *et al*. showed that the percentage of force loss of elastic chains ranged from 12 to 40% after 7 days.[23] In another study, Sen and Goswami examined the force decay properties of elastic chains from four companies, with the maximum degradation found in plastic chains (American Orthodontics®, USA), which was 46.23%.[24] The reason why polyurethane elastic chains had large force decay was due to a composite series arrangement of hard and soft domains of polyurethane structure when they were stretched.[25]

The results also implied that closed coil springs may have greater effect than elastic chains when cellular activity is considered, and alveolar bone resorption may be greater in the closed coil spring group than in the elastic chain group. This implication was supported by the study of Dixon *et al*. that showed the mean rates of space closure were 0.58 mm/month for elastic chains, and 0.81 mm/month for NiTi closed coil springs.[5] Furthermore, a recent study by Chaudhari and Tarvade also demonstrated that a significantly faster space closure was achieved by using NiTi closed coil springs when compared to the elastic chains although with more anchorage losses.[26]

The results concluded that TNF-α and TGF-β1 levels were found to be positively correlated with the rate of tooth movement. NiTi closed coil springs gave higher rate of canine retraction than elastic chains. The study also demonstrated an important clinical implication, which was even though closed coil springs are an expensive option for space closure, they have the advantage of being efficient. In contrary, elastic chains are cheaper, but an increased number of visits is required to obtain the same amount of space closure.

Conclusion

This study has shown that TNF-α and TGF-β1 are associated with the bone remodeling that occurs during canine distalization movement. Closed coil springs give higher TNF-α and TGF-β1 concentrations which make more bone remodeling to occur; therefore, closed coil springs can be considered as the treatment of choice.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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