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## **Comparison of Salivary Leptin Levels Between Underweight and Overweight Individuals and its Role in Orthodontic Tooth Movement**

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**Abstract**---Introduction: The Aim of this study is to compare the concentration of salivary leptin levels between underweight and overweight individuals and its significance in orthodontic tooth movement. Methods: Forty five underweight and overweight female subjects were taken in this study and they are divided into two groups, group I (underweight) and group II (overweight) depending on their mean body mass index. Angles class I malocclusion with bimaxillary protrusion were taken into this study. 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 (To), one hour after appliance

placement T2, and one month after appliance placement (T3). Results: Overweight individuals show increased salivary leptin concentration three times greater than underweight individuals. Orthodontic tooth movement is decreased in overweight individuals when compared with underweight individuals. Conclusions: Increased salivary leptin levels showed a decreased tooth movement in overweight individuals than underweight individuals who showed decreased salivary leptin levels. Obese individuals require more orthodontic treatment duration time when compared with lean individuals.

**Keywords**---inflammatory mediator, orthodontic tooth movement, overweight, salivary leptin, underweight.

## Introduction

Leptin is a obese hormone produced primarily by adipocytes and in small quantities by placenta, lung, gastric fundic mucosa, osteoblast, salivary glands. Etc. The primary function of leptin is the regulation of adipose tissue mass through central hypothalamus mediated effects like hunger, energy balance, physical exercise etc.<sup>1-4</sup> Leptin plays a dual role as a hormone and also like a cytokine.<sup>5</sup> Leptin is a proinflammatory cytokine which plays a major role in orthodontic tooth movement. Cytokines is a inflammatory mediator which is responsible for orthodontic tooth movement. Leptin shares its structural and functional quality similar to interleukin-6.<sup>6</sup>

Leptin has many regulatory functions like energy balance, bone metabolism, wound healing, immune and inflammatory response.<sup>7</sup> In bone metabolism leptin plays a role in regulating bone remodeling, resorption and new bone formation. Leptin plays 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.<sup>8</sup>

Leptin concentration is expressed in pulp, GCF samples and serum.<sup>9</sup> Recent studies evaluated leptin levels in orthodontic tooth movement and leptin plays a major role as a mediator in orthodontic tooth movement and leptin levels decreased during orthodontic tooth movement.<sup>10</sup> Recent studies also evaluated salivary leptin levels between normal weight and overweight individuals,<sup>11</sup> orthodontists have a major role in treating underweight patients also, till now no other studies were published comparing lean underweight individuals with overweight patients. Moreover analyzing leptin levels in salivary samples is non invasive, and studies reported that there is a strong positive correlation between serum and salivary leptin concentration.<sup>12</sup>

The prevalence of overweight and obese people are increasing worldwide. Obese people will have altered growth and development, bone metabolism, and pubertal growth.<sup>13</sup> Thus the aim of the present study is to evaluate the leptin concentration in lean underweight individuals and overweight individuals and its relationship with orthodontic tooth movement.

## Materials and Methods

Forty five female subjects undergoing orthodontic treatment were taken in this study. The subjects were categorized into two groups, Group 1 (underweight patients) and Group II (overweight) patients. Subjects were divided depending upon their mean body mass index given by WHO.<sup>14</sup> Underweight patients BMI ranges below 18.5 kg /m<sup>2</sup>. and Overweight patients BMI range was between 25 and 30 kg /m<sup>2</sup>.

Inclusion criteria:

- Angles class I malocclusion with bimaxillary protrusion
- Only Female subjects were taken in this study.
- First premolar extraction cases were taken in this study, and canines require active distalisation.

Exclusion criteria:

- No history of any systemic illness.
- Patients with any periodontal problems like diabetes, osteoporosis are excluded in this study.
- Patients with any Salivary gland related disorders

Informed consent was obtained from all patients and ethical clearance was obtained from institutional ethical committee. Salivary samples were collected from all the subjects before starting the treatment T0, after starting the treatment T1, and one month after treatment. T2. Unstimulated whole saliva is taken from the subjects using passive drooling method.<sup>15</sup> Salivary samples were refrigerated and stored at -80 degree Celsius storage box.

Active force is applied by lacebacks for distalisation of canine teeth after strap up with 0.014 niti wire. Force is measured and standardised using dontrix gauge. Impression is taken and study model is taken before starting the treatment and after the end of three months after distalisation. Third palatal rugae used as a standard reference point to measure the distance of canine tooth movement.<sup>16</sup> Measurement is made from canine distal surface point to premolar mesial surface point. It is measured using vernier caliper instrument by a single examiner. Salivary leptin concentration is measured using ELISA kit Ray biotech Inc, Germany.<sup>17</sup>

## Statistical Analysis

Student t test were used to compare the mean salivary leptin concentration and rate of tooth movement between underweight and overweight patients. To test the normality of the data 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 three times greater in overweight individuals when compared with underweight individuals at all three time intervals. The difference is statistically significant (P value = 0.00 ). Table 1

Table 1  
Mean and standard deviation of salivary leptin levels between Underweight and overweight individuals.

|                | Group       | N  | Mean  | Std. Deviation | P value |
|----------------|-------------|----|-------|----------------|---------|
| T <sub>0</sub> | Underweight | 45 | 246.2 | 96.83          | 0.001   |
|                | Overweight  | 45 | 756.1 | 303.09         |         |
| T <sub>1</sub> | Underweight | 45 | 334.2 | 54.69          | 0.001   |
|                | Overweight  | 45 | 985.2 | 354.45         |         |
| T <sub>2</sub> | Underweight | 45 | 223.2 | 98.34          | 0.001   |
|                | Overweight  | 45 | 764.2 | 259.39         |         |

After force application salivary leptin levels increases in both the groups at (T<sub>1</sub>). And after one month at T<sub>2</sub>, leptin levels starts to decrease similar like cytokine levels. Repeated measures of ANOVA test was used to compare the salivary leptin concentrations within the groups. The difference was statistically significant (Table 2, 3).

Table 2  
Repeated measures 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 <sub>0</sub> | T <sub>1</sub>          | -88.20*         | 23.770     | .014    |
|                | T <sub>2</sub>          | 23.03*          | 18.56      | .001    |
| T <sub>1</sub> | T <sub>2</sub>          | 111.064*        | 27.09      | .001    |

\*. The mean difference is significant at the .05 level.

Table 3  
Repeated measures of ANOVA test were used to compare the leptin concentrations in overweight group

| Time intervals | Comparison Within group | Mean Difference | Std. Error | P value |
|----------------|-------------------------|-----------------|------------|---------|
| T <sub>0</sub> | T <sub>1</sub>          | -229.1*         | 47.818     | .044    |

|                |                |         |        |      |
|----------------|----------------|---------|--------|------|
|                | T <sub>2</sub> | 8.16*   | 01.984 | .001 |
| T <sub>1</sub> | T <sub>2</sub> | 221.08* | 67.597 | .001 |

\*. The mean difference is significant at the .05 level.

The rate of orthodontic tooth movement was lesser in overweight group compared to normal weight group which was statistically significant (P value = 0.01), Table IV.

Table 4  
Rate of tooth movement between normal weight and overweight individuals

|                        | Group       | N  | Mean | Std. Deviation | P value |
|------------------------|-------------|----|------|----------------|---------|
| Rate of tooth movement | Underweight | 45 | 2.89 | 0.56           | 0.001   |
|                        | Overweight  | 45 | 1.25 | 0.43           |         |

Orthodontic tooth movement was correlated to the mean leptin concentration between T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> in both the groups. The results showed that there was a positive correlation of leptin levels with tooth movement, with greater significance in overweight groups when compared with normal weight groups. (Table 5, 6).

Table 5  
Pearson correlation test for overweight group

|                                                                                      |                     | Mean leptin concentration between T <sub>0</sub> , T <sub>1</sub> , T <sub>2</sub> | Rate of tooth movement |
|--------------------------------------------------------------------------------------|---------------------|------------------------------------------------------------------------------------|------------------------|
| Mean leptin concentration between T <sub>0</sub> , T <sub>1</sub> , T <sub>2</sub> . | Pearson Correlation | 1                                                                                  | 0.498                  |
|                                                                                      | Sig.                |                                                                                    | 0.019                  |
|                                                                                      | N                   | 45                                                                                 | 45                     |
| Rate of tooth movement                                                               | Pearson Correlation | 0.498                                                                              | 1                      |
|                                                                                      | Sig.                | 0.019                                                                              |                        |
|                                                                                      | N                   | 45                                                                                 | 45                     |

\*\*. Correlation is significant at the 0.05 level .

Table 6  
Pearson correlation test for normal weight group

|                           |                     | Mean leptin concentration between T <sub>0</sub> , T <sub>1</sub> , T <sub>2</sub> . | Rate of tooth movement |
|---------------------------|---------------------|--------------------------------------------------------------------------------------|------------------------|
| Mean leptin concentration | Pearson Correlation | 1                                                                                    | 0.457                  |

|                                                             |                                |                |       |
|-------------------------------------------------------------|--------------------------------|----------------|-------|
| between<br>T <sub>0</sub> ,T <sub>1</sub> ,T <sub>2</sub> . | Sig.                           |                | 0.013 |
|                                                             | N                              | 45             | 45    |
| Rate of tooth<br>movement                                   | Pearson<br>Correlation<br>Sig. | 0.457<br>0.013 | 1     |
|                                                             | N                              | 45             | 45    |

## Discussion

Leptin is a dual hormone which plays a major role as a cytokine and also a hormone. Leptin hormone is secreted by many cells like gastric mucosa, lung, placenta, saliva etc. After the discovery of leptin by Zhang et al in 1994,<sup>18</sup> numerous studies about leptin were published and leptin proves to have numerous role in human body. Leptin acts as a satiety centre through centrally mediated pathway through hypothalamus and it acts as a hunger hormone. Leptin shares its structural and functional quality similar to interleukin -6, so leptin acts like a cytokine and plays a major role in inflammation. Leptin has a strong correlation with body mass index, higher leptin will be secreted in patients with more visceral body fat.<sup>19</sup>

In this study female subjects were taken in order to avoid bias, and also female subjects will have more visceral adipose tissue when compared with abdominal fat tissue.<sup>20</sup> Only females are included in this study. Body mass index were calculated according to the chart released by WHO, and we categorised the individuals as overweight and underweight depending upon their height and weight chart and WHO reference data.<sup>14</sup> Angle's class I malocclusion with bimaxillary protrusion was taken in to this study. Extraction was carried out and appliance was placed with initial arch wire 0.014 niti. The force was standardized with Dontrix gauge, and active lacebacks were applied with five or six turns for all the subjects. We used active lacebacks to distalise the canine, when compared with elastomeric chain which delivers heavy forces.

Numerous study was conducted on GCF leptin levels, leptin levels in pulp samples etc. there is a strong positive correlation with serum and saliva leptin levels. So in our study we took saliva samples and moreover collection of saliva samples is easy and non invasive, so we compared salivary leptin levels in our study. Unstimulated whole saliva were collected with passive drooling technique, leptin also shows circadian rhythm,<sup>21</sup> so we collected salivary samples at 9.00a.m. Bias during saliva collection is eliminated. After samples collection, they are centrifuged and supernatant is stored for leptin detection.

ELISA test is used as a standard measuring technique to measure the quantity of leptin secreted in saliva.<sup>17</sup> Salivary leptin is a serum exudate, and these antibodies can be diagnosed very efficiently by ELISA test. Study models were taken before and after treatment, and third palatal rugae were used as a standard reference point to measure the tooth movement.<sup>16</sup> Measurements were made by a single examiner to reduce the bias, and distal contact point of right canine to the

mesial contact point of right premolar were measured and rate of tooth movement were calculated in this study.

After force application, cytokines as a inflammatory mediator will start to increase rapidly and then it starts to decline there on. Previous studies showed that after force application there is a rise of pro inflammatory cytokines in first twenty four hours and later the system stabilizes and reaches a new physiological hemostasis until the next activation.<sup>22</sup> In our study we measured salivary leptin levels before, one hour and one month after force application. Leptin levels showed a peak rise after force application similar like cytokines, proving its role as a cytokines. And later the leptin levels starts decreasing after a month both in underweight and overweight patients.

Rate of tooth movement is faster in underweight patients when compared with the overweight patients. Previous studies showed that there is higher mineral bone density in overweight patients,<sup>19,13</sup> this could be a possible reason that in overweight patients because of increased mineral density orthodontic movement is lesser. Moreover in overweight individuals increased fat mass results in increased serum leptin levels,<sup>12</sup> which could have a strong influence in bone metabolism. This might have further effect in rate of tooth movement in those individuals.

Obesity is a global problem worldwide and orthodontist have a role in treating those patients. This study paves the way that orthodontic treatment duration will be greater in underweight individuals when compared with the overweight individuals. Further studies at the molecular level are necessary to determine the exact biological role of leptin in tooth movement.

## Conclusions

- Overweight individuals showed three times higher leptin concentration than underweight individuals.
- Rate of tooth movement is lesser in overweight individuals.
- Rate of tooth movement is faster in underweight individuals.
- Salivary leptin plays a role as a mediator of orthodontic tooth movement, similar like cytokines. Duration of orthodontic treatment may be considerably less in underweight patients when compared with overweight patients.

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