Original Article

Assessment Of Gingival Crevicular Fluid (GCF) For Leptin Levels In Subjects Undergoing Orthodontic Tooth Movement

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Abstract

Background: Tooth movement in orthodontics is governed by the forces on the periodontal ligament and associated remodeling in the alveolar bone. Assessing the GCF biomarkers levels can help judge the cell response during tooth movement. Leptin maintains bone levels and is vital during tooth movement.

Aim: The present study aimed to assess the leptin levels in GCF (gingival crevicular fluid) in subjects undergoing orthodontic tooth movement and to gauge the leptin role as a mediator during tooth movement.

Methods: In 50 subjects undergoing tooth movement as a part of orthodontic treatment, GCF was collected and leptin levels were assessed before the start of the treatment (I), 6 hours following the retraction force on the right canine (II), no retractive force on left canine (III), and after 21 days from the right (IV) and left canine (V). The data was assessed and results were formulated.

Results: Leptin levels in the GCF were similar in the study participants before treatment, 6 hours, and 21 days control. However, at the test site, the concentration of leptin in GCF at 6 hours was statistically higher. On the 21st day, leptin levels in GCF reduced slightly at the test site compared to controls. However, the difference was not statistically significant.

Conclusion: The study, within its limitations, concludes that leptin is a mediator of tooth movement with increased levels in early phases with continuous and constant orthodontic forces. However, with maintained orthodontic forces for long period, the baseline value of leptin is restored.

Keywords: GCF, gingival crevicular fluid, leptin, NiTi coil, orthodontic tooth movement

INTRODUCTION

Acute inflammatory reaction in the periodontal tissues results as an early response to the orthodontic tooth movement secondary to mechanical stress. This mechanical stress is considered to induce a cellular response in the periodontal ligament (PDL) to induce the formation of biologically active products including the cytokines and enzymes leading to the remodeling of the connective tissue.[1] Hence, biochemical assessment of the GCF presents a non-invasive modality to assess the cellular response undergoing PDL in a subject with active tooth movement in the oral cavity. GCF is comprised of various constituents including alkaline phosphatase, osteocalcin, β_2 microglobulin, TNF (tumor necrosis factor) α and interleukins such as IL-6 and IL-1β which have been found to have higher values in subjects undergoing orthodontic tooth movement as compared to the controls.[2]

Leptin is a hormone falling under the classification of the cytokines owing to them sharing functional and structural similarities with the cytokines having long-chain helices such as oncostatin M, granulocyte colony-stimulating factor, leukemia inhibitory factor, IL-12, IL-11 and IL-6. Hence, leptin is classified as a cytokine. Leptin in humans is secreted from the adipose tissues.[3] Leptin has also been shown to manage the host response to the infectious and inflammatory stimuli initiating the immune system and increasing the phagocytosis by macrophages and production of cytokine.

Hence, an increase in leptin levels during infection and inflammation confirms its role in host defense and immune responses.[4]

Previous literature data confirms a relationship between leptin levels the periodontal disease. However, the levels of leptin have also been seen in the healthy and initially inflamed gingiva, various authors advocate its role in the development of periodontal diseases.[5] One literature study has demonstrated a progressive decrease in leptin levels with the progression of periodontal disease. In the recent literature, the role of leptin levels has been established in the formation of bone by its effect on osteoblast differentiation and proliferation along with increasing the life of primary osteoblasts and inhibition of apoptosis.[6] Leptin has also been shown to act on the hypothalamus and show anti-osteogenic effects. At high concentrations, leptin helps in protection from infection and inflammation along with maintaining bone levels.[7]

With the alterations in the stress-strain distribution in the periodontium following the application of orthodontic forces, bone remodeling takes place in terms of apposition and resorption. Also, during orthodontic treatment, a damage repair process with inflammatory reactions including macrophages, leukocytes, and high vascularity with immune system involvement is seen.Assessing the GCF biomarkers levels can help judge the cell response during tooth movement non-invasively.[8] Hence, the present study aimed to assess the leptin levels in GCF (gingival crevicular fluid) in subjects undergoing orthodontic tooth movement and to gauge the leptin role as a mediator during tooth movement.

MATERIALS AND METHODS

The present clinical study aimed to assess the leptin levels in GCF (gingival crevicular fluid) in subjects undergoing orthodontic tooth movement and to gauge leptin's role as a mediator during tooth movement.

The study included 50 subjects from both genders between the age of 15-20 years undergoing Orthodontic treatment at the Institute. The study had 26 males and 24 females undergoing Orthodontic treatment. The inclusion criteria for the study were the subjects undergoing orthodontic treatment needing distalization of canine and extraction in the maxillary arch, no history of medical drugs affecting the leptin levels, having healthy periodontium, no radiographic bone loss, no/minimal bleeding on probing, probing depth of ≤2mm, no history of anti-microbial or anti-inflammatory drugs within 1 month of the study, normal BMI (body mass index), and gave consent. The exclusion criteria were subjects who were not willing to participate in the study. After explaining the detailed study design, informed consent was taken from all the subjects in both written and verbal format.

Before the start of the treatment, GCF samples were collected from all the subjects from the maxillary left canines followed by the extraction of the 1st premolars. NiTi coil spring of 9mm and SS (stainless steel) wire of 17X25 was used to retract the left canine following the leveling of the maxillary arch. The NiTi spring delivers a continuous and light force of 150-200 grams. On the opposite side, on the right side, no retraction force was applied. GCF collection was done with the periopaper strips and periotron was used to measure the GCF.

In the present study, 5 samples of GCF were collected from all the participants. Pre-treatment samples were collected from the right maxillary canine and were named sample-I, following the alignment of the maxillary arch, NiTi coil spring, and SS (stainless steel) wire application to the left canine, after 6 hours of the force application. GCF was collected from both the right canine (sample II) and the left canine (sample III). After 21 days of force application, the sample was again collected from the right maxillary canine (sample-IV) and left maxillary canine (sample-V). The samples were collected at 4-6 hours as it is the time when vital cellular functions including differentiation occur and also second messengers are released. Again at 21 days, orthodontic appliances are reactivated following a repair and regeneration period of periodontium making it vital to collect GCF samples on the 21st day.

After GCF sample collection with the periostrip, the samples were kept in the Eppendrof tube and paraffin was used for isolation to prevent evaporation. These tubes were then placed in a cyclotron to allow the solid particles to settle down. Each sample was then stored at -80^{o}C till further assessment. ELISA (enzyme-linked immunosorbent assay) was used to assess the leptin levels in GCF and levels were expressed in a picogram (pg). In each sample, the concentration was calculated by division of leptin amount to sample volume (pg/microlitre).

The data gathered was analyzed statistically using the SPSS software version 22.0; Chicago, IL, USA software and one-way ANOVA (analysis of variance) and Turkey's test. The data were expressed in the means and standard deviations.

RESULTS

The present clinical study aimed to assess the leptin levels in GCF (gingival crevicular fluid) in subjects undergoing orthodontic tooth movement and to gauge leptin's role as a mediator during tooth movement. The study included 50 subjects from both genders between the age of 15-20 years undergoing Orthodontic treatment at the Institute. The study had 26 males and 24 females undergoing Orthodontic treatment.

The study results showed that leptin concentration in the GCF were similar before the start of the treatment, 6 hours for control teeth, and on the 21st day. However, an increase in the GCF concentration of leptin was seen at the test site of the tooth. This increase was statistically significant with $p<0.01$ as assessed with the ANOVA (analysis of variance) and the p-value was <0.05 as assessed from Turkey's HSD (honestly significant difference). Leptin concentrations in the GCF on the 21st day for the test tooth showed a reduction compared to the controls. However, this difference was statistically non-significant with $p>0.05$. Also, it was noted that higher leptin concentration was seen in females compared to males after body mass was corrected.

On assessing the pre-treatment leptin levels in the GCF of the study participants, it was seen that in controls, leptin concentration was 0.77±0.13, 0.77±0.09 and 0.76±0.07 at baseline, 6 hours after the application of the force, and 21st

day of the force application respectively. For the cases tooth, the leptin GCF concentrations were 0.77±0.13, 0.88±0.06 and 0.72±0.07 respectively at baseline, 6 hours after the force application and 21st day of the force application as shown in Table 1.

Concerning the leptin concentrations in the GCF of different study samples, each sample had 50 sites. It was seen that mean values for samples I, II, III, IV and V were 0.7514±0.115, 0.8606±0.086, 0.7502±0.076, 0.7014±0.095 and 0.7426±0.094 respectively. This difference was statistically significant with $p<0.001$ as depicted in Table 2.

DISCUSSION

The present clinical study aimed to assess the leptin levels in GCF (gingival crevicular fluid) in subjects undergoing orthodontic tooth movement and to gauge leptin's role as a mediator during tooth movement. Dilsiz A et al[9]in 2010 in their study assessed the GCF levels after application of the heavy force on the teeth. An increase in the leptin concentration during inflammation and infection depicts and confirms its role in the network of cytokines and had a part in the host defense mechanism. Leptin does not directly affect bone metabolism by regulating the HPG axis and production of estrogen, but also directly affects bone metabolism as suggested by Ahima RS[10] in 2000.

Serum leptin levels in the fetus show a negative correlation with the bone markers associated with the resorption showing the leptin effect for decreasing the bone loss and increasing the bone mass as reported by Alhashimi N et al[11] in 2001. Also, Ren Y et al[12] in 2002 suggested that leptin concentration had a significant correlation with the area of the bone and not with the bone mineral content showing leptin effect on periosteum and cortical bone during puberty. These findings suggest that leptin has a marked role in the remodeling of the alveolar bone which is vital during orthodontic tooth movement.

The leptin levels in GCF are lower in smokers compared to non-smokers with a significant difference. It has also been reported by the previous study of Karthikeyan and Pradeep[13] in 2007 that in healthy gingiva, leptin levels are higher compared to subjects with periodontitis. However, gingiva does not have any adipocytes. This can be explained by the study of Armitage GC[14] in 2000 where authors suggest that it could be due to leptin entrapment in the gingiva by microvasculature diffusion. Also, leptin levels in gingiva during health can be a part of a host defense mechanism like sepsis. In gingival inflammation, leptin levels are decreased secondary to vascular network expansion by the VEGF (vascular endothelial growth factor) which could increase the leptin removal rate from the gingiva.

E-chain was used for canine retraction in the previous study by Dilsiz A et al[9] in 2010, whereas, Sueri et al[15] in 2006 suggested that heavy forces are exerted by the E-chain of nearly 380 grams. This can cause undermining resorption of the alveolar bone leading to delay in tooth movement. Also, E-chain imbibes the fluids in the oral cavity and loses its magnitude of force before the next activation appointment leading to an interruption in the values of the forces.

The present study utilized the NiTi coil springs for canine retraction which cause delivery of a constant and light force of nearly 150 grams to 200 grams causing optimum continuous tooth movement and direct bone resorption. The study results showed that no change in the leptin concentration was seen in the control tooth between pre-treatment, 6 hours and 21 days. However, in the test tooth, a statistically significant increase in the GCF concentration of leptin was seen 4-6 hours after the force application which is similar to what was seen in the acute sepsis cases. After the retractive force application to the teeth using the NiTi coil spring, acute inflammatory alterations are seen in periodontal and gingival tissues. After 21 days of the force application, a decrease in the leptin GCF concentration was seen in test teeth with NiTi coil spring compared to the baseline values with a non-significant difference. This is in line with Samuels RH[16] in 1998 where authors reported that optimal forces are delivered by NiTi coil spring of magnitude 150-200 grams which is in contrast to the heavy force of 380 grams applied by the E-chain which is also suggested by Wichelhaus A et al[17] in 2010 along with easy and quick recovering of the investing tissues of teeth, undermining resorption, aseptic necrotic area, and minimal hyalinization. The study results depict that gingival conditions do not degrade but rather improve confirmed by the return of the leptin levels of GCF on the 21st day to the baseline values.

CONCLUSION

Considering its limitations, the present study concludes that optimal, continuous and constant orthodontic forces increase the GCF leptin concentration in the early stages of the treatment. On the maintenance of optimal orthodontic forces for a long time, gingival tissues heal fast and reach normal or baseline values of leptin in GCF. This confirms the role of leptin as a mediator in orthodontic tooth movement. However, future studies are needed to assess leptin levels in GCF with different force magnitudes in a longitudinal fashion to establish the role of leptin in the progression of periodontal disease to clear the role of leptin during the progression of periodontal disease and orthodontic tooth movement.

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TABLES

Tooth	**Pre-treatment**	**6-hours after force**	**21 days after the force**	**p-value**
Controls	0.77±0.13	0.77±0.09	0.76±0.07	**<0.001**
Cases		0.88±0.06	0.72±0.07	

Table 1: GCF levels in the control and test teeth in the study subjects during the study duration

Groups	**Number (n)**	**Mean± S. D**	**P-value**
Sample -I	50	0.7514±0.115	**<0.001**
Sample -II	50	0.8606±0.086	
Sample -III	50	0.7502±0.076	
Sample -IV	50	0.7014±0.095	
Sample -V	50	0.7426±0.094	

Table 2: Leptin levels in the various study samples