Leptin Levels in Gingival Crevicular Fluid During Orthodontic Tooth Movement

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ABSTRACT

Objectives: To test if leptin can be detected in the gingival crevicular fluid (GCF) around moving teeth, and to determine whether any changes occur during orthodontic tooth movement.
Materials and Methods: An upper canine requiring distal movement served as the test tooth; the contralateral canine was used as a control tooth. The control tooth was included in the orthodontic appliance, but was not subjected to the orthodontic force. GCF sampling from the distal sites of the test and control teeth was done at baseline, 1 hour, 24 hours, and 168 hours.
Results: Leptin concentrations of the test teeth decreased in a time-dependent manner. When compared with the baseline measurement, the decrease was significant at 168 hours ($P < .05$).
Conclusions: The concentration of leptin in GCF is decreased by orthodontic tooth movement; the results of the present study also suggest that leptin may have been one of the mediators responsible for orthodontic tooth movement. (*Angle Orthod.* 2010;80:504–508.)

KEY WORDS: Leptin; Gingival crevicular fluid; Canine distalization

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INTRODUCTION

During orthodontic tooth movement, the early response of periodontal tissues to mechanical stress is an acute inflammatory reaction. Mechanical stress from orthodontic appliances is believed to induce cells in the periodontal ligament (PDL) to form biologically active substances, such as enzymes and cytokines, responsible for connective tissue remodeling.[1–9] Therefore, biochemical analysis of the gingival crevicular fluid (GCF) has provided a noninvasive model for investigating the cellular response of the underlying PDL during orthodontic tooth movement in vivo.[10] In the GCF, several substances such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, β_2 microglobulin, osteocalcin, and alkaline phosphatase have been found to be significantly elevated in teeth undergoing orthodontic forces compared with untreated controls.[3,4]

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Leptin, a polypeptide hormone, has been classified as a cytokine.[11] Leptin and its receptor share structural and functional similarities with members of the long-chain helical cytokines: IL-6, IL-11, IL-12, leukemia inhibitory factor, granulocyte-colony–stimulating factor, and oncostatin M.[12] Thus, leptin should be classified as a cytokine. Circulating leptin in humans is mainly secreted from adipose tissue.[12]

It has been suggested that leptin orchestrates the host response to inflammatory and infectious stimuli as it stimulates the immune system by enhancing cytokine production and phagocytosis by macrophages.[13] Thus, the overall increase in leptin during inflammation and infection indicates that leptin is part of the immune response and host defense mechanisms.

Previous studies have suggested a relationship between periodontal disease and leptin levels. Since the presence of leptin within healthy and marginally inflamed gingivae has been demonstrated,[14] several studies have observed that the levels of GCF leptin activity may play an important role in the development of periodontal disease.[15] Karthikeyan et al[15] reported that leptin levels decreased progressively in GCF as periodontal disease progressed.

Recently, it has been suggested that leptin plays a significant role in bone formation by virtue of its direct effect on osteoblast proliferation and differentiation,

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and in prolonging the life span of human primary osteoblasts by inhibiting apoptosis.[16] Leptin is also involved in antiosteogenic effects by acting centrally on the hypothalamus.[17] Thus, leptin at high local concentrations protects the host from inflammation and infection and maintains bone levels.

A remodeling process (resorption and apposition) takes place in periodontal tissues induced by the changes in the stress-strain distribution in the periodontium after the application of orthodontic forces. Furthermore, a local damage-repair process with inflammation-like reactions, including high vascular activity with many leukocytes and macrophages and involvement with the immune system may occur during orthodontic tooth movement.[18]

However, to date, leptin concentration in GCF during orthodontic tooth movement has not been explored. Hence, the purpose of the present study was to test if leptin can be detected in GCF around moving teeth, and to find if any changes occur during orthodontic tooth movement.

MATERIALS AND METHODS

Twenty-two orthodontic patients (11 girls and 11 boys; mean age, 14.4 ± 1.1 years) were selected to participate in this study according to the following criteria:

- Orthodontic treatment requiring upper first premolar extraction and distal movement of the canines
- Good health
- Normal body mass index, according to the WHO chart
- No use of anti-inflammatory drugs within the month preceding the study
- No history of antimicrobial therapy within the previous 6 months
- Healthy periodontal tissues with generalized probing depths of $\leq$2 mm, with minimal bleeding
- No radiographic evidence of periodontal bone loss

Patients' rights were protected, and informed consent was obtained according to the Atatürk University Faculty of Dentistry Ethical Committee Board.

After the upper first premolars were extracted, all subjects underwent a session of scaling and polishing, and they all received oral hygiene instructions before placement of the orthodontic appliance. Moreover, the subjects were not allowed to take medications or mouthwashes. Fixed orthodontic appliances were placed 1 week following the extractions.

Experimental Design

For each subject, a canine undergoing distal movement was chosen as the test tooth, and the contralateral canine served as the control tooth. Test and control sites were selected from the same subjects for eliminating individual differences. Orthodontic brackets were placed in both arches. Bands were also placed on the upper first molars. After leveling the maxillary arch, the first premolars were retracted along an archwire with an elastomeric chain on a plain, stiff, 0.016×0.016-inch wire. The chain delivered an initial force of 250 g.

Impressions for study models were also taken at baseline and 168 hours, and the distances between the distal contact point of the test canines and the mesial contact point of the second premolars were measured with an electronic digital caliper (Max-Cal, Japan Micrometer Ltd, Tokyo, Japan) with an accuracy of 0.01 mm. The distances were measured 10 times, and the method error was determined.

Periodontal Examination and GCF Collection

For each subject, the plaque index,[19] gingival bleeding within 15 seconds after probing with a 20-g controlled-force probe (FP 32 Software version 4, Florida Probe, Gainesville, Fla), and probing-depth scores were recorded. These clinical periodontal parameters were assessed twice: at the baseline and at the end of the study. The same investigator collected all clinical data.

GCF collection was performed before periodontal probing to avoid mechanical irritation or bleeding by penetration of the probe. GCF was sampled four times for each patient from the distal of each canine with gingival fluid collection strips (Periopaper-ProFlow Inc, Amityville, New York). All GCF samples were collected at 9 AM during the study period. Supragingival plaque, if present, was removed from these teeth for sampling. The teeth were gently dried with an air spray and isolated with cotton rolls. A saliva ejector was used to avoid salivary contamination. 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 held within the distopalatal crevice for 30 seconds. The procedure was repeated once more with the third and fourth strips. Strips contaminated by blood or saliva were discarded. The strips were transferred for volume determination to the chairside electronic gingival-fluid measuring device (Periotron 8000, Oraflow Inc, Plainview, New York), which was calibrated using known volumes of phosphate-buffered saline. After this measurement, the strips were transferred to the Eppendorf tubes (Microcentrifuge tubes, ISOLAB, Wertheim, Germany) and isolated with Parafilm® M, (SPI Supplies Inc, West Chester, New York) to avoid evaporation. Each sample was stored at -80°C until the assay was performed.

Leptin Analysis

Each strip was eluted twice with 100 μL Hank's balanced salt solution containing 0.5% bovine serum

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Table 1. Mean and Standard Deviations of Gingival Crevicular Fluid Volume (μL) in the Test and Control Teeth Throughout the Study Period

	Baseline	1 h	24 h	168 h	*P* value (Friedman test)
Control teeth	0.88 ± 0.31	0.87 ± 0.28	0.88 ± 0.29	0.89 ± 0.26	$P > .05$
Test teeth	0.87 ± 0.29	0.89 ± 0.33	0.88 ± 0.25	0.87 ± 0.30	$P > .05$

albumin by centrifugation (3000 × *g*; 4°C) for 15 minutes. Leptin concentration was measured by commercially available enzyme-linked immunosorbent assay. The assays were conducted according to the manufacturer's instructions. For leptin assays, high-sensitivity kits (BioSource International Inc, Camarillo, Calif) were used to quantitatively detect low levels of leptin, which was bound to antileptin, monoclonal-coating antibody absorbed by the microwells. The second polyclonal antibodies were added and, after incubation, colored products were formed in proportion to the amount of leptin present in the sample. The reactions were measured at 450 nm. The total leptin was determined in picograms (pg) and calculation of the concentration in each sample was performed by dividing the amount of leptin by the volume of the sample (pg/μL).

Statistical Analysis

Descriptive statistics including means and standard deviations were calculated for GCF volume and GCF leptin levels of the test and the control teeth. The Wilcoxon test was chosen to compare GCF volume and GCF leptin levels of the test and control teeth for each time point. Repeated measurements were tested using the Friedman test. In addition, the results within each group were analyzed by the Wilcoxon test. The data thus collected were assessed using SPSS 16.0 statistical software (SPSS Inc, Chicago, Ill).

RESULTS

In all patients, plaque accumulation was minimal throughout the study and gingival health was excellent. Furthermore, probing depths remained less than 2 mm at all times throughout the study period, and there was no gingival bleeding on probing. The test teeth underwent a mean distal movement of 1.56 ± 0.64 mm. No displacement was detected in the control teeth.

The mean volume of GCF collected from the control teeth was similar to that collected from the test teeth (Table 1; Figure 1). Leptin was detected in all GCF samples. The levels of leptin in the distal sites of the test and control teeth are shown in Table 2.

GCF leptin concentrations were similar in the test and control teeth at baseline without statistically significant differences. Leptin concentrations of the test teeth decreased in a time-dependent manner during the study period. When compared with baseline, the decrease was statistically significantly at 168 hours ($P < .05$). In addition, there was a statistically significant difference in the leptin concentrations between the test and control teeth at 168 hours ($P < .05$; Figure 2).

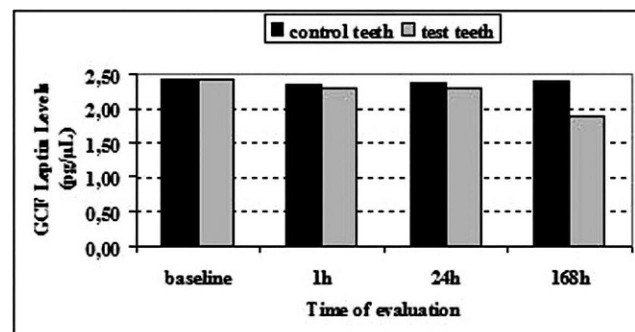

Figure 1. Gingival crevicular fluid volume (μL) during orthodontic tooth movement.

DISCUSSION

We designed a short-term, prospective study to investigate the relationship between GCF leptin levels and orthodontic tooth movement. The results show a significant decrease in GCF leptin levels of the test teeth, while the control teeth did not demonstrate any such decrease.

To our knowledge, the present clinical trial is the first report to determine the leptin concentrations in GCF during orthodontic tooth movement, although there have been studies to determine levels of biologically active substances such as hormones, enzymes, and cytokines in the GCF during tooth movement.[1–9] The analysis of GCF is a useful and promising method to monitor the changes at a single site during a certain method.[4,5,10] We used the same time intervals of 0, 1, 24, and 168 hours after initiation of orthodontic tooth movement as did previous studies of GCF samples.[1,3,6,7]

It has been reported that maintenance of oral hygiene is possible during orthodontic treatment.[20] However, other studies have not shown similar results in terms of gingival conditions.[21] The patients in our study showed similar gingival conditions for both the test and control teeth. This is probably due to the oral hygiene instructions given to each participant before treatment and followed by further reinforcement about gingival health throughout the study.

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Table 2. Mean and Standard Deviations of Levels of GCF[a] Leptin (pg/μL) in the Test and Control Teeth Throughout the Study Period

Groups	Baseline	1 h	24 h	168 h	*P* value (Friedman test)
Control teeth	2.42 ± 0.66	2.34 ± 1.02	2.38 ± 0.69	2.40 ± 1.04	$P > .05$
Test teeth	2.42 ± 0.90	2.28 ± 1.03	2.30 ± 1.01	1.88 ± 0.94*,**	$P > .05$

[a] GCF indicates gingival crevicular fluid.
* $P < .05$; significant difference between groups.
** $P < .05$; significant different from baseline value.

GCF is inflammatory, and exudates are found in the gingival sulcus. As an exudate, the amount of fluid in any crevicular location tends to increase with inflammation and capillary permeability.[10] Egelberg[23] has indicated that there was a significant positive correlation between GCF volume and periodontal inflammation. In the present study, the GCF volume from the test teeth was similar to that from the control teeth throughout the study. Previous studies have reported that GCF volume is not influenced by orthodontic tooth movement, but instead is influenced by inflammation of the periodontal tissue[1,4]; our results confirm this. In contrast, Samuels et al[9] reported that, although no gingival health changes occur, GCF volume can increase significantly during tooth movement. One explanation for this difference might derive from the different methods used for GCF collection.

Leptin is a product of the obesity gene that is released primarily by adipose tissue, and it is strongly correlated with body weight and body fat mass.[11,12] Therefore, only patients who had a normal body mass index were recruited for this study.

Leptin has been reported to influence various biological mechanisms, including the immune and inflammatory response, hematopoiesis, angiogenesis, bone formation, and wound healing[17]; it is also believed to have an anti-inflammatory action.[13] It has been reported that serum leptin levels were increased by surgical stress[24] and acute sepsis.[25] In these states, increased stress-induced hormones and cytokines, such as cortisol, TNF-α, IL-1, and IL-6 have been thought to cause the increment of serum leptin level.[24,25]

It is assumed that leptin has a role in protecting gingival tissues.[26] The higher concentrations of GCF leptin levels seen in periodontal health could be protective, as leptin stimulates the immune system[27] and enhances bone formation by acting directly on osteoblasts.[28] As periodontal disease progresses, the protective role of leptin on the gingiva is lost owing to a decrease in the leptin level.

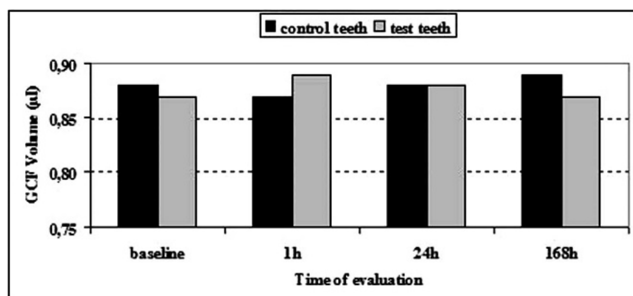

Figure 2. Leptin levels in the crevicular fluid volume (pg/μL) during orthodontic tooth movement.

This study is the first to assess the concentration of leptin levels in GCF during orthodontic tooth movement. We found a decrease in GCF leptin levels 1 and 24 hours after the initiation of tooth movement and a significant decrease at hour 168. This decrease is not surprising because periodontal changes take place during orthodontic treatment. Changes in the stress-strain distribution in the periodontium after the application of orthodontic forces trigger remodeling processes. These forces compress the PDL fibers and reduce the PDL space in the pressure area. At the tension site, PDL fibers are stretched, and orthodontic force results in widening of the periodontal membrane.[19] As initially described by Rygh,[29] bone remodeling determined by tooth movement is a continuous process characterized by bone resorption in the compression sites and bone deposition in the tension sites. However, another study reported that both bone resorption and deposition can be present in any tension site, as well as in any site of compression.[30] In the PDL, hyalinization of the most compressed area induced by compressive forces has been reported. This hyaline zone is described as an area of focal aseptic necrosis.[2] This decrease in GCF leptin concentration might be consequent to the tissue resorption in both the compressed and tensional sites,[30] or even secondary to possible cell necrosis in the PDL during orthodontic treatment.[2]

Significantly decreased levels of leptin concentration might result from the presence of inflammation adjacent to the teeth undergoing movement. It has been shown previously that orthodontic tooth movement may therefore show local traits of a damage/repair process with inflammation-like reactions: high vascular activity, many leukocytes and macrophages, and involvement of the immune system.[18]

CONCLUSIONS

- The concentration of leptin in the GCF is decreased by orthodontic tooth movement.

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- Leptin may be one of the mediators associated with orthodontic tooth movement.

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