Original Article

Tumor Necrosis Factor–α Levels during Two Different Canine Distalization Techniques

Seniz Karacay[a]; Işıl Saygun[b]; Ali Osman Bengi[c]; Muhittin Serdar[d]

ABSTRACT

Objectives: To compare levels of tumor necrosis factor (TNF)-α while applying continuous and heavy interrupted forces.

Materials and Methods: A hybrid retractor was used in the first group. In the second group, rapid canine distalization through periodontal distraction was performed. Gingival crevicular fluid samples were collected from the distal sides of the canine teeth before attaching the appliances and at 1 hour, 24 hours, and 1 week after the force was applied.

Results: In the hybrid reactor group, concentration of TNF-α decreased at 1 week according to 24-hour measurements. In the rapid canine distalization group, it severely increased at 1 hour. In the evaluation of between-group differences, significantly higher values were determined in the rapid canine distalization group at 1 hour and 1 week.

Conclusions: Heavy interrupted force induces a rapid release of TNF-α, and the tissue response continues for a longer time period. To avoid the harmful effects of heavy interrupted force, there might be feedback mechanisms that prevent the mediators from increasing excessively.

KEY WORDS: TNF-α; Cytokine; Gingival crevicular fluid; Periodontal distraction; Hybrid retractor; Heavy interrupted force; Continuous force

INTRODUCTION

Orthodontic forces cause acute inflammatory reactions, vascular changes, and migration of leucocytes.[1–9] A number of previous studies have been focused on certain cytokines and enzymes in the gingival crevicular fluid (GCF). In many previous studies, alkaline phosphatase,[4] lactate dehydrogenase,[5] aspartate aminotransferase,[6] prostaglandin E,[2,10–12] interleukin (IL)-1β,[2,10,13–16] IL-6,[15] IL-8,[17] tumor necrosis factor (TNF)-α,[15–18] β-glucuronidase,[16] and transforming growth factor[19] have been evaluated.

TNF-α is a typical mediator of inflammatory response that has been shown to be involved in the process of bone resorption.[18,20–22] TNF-α plays a prominent role in the mechanism controlling the appearance of osteoclasts at compression sites.[23–26] Lowney et al[18] revealed elevation of TNF-α attributed to orthodontic force. This cytokine is produced primarily by activated monocytes and macrophages but also by osteoblasts and has been proven to be an activator of osteoclastic bone resorbtion.[27,28] However, Alhashimi et al[22] performed in situ hybridization to measure the messenger RNA (mRNA) expression of IL-1β, IL-6, and TNF-α at 3, 7, and 10 days after application of orthodontic force on molars of rats. However, they could not detect mRNA expression of TNF-α, though induction of IL-1β and IL-6 was observed to reach maximum on day 3 and declined thereafter. They explained this absence by the feedback mechanism caused by increased TNF-α protein levels and species-related differences.

Canine teeth can be distalized with a segmented arch or by applying force with an elastic or closed coil spring on a continuous arch. In 1998, Liou and Huang[29] introduced rapid canine distalization tech-

[a] Assistant Professor, Dental Science Center Department of Orthodontics, Gulhane Military Medical Academy, Ankara, Turkey.

[b] Associate Professor, Dental Science Center Department of Periodontics, Gulhane Military Medical Academy, Ankara, Turkey.

[c] Associate Professor, Dental Science Center Department of Orthodontics, Gulhane Military Medical Academy, Ankara, Turkey.

[d] Associate Professor, Dental Science Center Department of Bioichemistry, Gulhane Military Medical Academy, Ankara, Turkey.

Corresponding author: Dr Seniz Karacay, Dental Science Center Department of Orthodontics, Gulhane Military Medical Academy, Gn Tevfik Saglam Cad, Ankara, Etlik 06018, Turkey (e-mail: senkaracay@yahoo.com).

Accepted: February 2006. Submitted: January 2006.

 DOI: 10.2319/120905-430

nique to orthodontics literature. Since then, several researchers have reported on this technique and described it as a phenomenon of periodontal ligament distraction.[29–34]

During orthodontic tooth movement, changes in the periodontium occur depending on the magnitude, direction, and duration of the force applied. In this study, we compared the levels of TNF-α when a continuous force was applied with a hybrid retractor and a heavy interrupted force was applied with a tooth-born distractor. A few case reports and studies have been presented about rapid canine distalization in the past decade.[29–35] However, to our knowledge, none of them evaluated the alterations of the cytokine levels in the GCF.

MATERIALS AND METHODS

Ten patients (4 boys, 6 girls; mean age 15.4 ± 0.8 years) who needed extraction of the upper first premolars for orthodontic treatment and had not taken antibiotics and anti-inflammatory drugs within the past 3 months were selected. Patients were also evaluated for periodontal health by the plaque index (PI), gingival index, pocket depth (PD), and bleeding on probing (BOP). Patients' rights were protected, and informed consent and assent were obtained according to the Gulhane Military Medical Academy Ethical Committee Board.

Groups were constructed according to anchorage requirements. The hybrid reactor group (HRG) consisted of five patients who needed moderate anchorage. Their canines were distalized with a hybrid retractor with continuous force. The rapid canine distalization group (RDG) consisted of five patients who needed maximum anchorage control. Rapid canine distalization through periodontal distraction was preferred in this group, as this technique prevents anchorage loss.

Canine Distalization With Hybrid Retractor

One week after the extraction of first premolars, canine brackets with vertical slot and molar bands were attached. Upper canines were distalized with a continuous force by the hybrid retractor, which is a segmented and prefabricated appliance.[36]

Rapid Canine Distalization Through Periodontal Distraction

After the first premolar extraction, a vertical osteotomy was performed to the buccal and lingual of the interseptal bone between the canine and first premolar teeth. Two vertical osteotomies were connected with an oblique osteotomy extending toward the base of the interseptal bone to weaken the resistance. Osteotomies were performed inside the socket. Surgery was not uncomfortable for the patients. They were instructed not to take any drug, as it might affect the cytokine levels in GCF.

One week later, the distraction device that was constructed individually for each patient was cemented on the canine and molar teeth. The patient activated the distractor 180° two times a day at 12-hour intervals (9 AM and 9 PM). The appliance performed heavy intermittent force. Details about the construction of the distractor and surgical procedure were described in the previous report of Sayın et al.[30]

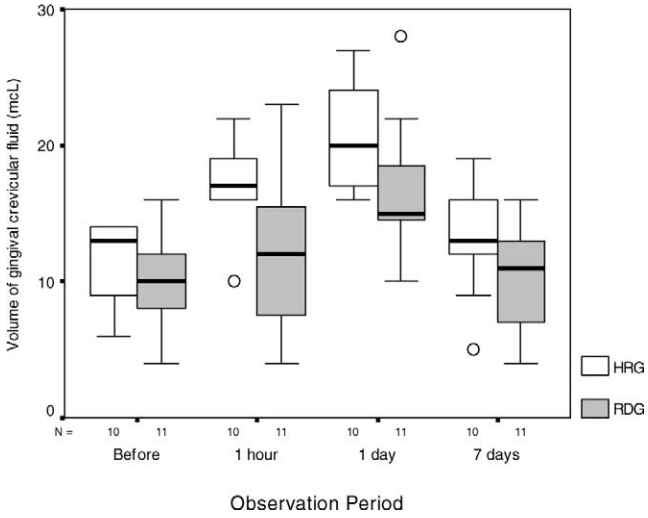

Figure 1. Alterations in the volume of gingival crevicular fluid during the observation period.

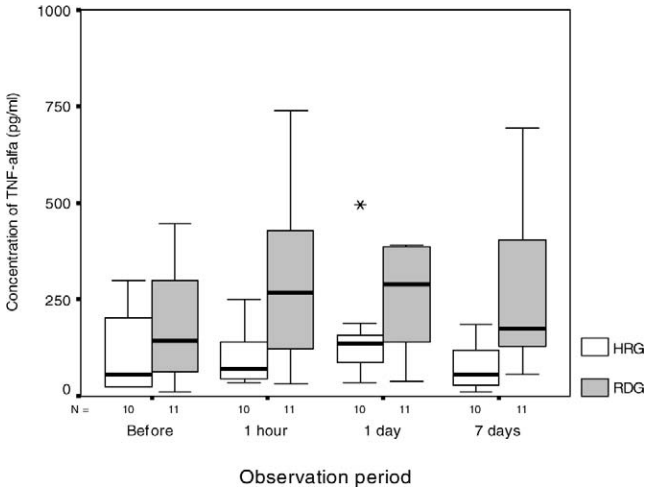

Figure 2. Alterations in the concentration of tumor necrosis factor–α during the observation period.

GCF Collection

GCF samples were collected from the distal sides of canine teeth before attaching the appliances and at

Table 1. Plaque Index (PI), Gingival Index (GI), Probing Depth (PD), and Bleeding on Probing (BOP) Revealing the Periodontal Condition Before Treatment[a]

Groups	PI	GI	PD	BOP
HRG	1 (0.75–1.31)*	1 (0.68–1.25)	1.5 (1.25–1.56)**	0.5 (0.25–0.75)*
RDG	0.5 (0.25–1)*	0.75 (0.25–1.25)	1 (0.75–1.25)**	0 (0–0.5)*

[a] Values in the parentheses reveal the 25–75 percentiles (quarters). HRG indicates hybrid reactor group; RDG, rapid canine distalization group.

* Significant difference between the groups ($P < .05$, Mann-Whitney *U*-test); ** significant difference between the groups ($P < .01$, Mann-Whitney *U*-test).

1 hour, 24 hours, and 1 week after the force was applied. Patients in the RDG were activating their appliance at 9 AM, and GCF samples were collected at 10 AM at 24 hours and at 1 week (1 hour later than activation).

Before the GCF collection, supragingival plaque was removed and the canine teeth were isolated with cotton and were dried. Samples were collected from the distobuccal and distopalatal sides with four paper strips (Periopaper-ProFlow Inc, New York, NY). The first strip was inserted into the distobuccal crevice to a level 1 mm below the gingival margin for 30 seconds. After a 1-minute interval, a second strip was inserted into the distopalatal crevice for 30 seconds. The procedure was repeated once more with the third and fourth strips. To determine the amount of collected GCF, weights of Eppendorf tubes were measured before and after the collection procedure with an electronic scale, and differences were calculated. Eppendorf tubes were stored at −80°C until analysis.

TNF-α Assay

The amount of TNF-α in GCF was assayed with an enzyme-linked immunosorbent assay (ELISA) kit (Cytelisa Human TNF-α, Cytimmune Sciences Inc, Rockville, Md) with recombinant TNF-α monoclonal antibody as a standard. All assay procedures were carried out according to the manufacturer's instructions. GCF samples were eluted from strips by a centrifugal method. Elution was carried out with the addition of 200 μL ELISA test buffer. Strips were then removed and the fluid was extracted for analyses. The amount of crevicular TNF-α in each sample was determined from TNF-α standard calibration curves. The peroxidase-substrate color reaction was read on a plate reader (Model EL312 Bio-Tek, Winooski, Vt) set to a wavelength of 490 nm.

The calibration curve was plotted by regression analysis, and the optical density of each sample was used to estimate the concentration of TNF-α (pg/μL). This was corrected for the original volume of GCF, and the results were expressed as pg TNF-α/μL. Mean intra- and interassay coefficients of variations for TNF-α measurement were 7.9% and 11.4%, respectively. The sensitivity was 0.87 pg/mL and the range of detection was from 8 to 500 pg/mL. The mass of the fluid on each strip was converted to a volume (mL) by assuming that the density of GCF was 1 and mass (mg) was converted to the volume (mL). TNF-α in the samples was determined (pg) and calculation of TNF-α concentration in each sample was performed by dividing the amount of TNF-α by volume of the sample.

Statistical Evaluation

Statistical analyses were performed by the SPSS (SPSS Inc, Chicago, Ill) statistical program, and the results were shown as median and 25–75 percentiles in the parentheses (quarters). The differences between the groups were compared by Mann-Whitney *U*-test, and the results within each group were analyzed by Wilcoxon signed rank test.

RESULTS

Clinical Parameters

The PI ($P < .05$), PD ($P < .01$), and BOP ($P < .05$) were significantly higher in the HRG (Table 1).

GCF Volume (Figure 1)

In the HRG, within-group analysis showed a statistically significant increase at 1 hour and 24 hours ($P < .01$) (Table 2). In the comparison of findings at 24 hours and 1 week, the volume of the GCF decreased ($P < .01$).

In the RDG, alteration in GCF volume at 1 hour was not statistically significant, but the comparison of the findings at 24 hours and 1 hour showed significant increases ($P < .01$ and $P < .05$, respectively). However, GCF volume was significantly lower at 1 week according to 24-hour measurements ($P < .01$).

A significantly lower volume of GCF was determined at 1 hour and 24 hours in the RDG when the groups were compared with each other ($P < .05$).

Concentration of TNF-α in the Collected GCF Samples (Figure 2)

In the HRG, a statistically significant decrease was observed at 1 week when compared with 24 hours (*P*

Table 2. Gingival Crevicular Fluid Volume (μL)[a]

Groups	Initial	1 h	24 h	1 wk
HRG	13 (8.75–14)	17 (14.5–19.25)**,*****	20 (16.75–24)**,*****	13 (11.25–16)****
RDG	10 (7–12)	12 (7–17)*****	15 (14–20)*,***,*****	11 (7–13)****

[a] Values in the parentheses reveal the 25–75 percentiles (quarters). HRG indicates hybrid reactor group; RDG, rapid canine distalization group.

* Significantly different from initial value ($P < .05$, Wilcoxon signed rank test); ** significantly different from initial value ($P < .001$, Wilcoxon signed rank test); *** significantly different from 1 hour ($P < .05$); **** significantly different from 24 hours ($P < .01$); ***** significant difference between the groups ($P < .05$, Mann-Whitney *U*-test).

Table 3. Concentration of Tumor Necrosis Factor–α (pg/μL)[a]

Groups	Initial	1 h	24 h	1 wk
HRG	57.48 (24.7–216.3)	69.06 (41.67–162.76)***	137.58 (76.1–165.39)	56.38 (23.4–119.57)**,****
RDG	142.85 (49.66–357.68)	268.57 (91.9–483.5)*,***	290.61 (85.71–389.06)	174.24 (121.97–602.96)****

[a] Values in the parentheses reveal the 25–75 percentiles (quarters). HRG indicates hybrid reactor group; RDG, rapid canine distalization group.

* Significantly different from initial value ($P < .05$, Wilcoxon signed rank test); ** significantly different from 24 hours ($P < .01$); *** significant difference between the groups ($P < .05$, Mann-Whitney *U*-test); **** significant difference between the groups ($P < .01$, Mann-Whitney *U*-test).

$< .01$) (Table 3). In the RDG, an increase at 1 hour was statistically significant when compared with the initial value ($P < .05$).

In the evaluation of between-group differences, significantly higher values were determined in the RDG at the 1 hour ($P < .05$) and 1 week measurements ($P < .01$).

DISCUSSION

Chemical analysis of GCF is a useful and promising method to monitor the changes at a single site during a certain period and to investigate the response of dental and paradental tissues to orthodontic tooth movement.[13,16,17] This method has been used especially for human studies, as it is a noninvasive method and repetitive sampling from the same side is possible.[3–10,13–19] Cytokines are local mediators released by cells of the immune system in response to stimulations, and orthodontic forces generate several cytokines that affect the formation and resorption of alveolar bone.[1,3,10,13–19] Mechanical stress triggers the biological response of the periodontal ligament and plays an important role in remodeling. It is likely that not only the nature but also the magnitude of mechanical stress affects the regulation of homeostasis in periodontal ligament. Different magnitudes of tensile force induce different responses from periodontal ligament cells.[37,38]

Rapid canine distalization has been reported as a promising technique to shorten the duration of orthodontic treatment.[29–34] In this technique, osteotomies were performed inside the socket of the extracted first premolar to weaken the resistance of the interseptal bone. Thus, the buccal and distal bone bends and moves with the canine and provides rapid canine distalization through the distraction of the periodontal ligament. Light continuous force cannot keep the bending of the bone; therefore, heavy interrupted forces must be applied in this technique.[31] In this study, we aimed to evaluate the levels of TNF-α during rapid canine distalization so as to determine tissue response to the heavy interrupted orthodontic force.

GCF is an exudate derived from a variety of sources, including microbial dental plaque, host inflammatory cells, host tissue, and serum.[4] The amount of this fluid tends to increase with inflammation and capillary permeability. Some previous studies revealed that GCF volumes were influenced by the presence of an orthodontic appliance independently of the existence of a clinically detectable dental movement.[4,7]

In our study, it was observed that the volume of GCF was lower in the RDG at all the phases, and the between-group differences revealed a significantly lower volume at 1 hour and 24 hours. It has been shown that a decreased vascular supply occurs when heavy orthodontic force are applied.[33] Thus, a lesser volume of GCF in the RDG may depend on reduced vascular supply because of the heavy force. On the other hand, Perinetti et al[4–6] suggested that inflammation rather than orthodontic tooth movement has an effect on the volume of GCF. In our study, initial PI, PD, and BOP were higher in the HRG, revealing more tendencies to gingival inflammation. Orthodontic appliances might have elevated the volume of GCF by developing a mild gingival inflammation in this group.

Concentration of TNF-α increased at 24 hours and declined at 1 week in the HRG. This increase was not statistically significant, but a decrease at 1 week was significant according to the measurement at 24 hours.

The reason for this decrease may be the adaptation of the tissues to the light continuous force at 1 week. In the RDG, the concentration of TNF-α increased significantly at 1 hour. However, in the HRG, the concentration of TNF-α slightly decreased at 1 hour. This was probably because of the incomplete diffusion of the cytokine into the sulcus in 1 hour. Hyalinization should be expected to occur as the magnitude of force increases. In the RDG, heavy forces caused a hyalinization tissue at the distal of the canine and an acute reaction occurred, which induced rapid secretion and a higher concentration of TNF-α. Ozaki et al[38] demonstrated that a strong mechanical stress decreased alkaline phosphatase (ALP) activity, resulting in an acceleration of the production of inflammatory cytokines. Similarly, in our study, because of the heavy orthodontic force, rapid and more production of TNF-α was found.

Although the appliance was activated two times a day in the RDG, no remarkable alteration was observed at 24 hours and 1 week. Tzannetou et al[16] evaluated the changes in IL-1β and β-glucuronidase during rapid palatal expansion by applying heavy forces. They observed severely elevated levels of IL-1β 24 hours after the activation of the appliance, and this elevation continued during the activation period of 3 weeks. However, they observed elevation in β-glucuronidase after the second week of activation, and it increased further at the third week. In our study, the level of TNF-α continued increasing at 24 hours, but at 1 week it was observed that the concentration declined. However, these alterations were not statistically significant. This decrease might be attributed to the adaptation of periodontal tissues to the orthodontic force, and there might be feedback mechanisms that prevented the mediators from increasing excessively, thereby avoiding any harmful consequences.[13–22]

CONCLUSIONS

- Periodontal tissue response to continuous force begins later than heavy interrupted force and declines at 1 week because of tissue adaptation to orthodontic force.
- Heavy interrupted force induces rapid release of the TNF-α from periodontal cells, and tissue response continues for a longer time.
- Rapid canine distalization technique is not a harmful method for periodontal ligament cells, as feedback mechanisms prevent the excessive increase of the cytokines.

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