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Evaluation of 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) and to determine whether any changes occur during orthodontic tooth movement.

Materials and Methods: Thirty patients were included in the study in which canine retraction was carried out with a NiTi closed coil spring on either side. GCF samples were collected before the commencement of canine retraction, on the first, Six hrs and 21st day after application of force and were analysed for leptin levels by the ELISA technique. An upper canine requiring distal movement served as the test tooth; thecontralateral canine was used as a control tooth. The control tooth was included in the orthodonticappliance,but was not subjected to the retractive orthodontic force. GCF sampling from the distal sites of the test and control teeth was done at baseline, 1 hour, 6hours, and 21st day.

Results: Leptin concentrations of the test teeth decreased in a time-dependent manner. When

Compared with the baseline measurement, the decrease was stastically significant at 6hrs and 21st day (P <.001)but didn't reach baseline value explaining inflammatory reactions immediately after forces applied .

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.

Keywords: Leptin, Gingival crevicular fluid, Canine distalization, Retraction coil springs

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 remodelling[1] (Nishijima Y et al 2006)[2]. Leptin, a polypeptide hormone has been classified as a cytokine (Zhang et al 1994[3]). Leptin is a highly hydrophilic protein that circulates in plasma as a 16-kDa protein. It is produced in adipose tissue and also recently described to be synthesized by placental tissue. Plasma

concentration of leptin is positively correlated to body fat mass, and administration of recombinant leptin to mice indicates that leptin participates in the regulation of food intake and energy expenditure.

Leptin is released primarily by adipose tissue, and it is strongly correlated with bodyweight and body fat mass[4]. Leptin has been reported to influence various biological mechanisms, including the immune and inflammatory response, haematopoiesis, angiogenesis, bone formation, and wound healing, it also has an anti-inflammatory action.It has been reported that serum leptin levels were increased by surgical stress and acute sepsis. 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[5]. Earlier findings concluded that leptin at high local concentrations protects the host from inflammation and infection as well as maintaining bone levels. It has been also suggested that leptin plays a significant role in bone formation by its direct effect on osteoblasts (Alparslan et al 2010)[6,7]. Leptin is also involved in antiosteogenic effects by acting centrally on the hypothalamus[8].

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[9].

Gingival crevicular fluid (GCF) can be defined as anosmotically mediated pre-inflammatory exudate (Alfano,1974[10] Barbieri,2013[11]; Cimasoni, 1983[12]) various molecules are enhanced during mechanical stress resulting in an initial inflammatory response. Biochemical markers of bone remodelling provide a potentially important noninvasive clinical tool for assessing and monitoring bone metabolism (Pender, 1994[13]). The purpose of this study was to test the levels of leptin in GCF around a moving tooth and to find if any changes in leptin level occur during Orthodontic tooth movement after applying constant continuous force.

Material and methods

Experimental Design

The study consists 30 orthodontic subjects including 13boys and 17 girls in the age group of 13-20 years attending the 0utpatient Department of Orthodontics and Dentofacial Orthopedics and private dental practice. Patients' rights were protected, Comprehensive procedural information was given to all patients and written informed consent obtained. Subjects those who fulfilled the following criteria were only included in the study Orthodontic patients requiring maxillary 1st PM extraction and distal movement of canines.

- Good health Normal body mass index No use of anti-inflammatory drugs within the month preceding the study
- No history of antimicrobial therapy within previous 6 months Healthy periodontal tissues with generalized probing depth of less than or equal to 2 mm with minimal Healthy periodontal tissues with generalized probing depth of less than or equal to 2 mm with minimal bleeding[14].
- No history of chronic medication that may have effect on leptin levels (oral contraceptives and antipsychotics)
- No radiographic evidence of periodontal bone loss.

Patients who have signed the informed consent Oral prophylaxis was done for all subjects following which oral hygiene instructions were given before placement of orthodontic appliances. To avoid leptin derived from obese subjects biasing the estimation of leptin

concentration; these subjects were excluded from the study by selecting only subjects with a normal body mass index (18.5–22.9 kg/m2) according to a chart for the Asian population given

Gingival Fluid Collection

All the GCF samples were collected around 10 am. GCF collection was performed before periodontal probing to avoid mechanical irritation or bleeding by penetration of probe. Supra gingival plaque if present at the time of sampling was removed. The teeth were gently dried with air spray and isolated with cotton rolls. Retraction of cheeks was done with cheek retractor. Salivary ejector was used to avoid salivary contamination.

GCF was collected using gingival fluid collection strips (Perio paper). The first strip was inserted into the disto buccal crevice of maxillary right canine to a level 1mm below the gingival margin and held in place for 30seconds. After 1 minute the second strip was inserted into the distopalatal crevice and held in place for 30 seconds. Extra care was taken to avoid blood and saliva contamination. Strips contaminated with blood or saliva was discarded.

Timing of the Sample

Totally 5 GCF samples are collected from each subject.Pre treatment from disto buccal and disto palatal crevice of right side maxillary canine. (GROUP A) After maxillary arch is aligned upto 17 x25 SS wire stage, retractive force is applied with 9mm size NiTi coil spring to the maxillary left side canine and not to the right side canine. 6 hours after applying this distal retractive force GCF is collected from both maxillary right (GROUP B) and maxillary left canine (GROUP C). After 21 days GCF is again collected from the maxillary right (GROUP D) and maxillary left (GROUP E) canines.

4- 6 hours is the critical time period during tooth movement when second messengers are released that are very important for cellular functions including differentiation and 21st day is the one in which the appliance is usually reactivated after giving the periodontal tissues a time period for repair and regeneration.

Each strip was eluted twice with 100 microlitres of Hanks Balanced Salt Solution containing 0.5% Bovine Serum albumin by centrifugation (3000 x g; 4oc) for 15 minutes. Leptin concentration was measured by commercially available enzyme linked immunosorbent assay. The assay was conducted according to the manufacturer's instructions. For leptin assays high sensitive kits 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 coloured 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). The calculation of concentration in each sample was performed by dividing the amount of leptin by the volume of the sample (µg/ microlitre).

Statistical analysis

Descriptive statistics including means and standard deviations were calculated for GCF leptin levels of the test and the control teeth. The Oneway Anova was chosen to compare GCF leptin levels of the between the groups for each time point. Repeated measurements were tested using the Friedman test. Both Groups were compared using the Students *t* test. In addition, the results within each group were analysed. 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 distal movement. No displacement was detected in the control teeth.

The mean leptin levels of GCF collected from the control teeth was similar to that collected from the test teeth. The levels of leptin in the distal sites of the test and control teeth are shown in (Table 1,fig 1). 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 21 days (P <0.001). Repeated measurement analysed using friedman test In addition, there was a statistically significant difference seen during students *t* testin the leptin concentrations between the test and control teeth at 21 days(P <0.001)(Table 3).

Mean and Standard Deviations of Levels of GCF Leptin (pg/µL) in the Test and Control Teeth Throughout the Study Period

Table No1

	MEAN	MEAN SE	SD	p VALUE
GROUP A	0.733	0.0188	0.101	
GROUP B	0.863	0.0178	0.096	
GROUP C	0.759	0.0166	0.089	< 0.0001*
GROUP D	0.704	0.0213	0.115	
GROUP E	0.747	0.0191	0.103	

*-significant difference between groups at different time interval

	Rank sum	Mean rank	p VALUE
GROUP A	71.5	2.47	
GROUP B	145.0	5.00	
GROUP C	97.5	3.36	< 0.0001*
GROUP D	42.5	1.47	
GROUP E	78.5	2.71	

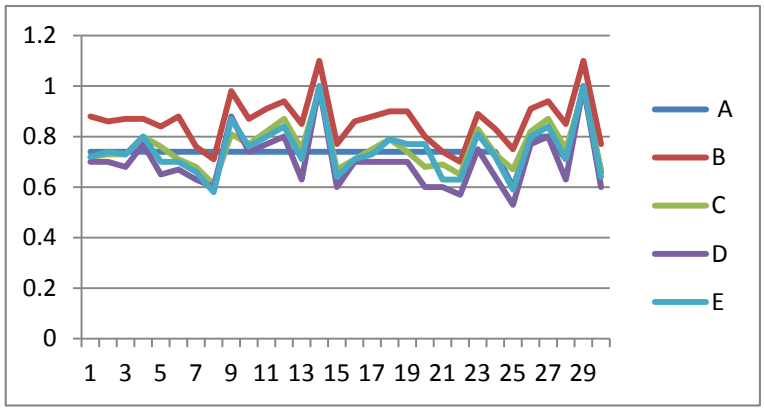
*significant difference from baseline value.

Table no 3

t statistics	16.83
Df	28
p-value	<0.0001*

*significant difference between test and control groups

Fig no 1- indicates GCF leptin levels at different time interval.



Discussion

The study was conducted to investigate the relationship between GCF leptin levels in orthodontic tooth movement. The key to successful orthodontic tooth movement is good periodontal health, oral hygiene and optimal orthodontic force. Tooth movement induced by force application is characterised by remodelling changes in the dental and periodontal tissues (Krishnan and Davidovitch, 2006[15]).Orthodontic force changes periodontal tissue vascularity leading to the synthesis of various signalling molecules and metabolites. The released molecules generate cellular responses around the teeth, providing a favourable microbiological environment for tissue deposition or resorption (Bartzela, 2009[16] Leptin is also involved in anti-osteogenic effects by acting centrally on the hypothalamus (Karthikeyan and Pradeep, 2007a[17], 2007b[18]). Thus, leptin has a dual effect on bone, acting by two independent mechanisms.This study shows an increase in GCF leptin levels at 4-6 hours at the test tooth site that is statistically significant. This corresponds to the fact that GCF leptin levels increase in acute sepsis. When

a retractive force is applied to the tooth with NiTi coil springs, acute inflammatory changes occur in the gingival and periodontal tissues. 4- 6 hours is the critical period when all the second messengers necessary for cellular differentiation and thereby tooth movement are released into the periodontal environment thus the study was in accordance with researchers.

After 21 days, the test tooth with NiTi coil springs still in place shows a little decrease in GCF leptin concentration than the control side and pretreatment (Baseline) values. But this shows statistically no difference. This indicates that as the forces exerted by the NiTi coil springs are optimal (150-200 gm) unlike E- chain that exerts heavy force (380 gm), the investing tissues of the teeth recover easily and quickly with minimal hyalinization, aseptic nectrotic area and undermining resorption. In other words, gingival condition do not worsen but recovers which is indicated by returning of GCF leptin levels to normal or base values at 21 days.[19,20]

Leptin at a high concentration locally protects the host from inflammation and infection and maintains bone levels (Włodarski and Włodarski, 2009[21]). The concentration of leptin in GCF is decreased by tooth movement; the results of the study also suggested that leptin may have been one of the mediators responsible for orthodontic tooth movement (Dilsiz 2010[22]). Leptin can influence the bone remodelling via a direct signalling from the brain;though leptin's action works toward reducing cancellous bone, it appears to increase cortical bone (Ducy 2000[23]). In the present study retraction of canine was done with NiTi coil springs that deliver light constant continuous force (150-200 gm).[24] This leads to direct resorption and optimal rate of continuous tooth movement[25]. In the current study, timing of sample collection is 6 hrs and 21 days after retractive force application with NiTi coil springs here there was decreased levels of leptin concentration might be due inflammation similar to the studies done by Dilsizand Ducy et al

Summary & Conclusion

From the findings observed in this study it can be concluded that

When constant, continuous and optimal orthodontic forces are applied concentration of leptin in GCF is decreased in early acute stages. When the orthodontic forces are maintained within optimal range for longer period, the gingival tissues recover quickly restoring the normal or baseline leptin values in GCF. Girls have more leptin concentration in GCF than boys that may be due various hormonal factors. Orthodontic tooth movement can be carried out without any significant destructive changes in investing tissues of the teeth provided oral hygiene is properly maintained. Leptin is one of the mediators of orthodontic tooth movement Future studies are required to evaluate the levels of leptin in GCF under various force magnitudes over a long period and to clarify the protective role of leptin in periodontal disease progression. Future interventional studies involving leptin administration are expected to further clarify the pharmaco therapeutic role of leptin in orthodontic tooth movement and periodontal disease progression.

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