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Evaluation Of Leptin Concentration With Orthodontic Tooth Movement
Running Title: Leptin Concentration With Orthodontic Tooth Movement

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Abstract

Introduction: The biological markers measurement in the GCF and their relationship with clinical effect can be helpful for supervising and forecasting the orthodontic treatment effect. PGE2 is one of the initial biomarker for bone resorption, which can be used to supervise orthodontic tooth movement.

Aim: This research aim to evaluate the association of leptin level on orthodontic tooth movement.

***Material and method*:** An observational prospective study was carried out on 40 participants attending Department of Orthodontics School of Dental Sciences In present study forty orthodontic patients were included. Concentration of leptin was assessed at baseline (T0), 1 hr after application of force (T1), 24 hours later (T2), 15 days after application of force (T3), and after 2 months of orthodontic force (T4). GCF was collected with help of strips of filter paper, from sulcus of gingiva on right maxillary canine's distal aspect. Distalized tooth movement was evaluated by measuring the difference on dental casts, at baseline and 2 month following force application. Obtained data was statistically evaluated. The obtained data was statistically evaluated with Chicago with "t" test and one-way

ANOVA with Bonferroni correction. Kolmogorov Smirnov test was used to check normality of distribution of data.

Results: Average leptin concentrations in GCF raises from baseline (T0, 296.234 picograms/micro liter) to 1 hours after application of force (T1, 393.865 pg/µL), then increased to peak at after 24 hours (T2, 938.82 pg/µL), and reduced to a lowest amount after 15 days (T3, 31.28 pg/µL) and again raises after 2 months (254.42 pg/µL), related to the base line value (T0), and this was statistically considerable ($P < 0.05$). A Leptin level at different time points was statistically considerable excepting for value at T2. There was a considerable association of the in general average levels of leptin to degree of movement of tooth.

Conclusion: It was observed that, there was a biphasic variation in leptin level of GCF while orthodontic tooth movement. There was a considerable association among the degree of tooth movement with leptin level in GCF.

Keywords: GCF, leptin concentration, orthodontic force, tooth movement

Introduction

Orthodontics is a branch of dentistry that deals with correction of malocclusion. From past several decades, numbers of patients undergoing orthodontic treatment have increased enormously.

Orthodontic tooth movement (OTM) are difficult and consists of relations among the periodontal ligament (PDL) cells and alveolar bone cells, together with numerous intercellular manners. The biochemicals, cellular and molecular actions that happen during OTM have been broadly deliberated. At the time of orthodontic procedure, the applied forces on the tooth alter the extracellular matrix of PDL's and which may modify the cellular form and cytoskeletal arrangement. These modifications may alter the flow proportion and composition of the gingival crevicular fluid (GCF). [1]

The GCF is a transudate of interstitial tissues produced by an osmotic gradient. [2] It was found that remodelling occurs with markers. The host-derived substances in the GCF consist of antibodies, cytokines, and tissue degradation products. The biological markers measurement in the GCF and their relationship with clinical effect can be helpful for supervising and forecasting the orthodontic treatment effect. [1]

Mechanical stimulation results into inflammatory response inside the periodontal tissues, which consecutively activate the biological course related with remodelling of bone.[1, 2] It has been observed that orthodontic force application trigger a multilevel cascade of signal transduction pathways, as well as the prostaglandin E2 (PGE2) pathway, which consecutively initiates structural and functional alterations in cell membrane, extracellular, and cyto-skeletal proteins. PGE2 is one of the initial biomarker for bone resorption, which can be used to supervise OTM.[3]

Cytokines are one amongst the local biochemical mediators of tooth movement and are secreted chiefly by a dipocytes. Cytokines have multiple biologic activities together with bone remodeling, and thus, they play a major role in tooth movement.[4] Leptin is categorised as acytokine, and it is a polypeptide hormone that shows a considerable role in raise of phagocytosis by macrophages in reaction to initial stress/strain made by orthodontic forces.[1] Leptin indicates a substantial part in bone formation by means of its direct outcome on

proliferation of osteoblast, differentiation and in enhancing the life span of human primary osteoblasts. [4] Leptin encourages the immune system by boosting phagocytosis by macrophages and cytokine production. [3] GCF is conversely a superior option for evaluating biomolecules or mediators as test collection is sensitive, simple, suitable, repetitive and non invasive. Quantitative assessments of mediators in GCF imitate biochemical mechanisms related with orthodontic tooth movement. [5]

Studies regarding the role on leptin concentration on tooth movement are vary scares therefore, this research was done to compare mean levels of leptin to the degree of tooth movement by evaluating the concentration of leptin at different time points at the time of application of orthodontic force.

Materials And Method

Forty orthodontic patients of both genders with equal sample size were included for the present observational prospective study. This research was done in Orthodontics department after obtaining approval from institutional ethics committee of ITS Centre for Dental Studies and Research, Ghaziabad, UP with Ref no. Director-PG Studies/ITSCSR/L/2018/017. This study was done form April 2018 to December 2019. Informed consent was attained from all the participants. Sample size was calculated according to Dilsiz *et al* study with power (1-ß error) = 0.8, ∝ error chances = 0.05 and effect size $f = 0.28$. Participant's average age was 20.6+_1.75 and in the age ranges of 18-24 years. [6]

Inclusion criteria was; patients with Angle's class I malocclusion with ANB angle 2° ±2 and class I skeletal base, maxillary crowing more than 6 mm which requires canine distalisation following extraction of all 1st premolars; healthy patients with healthy gingival and peridontium; absence of craniofacial anomalies. Exclusion criteria were subjects with presence of oral habits and subjects not willing to participate in the study.

Fixed orthodontic alignment was initiated with initial arch wire of 014-inch NiTi .After 2 weeks following extraction of all 1st premolars, distalisation of canine was initiated with active lace-backs, from canine to 1st molar using 0.009" SS wire. The force application was made consistent by placing an equivalent number of turns (four turns) for every participant. Trans-palatal arch on 1st molar was used as an anchorage.

Using strips of filter paper (Periopapers, Ora flow Inc, New York,) gingival crevicular fluid was obtained from the sulcus of gingiva on distal aspect of the right maxillary canine. Patients were instructed to maintain good oral hygiene to evade gingival inflammation during the study stage. This was confirmed by evaluating the bleeding on probing and probing depth at each time before collecting sample. Blood and saliva contaminated samples were kept away. The GCF sample was acquired in morning time at each recall visit. Concentration of leptin was assessed at baseline (T0), 1 hour after application of force (T1), 24 hours later (T2), 15 days after application of force (T3), and 2 month after application of orthodontic force (T4). Base line acts as control.

Leptin Analysis

Every filter paper strip was used two times with 100 µL Hank's balanced salt solution including 0.5% bovine serum albumin by centrifugation (3000 × *g*; 4°C) for fifteen min. Leptin levels was evaluated using enzyme-linked immune sorbent method a commercial product. The analyses were carried out in line with the manufacturer instructions. For leptin

evaluation, high-sensitivity kits (Bio Source International Inc, Camarillo, Calif) were included to quantitatively perceive low on concentrations of leptin,

Measurement of rate of tooth movement

Casts were made from alginate impression at baseline (T0) and after 2 month after orthodontic force application (T4). The tooth movement rate was evaluated as the quantity of distal movement of the maxillary canine at the end of two months. Amount of canine movement was calculated using Vernier caliper at baseline and after 2 month on cast. Difference amongst these two readings was considered as the degree of tooth movement. All the procedure was done by trained single operator. Error was measured with Dahlberg's formula.[11]

Method error = $\sqrt{\sum d^2/N}$,where "d" is the distinction amongst two observations made from the similar constraint and "n" is the number of subjects. Method error was established to be 0.02 mm.

Statistical analysis

The obtained data was statistically evaluated with SPSS software version 21, Chicago with "t" test to calculate average GCF leptin level and degree of tooth movement. Mean leptin concentrations at the 5 time points were calculated with one-way ANOVA with Bonferroni correction. Kolmogorov Smirnov test was used to check normality of distribution of data (Table-1). By means of Pearson's correlation coefficient, mean degree of tooth movement was measured, and it was associated to average leptin levels.

Result

Table 1 indicates T0 with difference df of 18 and insignificant result. Table 2 indicates average GCF leptin levels at different points of time. Average leptin concentrations in GCF raises from baseline (T0, 296.234 picograms/micro liter) to 1 hours after application of force (T1, 393.865 pg/µL), then increased to peak at after 24 hours (T2, 938.82 pg/µL), and reduced to a lowest amount after 15 days (T3, 31.28 pg/µL) and again raises after 2 months (254.42 pg/µL), relevant to the base line reading (T0), and this was statistically considerable ($P < 0.05$).

Table 3 indicates comparison of average GCF leptin level at several time points. It was statistically considerable (P<0.005) excepting for value at T2 which was not statistically considerable in contrast to findings at T0 and T1. This could be because of increase in 95% confidence interval at T2. Table 4 indicates, a significant association in general about average levels of leptin to degree of tooth movement (correlation coefficient = 0.564).

Discussion

OTM is consists gene expression with cellular and molecular events in alveolar bone and periodontal tissue following orthodontic force application. [7] Orthodontic forces cause an initial inflammatory response subsequently perpetual bone and tissue remodelling with bioactive mediator's release. [5, 6]

Alaguselvaraj et al assessed the leptin level in GCF while OTM. They concluded that Leptin is one of the mediators of OTM.[4] Jayaprakash et al assessed the various cytokines in GCF during OTM. They concluded that alterations in cytokines in GCF are related with OTM. [7] Jain et al assessed the Leptin levels in GCF while OTM at 1 hr, 1 day, and 7days

after the beginning of tooth movement.They concluded that Leptin may be one of the mediators related with OTM. [8] Dharma et al evaluated the salivary leptin concentration among various types of facial skeletal pattern. They concluded that salivary leptin concentrations are elevated in patients with various facial skeletal types in comparisons to Class I. [9] These findings are in agreement with our results.

Soares Bonatoa et al to estimated orofacial pain, tooth movement, and leptin, tumor necrosis factor (TNF)–a cytokine, interleukin (IL)–1b, and levels in the GCF at the time of orthodontic procedure in obese adolescents. They concluded that orofacial pain, while movement, and leptin, a cytokine levels influence in GCF during orthodontic procedure in obese adolescents. [10]

Drummond et al assessed whether orthodontic appliance *per se* or OTM can provoke noticeable alteration in GCF level. They concluded that GCF concentration does not seem to be a dependable biomarker for tissue remodeling while orthodontic procedure. [2] This is in contrast to our findings

Schröder et al suggested that raised leptin levels in obese patients may enhance orthodontic tooth movement. The raised expression of pro inflammatory factors and RANKL as well as increased osteoclasto genesis can be guessed to accelerate bone resorption. [11]

Goutam et al evaluated the connection of OTM with the levels of leptin in GCF. They found a biphasic variation in GCF leptin levels and significant association between rates of tooth movement with GCF leptin concentration. [12] The results are in agreement with our findings. We observed bimodal changes in leptin levels with OTM. We can state that leptin has role in OTM.

The limitations of the current research were smaller sample size. Further researches needed on larger sample size to validate the results.

Conclusion

It was observed that, during application of one set of orthodontic force from baseline to one month postoperatively, there was a change in GCF leptin levels. Leptin levels in GCF are positively associated with degree of tooth movement.

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Legends for illustrations

Tables and graph

Table 1: *Test of normality with Kolmogorov -Smirnov*

	Statistic	Difference	df	Significance
T0	0.138	18		0.874

Table 2: *Mean GCF leptin level at a range of time point*

	GCF leptinlevels in picogms/micro litre (pg/μL)		95% confidence intervals
	Mean	Standard deviation	Upper Bound Lower Bound
T0 Baseline	296.234	79.64	269.58-322.654
T1 after 1 hour	393.865	58.75	362.54-425.23
T2 after 24 hours	938.82	1274.01	414.12-1463.52
T3 after 15 days	31.28	14.62	24.42-38.13
T4 after 2 months	254.42	112.64	184.78-324.06

Table 3: *Avarage GCF leptin concentration comparison of at various time of time*

Time points (T)	Time points (T)	Significance
0	1	0.001 **
	2	0.105
	3	0.000***
	4	0.000***
1	0	0.001 **
	2	0.154
	3	0.000***
	4	0.000***
2	0	0.106
	1	0.164
	3	0.015*
	4	0.058
3	0	0.000***
	1	0.000***
	2	0.013*
	4	0.000***
4	0	0.000***
	1	0.000***
	2	0.058

significance at 0.05 level, ** P>0.01 ,* highly significance (0.001), test used – ANOVA*

Table 4: *Association of GCF leptin level to degree of tooth movement*

Correlation	Amount of tooth movement	Mean
Amount of **movement of tooth**in mm		
Pearson Co-relation	1	0.625**
Sig.(2-tailed)		0.003
n	38	19
Pearson Correlation	0.564**	1
Sig.(2-tailed)	0.003	
*N*4040	*N*4040	*N*4040

***indicatessignificance , P<0.01 level, n=Number of subjects*