Correlation between Leptin and Chronic Periodontitis

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Abstract

Objective: One of the principle functions of leptin is the regulation of immunologic and inflammatory responses of the body. A recent study reported no existing correlations between leptin and periodontal disease. The present study aimed to investigate the role of leptin in chronic moderate to severe periodontitis in patients who attended the periodontics department at the dental faculty of ShahidBeheshti Medical University.

Methods: This analytical study was conducted on 20 healthy gingival tissue samples (control group) and 20 gingival tissue specimens with moderate to severe periodontitis (experimental group). Patients with a mean age of 42.25 years including 55% women and 45% men participated in this study. Blood samples of 5ml were obtained from each individual and the serum was segregated and frozen at -20ºC. The gingival samples were obtained from segments provided during periodontal surgery and were cultured for 72 hours. The concentrations of leptin and IL-6 in the blood serum and the supernatant fluid of the cultured specimens were determined by ELISA. Paired T and Wilcoxon signed ranks tests were used to perform the statistical analysis.

Results: Leptin was not found in the gingival specimens of the experimental or control groups. The mean concentrations of IL-6 in the healthy and diseased gingival tissue samples were 36.72 ±81.08 pg/ml and 90.35 ± 29.71 pg/ml respectively. Although the experimental group showed a higher concentration of IL-6 compared with the control group, the Paired T test results confirmed by the Wilcoxon test revealed no significant statistical differences between these groups.

Conclusion: The findings of the present study revealed that leptin as an inflammatory protein does not play a role in periodontal disease. The absence of leptin in both clinically healthy gingiva and in gingival tissue with chronicmoderate to severe periodontitissuggests that gingiva is not a source for leptin production.

Key words: leptin, IL-6, chronic periodontitis

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Introduction:

Chronic periodontitis is recently defined as an infectious disease caused by inflammatory processes in the supportive tooth structures that will lead to tissue disjunction and destruction of the alveolar bone (1). Studies in the past two decades have investigated the role of immunological responses especially humoral responses in the pathogenesis of this disease (2). Some of the non-specific components of the immune system such as collagenase (MMP8),
the complement system, inflammatory cytokines such as Interleukin-1 (IL-1), Tumor necrosis factor (TNF) and Interleukin-6 (IL-6) are suggested to have a contributing role in this disease (3).

It's been recently found that a hormone-like protein called leptin, initially known for its effects on body weight regulation, body metabolism and reproduction, may also be part of some inflammatory diseases via its direct impact on the innate and adoptive immune responses (4). Leptin is a Greek word meaning slim. It is a protein based hormone that affects the body weight, metabolism and reproductive system. It has a molecular weight of nearly 16 kilodaltons and is encoded by the obesity gene (5). Friedman and colleagues found this gene in 1994 following their investigations at Rockefeller University. It was shown that the product of this gene directly affects obesity (6, 7). With the presence of leptin, food absorption and body weight will be reduced and energy expenditure will be elevated (8). During fasting and starvation, leptin mRNA and its level will be down regulated in the body which will induce a positive signal for food intake (9, 10). Defects in the expression of the leptin producing gene(ob) or its receptors (diabetes gene) will cause extreme obesity in rodents (11, 12). Adipocytes are the main cells responsible for leptin production but the gastric epithelial and the placental cells also produce a small amount of leptin (5, 7). It's been recently suggested that the gingival epithelium may also play a role in leptin production (13).

In a study by Johnson and Serio in 2001, the presence of leptin in healthy and diseased human gingival tissue was investigated for the first time. Their findings showed that leptin is present in healthy and marginal zones of inflamed gingival tissue and as the inflammation and gingival pocket depth proceed, the amount of leptin will be diminished. They considered gingiva as another source for leptin production along with the adipocytes (13). In a study by Karthikeyan and Pradeep it was demonstrated that with increased destruction of periodontal tissues leptin concentration will significantly decrease in the gingival crevicular fluid (GCF) (14). They also reported in another study that despite GCF, the concentration of leptin in the blood serum shows a direct correlation with chronic periodontitis (15). Furthermore, Shimada and colleagues found that non-surgical treatment of periodontal tissues will significantly reduce the concentrations of leptin, CRP and IL-6 in the GCF (16). Contradictory results achieved in different studies and as no other previous investigation in Iran has been performed on this topic; we aimed to evaluate the correlation between leptin and chronic moderate to severe periodontitis in patients who attended the periodontics department at the dental faculty of Shahid Beheshti Medical University.

**Methods:**

This analytical cross sectional study was performed on patients at the periodontics department of Shahid Beheshti dental faculty. Patients included 55% women and 45% men with a mean age of 42.25 years. Exclusion criteria were as follows:

a) malnutrition, presence of infectious diseases (except for periodontal disease), immune system deficiency, cancer, metabolic disease, mental retardation, menstruation, pregnancy and lactation

b) high drug use, antibiotic consumption and treatment with immunostimulants such as levamisole, H2 antagonists and interferons following the past two months

c) radiation therapy, intense psychological stress, hormonal discrepancies, drug addictions, treatment with immunosuppressive drugs such as corticosteroids, cytotoxic drugs and Cyclosporine A class of drugs in the past year

Specimens were divided into two groups. The experimental group included the gingival samples with moderate to severe periodontitis and samples in the control group did not show any clinical signs of gingival disease. Patients were comparable regarding their age groups, diet, and oral hygiene. Twenty patients were assigned in each group according to past studies. Nonprobability sampling was the technique carried out for patient selection. Blood samples
of 5ml were taken from each individual to determine the leptin concentration in the serum. The diseased and healthy gingival tissue samples were obtained from the same patient. Determination of periodontal disease was based on a pocket depth of 5mm or more measured by a periodontal probe and radiographic evidence of the disease. Healthy gingival tissue samples were obtained at a 3mm distance away from the gingival margin using the punch method. They had a dimension of 3mmx3mm and included the subepithelial connective tissue. Each individual gave 5ml of venous blood drawn from the arm. The blood samples were immediately centrifuged to segregate the serum which was frozen at -20ºC and was stored in sealed microtubes for later use in the experiment. Gingival samples obtained during periodontal surgeries were immediately transferred into sterile tubes containing 5 ml of FCS (10%) + RPMI-1640 (10gr/lit) + Amphotericin B (2.5µg/ml) + gentamicin sulfate (100µg/ml) and were kept in the fridge. Samples were cultured no later than a week after they had been prepared. They were initially rinsed several times in a sterile petri dish using FCS (10%) + RPMI-1640 (10gr/lit) + Amphotericin B (2.5µg/ml) + gentamicin sulfate (20µg/ml). Samples were then taken into another sterile petri dish and got cleaned off blood and tissue remnants and were segmented into 1mm² pieces using a dental blade. Each piece was placed in one well at 96-well plates (Nunc Denmark- provided by TubiNegin Company in Tehran) and 300 microliters of culture media containing FCS (10%) + RPMI-1640 (10gr/lit) + Amphotericin B (2.5µg/ml) + gentamicin sulfate (20µg/ml) was added. After 72 hours insulin syringes were used to extract the supernatant fluid on the cultured samples that were placed in sealed microtubes and kept frozen at -20ºC. After the total required samples were provided, the supernatant fluid samples were unfrozen and the concentrations of leptin and IL-6 were determined by ELISA. The concentration of IL-6 was measured to discover the degree of inflammatory processes in the tissues. The same procedure was performed on the blood serum which was also unfrozen and underwent ELISA testing.

Statistical analyses were administered by paired T-test and Wilcoxon signed ranks test.

**Results:**

Twenty patients including 11 women (55%) and 9 men (45%) participated in this study. Twenty clinically healthy gingival tissue samples, 20 gingival specimens with moderate to severe periodontitis and 20 samples of blood serum were taken for analysis. Leptin was not detected in either the healthy or diseased gingival tissue samples. The mean leptin concentration in the blood serum of the patients studied was 8903.27±4580.49 pg/ml.

The mean concentrations of IL-6 in the healthy and diseased gingival tissue samples were 81.08±36.72 and 90.35±29.71pg/ml respectively. Although a higher concentration was detected in the experimental group compared with the control group, the t tests did not find a significant statistical difference between these groups. The mean concentrations of IL-6 in the experimental and control groups based on the two sex groups are shown in graph 1.

![Graph 1](image)

**Graph 1- Comparison of IL-6 concentration in the experimental (samples with chronic periodontitis) and control groups**

It was noticed that IL-6 in all the samples taken from men showed the highest concentration of 100pg/ml but the range varied from 2-100pg/ml in women. This difference between the two sex groups was not statistically significant.
Statistical descriptions of the study variables are shown in Table 1.

**Table 1- Statistical descriptions of different study variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>24</td>
<td>55</td>
<td>42.25</td>
<td>8.27</td>
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<tr>
<td>Blood leptin concentration (pg/ml)</td>
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<td>13345</td>
<td>8903.27</td>
<td>4580.49</td>
</tr>
<tr>
<td>IL-6 concentration of healthy tissue (pg/ml)</td>
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<td>100</td>
<td>81.07</td>
<td>36.72</td>
</tr>
<tr>
<td>IL-6 concentration of diseased tissue (pg/ml)</td>
<td>2</td>
<td>100</td>
<td>90.35</td>
<td>29.71</td>
</tr>
<tr>
<td>BMI</td>
<td>19.3</td>
<td>27.3</td>
<td>22.83</td>
<td>3.22</td>
</tr>
<tr>
<td>Leptin concentration of healthy tissue (pg/ml)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leptin concentration of diseased tissue (pg/ml)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion:**

Leptin is a hormone-like protein that was initially known for its effects on body weight regulation and metabolism (7). Later studies discovered its impact on the immune system and its components such as the T cells, macrophages and endothelial cells. That is why it is sometimes referred to as a cytokine (7, 17). Some studies also refer to leptin as an acute phase protein in inflammatory processes (12, 18).

As leptin is suggested to play a role in some inflammatory diseases, this study aimed to investigate whether it would have a correlation with chronic periodontitis which is the most common inflammatory disease of the periodontal tissues. Leptin was not detected in the experimental (diseased gingival samples) or control (clinically healthy gingival tissue) specimens in our study. It should be noted that the mean leptin concentration in the blood serum of the patients was 8903.27±4580.49pg/ml, whereas IL-6 was found in noticeable concentrations in both of the study groups. As IL-6 is an inflammatory cytokine, active inflammatory processes (at the molecular level) must have been present in all the samples studied. Comparison of the concentrations of IL-6 in the experimental and control groups did not reveal significant statistical differences between them meaning that samples in both groups resembled equivalent inflammatory conditions. However, it should be noted that the concentration of IL-6 was greater in men than women which is a significant finding.

Considering the mean standard deviation of our results and to detect a difference of 40pg/ml when $\alpha = 0.05$ the power of the study was estimated around 87% based on the total number of the samples used.

In a study by Serio and Johnson in 2001, the correlation between leptin and periodontal disease was evaluated. They detected no significant differences regarding the concentrations of leptin in the healthy and disease-affected groups. They found the greatest leptin concentrations in healthy gingiva and concluded that as gingival inflammation proceeds the concentration of leptin will fall. They also found a reverse relationship between leptin and IL-6 and stated that leptin would be found in healthy gingival tissue and inflamed gingival margins and with progression of the inflammatory processes and increase in the pocket depth, its concentrations would be declined (4). We did not find a significant statistical difference in the leptin concentration between the experimental and control groups as its concentration was not detectable or measurable in any of the samples. Contradictory results between the findings of the present study compared with the previous study mentioned could be due to their immediate ELISA testing of the samples after they had been broken down by enzymes; whereas samples in our study had been cultured. So we measured the amount of the released leptin. Due to tissue decomposition in the previous study, leptin that had been stored in the cells and was released after enzymatic breakdown may have also been measured.
As leptin was not detected in any of our samples, it was not possible to evaluate any existing correlation between IL-6 and leptin but it was shown that despite leptin, IL-6 was found in noticeable concentrations in most of the samples. This is in agreement with the findings of the previous study to some extent. Moreover, the absence of leptin in all of our samples suggests that gingiva would not be a production source for leptin.

It should also be noted that the depth of the gingival sulcus and bleeding on probing were used to differentiate between healthy and diseased gingiva in the previous study. If the sulcular depth was equal to or less than 3mm (≤ 3) it was considered as healthy gingiva and if the gingival sulcus depth was equal or more than 3mm (≥ 3) and bleeding occurred on probing, it was recognized as diseased gingiva. Clearly, both groups may have received samples taken at a gingival sulcus depth equal to 3mm. The degree of inflammation in the diseased gingival samples was not also specified. It was not stated whether both the healthy and diseased gingival samples had been obtained from the same individual either.

We should point that due to ethical reasons the healthy gingival specimens in our study were obtained from the submarginal region. This could be another reason for the contrast in the results of the two studies. Despite the other investigation, BMI was also taken into account in our study.

In a study by Karthikeyan and Pradeep in 2007 it was reported that there would be a significant reduction in the leptin concentration in the gingival crevicular fluid (GCF) with increased destruction of periodontal tissues (14). The difference in their results compared with the present study could be due to different types of samples taken for analysis. They had performed their measurements on the GCF derived from gingival blood vessels whereas our study was conducted on gingival tissue samples. It should be noted that they did not also find significant leptin concentrations in their cases of gingival periodontitis.

Due to limited information provided by literature on this topic, further comparison of our findings with other investigations cannot be performed.

**Conclusion:**

The results of the current investigation reveal that leptin as an inflammatory protein does not play a role in periodontal disease. As somerefer to leptin as an acute phase protein in inflammatory processes and with regard to the chronic nature of gingival inflammatory diseases such as chronic periodontitis, its presence in such conditions may not be expectable.

The absence of leptin in both clinically healthy gingival samples and those with moderate to severe periodontitis suggests that gingiva cannot be a source for leptin production. Administration of further studies would be required to confirm such findings.

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**References:**


