Expression Of Leptin In Human Saliva After Orthodontic Force And Its Relationship With Body Mass Index.

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Abstract

Introduction: The Aim of this study is to compare the concentration of salivary leptin levels between underweight and normal weight individuals and its significance in orthodontic tooth movement.

Materials and Method: This observational study included forty-five underweight and normal weight female subjects and they are divided into two groups, group I (underweight) and group II (normal weight) depending on their mean body mass index given by WHO classification. All subjects underwent upper first premolar extraction, and distal force is applied with lacebacks to maxillary canine. Salivary leptin samples were collected before force application (T_o), one hour after appliance placement (T_1), and one month after appliance placement (T_2).

Result: Underweight individuals show decreased salivary leptin concentration than normal weight individuals. Orthodontic tooth movement is increased in underweight individuals when compared with normal weight individuals.

Conclusion: Orthodontic tooth movement is increased in underweight patients when compared with normal weight patients. Salivary leptin is two times greater in normal weight patients when compared with underweight patients.

Keywords: Salivary Leptin, Orthodontic Tooth Movement, Inflammatory mediator

INTRODUCTION

Orthodontic tooth movement involves active remodeling of the periodontal ligament in response to orthodontic force. Acute inflammatory reaction induced by orthodontic appliance, helps in release of inflammatory cytokines (IL-1, IL-6, TNF-α) and enzymes from periodontal ligament responsible for connective tissue remodeling. These inflammatory mediators rely on coordinated tissue resorption and formation in the surrounding bone and periodontal ligament.[1-4]

Leptin is a polypeptide hormone, produced by adipocytes, osteoblast cells, salivary gland etc.[5]Leptin is a proinflammatory cytokines which plays a major role in orthodontic tooth movement. Leptin shares its structural and functional similarity with interIeukin-6.[6] Leptin plays a dual role as a hormone and cytokine, as a hormone helps in maintaining energy balance and hunger, and as a cytokine helps in acute inflammatory process.[7]

Leptin has both stimulatory and inhibitory effects on bone metabolism. It prolongs the shelf life of human osteoblast cell growth and indirect suppressive effect on bone formation through the hypothalamus by central regulatory pathway. Biochemical markers of bone remodeling provide a potentially non - invasive clinical tool for assessing and monitoring bonemetabolism.[7]

Leptinconcentrationisexpressedin periodontal tissues,pulp,GCFsamples and saliva.[9] Recent studies evaluated leptin levelsin orthodontic tooth movement and leptin behaves like a cytokine and plays a major role as a mediator in orthodontic tooth movement.[10]

Recent studies also evaluated salivary leptin levels between normal weight and overweight individuals and showed

that orthodontic tooth movement is decreased in overweight individuals when compared with normal weight patients.[11] Analyzing leptin levels in salivary samples is non-invasive and moreover quantity of leptin expressed in saliva is more in nanograms (10^{-9}) when compared with GCF samples which is expressed in picograms (10^{-12}) and salivary leptin with tooth movement can be correlated much easily when compared with GCF samples.[12]

Orthodontists have a major role in treating underweight patients also and till now there has been no studies compared salivary leptin in underweight and normal weight individuals. Thus, the aim of the present study is to evaluate the leptin concentration in average normal weight individuals and underweight individuals and its relationship with orthodontic tooth movement.

MATERIALS AND METHODS

This was an observational study. Forty-five female subjects who reported for orthodontic treatment were selected. The subjects were categorized into two groups, Group 1 (underweight patients) and Group II (normal weight) patients. Subjects were divided depending upon their mean body mass index given by WHO.[4] Underweight patients BMI ranges below 18.5 kg /m2 and Normal weight patients BMI range was between 18.5 and 25 kg /m2.

Inclusion criteria

- Angle's class I malocclusion with crowding < 6mm.
- Only Female subjects were taken in this study.
- First premolar extraction cases were taken.

Exclusion Criteria:

- No systemic illness, periodontal problems, osteoporosis**,** craniofacial anomalies were excluded.
- Patients with any Salivary gland related disorders, parafunctional habits were also excluded.

Informed consent was obtained from all patients and ethical clearance was obtained from institutional ethical committee Unstimulated whole saliva was collected using passive drooling method making the patient to sit upright. samples were collected from all the subjects before starting the treatment T_O, after starting the treatment T_1, and one month after treatment. T_2.[5] Salivary samples were refrigerated and stored at -80degree Celsius storage box.

After extraction, patient strap up done with initial arch wire with 0.014 Niti. Canine is distalised using active lacebacks. Force is standardized using dontrix gauge by giving equal number of turns in all subjects both in test and control group. Study model impression is taken before and after three months after applying force. Third palatal rugae used as a standard reference point to measure the distance of canine tooth movement.[6] For measuring the tooth movement, distal surface of canine to premolar mesial point is taken. Salivary leptin concentration is measured using ELISA kit Ray biotech Inc, Germany.[7]

STATISTICAL ANALYSIS:

Student t test were used to compare the mean salivary leptin concentration and rate of tooth movement between underweight and normal weight patients. Shapiro Wilk's test was used and repeated measures of ANOVA were used to compare the data within the groups and to control the type I error, the Bonferroni correction was used. Pearson correlation test was done to measure the correlation between salivary leptin concentrations with rate of tooth movement. P value < 0.05 was considered to be statistically significant. The data thus collected were assessed using SPSS 16.0 statistical software (SPSS Inc, Chicago, III).

RESULTS

Mean leptin concentration was two times greater in normal weight individuals when compared with underweight individuals at all three-time intervals. The difference is statistically significant (P vaIue =0.00).
Table I.

Table I: Mean and standard deviation of salivary leptin levels between underweight and normal weight individuals.

	Group	N	Mean	Std. Deviation	P value
T_0	Underweight	45	246.2	96.83	0.001
	Normal weight	45	512.4	111.32	
T_1	Underweight	45	334.2	54.69	0.001
	Normal weight	45	682.4	123.13	
T_2	Underweight	45	223.2	98.34	0.001
	Normalweight	45	453.1	119.34	

After force application at T_1 leptin level increase in both the groups. At T_2 leptin levels starts to decline and reaches nearby baseline values T_0 Repeated measures of ANOVA were used to compare salivary leptin within the groups and it is statistically significant.

Table II: Repeated measure of ANOVA test were used to compare the leptin concentrations in underweight group.

Time intervals	Comparison Within group	Mean Difference	Std. Error	P value
T_0	T_1	-88.20*	23.770	.014
	T_2	23.03*	18.56	.001
T_1	T_2	111.064*	27.09	.001

The mean difference is signicant at 0.05 level

Table III: Repeated measure of ANOVA test were used to compare the leptin concentrations in Normalweight group

Time intervals	Comparison Within group	Mean Difference	Std. Error	P value
T_0	T_1	-229.1*	47.818	.044
	T_2	8.16*	01.984	.001
T_1	T_2	221.08*	67.597	.001

** The mean difference is signicant at 0.05 level

The rate of tooth movement is greater in underweight patients which is statistically significant
(P value =0.01)

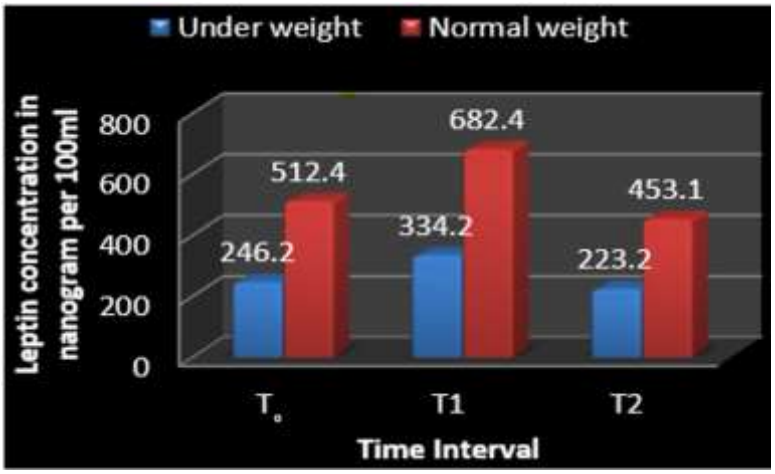
Table IV: Rate of tooth movement between normal weight and underweight patients.

	Group	N	Mean	Std. Deviation	P value
Rate of tooth movement	Underweight	45	2.3	0.51	0.001
	normalweight	45	1.2	0.42	

Orthodontic tooth movement was corelated with mean leptin concentration between underweight and normal weight individuals. There was a greater significance of tooth movement in underweight group when compared with normal weight (Table V,VI)

Table V: Pearson correlation test for underweight group

		Mean leptin concentration between T_0, T_1, T_2	Rate of tooth movement
Mean leptin concentration between T_0, T_1, T_2,	Pearson Correlation	1	0.48
	Sig.		0.01
	N	45	45
Rate of tooth movement	Pearson Correlation	0.48	1
	Sig.	0.01	
	N	45	45



Graph I : Mean salivary leptin concentration between underweight and normal weight individuals at three time intervals.

DISCUSSION

The study was conducted to investigate the relationship of salivary leptin in underweight and normal weight individuals with orthodontic tooth movement. Leptin plays a dual role as a hormone and cytokines, it shares structural and functional similarity with interleukin-6, thus leptin can be a one of the mediators of inflammation. Leptin have a dual role on bone metabolism. It has a both stimulatory and inhibitory effect on bone metabolism. After the discovery of leptin in 1994,[18] numerous studies were conducted regarding the role of leptin in inflammation.[19]

In our study to rule out any gender related bias, we have included only female subjects. Studies have shown that females have more adipose tissue when compared with males. Female subjects were categorized in group 1 (underweight) and group 2 (normal weight) depending upon the height and weight chart given by WHO classification.[20] Body mass index BMI is calculated according to WHO criteria.[14]

All subjects underwent orthodontic therapy. Salivary samples were collected before appliance treatment. Unstimulated whole saliva were collected using passive drooling method, patient were sit upright and samples were collected exactly at 9 am. Salivary leptin exhibits circadian rythm , so all samples were collected at same duration.[21]

There is a strong positive correlation between serum and salivary leptin.[21] Collecting saliva sample is non -invasive than collecting serum samples. Previous studies correlated GCF leptin levels with orthodontic tooth movement, here in our study we took salivary samples, the quantity of leptin expressed in saliva is in nanograms, but in GCF the quantity of leptin is expressed in picograms which is lesser in quantity.

ELIZA test is the standard method to diagnose the salivary leptin in underweight and normal weight individuals. saliva is a serum exudate and the antibodies can be determined easily using eliza test.[17]

Previous study also reported the leptin levels in GCF, pulp, periodontal tissues etc. All patients undergone fixed orthodontic therapy, and samples were collected before force application. Angles class 1 malocclusion with bimaxillary protrusion is selected, to rule out any bias in tooth movement. After strap up with o. O14 niti wire, and canine is distalised with active lace backs. In our study we standardized the force system with dontrix gauge and with equal number of turns in both the groups, so that equal force is given in all the samples.[16]

Study models were taken before and after three months of orthodontic treatment, and distal of canine to mesial of premolar is measured using digital vernier caliper. All the measurement were made by the single examiner to rule out any bias or to reduce inter examiner reliability.[15]

Third Palatal rugae provides a stable landmark to rule out the tooth movement and helps in determining the rate of tooth movement from mesial of premolar to distal of canine.[16]

Initially leptin concentration was low in both groups. but after force application leptin levels start to rise in first one hour both in underweight and normal weight patients at T_1. The rise of salivary leptin is two times lesser in underweight patients when compared with the normal weight patients. And later at T_2 (after one month) leptin levels come to baseline values in both the groups.

Leptin level showed a peak rise after force application similar like cytokines, proving its role as a inflammatory cytokines which play a role in mediators of tooth movement.[22]

Till now no studies compared underweight with normal weight individuals, though leptin is a obese gene, its minor quantities are secreted in saliva, stomach, lungs etc, in normal individuals also. In our study we evaluated leptin levels in underweight and normal weight individuals and its correlation with tooth movement, we found leptin levels is twice less in underweight patient when compared with normal weight patients, this could be potential factor of leptin excitatory effect on bone metabolism, Lesser leptin in underweight patients paves the way for more osteoclast resorption and more tooth movement in underweight patients. Rate of tooth movement is greater in underweight patients, this could be due to decreased mineral bone density in underweight patients.[19,13]

However further studies at molecular level are needed to determine the exact biological role of leptin in tooth movement.

CONCLUSION:

- Salivary leptin levels decreased in underweight patients which is two times lesser than normal weight patients.
- Orthodontic tooth movement is increased in underweight patients when compared to normal weight patients

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