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Changes of tumor necrosis factor-α, interleukin-10, and tartrate-resistant acid phosphatase5b in the crevicular fluid in relation to orthodontic movement

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Abstract

The aim of this study was to determine gingival crevicular fluid (GCF) expressions of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and tartrate-resistant acid phosphatase (TRAP) 5b in the mechanism of orthodontic tooth movement. Nine adolescents requiring canine distalization participated in the study. A canine undergoing distal movement served as the test tooth, and the contra-lateral canine served as the control. The clinical parameters were recorded and GCF samples were collected from the mesial and distal gingival crevices of teeth at baseline, 1 h, 24 h, 7 days, and 28 days following force application. GCF samples were analyzed using ELISA. The data were analyzed using three-way repeated measures analysis of variance. TNF-α and TRAP5b levels in distal and mesial sites of the test teeth were significantly higher than that at both sites of the controls. When compared with baseline values, increase was prominent at 1 h and 24 h. The IL-10 concentration decreased during experimental period at both sites of the control and test teeth. The results demonstrated that orthodontic forces evoke changes in the levels of TNF-α, IL-10, and TRAP5b during the initial stages of force application. The changes in local host response in periodontal tissues may be one of the triggers in regulating alveolar bone resorption during orthodontic tooth movement.

Keywords

bone remodeling, gingival crevicular fluid, interleukin-10, tartrateresistant acid phosphatase, tooth movement, tumor necrosis factor-alpha

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Introduction

Orthodontic tooth movement is achieved by a remodeling activity leading to bone resorption in the pressure sites and bone deposition in tension sites.[1] Bone remodeling that facilitates the movement of the teeth through bone is regulated by various inflammatory mediators, such as prostaglandins, cytokines, and chemokines.[2]

Cytokines are involved in initiating, amplifying, perpetuating, and resolving inflammatory responses, and classified as pro-inflammatory (PIC) and anti-inflammatory (AIC) key mediators, which play an important role in bone remodeling.[3] Numerous studies have shown that the pro-inflammatory cytokines,

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such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), are important promoters of bone resorption and tooth movement.[4,5] Anti-inflammatory cytokines, such as IL-4, -10, -12, -13, and -18, exert an opposite effect and contribute to inhibit bone resorption.[6] Thus, the balance between the activities of pro-inflammatory and anti-inflammatory cytokines plays a major role in progression of the periodontal inflammation.

TNF-α, which is released primarily by activated monocytes, macrophages, and osteoblasts, is a potent inducer of osteoclastic bone resorption.[7] In addition, orthodontic tooth movement has been reported to increase the levels of TNF-α in the gingival crevicular fluid (GCF) in humans.[8] It has been well established that the release of TNF-α promotes tissue resorption at pressure sites.

Interleukin-10 (IL-10) is produced primarily by T cells, B cells, dendritic cells, and monocytes/macrophages.[9] IL-10 plays an important role in inhibition of the secretion of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6). IL-10 has been implicated in the suppression of tissue destruction, and is expressed in both healthy and diseased human periodontal tissues.[10] Periodontal therapy has been shown to reduce the total amount of IL-10 in GCF by Gamonal et al.,[11] while some studies have found increased levels of IL-10 after periodontal therapy.[12,13] IL-10 total amounts were also reported to be similar in diseased and non-diseased sites and reported no changes following therapy.[14] Changes in IL-10 levels in response to mechanical stimulation remain a controversial subject as well. Alhashimi et al.,[15] and more recently Grant et al.,[16] could not detect IL-10 during orthodontic tooth movement, while Garlet et al.[17] reported that tension sites exhibited higher expressions of IL-10 during orthodontic tooth movement.

Tartrate-resistant acid phosphatase, especially isoenyzme 5b (TRAP5b), is secreted into the circulation by osteoclasts during bone resorption and has been considered a potentially useful marker of bone resorption.[18] The early inflammatory process at compression sites is caused by the constriction of the periodontal microvasculature, resulting in a focal necrosis known by its histological appearance as hyalinization, and compensatory hyperemia occurs in the adjacent periodontal ligament and pulpal vessels.[19,20] These necrotic sites release various chemo-attractants that draw giant, phagocytic, TRAP-positive cells to the periphery of the necrotic PDL.[21] These cells resorb the necrotic periodontal ligament, as well as the underlying bone and cementum. The release of pro-inflammatory cytokines and lysosomal enzymes that promote tissue resorption at compression and tension sites during orthodontic tooth movement has been well documented. Increases in the lysosomal enzymes, acid phosphatase, and TRAP are also localized at compression sites.[22]

The biological basis of tooth movement induced by mechanical stress has been studied over years, and some doubts still remain. The knowledge of molecular and cellular mechanisms that regulate the movement of the teeth can be used to accelerate the treatments, and reduce the side effects associated with orthodontic treatment such as pain, root resorption, pulpal changes, decalcification, and periodontal diseases. Investigating the GCF pro-/anti-inflammatory response in orthodontic tooth movement may also provide important data about inflammatory bone loss and new treatment strategies. Hence, we aimed to outline changes in GCF composition for three different groups of mediators in tooth movement at sites of pressure and tension in the same patients.

Since orthodontic tooth movement may affect the periodontium, a full understanding of the biologic response of periodontal tissues to orthodontic forces is essential for achievement successful orthodontic treatment in periodontally-compromised patients. Furthermore, unfolding the cytokine mechanisms of orthodontic tooth movement may help to create different treatment approaches in orthodontics. Accelerating methods for tooth movement, such as the administration of biological molecules, may reduce the duration and risks of orthodontic treatments. In consequence, this study aims at identifying the role of cellular mechanisms of orthodontic tooth movement. Therefore, the objective of this study was to determine the involvement of IL-10, TRAP 5b, and TNF-α in the GCF during orthodontic force application.

Materials and methods

Subject population

The study was undertaken in the Orthodontics and Periodontology Departments of the Faculty of Dentistry, Gazi University. Nine adolescents (age range, 13–17 years; mean age, 14.5±1.33 years) who required fixed orthodontic treatment were

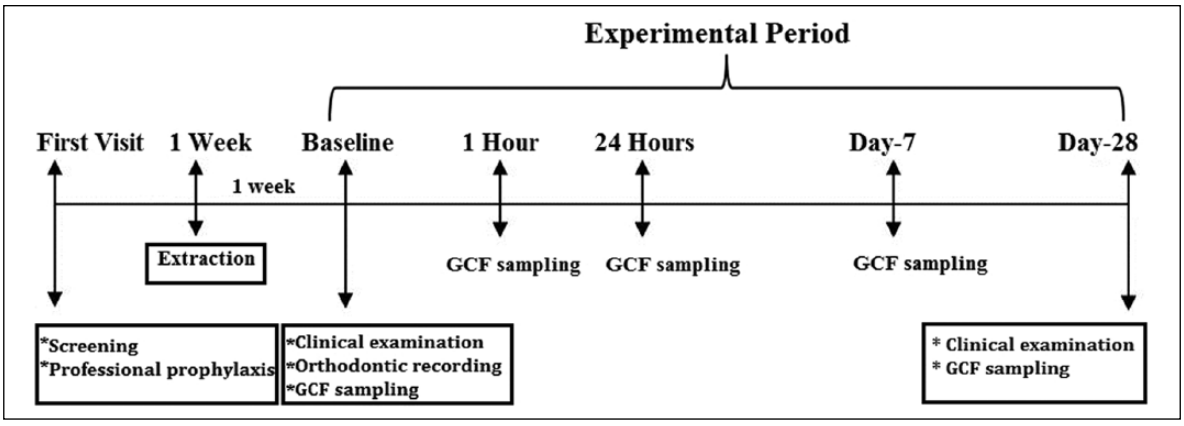

Figure 1. Study protocol performed in the study.

selected to participate in this study according to the following criteria: (1) good general health; (2) lack of antibiotic therapy during the previous 6 months; (3) no use of anti-inflammatory drugs in the month preceding the study; (4) healthy periodontal tissues with generalized probing depths of <3 mm; (5) no radiographic evidence of periodontal bone loss; (6) no received periodontal therapy within 6 months preceding the study; (7) requirement of extraction of four first-premolars and canine distalization as part of orthodontic treatment plan; and (8) all were never-smokers. All eligible subjects were thoroughly informed of the nature, potential risks, and benefits of their participation in the study and informed consent was obtained from the parents of patients. This study protocol was approved previously by Ethical Board of Gazi University. The study was performed in accordance with the Helsinki Declaration of 1975,[23] as revised in 2008.[24]

Experimental design

The schedule of the study is presented in Figure 1. All subjects underwent a first evaluation for periodontal status and received oral hygiene instruction, full mouth supragingival scaling, and polishing 2 weeks before the trial had been initiated. Patients were given detailed oral hygiene instructions and maintained self-performed oral hygiene throughout the study. Supragingival scaling was performed for each patient in all groups using hand instruments and ultrasonic devices. Following full mouth professional scaling and polishing, first premolars were extracted. Subjects were re-called for orthodontic treatment 1 week after the extraction of first premolars. Before orthodontic treatment, periodontal examination and orthodontic records of the patients were obtained and GCF sampling was performed (baseline). Initial orthodontic records included facial photographs, and digital panoramic and cephalometric radiographs and models. The entire orthodontic appliance was placed in a single clinical session by the same orthodontist. Patients were given detailed oral hygiene instructions and maintained self-performed oral hygiene throughout the study.

Orthodontic appliance

For each subject, canines in both the upper and lower sextants undergoing distalization were randomly chosen as the test tooth (TT), and the contralateral canines served as the control tooth (CT). The control teeth were free of distal force. Orthodontic brackets (Omni Roth, GAC International, Inc., Bohemia NY, USA) were placed on the buccal surfaces of both test and control teeth. Molar bands were placed on the upper first molars. Segmental 0.016- $\times$ 0.022-inch Sentalloy archwires were placed on the test canines, and the distalization forces were applied by Sentalloy closed coil springs with eyelets (10-000-02 Medium, GAC International, Inc., Bohemia NY, USA). Ligation was performed from the canine brackets towards the molar bands with a continuous force of 150 g which was verified by a calibrated orthodontic force gauge. During the study period no orthodontic activation was performed.

Periodontal examination

All examinations were conducted by a single, experienced dental examiner who was blinded. The whole mouth recordings in each participant served

as a basis for the clinical periodontal diagnosis. The following parameters were recorded in a sequential order by the same investigator at baseline (immediately prior to orthodontic treatment) and at the 28th day: plaque index (PLI),[25] gingival index (GI),[26] pocket probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). Third molars were excluded from the analysis. All clinical parameters were measured at six sites per tooth (mesio-, mid-, disto-buccal and mesio-, mid-, disto-palatal) using a Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Village, IL, USA) calibrated in millimeters. Orthodontic treatment was not scheduled if the patient could not demonstrate an adequate standard of supragingival plaque control (individuals with plaque index and gingival index average less than 1).

The Plaque Index System Scores Criteria[25] are as follows: 0, no plaque; 1, a film of plaque adhering to the free gingival margin and adjacent area of the tooth; 2, moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin, which can be seen with the naked eye; and 3, abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

The Gingival Index System Scores Criteria[26] are as follows: 0, normal gingiva; 1, mild inflammation, slight change in color, slight edema, no bleeding on probing; 2, moderate inflammation, redness, edema and glazing, bleeding on probing; and 3, severe inflammation, marked redness, edema, ulceration, tendency to bleed spontaneously.

GCF collection

GCF was sampled separately from the mesial (tension) and distal (pressure) gingival crevices of each canine both at the test and control teeth at baseline, i.e. immediately prior to orthodontic treatment and 1 h, 24 h, 7 days, and 28 days after the application of orthodontic force. The sampling was performed before clinical measurements by blinded experienced periodontist.

After removal of supragingival plaque with sterile curettes, the sampling sites of the test and contra-lateral test teeth were gently washed, air-dried, and isolated with sterile cotton rolls. The standardized sterile paper strips (Periopaper-ProFlow Inc., Amityville, NY, USA) were inserted into the gingival crevices until mild resistance for 30 s.[27] Strips contaminated with saliva or blood were excluded from the sampled groups. The paper strips from the mesial and distal sites of each tooth were sealed in polypropylene containers separately and stored at –20°C until analysis. GCF volume in each paper strip was detected by Periotron 8000 (Periotron 8000, Oraflow Inc., Smithtown, NY, USA).

ELISA analysis

Each tube containing sample strips was eluted with 250 μL Hank's buffer containing 0.1% bovine serum albumin (Sigma, St Louis, MO, USA) by centrifugation (2,000 × g; 4°C) for 5 min. After removal of the strips, the supernatants were divided into three aliquots for the determination of each cytokine. TNF-α, IL-10, and TRAP5b concentrations were measured by commercially available enzyme-linked immunosorbent assay. For TRAP5b analysis, high sensitive kits (Medac, Webel, Germany) were used to quantitatively detect levels of TRAP5b. The amounts of IL-10 and TNF- α were determined using ELISA assays (RayBiotech Inc., Norcross, GA, USA). The assays were conducted according to the manufacturer's instructions. After the color development was stopped, the optical density was measured at 450 nm. The total TNF-α, IL-10, and TRAP5b were determined in picograms (pg) and calculation of the concentration in each sample was performed by dividing the amount of TNF-α, IL-10, and TRAP5b by the volume of the sample (pg/μL).

Statistical analysis

To compare groups, sites, and times for levels of TNF-α, levels of IL-10, levels of TRAP5b, and GCF volume, the data were analyzed using three-way repeated measures analysis of variance for nested designs by PROC GLM SAS 9.0. This test statistics was used to determine if there was any significant variation in levels of TRAP5b, levels of IL-10, levels of TNF-α, and GCF volume between groups, between sites nested under group, and between times nested within each site. Groups, site nested under groups, and time nested within each site were fixed factors in the analysis. The differences between baseline and day 28 of PLI, GI, pocket depth, clinical attachment level, and bleeding on probing values were evaluated by Wilcoxon signed rank test by the statistical software package (SPSS v.15.0 Inc., Chicago, IL, USA). Dots on the graphs show the outliers for the dataset for each

Table 1. The median and minimum-maximum values of clinical parameters (full mouth) at baseline and on day 28.

	PLI	GI	PD (mm)	CAL (mm)	BOP (%)
Baseline					
Median	1	1.14	2.11	2.11	0.25
Minimum	0.26	0	1.25	1.25	0
Maximum	1	1.4	2.91	2.91	0.33
Day 28					
Median	1	1.14	2.26	2.26	0.25
Minimum	0.56	0	1.25	1.25	0
Maximum	1	1.4	2.91	2.91	0.33

BOP, bleeding on probing; CAL, clinical attachment level; PD, pocket probing depth; GI, gingival index; PLI, plaque index.

variable. $P < 0.05$ was considered to be statistically significant.

Results

Clinical parameters

Plaque accumulation was minimal and gingival health was excellent in all subjects throughout the study. Tables 1 and 2 show the data concerning the clinical parameters. At baseline, clinical parameter scores were similar, without any statistically significant differences, irrespective of the experimental groups or sites ($P > 0.05$) (Table 2). Also no statistically significant differences were found in any clinical parameters between baseline and day 28 ($P > 0.05$). The differences were significant between baseline and day 28 at TT pressure site regarding PLI and GI ($P < 0.05$). Significant differences were also found between baseline and day 28 at both TT and CT pressure sites regarding BOP ($P < 0.05$).

GCF volume

Table 3 shows the data concerning the GCF volumes. The baseline mean volumes of GCF of the test and the control teeth were similar. There were statistically significant differences in the GCF volume between test and control teeth at 1 h ($P < 0.05$). At 24 h, statistically significant results were found between the groups at the pressure sites ($P < 0.05$), a slight increase was seen in the TT group. A statistically significant difference between tension and pressure sites was detected only for the TT group at 24 h, with the GCF volume of the distal site higher than that of the tension site ($P < 0.05$). No significant differences were found between tension and pressure sites within the control group in any time point ($P > 0.05$).

Table 2. The mean values of clinical parameters (sample sites) at baseline and on day 28.

	Groups	Baseline	Day 28
PLI	TT pressure site	2.69 (0.75)*†	1.67 (0.81)
	TT tension site	2.46 (1.10)	1.54 (0.99)
	CT pressure site	2.77 (0.60)	1.73 (0.86)
	CT tension site	2.12 (0.92)	1.42 (0.82)
GI	TT pressure site	0.90 (0.56)†	0.37 (0.35)
	TT tension site	0.48 (0.52)	0.29 (0.37)
	CT pressure site	0.63 (0.62)	0.35 (0.38)
	CT tension site	0.44 (0.37)	0.21 (0.30)
PD (mm)	TT pressure site	2.45 (1.53)	2.24 (0.46)
	TT tension site	2.22 (0.57)	2.06 (0.55)
	CT pressure site	2.26 (0.49)	2.19 (0.67)
	CT tension site	2.11 (0.47)	2.01 (0.51)
CAL (mm)	TT pressure site	2.45 (1.53)	2.24 (0.46)
	TT tension site	2.22 (0.57)	2.06 (0.55)
	CT pressure site	2.26 (0.49)	2.19 (0.67)
	CT tension site	2.11 (0.47)	2.01 (0.51)
BOP (%)	TT pressure site	7.69 (9.37)†	2.27 (7.54)
	TT tension site	4.67 (9.65)	1.92 (6.93)
	CT pressure site	6.67 (8.87)‡	2.08 (7.22)
	CT tension site	4.17 (9.73)	2.02 (5.72)

*Normally distributed data are expressed as means and standard deviations. Friedman test was used for comparisons. $P < 0.05$ was considered to be statistically significant.
†The differences were significant between baseline and day 28 at TT pressure site ($P < 0.05$).
‡The differences were significant between baseline and day 28 at CT pressure site ($P < 0.05$).
TT: Test teeth; CT: Control teeth; PLI: Plaque index; GI: Gingival index; PD: Pocket depth; CAL: Clinical attachment level; BOP: Bleeding on probing.

Levels of TNF-α in GCF

At pressure sites, on days 1 and 7, significant differences were observed in TNF-α levels between the TT group and the CT group ($P < 0.05$). In the TT group, pressure sites showed statistically significant increase at 1 h when compared with the initial value ($P < 0.05$), and a decrease was noted statistically significant at 7 days and 28 days when compared with 1 h ($P < 0.05$) (Figure 2).

Levels of IL-10 in GCF

At pressure sites, the GCF level of IL-10 was greater than those at tension sites in both groups. However, no statistically significant results were

Table 3. Three-way nested repeated ANOVA test results for levels of GCF volume.

Source	DF	Type I SS	Mean square	F value	Pr>F
Group	1	9.52200000	9.52200000	8.75	0.0035
Site	1	6.34688889	6.34688889	5.83	0.0168
Time	4	43.33422222	10.83355556	9.96	<0.0001
Site (group)	1	0.02688889	0.02688889	0.02	0.8753
Time (site)	4	1.81088889	0.45272222	0.42	0.7969
Model	11	61.0408889	5.5491717	5.10	<0.0001
Error	168	182.8211111	1.0882209		
Corrected total	179	243.8620000			

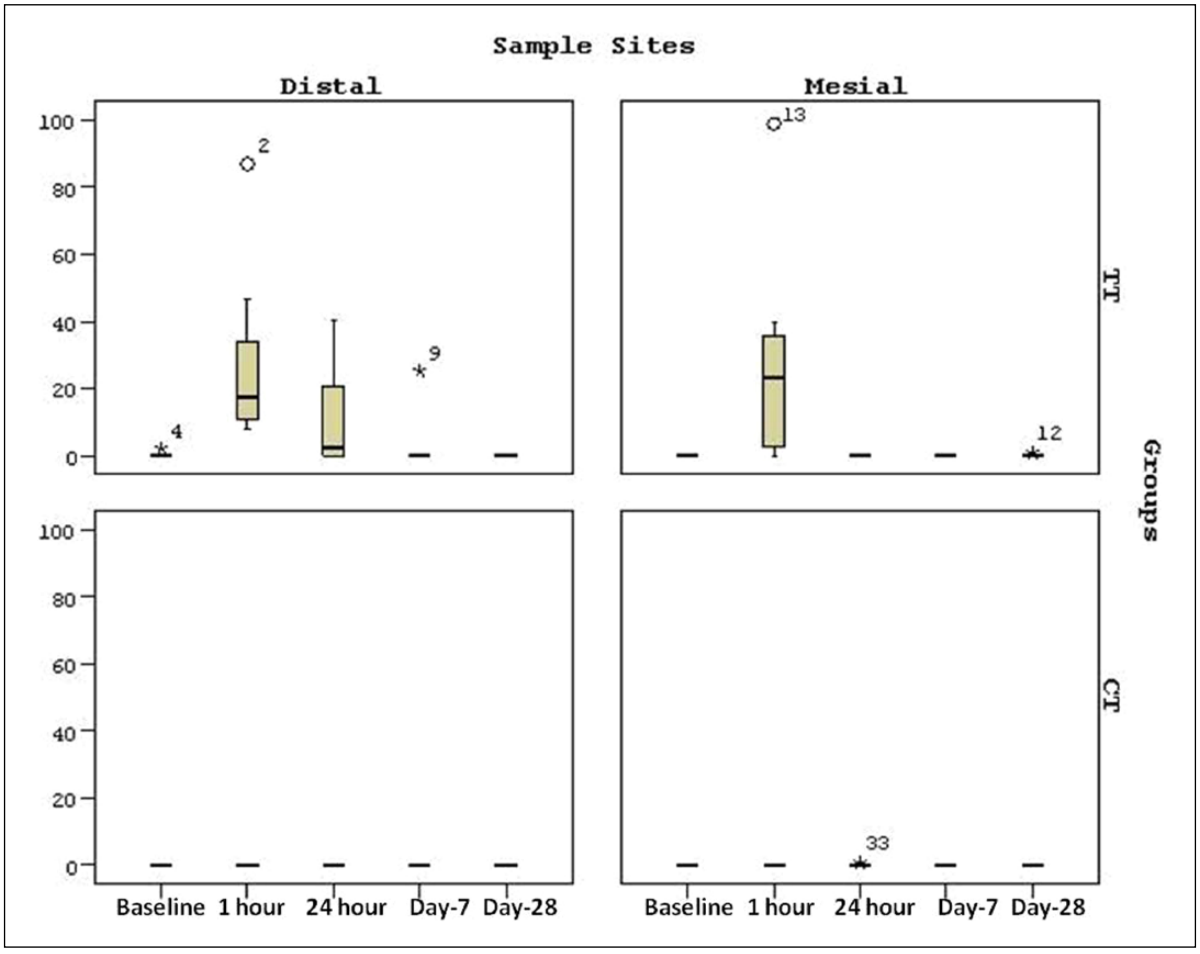

Figure 2. Levels of TNF-α concentration (pg/μL) in GCF for each group, each site, and each time point. Dots on the graphs show the outliers for the dataset for each variable. $P < 0.05$ was considered to be statistically significant. CT, control teeth; TT, test teeth.

found between the groups during the whole period of observation ($P > 0.05$). The IL-10 levels did not show a statistically significant change over time within the groups ($P > 0.05$) (Figure 3).

Levels of TRAP5b in GCF

No significant differences were found between tension and pressure sites within both groups by time point ($P > 0.05$). GCF TRAP5b levels did not differ significantly from the baseline to the end of the experimental period at the control teeth ($P > 0.05$). The results obtained for the TT group showed an increase of GCF levels of TRAP5b at the pressure sites until it reached a maximum on day 7. This increase was statistically significant on day 1 ($P < 0.05$). Then, after 28 days TRAP5b

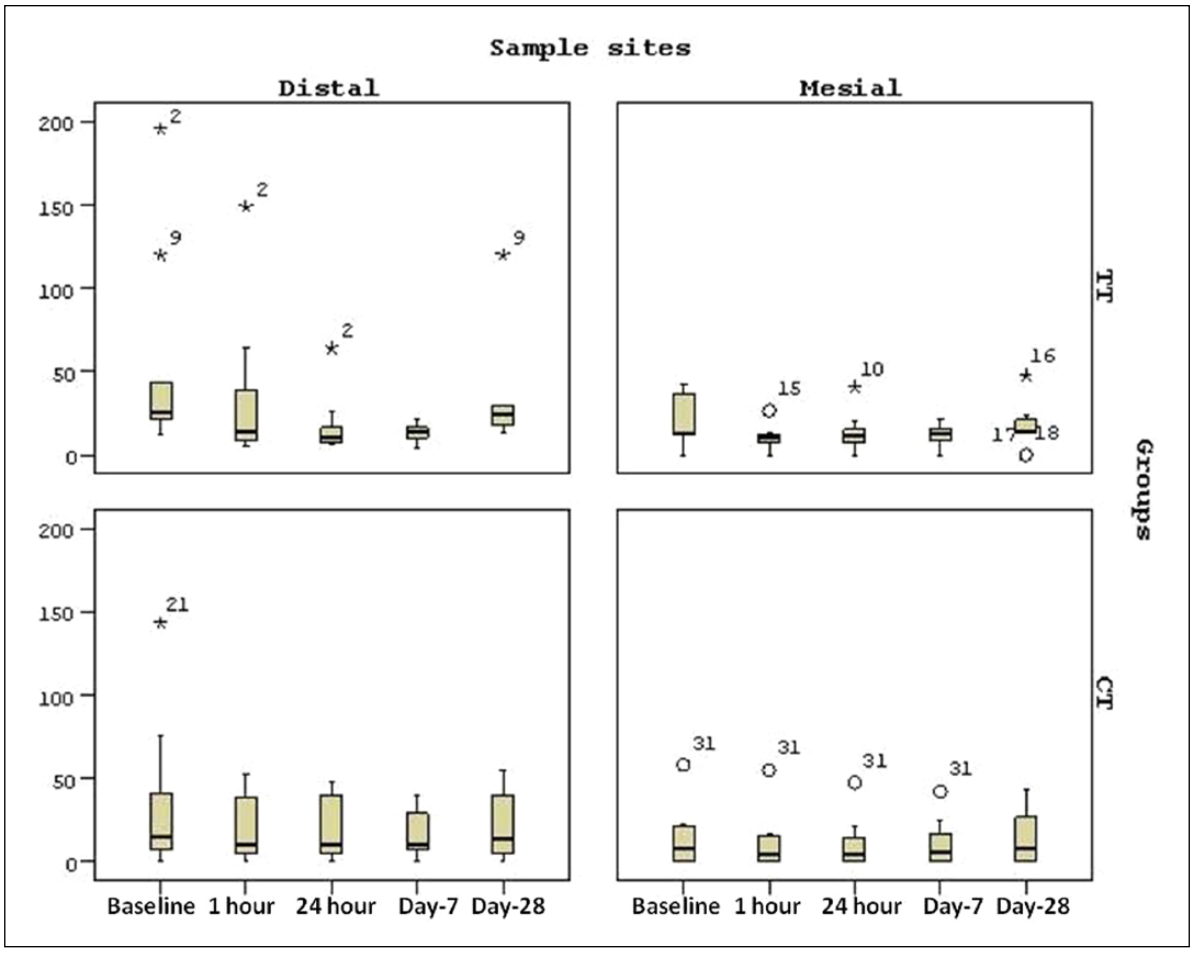

Figure 3. Levels of IL-10 concentration (pg/μL) in GCF for each group, each site, and each time point. Dots on the graphs show the outliers for the dataset for each variable. $P < 0.05$ was considered to be statistically significant. CT, control teeth; TT, test teeth.

levels decreased significantly when compared with 1 day and 7 days ($P < 0.05$) (Figure 4).

Discussion

Molecular and cellular events that regulate osteogenesis during tooth eruption and orthodontic treatment require different biological processes that make their mechanisms unique. Investigation of the differential regulation of these processes may be helpful in highlighting the complexity of bone remodeling during tooth movement. The up- and downregulation of cytokines in GCF have been studied to provide a non-invasive way to show their involvement during orthodontic tooth movement. The present study was an attempt to investigate the efficacy of TNF-α, IL-10, and TRAP5b levels in early stage of tooth movement with continuous orthodontic force.

Orthodontic tooth movement consists of three phases over a certain period of time: the initial phase – initial tooth movement; the lag phase – delayed tooth movement; and the postlag phase – a linear increment of tooth movement. The initial phase is characterized by immediate and rapid movement and occurs 24–48 h after the first application of force to the tooth. The second phase of arrest in tooth movement lasts 4–20 days. The postlag phase follows the lag phase and comprises the real tooth movement. Each of these phases can be characterized using suitable biomarkers. Although different mediators were analyzed during orthodontic tooth movement at different time intervals in previous *in vivo* studies,[2,8,28] in order to evaluate true orthodontic tooth movement, at least 1 month for an observation period is required. Therefore, we aimed to investigate the levels of TNF-α, IL-10, and TRAP5b over a 1-month period as similar studies.

Previous studies have demonstrated that plaque accumulation depends on the site.[29] The upper canines were selected in this study because these

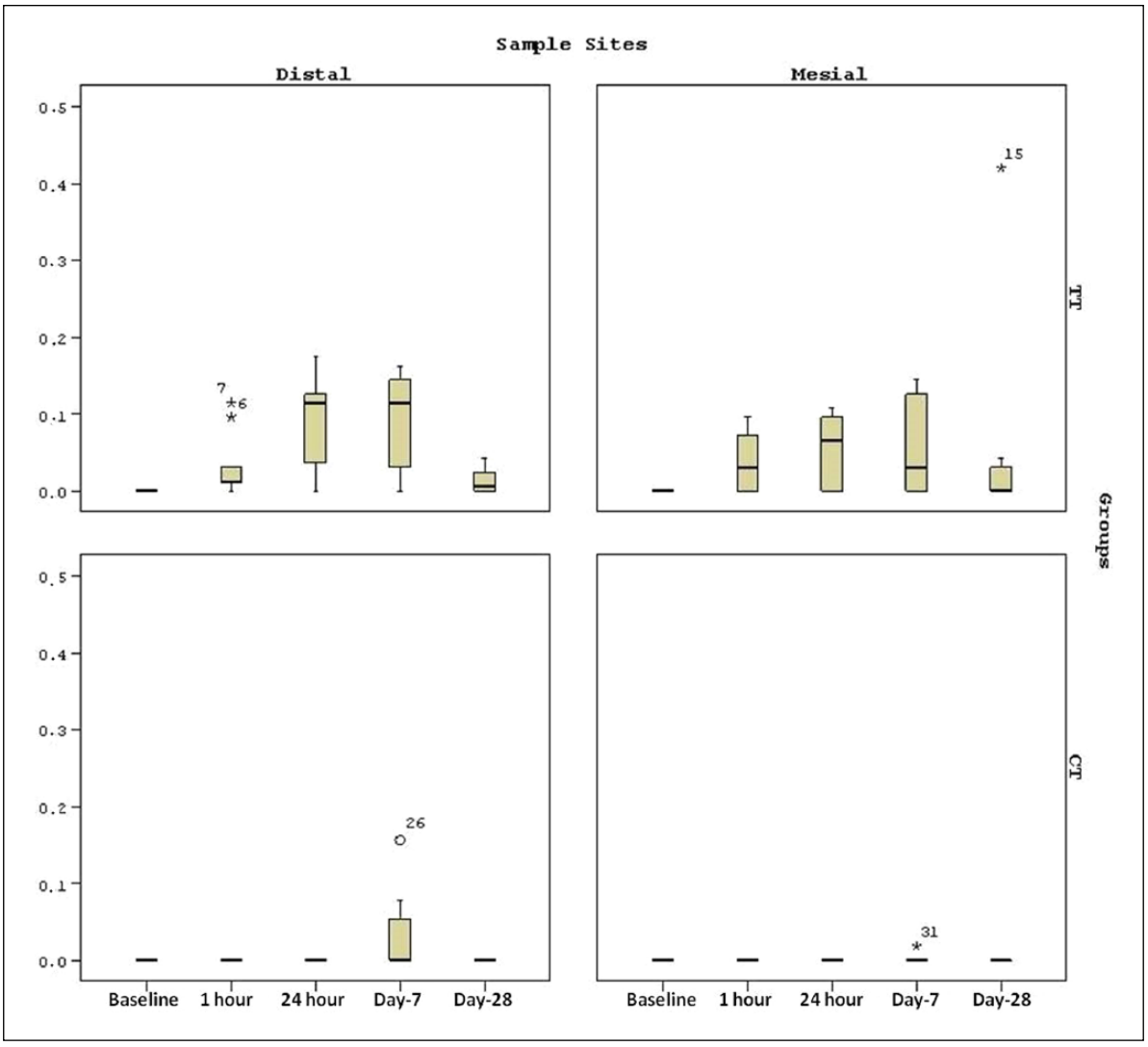

Figure 4. Levels of TRAP5b concentration (pg/μL) in GCF for each group, each site, and each time point. Dots on the graphs show the outliers for the dataset for each variable. $P < 0.05$ was considered to be statistically significant. CT, control teeth; TT, test teeth.

teeth are accessible and easily cleaned. Test and control sites were selected from the same subjects in order to eliminate individual differences.

The early phase of orthodontic tooth movement involves an acute inflammatory response, characterized by vasodilatation and the migration of cells out of periodontal ligament capillaries. Since inflammatory mediators trigger tissue remodeling and the release of these inflammatory mediators at the site of inflammation induces bone remodeling process consequent to tooth movement, the monitoring of the GCF volume has been proposed as a predictable biomarker of tissue changes during orthodontic tooth movement.[30] During the last decades, the changes of GCF composition during orthodontic tooth movement have been extensively studied.[8,31] It has been reported that GCF analysis is a non-invasive, effective and useful method to monitor the response of periodontal tissues to orthodontic tooth movement.[8,31] GCF volume and composition can affect by inflammation of gingiva. In addition, expressions of inflammatory mediators in GCF are directly influenced by type of mechanical stress (tension versus pressure). In the present study, significant increases in GCF volume were found at canines under orthodontic force, although no clinically gingival health changes occur according to clinical parameters. The results demonstrated a statistically significant change in the pressure side than tension of TT group at 24 h. However, conflicting results have been reported regarding the effects of orthodontic tooth movement on the GCF

volume.[32,33] Perinetti et al. showed that gingival inflammation affects GCF volume rather than orthodontic tooth movement.[32] On the other hand, Karacay et al. suggested that GCF volume decreased after heavy orthodontic force application.[33] These results could be attributed to the different methods of GCF sampling and laboratory techniques as well as variations in the monitoring time following the application forces and study design.

Various studies have evaluated the levels of TNF-α in GCF to explain the involvement of this cytokine in the mechanism of orthodontic tooth movement.[7,8,33] Consistent with literature, in the present study the TNF-α level significantly increased at 1 h on the canine to which force was applied followed by a significant decrease at 7 and 28 days.[8,33] Our study demonstrated that TNF-α levels were correlated with the speed of tooth movement, since TNF-α expression showed a rapid onset following the application of distalizing force, then slowed over 7 and 28 days. Also in agreement with the literature, we found that TNF-α levels were different at tension and compression sites on the test teeth indicating osteoclast response to mechanical stress.[2]

The synthesis of monocyte-derived pro-inflammatory cytokines is inhibited by IL-10.[34] In line with previous studies we found the negative correlation between IL-10 levels and TNF-α levels.[35,36] These results showed that TNF-α production is inhibited anti-synergistically by IL-10. Therefore, the presence of IL-10 in GCF could perform a relevant role in the regulation of local immune response.

It has been revealed that the release of collagen from bone tissue may be related with the secretion of TRAP from osteoclasts during bone resorption.[37] The levels of TRAP correlates with the resorption amount of bone, therefore serum TRAP is a potentially useful resorption marker. In this study, the levels of TRAP5b in the test teeth were increased in comparison to the control teeth in both mesial and distal sites at 24 h. Studies have reported that expression of TRAP was detected in the compression sites of the alveolar bone during orthodontic tooth movement, in accordance with current results.[22]. Although animal tooth movement experimental studies have found that serum TRAP5b activity could become elevated at pressure sites during orthodontic tooth movement,[22] a limited number of studies had investigated TRAP activity in a human model during orthodontic tooth movement.[38] Controlled experimental studies are required to validate the use of TRAP as a biomarker to monitor orthodontic tooth movement.

Mechanical forces in different duration, magnitude, and direction are important factors in orthodontic treatment because they evoke biological changes in the periodontal tissues and cause bone remodeling. Evaluation of these changes can be analyzed using GCF, which is a non-invasive and useful method. In this study, continuous orthodontic forces revealed changes in the levels of TNF-α and TRAP5b at the early stages of inflammation between the test and the control teeth. Levels of IL-10 concentration were higher in the control teeth than that in the test teeth, and revealed decreases in a time-dependent manner at both sites of the test and control teeth. Therefore the presence of IL-10 in GCF could play a relevant role in inhibiting the local immune response. Based on the results of this study, in the periodontitis patients undergoing orthodontic treatment the speed and force of orthodontic tooth movement should be set taking into account. Furthermore, the biological changes following orthodontic force application in early and late phases should be evaluated before the orthodontic treatment. Also further studies are needed to determine the activation of these mediators in longer time intervals in relation to the rate of tooth movement.

Declaration of conflicting interests

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