An Evaluation of Serum and Salivary Adipokines (Leptin and Resistin) Levels in Periodontal Health and Disease

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ABSTRACT

Background: With the start of the current century, increased the interest in the role of the adipose tissue derived substances that named adipokines in the inflammatory diseases of the human being including the inflammatory periodontal disease, but scientific evidences were not clearly demonstrate the association between these adipokines and periodontal pathologies.

Materials and Methods: Forty two subjects male only with normal body mass index were selected for the study with an age ranged (30-39 years). Samples were divided into three groups of 14 subjects in each group based on clinical periodontal parameters; clinically healthy gingiva (group I), gingivitis group (group II) and chronic periodontitis patients group (group III), from whom saliva and serum samples were collected for estimating the levels of leptin and resistin using Enzyme-Linked Immuno Sorbent Assay (EUSA).

Results: The results showed that the serum level of leptin and resistin were significantly higher in chronic periodontitis patient (9.81 ng/ml, 6.55 ng/ml) respectively as compared to gingivitis and healthy control groups (leptin; 8.10 ng/ml, ng/ml, resistin; 5.85 ng/ml, 5.45 ng/ml) respectively. On the other hand the level of leptin in saliva of patients with chronic periodontitis (0.17 ng/ml) was significantly lower than that of its saliva levels in gingivitis and healthy control groups (0.21 ng/ml, 0.29 ng/ml) respectively. Whereas, salivary resistin levels was significantly higher in chronic periodontitis patient(14.45 ng/ml) when compared to the gingivitis group (11.59 ng/ml) and the health control group (6.43 ng/ml).

Conclusions: Concomitant raise in serum leptin, serum resistin and salivary resistin, while a sensible reduction in salivary leptin with conversion from periodontal health state to periodontal disease state. These finding may draw a suggestion on the role of leptin and resistin in the relation between periodontal disease and the systemic health since the increase in their level were associated with a various systemic pathologies.


INTRODUCTION

Periodontal disease is a chronic microbial and inflammatory process characterized by the presence of sulcular pathogenic bacteria, impaired host immune response and destruction of the connective tissue attachment (1). Biochemical signaling involving three biological phases (inflammation, connective tissue degradation and alveolar bone turnover) contributes to the clinical morbidity observed in the affected tissues, in these biological phases, circulating molecules have been detected at elevated levels in the whole saliva and gingival crevicular fluid (GCF) of patients with periodontal disease making them putative biomarkers of the disease (2-4).

It has been proposed that appropriate cytokine production results in protective immunity, while inappropriate cytokine production leads to tissue destruction and disease progression (5). Adipose tissue participates in the regulation of energy homeostasis and is an active endocrine organ that secretes more than 50 biologically active substances, collectively termed adipokines. Adipokines such as the hormone-like proteins: leptin, resistin and adiponectin (6).

Leptin is a non-glycosylated peptide hormone that has been classified as a cytokine as it shows structural similarities to the long chain helical cytokine family interleukin (IL)-6 (7). Ahima and Flier suggested that leptin orchestrates the host response to infectious and inflammatory stimuli as it stimulates the immune system by enhancing pro-inflammatory cytokine production and phagocytosis by macrophages (8).

It was shown that leptin synthesis is increased by a number of inflammatory stimuli, including interleukin (IL)-1, IL-6, tumor necrosis factor alfa (TNF-α), and lipopolysaccharid (LPS) (9). As the gingival disease progressed, there is significant decrease in the gingival leptin concentration, GCF leptin concentration and significant increase in the plasma leptin concentration (10,11).

Resistin is a polypeptide hormone that is expressed abundantly in adipose tissues of mice; however, in humans, resistin is expressed at very low levels in adipocytes (12). Human resistin acts as a pro-inflammatory molecule and stimulates the synthesis and secretion of TNF-α and interleukin (IL-12) (13).

In addition to TNF- α and IL-6, resistin may participate in inflammatory processes caused by bacterial infections (14). Periodontitis was significantly associated with increased levels of resistin, after adjustment for gender, smoking, fasting glucose, and body mass index (BMI) (15,16).

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Potential role for resistin in bone metabolism was suggested by increased resistin levels that coincided with osteoclast differentiation (17). This study aimed to evaluate the salivary and serum levels of leptin and resistin in periodontal health and disease (individuals with clinically healthy gingiva, gingivitis and chronic periodontitis).

MATERIALS AND METHODS
A total of 42 males with normal body mass index according to WHO criteria were participated in the study. They were divided into three groups, based on clinical examination according to Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL).

Group I (control group): Fourteen individual with clinically healthy gingiva, and this was defined by GI scores (<0.7) and with no periodontal pocketing or attachment loss. For standardization of biochemical parameters.

Group II Gingivitis Patient: Fourteen individual with gingivitis, and this was defined by GI scores (>0.7) and with no periodontal pocketing or attachment loss.

Group III Chronic Periodontitis: Fourteen patients with chronic periodontitis, and this was defined by the presence of at least four sites with PPD (≥4mm ) with CAL (≥1mm).

All participants were instructed not to eat or drink (except water) at least 1 hour prior to collection of saliva. The subject should sit in a relaxed position. Samples were collected between 9-12 am. After the subject rinse his mouth several times by bottled water and then wait for 1-2 minutes for water clearance, 5ml of whole unstimulated mixed saliva was collected into polyethylene tubes. Saliva should not be spat into the tube and samples containing blood were discarded, also the patient shouldn’t swallow during the. Saliva then centrifuged at 4000 round per minute (rpm) for 15 minutes; this was done within one hour after collection to eliminate debris and cellular matter, the clear supernatant was separated by micropipette and transferred to eppendorf to be stored at (-20 ºC) till being analyzed.

Four ml of blood was collected from the antecubital fossa by venipuncture using 5 ml disposable syringes and immediately transferred to lab.

Samples were collected between 9-12 am. Blood sample was allowed to clot at room temperature and after one hour, serum was extracted from blood by centrifuging at 3000 rpm for 5 min. The extracted serum was immediately transferred to eppendorf and stored at (-20 ºC) till being analyzed.

The Demeditec leptin and resistin Enzyme-Linked Immuno Sorbent Assay (ELISA) kits were used for quantitative measurement of their serum and salivary levels.

Oral examination was done using periodontal probe on all teeth except 3rd molar on four surfaces. The collected data include: plaque index system, Gingival index, Bleeding on probing, probing pocket depth and Clinical attachment level.

RESULTS
The standard deviation (SD), standard error (SE), minimal (Min.) and maximal (Max.) values were obtained for each group. ANOVA table test was performed for Analysis of variance. The study had revealed that there is a highly significant difference in serum leptin levels at P< 0.01 among the studied groups as shown in table (1) and with mean value of (5.61) in group I, (8.10) in group II and (9.81) in group III.

Table (1): Serum Leptin Levels (ng/ml) among Three Studied Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5.61</td>
<td>1.19</td>
<td>0.32</td>
<td>4.07</td>
<td>7.71</td>
</tr>
<tr>
<td>Group 2</td>
<td>8.10</td>
<td>0.97</td>
<td>0.26</td>
<td>6.54</td>
<td>9.81</td>
</tr>
<tr>
<td>Group 3</td>
<td>9.81</td>
<td>1.15</td>
<td>0.31</td>
<td>8.34</td>
<td>11.64</td>
</tr>
</tbody>
</table>

** Highly significant difference at P<0.01.

Table (2) showed that the mean salivary leptin in group I had the largest value (0.29 ng/ml) followed by group II (0.21 ng/ml ) and the Group III had the smallest value (0.17 ng/ml) and also it revealed that there is highly significant difference between mean salivary levels at P< 0.01.

Table (2): Salivary Leptin Levels (ng/ml) among Three Studied Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.29</td>
<td>0.04</td>
<td>0.01</td>
<td>0.22</td>
<td>0.36</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.21</td>
<td>0.04</td>
<td>0.01</td>
<td>0.13</td>
<td>0.27</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.17</td>
<td>0.04</td>
<td>0.01</td>
<td>0.10</td>
<td>0.23</td>
</tr>
</tbody>
</table>

** Highly significant difference at P<0.01.

There is a significant difference in mean serum resistin levels at P<0.05 with highest mean value in chronic periodontitis patient (group III) (6.5 ng/ml) and lowest mean value in healthy control group (group I) (5.45ng/ml) that was seen in table (3).
Periodontitis individuals presented the lowest serum leptin levels than periodontitis patients and stating that periodontitis affected the circulating levels of leptin in favor of pro-inflammation. A recent study have stated that serum level of leptin was significantly higher in chronic periodontitis patients when compared to healthy controls suggesting that chronic periodontitis upregulated the circulating level of leptin in subjects with normal BMI. There was an association of periodontal conditions with serum leptin levels, since its levels was influenced by oral bacteria.

Regarding salivary level of resistin the present study revealed that the level of salivary resistin in chronic periodontitis group (14.45) was significantly higher at P<0.01 than the other two groups with lowest level in healthy group (6.43), LPS stimulation in human. Controversy was also seen in regard to association between circulating leptin and periodontitis as the result of this was disagreed with Davies et al who stated that the level of serum leptin was not significantly different between the aggressive periodontitis patients. All that was shown in table (4).

### Healthy subjects

A recent study has pointed out that serum levels of leptin and soluble leptin receptor do not vary between patients and with different periodontal conditions. The possible explanation for this is due to the fact that Porphyromonas gingivalis (P. gingivalis) which is a G -ve bacterium found in periodontal pockets of patients with periodontitis and strongly implicated in the pathogenesis of periodontal disease.

### DISCUSSION

Lipopolysaccharide (LPS) is virulent factor of the bacterium leading to the development of inflammatory responses that characterize periodontitis. Leptin potently enhanced P. gingivalis LPS induced IL-18 release suggesting a possible interaction between leptin and periodontal bacteria in modulating host.

Interestingly, this study showed that the immune responses. Leptin was shown to circulating level of leptin in serum was correlated positively with transition from periodontal health to disease. Many researchers have studied the relation of leptin to various forms of periodontal disease using different types of samples their results seemed to be in enhancing toll like receptor (TLR) expression and the secretion of cytokines in monocytes and may thereby potentiate immune responses to periodontal pathogens.

There are significant differences between leptin action in the circulation and locally within according to a certain degree with the result of the gastrointestinal lumen. Studies suggested the present study. This is in agreement with a study done by Gangadhar and his colleagues who declared that the serum leptin concentrations were found to increase as the gingival disease progressed. It also in agreement with Karthikeyan and Pradeep who suggest that greater the periodontal destruction, greater is the serum leptin concentration and the lowest serum leptin concentration was found in healthy individuals. A multifunctional role of leptin released locally within peripheral tissues and that secreted into saliva by the acinar cells of salivary glands, have brought to the forefront its importance in the processes of mucosal defense and repair along alimentary tract, including that of oral cavity and gingiva as salivary leptin potently stimulate the expression of two cytokines relevant to keratinocyte proliferation, salivary leptin increases proliferation of oral keratinocytes.

The present study come in line with Shimada et. al., who found that there was a significant sublingual salivary gland acinar cells against differences between healthy and chronic ethanol cytotoxicity, that exogenous leptin periodontitis patients in serum leptin levels. On other hand, Zimmermann et. al., showed that non-known to accelerate wound repair, it contributes to the inhibition of bacterial growth by mucins, since it prevents the reduction of salivary mucin synthesis evoked by oral bacterial LPS, that gingival leptin was known for its protective role in periodontal disease.

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### Table (3): Serum Resistin Levels (ng/ml) among Three Studied Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>5.45</td>
<td>0.60</td>
<td>0.16</td>
<td>4.70</td>
<td>6.30</td>
</tr>
<tr>
<td>Group2</td>
<td>5.85</td>
<td>0.97</td>
<td>0.26</td>
<td>4.37</td>
<td>7.50</td>
</tr>
<tr>
<td>Group3</td>
<td>6.55</td>
<td>1.45</td>
<td>0.39</td>
<td>4.71</td>
<td>8.85</td>
</tr>
</tbody>
</table>

*Significant difference at P<0.05.

** Highly significant difference at P<0.01.

### Table (4): Salivary Resistin Levels (ng/ml) among Three Studied Groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>6.43</td>
<td>0.81</td>
<td>0.22</td>
<td>4.63</td>
<td>7.89</td>
</tr>
<tr>
<td>Group2</td>
<td>11.59</td>
<td>1.60</td>
<td>0.43</td>
<td>8.65</td>
<td>13.77</td>
</tr>
<tr>
<td>Group3</td>
<td>14.45</td>
<td>1.88</td>
<td>0.50</td>
<td>11.60</td>
<td>17.84</td>
</tr>
</tbody>
</table>

** Highly significant difference at P<0.01.
Moreover, the result of the present study showed that there is a highly significant difference in mean salivary leptin levels among the studied groups at P < 0.01, with group I had the highest value and the Group III had the lowest one. This in contrast to what we have found in relation to circulating leptin level, but these finding be consistent with a study by Ducroc et al., as they showed that there are significant differences between leptin action in the circulation and locally within the gastrointestinal lumen (29).

Little information was known about the association between salivary leptin level and periodontal disease. Though a study showed that there were no differences in the distribution of leptin single nucleotide polymorphism (-2548G/A SNP) genotypes (leptin genetics) in saliva of 50 subjects between control and periodontitis subjects (40).

In regards to circulating resistin the result of the present study illustrated that there was a significant difference in mean serum resistin levels with highest mean value in chronic periodontitis patient and the lowest mean value in healthy control. These consequences were in accordance with a Japanese study which declared that increased serum resistin levels were significantly associated with periodontal condition in elderly people (15). Also it matched with Zimmermann and colleagues who found that serum resistin level was higher in periodontitis than in non-periodontitis (19). Coming in line with another Japanese study which stated that having periodontitis was significantly associated with an increased level of serum resistin (16). Recently, Duarte et al., have found that serum levels of resistin were significantly higher in chronic periodontitis subjects when compared to healthy controls. Suggesting that chronic periodontitis upregulated the circulating levels of resistin in subjects with normal BMI (26).

Even though there were number of researchers have disagreed in part or as whole with our findings. There was not much difference in the serum resistin levels between the chronic periodontitis and the healthy controls. Also the decrease in the resistin levels following nonsurgical periodontal therapy did not show any statistical significance (41). Serum resistin levels have not presented any significant differences between the periodontally healthy and ligature induced periodontitis in rats (42). Resistin concentrations in serum was not associated with periodontitis (43).

This rise in serum resistin in periodontal disease can be explained logically in the context of the following studies which showed that circulating resistin concentration was increased in response to LPS and/or leukotoxin that were produce by common periodontopathic bacteria; inflammatory cells such as monocytes and macrophages present in the periodontal tissue appear to be the major source of resistin (15). Resistin release from neutrophils was induced by both P. gingivalis and Escherichia coli (E. coli) LPS (44). Increased resistin in several inflammatory cells stimulated by periodontal pathogenic components such as LPS (45). There was an association of Periodontal conditions with serum resistin levels, since its levels was dramatically increased by LPS stimulation in humans (21,46-49).

Furugen et al showed that A. a expressed leukotoxin that induces extracellular release of human neutrophil derived resistin and suggested that increased prevalence and levels of A. a in periodontal patients contribute to their higher circulating levels of resistin (50).

Moreover, resistin was shown to mediate its proinflammatory effects via TLR-4 (51). The expression level of TLR-4 was higher in all periodontal patients than in healthy individuals (52). There was no data provided in the surveyed periodontal literature regarding local level of resistin in saliva of patients with periodontal disease. Even though, a number of studies have investigated the local resistin in GCF but not in the gingival tissue. The present study revealed that the level of salivary resistin in chronic periodontitis group was significantly higher than the other two groups with lowest level in healthy control group and that of gingivitis group was intermediate between them.

The level of salivary resistin were upregulated locally in the salivary glands in case of inflammation in patient with primary Sjögren's syndrome; and the levels of resistin correspond to the intensity of lymphocytic inflammation (53).

Based on this fact we may explain and illustrate that the salivary resistin were upregulated locally in the salivary glands in relation to inflammation caused be the inflammatory periodontal diseases.

REFERENCES
