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Evaluation of leptin concentration in Gingival Crevicular Fluid (GCF) during orthodontic tooth movement and its correlation to the rate of tooth movement

Bhadrinath Srinivasan, Arun Chitharanjan, Vignesh Kailasam, Vamsi Lavu[1] and Vani Ganapathy[2]

Abstract:

OBJECTIVES: Leptin, a polypeptide which is related to body fat regulation, is also found to have a role in the inflammatory reaction. The aim of this study is to assess the concentration of leptin in Gingival Crevicular Fluid (GCF) during orthodontic force application and to correlate its concentration to rate of tooth movement.

METHODS: Twenty orthodontic patients (10 males and 10 females) were selected for the study. Leptin concentration was measured at T0, before force application; T1, one hour after force application; T2, one day after force application; T3, one week after force application; T4, one month after force application. GCF was collected using filter paper strips from the distal aspect of gingival sulcus of the right maxillary canine distalized by an active lace-backs of tooth movement was measured on dental casts, before and one month after force application. One-way ANOVA with Bonferroni correction and Pearson's correlation test were used to analyze the data.

RESULTS: The mean GCF leptin concentration increased from T0 to T1, rose to a peak at T2, then declined to a minimum value at T3 and then increased to a value at T4, closer to the base line value (T0), and it was statistically significant ($P < 0.05$). There was positive correlation of the overall mean leptin concentration to rate of tooth movement (correlation coefficient = 0.634).

CONCLUSION: There was a biphasic change in GCF leptin concentration during one cycle of orthodontic force application. There was a positive correlation between the GCF leptin concentration and rate of tooth movement.

Keywords:

Bone remodeling, gingival crevicular fluid, leptin, tooth movement

Departments of Orthodontics and [1]Periodontics, Faculty of Dental Sciences, Sri Ramachandra Medical College and Research Institute, Porur, [2]Department of Biochemistry, Madras University, Chennai, Tamil Nadu, India

Address for correspondence:
Dr. Bhadrinath Srinivasan, Department of Orthodontics, Faculty of Dental Sciences, Sri Ramachandra Medical College and Research Institute, Porur, Chennai - 600 116, Tamil Nadu, India.
E-mail: sbhadrinath@yahoo.com

Introduction

Orthodontic tooth movement is the result of bone re-modeling, characterized by the inflammatory response of the periodontal ligament to mechanical stresses applied by an orthodontic appliance. Due to the inflammatory reaction, numerous cytokines and other inflammatory mediators are released, which play a vital role in bone re-modeling. Various cytokines such as interleukin-6, interleukin 1-beta, tumor necrosis factor ∝, B2 microglobulin, matrix metallo-proteinases, pentraxin -3, and enzymes such as, alkaline phosphatase, lactate dehydrogenase, have been shown to increase during orthodontic tooth movement.[1-5]

Leptin, a glycosilidated polypeptide secreted by adipose tissues, was originally

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identified as an obesity related hormone. Aberrations in the leptin signaling pathway give rise to obesity in animals and humans.[6] Leptin has a role in the host response to inflammatory and infectious stimuli, because it structurally and functionally resembles the cytokine family. In addition, it also stimulates the immune system, since it enhances cytokine production and phagocytosis.[7]

Leptin is found to have both centrally mediated and direct actions in bone metabolism, with both bone stimulatory and bone inhibitory activity.[8-14] Bone inhibitory activity is a central action through the hypothalamus, mediated by the sympathetic nervous system.[15] Leptin can be detected in the Gingival Crevicular Fluid (GCF) and its concentration in the GCF is found to be higher in subjects with healthy gingival tissues as compared to subjects with periodontal disease.[16-18] Leptin receptors were also detected in the gingiva and GCF both during health and disease.[19]

Dilsiz *et al*. evaluated leptin level in GCF during orthodontic tooth movement and reported a time-dependent decrease in leptin concentration following orthodontic force application.[20] However, correlating the levels to rate of orthodontic tooth movement was not done and would be of immense clinical significance, since leptin plays a major role in bone metabolism and inflammation, and has a major effect on the rate of tooth movement. Hence, the purpose of this study is not only to assess leptin concentration at various time points during one cycle of orthodontic force application but also to measure the rate of tooth movement and thus correlate mean leptin concentration to the rate of tooth movement.

Materials and Methods

This was an observational study. Twenty patients (10 males and 10 females) who reported for orthodontic treatment were selected. Sample size was determined based on previous work by Dilsiz *et al*.,[20] with power (1-ß error) = 0.8, ∝ error probability = 0.05 and effect size f = 0.28. Proposal of this study was approved by the Institutional Ethics Committee of Sri Ramachandra University, Chennai (No: IEC-NI/11/DEC/26/77, Dated: 21 January 2012). Informed consent was obtained from all the subjects participating in the study. Mean age of patients included in the study was 20.5 ± 1.93 years and the range was 19–24 years.

Inclusion/exclusion criteria for this study were as follows:

a. Patients with Class I skeletal base with ANB angle 2° ±2 presenting with Angle's class I malocclusion with crowding greater than 6 mm (assessed by the arch perimeter and Carey's analysis) that required maxillary canine retraction following all first premolar extraction
b. Subjects possessing healthy periodontal tissues having a generalized probing depth ≤2 mm, with no bleeding and without any radiographic evidence of crestal bone loss. Patients with minimal signs of gingival inflammation were excluded from the study
c. Subjects with any systemic illness and those under any medication, anti-inflammatory drugs, and antimicrobial therapy were excluded from the study
d. Subjects with craniofacial anomalies, previous history of orthodontic treatment, and trauma were excluded from the study
e. Subjects having any oral or parafunctional habit were excluded from the study.

All the subjects were started with pre-adjusted edgewise appliance with MBT prescription and 022" slot (Ortho Mini 2000, Ormco Corporation, Orange, California), two weeks after extraction of the first premolars. The initial arch wire used was 0.014" superplastic nickel titanium wire (AMC Inc, USA). A distal force was applied to the right maxillary canine (test site) using active lace-backs running from the canine to the first molar and made of 0.009" thickness stainless steel wire. The anchorage was maintained by a soldered trans-palatal arch on the first molars.

GCF collection

GCF was collected from the test site at five time intervals (T0, just before force application; T1, one hour after force application; T2, one day after force application; T3, one week after force application; T4, one month after force application, just before the next activation of active lace-backs) using filter paper strips (Periopapers, Ora flow Inc, New York,). Since GCF leptin concentration was measured for one full cycle of orthodontic force application (i.e., before the next force application), the base line served as control. Patients were given strict oral hygiene instructions to prevent gingival inflammation throughout the period of the study. This was ensured by checking probing depth and bleeding on probing each time before sample collection. It was also ensured that probing did not inflict any local tissue injury which might lead to alterations in GCF composition. Before placing the strips, the patient was asked to wash with water, the area of interest was air dried and then isolated with cotton rolls to avoid salivary contamination. For collection of GCF, filter paper strips were placed at the disto-buccal and disto-palatal gingival sulcus of right maxillary canine for 30 seconds. Samples contaminated with blood and saliva were discarded. The GCF sample was collected between 9 am and 12 pm in the day, as serum leptin concentrations are found to exhibit

circadian rhythmicity, peaking at 2400 hrs and reaching a nadir at 1000 hrs.[21]

After retrieval, Periotron 8000 (Ora Flow Inc., New York,) was used to quantify the amount of fluid absorbed by the filter paper. The filter paper strips were then stored in Eppendorf tubes, sealed with parafilm to make them air tight and immediately stored at -80°C. At the time of estimation of leptin concentration, the samples were diluted in 100 micro liter of Hanks Balanced Salt Solution with 0.5% of bovine serum albumin and centrifuged for 15 minutes. The centrifuged samples were then analyzed for leptin concentration with Enzyme Linked Immunosorbent Assay (ELISA) (Bio-source International Inc, Camarillo, Calif.). Colour change in the processed samples were read as net absorbance values with the help of fully automated ELISA reader at 450 nm. From these absorbance values, corresponding concentration of each sample was calculated using the standard curve. The calculated concentration of leptin was expressed in picograms/micro liter of the sample.

Measurement of the rate of tooth movement

Rate of tooth movement was calculated as the amount of distal movement of the maxillary canine at the end of one month. Distance between the mesial contact point of the right maxillary canine and the mesial contact point of the right maxillary first molar was measured at two time points, one before force application (T0) and at one month after force application (T4). Alginate impressions of the maxillary arch were made, and dental casts were poured before force application (T0) and one month after force application (T4). Measurement was made on the dental casts using a digital Vernier calliper which could measure up to 0.01 mm. Difference between these two values was taken as the rate of tooth movement. The measurements were repeated after 15 days by the same operator for all the dental casts and method error was calculated using Dahlberg's formula.[22]

$$\text{Method error} = \sqrt{\sum d^2/N}$$

where "d" is the difference between two measurements made from the same parameter and "n" is the number of subjects. Method error was found to be 0.02 mm.

Statistical analysis

The values were tabulated and subjected to statistical analysis using SPSS software version 15. (SPSS, Illinois, Chicago). Normality of distribution of data was tested using Kolmogorov Smirnov test [Table 1]. Gender comparison of mean GCF leptin concentration and rate of tooth movement was done using the "t" test. One-way ANOVA with Bonferroni correction was used to compare mean leptin concentration at the five time points. Average rate of tooth movement was calculated, and it was correlated to mean leptin concentration using Pearson's correlation coefficient.

Results

Mean GCF leptin concentration was least at T0 (318.63 picograms/micro liter), gradually increased at T1 (395.96 picograms/micro liter), reached a peak at T2 (1171.54 picograms/microliter), had a marked decline at T3 (30.33 picograms/microliter) and finally, a small increase at T4 (248.51 picograms/microliter) [Table 2 and Figure 1]. ANOVA revealed that most of the values were also statistically significant, $P < 0.05$, [Table 3] except for value at T2 which was not statistically significant when compared with values at T0 and T1. This could be explained by the increased 95% confidence interval at T2.

More importantly, the value at T4 was slightly lesser than the concentration at T0 [Figure 1]. This implied that leptin levels could reach baseline values within a four-week cycle following a single orthodontic activation.

Table 1: Test of normality

	Kolmogorov- Smirnov		
	Statistic	df	Significance
T0	0.147	20	0.927

Table 2: Mean GCF leptin concentration at various time points

	GCF leptin concentration in picogms/micro litre		Total no. of subjects (*n*)	95% confidence intervals
	Mean	Standard deviation		Upper Bound Lower Bound
T0	318.63	85.86	20	278.45-358.817
T1	395.96	68.79	20	364.76-429.15
T2	1171.54	1380.02	20	525.6-1817.41
T3	30.33	16.50	20	22.61-38.05
T4	248.5109	116.64	20	193.92-303.10

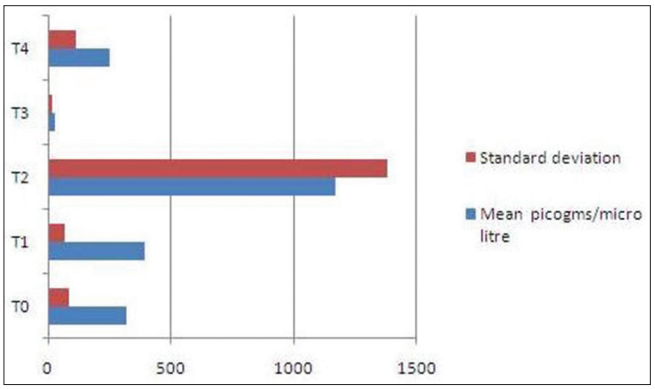

Figure 1: Mean and standard deviation of GCF Leptin Concentration at T0, T1, T2, T3 and T4

Also, mean leptin concentration and rate of tooth movement did not have any statistically significant gender variation ($P > 0.05$) [Table 4].

On correlating the mean rate of tooth movement with mean leptin levels, it was observed that there was a moderately strong correlation (correlation coefficient = 0.634) which was also statistically significant ($P = 0.003$) [Table 5].

Discussion

Numerous studies evaluated concentration of various chemical mediators such as, IL 1 B, RANKL, PGE2, Substance P, Tumour Necrosis Factor alpha etc., in GCF during orthodontic force application.[1,5,7]

Table 3: One Way ANOVA to compare mean GCF leptin concentrations at different time points

Time points (T)	Time points (T)	Significance
0	1	0.002**
	2	0.109
	3	0.000***
	4	0.000***
1	0	0.002**
	2	0.185
	3	0.000***
	4	0.000***
2	0	0.109
	1	0.185
	3	0.016*
	4	0.070
3	0	0.000***
	1	0.000***
	2	0.016*
	4	0.000***
4	0	0.000***
	1	0.000***
	2	0.070
	3	0.000***

*Indicates significance at 0.05 level, **Indicates significance at 0.01 level, ***Indicates significance at 0.001 level

All these studies showed an increase in the concentration of the mediators in GCF following force application and this increase in concentration of the mediators was attributed to inflammatory reaction of periodontal ligament to the orthodontic force.[5,23,24]

In this study, we have evaluated the concentration of leptin in GCF during one cycle of orthodontic force application. There was biphasic change in leptin concentration, with a peak one day after force application and reaching a nadir after one week. Then, one month before the next activation, it increased to a slightly higher value, which was lesser than the base line value.

Dilsiz *et al*., who evaluated leptin concentration in GCF before and after force application up to one week, reported a gradual decrease in GCF leptin concentration which is contradictory to our results.[20] The results in this study was also different from previous studies that measured other biomarkers during orthodontic tooth movement, where an exponential increase in concentrations of the mediators was observed after force application.

The initial increase in leptin concentration in the GCF at T1, T2 could be compared to increased secretion of any inflammatory mediator after force application. The initial increase in leptin concentration was followed by marked decrease one week after force application which could be due to increased expression of leptin receptors in the periodontal ligament following the initial increase in leptin concentration which, in turn, could have resulted in formation of more leptin -leptin receptor complexes, leading to a decreased concentration of leptin in the GCF resulting in decrease in GCF leptin concentration after 1 week.

One more objective in this study was to correlate the rate of tooth movement to the GCF leptin concentration; the correlation was found to be strong. The strong correlation could be attributed to its osseo-regulatory actions.

Table 4: Gender difference in Mean GCF leptin concentration at various time points and in rate of tooth movement

Time points	Gender	No. of subjects (*n*)	Mean GCF leptin concentration in picograms/microlitre	Standard deviation	Significance
T0	Female	10	313.62	98.22	0.802
	Male	10	323.65	76.54	
T1	Female	10	414.92	59.44	0.253
	Male	10	378.99	79.77	
T2	Female	10	851.78	724.88	0.313
	Male	10	10491.29	10807.72	
T3	Female	10	34.46	16.98	0.275
	Male	10	26.20	15.76	
T4	Female	10	245.64	115.08	0.916
	Male	10	251.38	124.34	
Rate of tooth movement	Female	10	0.88	0.34	0.149
	Male	10	1.09	0.28	

Table 5: Correlation of GCF leptin concentration to rate of tooth movement

Correlation	Rate of tooth movement	Average
Rate of tooth movement in mm		
Pearson Co-relation	1	0.634**
Sig.(2-tailed)		0.003
n	20	20
Average leptin concentration		
Pearson Co-rrelation	0.634**	1
Sig.(2-tailed)	0.003	
n	20	20

**Co-relation significant at 0.01 level, *n*=Number of subjects

Leptin's bone regulatory actions include proliferation and differentiation of osteoblasts and prolongation of the life span of human primary osteoblast by inhibiting apoptosis.[10,13] It also promotes growth of cultures of both primary osteoblasts and chondrocytes.[9,11,13,14] Leptin inhibits osteoclastogenesis, by down regulating RANK production from mononuclear cells.[15] Further, leptin decreases the production of osteoprotegrin which is an inhibitor of bone formation.[9]

Among the various biomarkers evaluated for orthodontic tooth movement, interleukin 1 beta was correlated to the rate of tooth movement.[25,26] There was a positive correlation between concentration of interleukin 1 beta and the rate of tooth movement.[25,26] Leptin is found to control the interleukin system by stimulating secretion of interleukin 1 ß s, interleukin 1 receptor antagonist, and expression of IL 1 receptor, which could also explain the positive correlation of GCF leptin concentration to rate of tooth movement.[27]

Bremen *et al*. reported that orthodontic treatment duration was greater in obese individuals.[28] Such individuals were found to have greater serum leptin concentrations and increased bone mineral density.[29,30] The common age group of orthodontic patients is usually early adolescence. Due to increasing prevalence of obesity among children,[31] there is a possibility of leptin playing a major role in orthodontic tooth movement and therefore the treatment outcome.

In this study, there were changes in GCF leptin concentration during orthodontic force application, and a positive correlation between rate of tooth movement and leptin concentration. Future long-term evaluation of leptin concentration during orthodontic tooth movement can reveal additional information. Also, relating leptin concentration to levels of other important cytokines during orthodontic tooth movement would help us find the specific role of leptin in orthodontic tooth movement.

Conclusion

1. The mean GCF leptin concentration assessed at five time points during one cycle of orthodontic force application showed a biphasic change, with initial increase followed by a decrease after one week and reaching a concentration lesser than the base line value after one month
2. There was an overall strong positive correlation between mean GCF leptin concentration and rate of tooth movement.

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Conflicts of interest

There are no conflicts of interest.

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