TNF-α, IL-1β AND IL-8 LEVELS IN TOOTH EARLY LEVELLING MOVEMENT
ORTHODONTIC TREATMENT

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Abstract

During orthodontic treatment, the early response of periodontal tissues to mechanical stress involves several metabolic changes that allow tooth movement. The aim of the present study was to determine tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 8 (IL-8) levels in GCF in tooth early levelling movement orthodontic treatment.

Seventeen patients, 8 female and 9 male individuals (aged: 16-20 years; mean 18.2 ± 1.4 years), participated in this study. Each subject underwent a session of professional oral hygiene and received oral hygiene instructions. Three months later, a fixed orthodontic appliance was placed. The patients were seen at baseline and between 1-7 days levelling of the teeth.

The concentration of TNF-α, IL-1β and IL-8 were shown in Tables 1. There were statistical differences between the observations at the 1-2 day of levelling for TNF-α, IL-1β and IL-8 (p<.05).

Orthodontic forced induces rapid release of the TNF-α, 1L-1β and IL 8 levels during tooth movement in gingival crevicular fluid (GCF). The results of this study support the hypothesis that proinflammatory cytokines play a potent role in bone resorption after the application of orthodontic force in short time.


Keywords: orthodontic treatment, bone remodeling, gingival crevicular fluid, TNF-α, IL-1β, IL-8.

Introduction

Orthodontic tooth movement is based on force induced periodontal ligament (PDL) and alveolar bone remodeling.1-4 Mechanical stimuli exerted on a tooth cause an inflammatory response in the periodontal tissue. Inflammatory mediators trigger the biological processes associated with alveolar bone resorption and apposition.1

An important breakthrough in bone biology was the identification of the role of cytokines in bone remodeling. Cytokines are involved in initiating, amplifying, perpetuating, and resolving inflammatory responses. They are key mediators for tissue damage and play an important role in tooth movement. Cytokines are classified as proinflammatory and anti-inflammatory. Proinflammatory ones are TNF-α, IL-1, interleukin 2, interleukin 6 and IL-8. Anti-inflammatory cytokines are interleukins 4, 10, and 13. The proinflammatory ones are alarm cytokines, inducing vascular dilatation with increased permeability and enhancing inflammatory response.5

TNF-α is a typical mediator of inflammatory response that has been shown to be involved in the process of bone resorption.6,7 TNF-α plays a prominent role in the mechanism controlling the appearance of osteoclasts at compression sites.8,9 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 resorption.10

IL-1β, a major physiologic form of IL-1, is mainly secreted by monocytes and partially by macrophages, endothelial cells, fibroblasts, and epidermal cells. This secretion is activated by various stimuli11 All these studies demonstrated that mechanical stimuli activate the release of inflammatory cytokines.

IL-8 is a potent proinflammatory cytokine that has a key role in the recruitment and activation of neutrophils during inflammation. It is secreted mainly by monocytes and is important in regulating alveolar bone resorption during tooth movement by acting early in the inflammatory response.12

Various researchers demonstrated elevated levels of cytokines in tooth movement.7,13 Alhashimi et al.7 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 rat molars. 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.

The level of chemokines was significantly increased at the early stages and decreased in 7–10 days, and it was concluded that the early inflammatory response was the main trigger for bone remodeling processes.1 Stashenko et al.14 examined the cytokine levels of IL-1α, IL-1β, and TNF-α in bone resorption and indicated that IL-1β is an important mediator in the mechanism of bone resorption.

The purpose of the present study was to determine TNF-α, IL-1β and IL-8 levels in tooth early levelling movement orthodontic treatment and to evaluated the effect of orthodontic forces on periodontal tissues.

Materials and Methods

8 female and 9 male individuals (age 16-20 years; mean 18.2 ± 1.4 years), who attended to Dicle University, School of Dentistry, Department of Orthodontics and diagnosed for the extraction of their first premolar(s) were participated in this study. The inclusion criteria for the participants were: (1) a healthy systemic condition, (2) no use of anti-inflammatory drugs in the four months preceding the beginning of the study, (3) the need for extraction treatment with fixed appliances involving distalization of at least one maxillary canine, (4) probing depth values (measured as the distance from the bottom of the sulcus to the most apical portion of the gingival margin) not exceeding three mm in the whole dentition, (5) no loss of periodontal attachment (measured as the distance from the bottom of the sulcus to the cemento-enamel junction) exceeding two mm in any interproximal site, and (6) no radiographic evidence of periodontal bone loss after a full-mouth radiographic periapical examination. Informed consent was obtained from the patients and the parents of patients under 18 years of age.

Clinical procedures

Maxillary first premolars were extracted for each participant and, all subjects received repeated oral hygiene instructions (OHIs), which included the correct use of a toothbrush and an interdental brush during the following three months. At the end of this period, before orthodontic treatment with full brackets (Omni Roth, GAC International, Inc, Bohemia NY, USA) was initiated, GCF sampling was performed (baseline). GCF was collected from the mesial aspect of the upper canines in this study. At this appointment orthodontic treatment was begun with 0.014” Nitinol arch wires (GAC International, Inc, Bohemia NY, USA). On the second appointment, patient was instructed to brush their teeth and not to eat anything three hours before other sampling. The forces that are applied on the tooth were still active. Other days (3rd, 4th, 5th, 6th, 7th) sampling was performed with the same procedures.

GCF sampling

Sample collections were done in the early hours of the day. GCF sampling was obtained with paper strips (Periopaper, Pro Flow, Amityville NY, USA) using the method described by Rudin et al.15 Sampling was performed only on the vestibular sides of the tooth to prevent salivary contamination (sample sites were isolated with cotton rolls, plaque was removed and the tooth surfaces were air dried). Paper strips were placed into the sulcus and, after waiting 30 seconds, an apparatus (periotron
8000, Ora Flow Inc, Plainview NY, USA) was used for determining the GCF volume. Saliva and blood contamination was important so contaminated samples were excluded from the study. Paper strips were stored in sterile tubes at -20°C until the day of experimentation. GCF sampling was done before all other clinical examinations were performed to prevent increase in fluid volume. Before examination of the GCF, 1000µl sterile NaCl (9mg/ml) was added to paper strips and the GCF was diluted at 3000g at +5°C for 20 minutes.

**Cytokines analysis**

The kit and the machine used for measuring TNF-α, IL-1β and IL-8 were immunoassay system (Immumile 1000, Diagnostic Products Corp., Los Angeles CA, USA) (Figure 1). For the manual dilution of patient samples, TNF-α, IL-1β and IL-8 free non-human buffer matrix was used. The amount of TNF-α, IL-1β and IL-8 detected in each sample was compared with an TNF-α, IL-1β and IL-8 standard curve that demonstrates a direct relationship between optical dentistry and cytokine concentration. The total amount of TNF-α, IL-1β and IL-8 was determined in picograms.

**Statistical evaluation**

Repeated one way ANOVA was used to determine differences between the GCF TNF-α, IL-1β and IL-8 cytokine levels in experiment days (Table 1). Paired student t test was used to understand the significant differences between the groups at a time interval, by taking the differences between two adjacent points for TNF-α, IL-1β and IL-8 concentrations (Table 2). Descriptive measurements (mean, standard deviation, median, minimum and maximum) of the volume of the gingival crevicular fluid are given in Table 3. All of the statistical evaluations were made using SPSS 10.0 software program.

**Results**

The concentration of TNF-α, IL-1β and IL-8 were shown in Table 1. There were statistically significant differences between the observations at the 1-2 day of levelling for TNF-α, IL-1β and IL-8 (p<.05). Moreover, there was no statistically significant difference was detected in GCF levels of TNF-α, IL-1β and IL-8 between the days of 3-7 day of leveling (p>.05).

The comparison of two adjacent concentrations of TNFα ,IL-1β and IL-8 were shown in Table 2. The comparison between two adjacent observations were not statistically different from each other (p>.05).

Gingival crevicular fluid volumes were given in Table 3. The GCF volume was greater
between 1-3 days of levelling and it returned to base line level after 3 days. No statistically significant differences were found between the groups (p>.05).

Discussion

Orthodontic tooth movement occurs by the remodeling of the alveolar bone as a result of the force exerted on the periodontium. When a force, greater than capillary blood pressure, is directed on the tooth; a hyaline zone occurs in the direction of force and a tension site occurs on the opposite side. This hyaline zone, free of cells, is necrosed by osteoclasts that originate from the tension site. On the tension side, osteoblasts occur in the bone-apposition process.8 These are well-known histological signs. Proinflammatory cytokines play important roles in bone and root resorption. Determining the levels of various cytokines during various phases of orthodontic treatment undoubtedly contributes to our understanding of the underlying mechanisms of tooth movement. Biologic aspects of tooth movements should be clearly determined.

IL-1β and TNF-α affect bone metabolism directly. At extremely low concentrations, IL-1β and TNF-α have been implicated in the process of bone remodeling through specific receptors on the bone cell population.13 Monocytes and macrophages do not constitutively produce IL-1β or TNF-α, but on “activation” they synthesize and release these cytokines Davidovitch et al.1 have ocalized induced levels of IL-1β and TNF-α in the periodontium of teeth undergoing movement.

IL-8 is believed to play an important role in the pathogenesis of various forms of periodontitis, and high levels were detected in such subjects.17 Because periodontal tissues are remodeled at both tension and pressure sites during tooth movement, the increased amount of IL-8 at both sites just after application of mechanical forces might be a sign of neutrophil reaction in the area. After the acute response, IL-8 stimulation might be continued at the tension sites. Therefore, under the influence of mechanical forces, pressure sites indirectly contribute to the production of IL-8. Thus, we thought that the production of IL-8 is regulated differentially at tension and pressure sites and probably plays a major role in the initial stage of remodeling. Similar results were shown by Takahashi et al.18, who tried to clarify the hypothesis that the expression of matrixmetalloproteinase-8 (MMP-8) and matrixmetalloproteinase-13 (MMP-13) are regulated differently by tension and compression. Alternatively, because of the less active IL-8 production at pressure sites in this study, it was concluded that the initial force might be too heavy for a period and that decreased the cellular activity.

The gingival sulcus was selected as the testing site because of its continuity with the PDL and its accessibility within the oral cavity. The prediction that compression of the PDL in humans could result in the migration of biochemical products into the gingival sulcus is the basis of our experimental design. Previous in situ techniques for biochemical analysis of the gingival crevice involved sampling crevicular fluid using paper strips.7 The paper strip method was used in this study. The upper canines of all patients were monitored in this study because these teeth are accessible and easily cleaned.19

It has been shown that levels of biochemical markers in GCF might depend on different collection sites.20 For this reason, the canines were used as both the test and control teeth in this study. The control data were collected at baseline, which were obtained before any force was applied. Serra et al.9 stated that age and sex does not increase enzymatic activity so age and sex differences were not considered in this study.

Study were designed to examine the GCF levels of TNF-α, IL-1β and IL-8 during early levelling movement orthodontic treatment. The results clearly demonstrated that TNF-α, IL-1β and IL-8 levels were increased during study period.

King et al.21 described an early phase of bone resorption (3-5 days), its reversal (5-7 days), and a late phase (7-14 days) of bone deposition in rats. A similar bone cycle has also been reported in humans,17 but in humans this timing seems to be longer than in rats. These studies might support our finding of increased IL-1β and TNF-α levels in GCF.

In the early stages of orthodontic force application it has been shown that many PDL cells stain positively for IL-1β.7 Also, Lynch et al.22 reported that in the early stages of tooth movement...
movement (12 and 24 hours) many PDL cell types stained positively for IL-1β. Lowney et al. demonstrated that TNF-α plays a pivotal part in the assessment of orthodontic tooth movement.

Tzannetou et al. used low and high forces to the maxillary molars to expand the palate. Low forces were produced by separator placement and higher forces by a palatal expansion device. They observed high levels of IL-1β levels with both of the force levels. Also Kee-Joon Lee et al. demonstrated that the mean concentrations of IL-1β increases in the first 24 hours after continuous and interrupted forces. All of these studies examined GCF in short times as compared to the present study. They found that especially in the first 24 hours, cytokine levels increased and than equilibrium is reached which is higher than the baseline levels.

TNF-α, IL-1β and IL-8 concentration showed highest levels in the pressure sites immediately in the first and 24th hour. This might be caused by an early upregulation of chemotactic activities directly after mechanical force application. This is in accordance with Davidovitch et al.

Who showed the acute inflammatory response in the initial phase of tooth movement. The increased production of the other mediators in tooth movement has been shown in several studies.

Conclusions

The health condition of the periodontium is important during orthodontic treatment. Forces in different directions, duration, and magnitudes cause changes in the periodontal tissues. Orthodontic forced induces rapid release of the TNF-α, 1L-1β and IL-8 levels during tooth movement. Levelling of the teeth evoked increases both in the TNF-α, 1L-1β and IL-8 levels in the periodontal tissues that can be detected in GCF. The results of this study support the hypothesis that proinflammatory cytokines play a potent role in bone resorption after the application of orthodontic force in short time.

Declaration of Interest

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