Impact of Weight Reduction on Adiponectin and TNF-α Levels in the Gingival Crevicular Fluids of Obese Patients with and without Periodontal Disease

Lalith Vivekannada, Mohamed Faizuddin

ABSTRACT

Aim: The aim of the current study was to assess the impact of weight loss on the gingival crevicular fluid (GCF) levels of adiponectin and TNF-α in obese patients with periodontal disease.

Materials and methods: Sixty obese subjects were recruited into the study and were divided into three groups of 20 each. Group 1—periodontally healthy obese patients, group 2—obese patients with gingivitis, group 3—obese patients with periodontitis. The GCF levels of adiponectin and tumor necrosis factor α (TNF-α) were evaluated using ELISA, and the same was correlated with the probing pocket depth (PPD) and clinical attachment loss (CAL) at baseline and after attainment of ≥10% weight reduction.

Results: Reductions in body weight resulted in elevated adiponectin and reduced TNF-α levels in the GCF. Adiponectin levels showed significant positive correlations and TNF-α negative correlation with improved periodontal parameters.

Conclusion: Weight reduction has an impact on obesity-related inflammatory changes on periodontal disease.

Clinical significance: Obesity can be a potential risk factor for periodontitis, weight reduction measures can considerably reduce the inflammatory burden on the periodontium.

Keywords: Adiponectin, Gingivitis, Obesity, Periodontal disease, TNF-α.

INTRODUCTION

Periodontitis is a bacterially induced chronic inflammatory disease that destroys the connective tissue and bones that support the teeth.1 Periodontitis is considered a multifactorial disease with obesity as an emerging risk factor.2

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems2 and with a body mass index (BMI) ≥30.0 kg/m². Today obesity is considered a disease and implicated as a risk factor of various other disease conditions such as hypertension, type 2 diabetes, dyslipidemia and coronary heart disease.3

Human adipose tissue is not only a storage organ for excess energy but also an active endocrine organ is known to produce and release a number of bioactive proteins known as adipokines namely leptin, TNF-α, interleukin-6 (IL-6), IL-8, monocyte chemoattractant protein (MCP)-1 and adiponectin.4

Adiponectin is a recently discovered adipocytokine, also referred to as gelatin-binding protein-28. It is a 244 amino acid protein, which is specifically and highly expressed in human adipose cells.5 Adiponectin is postulated to be associated with the modulation of inflammatory responses. It attenuates the inflammatory response mediated byTNF-α and inhibits macrophage phagocytic activity and TNF-α production.6

Adiponectin levels have been reported to be inversely correlated with body fat percentage. Adult obese individuals have shown lower plasma levels of adiponectin than the normal healthy individuals.7 This observation suggests that low levels of adiponectin may render obese individuals susceptible to develop long-term complications like insulin resistance, endothelial dysfunction, and atherosclerosis.5
Many studies have reported an association between obesity and periodontal disease in both animal models and humans.\textsuperscript{8-10} While the exact mechanisms of this causal associations are not fully understood, obesity-mediated inflammatory changes and oxidative stress may be important in these contexts.\textsuperscript{11} Gonçalves et al. in a recent study note that obesity may modulate systemic and periodontal levels of adipokines in favor of pro-inflammation.\textsuperscript{12} Furthermore, the beneficial effects of adipokines on periodontal health have been reported by a number of studies.\textsuperscript{13,14} Thus, corrections of these inflammatory changes may have putatively positive effects on blunting the effects of obesity on periodontal health.

A study conducted on elderly Japanese population indicated that patients suffering from periodontitis showed decrease serum levels of adiponectin compared to healthy individuals.\textsuperscript{15} In another interventional study periodontal condition improved with an increase in serum adiponectin levels after nonsurgical periodontal therapy.\textsuperscript{16}

The above-mentioned studies indicate that adiponectin is associated with periodontal disease among obese individuals. However, its exact role in the etiopathogenesis is not clearly understood. In the studies conducted so far, adiponectin levels have been estimated in serum rather than GCF which provides a unique window and reflects the biological condition of the periodontal tissues.\textsuperscript{17} To our knowledge, no attempt has been made to estimate the relationship between adiponectin and TNF α levels in the GCF of obese individuals suffering from periodontal disease before and after weight loss.

Hence the present investigation was proposed to evaluate the impact of weight reduction on adiponectin and tumor necrosis factor α levels in the gingival crevicular fluid (GCF) and thereby on the periodontal status of obese patients with and without periodontal disease.

**MATERIALS AND METHODS**

This study was conducted to assess the association between obesity and periodontal disease in the Indian population. The study was conducted at the MR Ambedkar Dental College and Hospital, Bengaluru, Karnataka, India. The subjects were referred from Bangaluru-based health clubs promoting weight loss through lifestyle modifications. All the study participants signed an informed consent form, and the study was approved by the institutional ethics committee. All the parameters were evaluated by the principal investigator. A total of 124 subjects obese subjects aged between 25 to 65 years were evaluated out of which 60 subjects full filled the inclusion and exclusion criteria (Flow chart 1). The 60 subjects participating in the study were age and gender-matched and were divided into three groups of

**Flow chart 1: Depicting the selection and recruitment of patients**

Assessed for eligibility (n = 124)  
- Found eligible (n=103)  
  - Group–1 (n = 41) periodontally healthy subjects  
  - Group–2 (n = 32) subjects with gingivitis  
  - Group–3 (n = 30) subjects with periodontitis  

Subjects who agreed to participate in the study (n = 89)  
- Group–1 (n = 35)  
- Group–2 (n = 28)  
- Group–3 (n = 26)  

Subjects who co-operated for the GCF collection(N=81)  
- Group–1 (n = 30)  
- Group–2 (n = 25)  
- Group–3 (n = 26)  

Adiponectin and TNF α detected in the GCF samples (N=60)  
- Group–1 (n = 20)  
- Group–2 (n = 20)  
- Group–3 (n = 20)
20 each. Group 1—periodontally healthy obese patients, group 2—obese patients with gingivitis, group 3—obese patients with periodontitis. Subjects taking medications for weight reduction were excluded from the study. Subjects included in the study were advised on oral hygiene techniques to manage their condition.

Furthermore, subjects requiring immediate interventions for gingivitis and periodontitis were excluded from the study. Subjects with a body mass index (BMI) ≥ 30 kg/m² were considered obese in the study. In the study, subjects with ≤ 5% sites with bleeding on probing, probing pocket levels of ≤ 3 mm were considered as periodontally healthy (group 1). Subjects with bleeding on probing ≥ 50% of sites, ≥ 20 teeth excluding third molars with minimal or no attachment loss and probing depth (PD) of ≤ 3 mm were included into the gingivitis group (group 2). Subjects in the periodontitis group had bleeding on probing ≥ 80% of sites, ≥ 20 teeth excluding the third molars with at least 8 teeth showing ≥ 3 mm of clinical attachment loss and ≥ 5 mm of probing pocket depth (group 3).

In all the obese individuals, weight was measured to the nearest 0.1 kg, height was measured to the nearest 0.5 cm, and BMI (kilograms per meter squared) was computed. Waist:hip ratio (W/H) was defined as the minimal abdominal circumference between the xiphoid process and the iliac crests (waist) with subjects standing and breathing normally, divided by the hip circumference, measured on the greater trochanters. The patients were also subjected to routine blood investigations and lipid profile. The duration of the study lasted for a period of 3 months to 18 months depending on the reduction in weight of ≥ 10% for the individual.

Assessments of BMI, W/H ratio, assessments of bleeding index (Ainamo and Bay), plaque index (Silness and Loe), probing depth (using UNC 15 probe) and clinical attachment loss (with CEJ as the reference using UNC 15 probe) were evaluated at baseline (before weight reduction) and after attainment of ≥ 10% reduction in body weight. Furthermore, GCF levels of adiponectin and TNF-α were also assessed using commercially available human enzyme-linked immunosorbent assay (ELISA) kits. (RayBiotech, Inc. 3607 Parkway Lane, Suite 200, Norcross GA 30092) techniques at baseline and after attainment of the primary endpoint by the individual subject. (reduction in ≥ 10% body weight).

GCF was collected by asking the subjects to rinse their mouth vigorously with water to cleanse the teeth of loosely adherent debris. Samples of GCF were obtained from the sites by placing color-coded, calibrated, volumetric, microcapillary pipettes with 0.5 μL range. In groups 1 and 2 the site was selected randomly and in the group 3 the site with the deepest pocket was selected and was isolated with cotton rolls. Volumetric micropipettes were placed extra crevicular at the entrance of the gingival crevice, and the GCF samples were collected from each patient. The pipettes contaminated with blood or saliva was discarded. The pipette with collected fluid was wrapped in a sterile aluminum foil to prevent oxidation, placed in a plastic vial and immediately stored at −70°C until analyzed for adiponectin and TNF-α with commercially available ELISA kits.

### Statistical Analysis

Results are presented as mean ± SD and proportions are presented as percentages. Analysis of variance (ANOVA) was used to compare means across the three groups of the study. T-tests were used to compare the means between two groups. Pearson’s correlation was used to assess the relationship between the concentrations of adiponectin and TNF-α in the GCF and the periodontal parameters. Statistical significance is considered when \( p < 0.05 \). All the tests were done using SSP version 19.0. All the study participants signed an informed consent form and the study was approved by the institutional ethics committee.

### RESULTS

The study included a total of 60 subjects. All the study participants achieved the primary endpoint of ≥ 10% reduction in body weight in a period of 3 to 18 months post-enrollment. Table 1 presents the demographic profiles and the baseline characteristics, and the values are represented as mean of 20 participants, of the age, body weight, BMI, W/H ratio of the study subjects. Subjects in all the three groups were adherent to weight reduction programs and attained significant reductions in weight in 3 to 18 months from the baseline. Table 2 presents a significant change in the weight in all the three groups (\( p < 0.05 \)), the values are represented as mean of 20 participants.

The distribution of BMI and W/H ratio before and after weight reduction is as shown in Tables 3 and 4, and

<table>
<thead>
<tr>
<th>Table 1: Demographic profiles and the baseline characteristics of Age, Body weight, BMI, W/H ratio of the study subjects. (mean of 20 participants in each group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
</tr>
<tr>
<td>(n = 20)</td>
</tr>
<tr>
<td>Age in years</td>
</tr>
<tr>
<td>Body weight</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
</tr>
</tbody>
</table>
the values are represented as mean of 20 participants. The comparison of the mean BMI and W/H ratio before and after weight reduction showed statistically significant changes in the BMI between the groups (group 1 : \( p = 0.0001 \), group 2 : \( p = 0.0001 \) and group 3 : \( p = 0.001 \)). However, the changes in the W/H ratio was not significant in all the three groups (group 1 : \( p = 0.601 \), group 2 : \( p = 0.716 \), group 3 : \( p = 0.767 \)). As shown in Table 5, significant improvements from baseline were noted with respect to bleeding index, plaque index, probing depth and clinical attachment loss in all three groups. Furthermore, significant elevations in GCF levels of adiponectin were noted along with significant reductions in the levels of TNF-α (Table 6).

GCF adiponectin levels showed significant negative correlations (\( p < 0.005 \)) with probing pocket depth as shown in Table 7, and subsequently as demonstrated in Graphs 1 to 6 in all the groups. In patients with periodontitis, GCF adiponectin levels showed significant negative correlations (\( p = 0.0001 \)) with clinical attachment levels as shown in Table 8 and Graphs 7 and 8.

**Table 2: Reductions in body weight from baseline (mean of 20 participants in each group)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight at baseline (kg)</th>
<th>Body weight at endpoint (kg)</th>
<th>Percentage weight loss (%)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>79.25 ± 5.00</td>
<td>65.42 ± 4.35</td>
<td>17.45%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 2</td>
<td>83.33 ± 7.94</td>
<td>69.89 ± 6.96</td>
<td>16.12%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 3</td>
<td>91.25 ± 13.05</td>
<td>76.82 ± 11.32</td>
<td>15.81%</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 3: Reductions in BMI from baseline (mean of 20 participants in each group)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BMI at baseline</th>
<th>BMI at endpoint</th>
<th>Percentage changes (%</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>32.05 ± 2.16</td>
<td>65.42</td>
<td>18.28%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 2</td>
<td>32.42 ± 2.54</td>
<td>69.89</td>
<td>16.40%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 3</td>
<td>36.23 ± 5.13</td>
<td>76.82</td>
<td>15.89%</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 5: Changes in periodontal parameters after ≥10% weight reduction (mean of 20 participants in each group)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Difference from Baseline Mean ± SD</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td>Group 1 : 0.19 ± 0.06</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Group 2 : 1.58 ± 0.22</td>
<td>1.29 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Group 3 : 1.51 ± 0.30</td>
<td>1.11 ± 0.23</td>
</tr>
<tr>
<td>Probing depth</td>
<td>Group 1 : 0.99 ± 0.35</td>
<td>0.69 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Group 2 : 1.53 ± 0.78</td>
<td>0.78 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Group 3 : 1.60 ± 0.98</td>
<td>0.90 ± 0.45</td>
</tr>
<tr>
<td>Bleeding index</td>
<td>Group 1 : 1.20 ± 0.53</td>
<td>1.57 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Group 2 : 2.59 ± 0.25</td>
<td>2.27 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Group 3 : 3.65 ± 0.35</td>
<td>2.97 ± 0.24</td>
</tr>
<tr>
<td>Clinical attachment level</td>
<td>Group 3 : 1.66 ± 0.65</td>
<td>0.85 ± 0.47</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Obesity is a very prevalent chronic condition and has been suggested to increase the susceptibility for periodontitis. Interventions to reduce weight like dieting, exercise might decrease the inflammatory burden caused by obesity. This will definitely have a positive impact on the periodontal status as well. In our study, we have evaluated the impact of weight reduction on adiponectin and tumor necrosis factor α levels in the GCF of obese patients with and without periodontal disease.

An important aspect of this study was that there was no attrition. All the 60 subjects enrolled for the study completed it, this can be attributed to the high motivational levels of the subjects to adhere to the weight reduction.

Erik et al. has suggested that a minimum of 10% weight reduction is needed for general improvement of circulating adiponectin and markers of inflammation in obese patients. Accordingly in our study the percentage weight loss in all the three groups was more than 10% in a duration of 3 to 18 months and was statistically significant, group 1–17.45% with \( p \) value 0.0001, group 2–16.12% \( p \) value 0.0001 and group 3–15.81% with a \( p \) value of 0.001.

**Table 4: Reductions in W/H ratio from baseline (mean of 20 participants in each group)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>W/H at baseline</th>
<th>W/H at endpoint</th>
<th>Percentage changes (%)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.93 ± 0.06</td>
<td>0.92 ± 0.06</td>
<td>1.0%</td>
<td>0.601</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.93 ± 0.10</td>
<td>0.93 ± 0.07</td>
<td>0%</td>
<td>0.767</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.96 ± 0.12</td>
<td>0.95±0.09</td>
<td>1.04%</td>
<td>0.767</td>
</tr>
</tbody>
</table>

**Table 6: Changes in GCF adiponectin(ng) and TNF-α (pg) levels after >10% weight reduction (mean of 20 participants in each group)**

**Table 7: Pearson’s correlation of GCF adiponectin levels and probing pocket depth (PPD)–before and after weight reduction**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adiponectin GCF</th>
<th>TNF-α GCF</th>
<th>( R ) value</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>838.04±46.39</td>
<td>938.41±28.60</td>
<td>-0.158</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 2</td>
<td>915.22±26.83</td>
<td>969.66±36.06</td>
<td>-0.193</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group 3</td>
<td>815.15±38.31</td>
<td>951.69±44.28</td>
<td>-0.193</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adiponectin &amp; PPD</th>
<th>( R ) value</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>-0.158</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>-0.040</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>-0.005</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>
In our study, the comparison between the mean BMI before and after weight reduction showed a statistically significant change ($p < 0.05$) among all the three groups. The percentage difference in the W/H before and after weight reduction in all the three groups was not statistically significant, group 1–1.0% with $p$ value 0.601, group 2–0% $p$-value 0.716 and group 3–1.04% with a $p$ value of 0.767. Saito et al. used WHR as an indicator of...
abdominal obesity instead of WC. They reported that participants with lower WHR values had less risk of periodontitis. In another study by Saito et al., the highest quintile of mean PPD was significantly associated with WHR in a multivariate analysis, whereas CAL did not reach statistical significance when correlated to WHR. In our study, the average CAL and PPD were significantly higher in participants with higher WHR. Our results were in concordance with that documented by Wood et al.

The PI, BI, PD, and CAL show a statistically significant change before and after weight reduction. The changes in the periodontal parameters without any intervention and just maintaining good oral hygiene and reemphasizing on the oral hygiene measures indicate that the mere reduction in the weight might bring about a reduction in the inflammatory burden on the periodontium. The changes seen in this study shows that the motivational level of the participants to reduce weight was high and so the reduction in the inflammatory burden on the periodontium.

An increase in body mass index might be a potential risk factor for periodontitis. In our study the Group 3 subjects belonging to the periodontitis group showed a percentage difference of 48.79% improvement in the CAL, suggesting that the BMI may be a potential risk factor for periodontitis. This is in accordance with the study conducted by Ekuni et al. in the Japanese population. There are several studies which support the results obtained by us in this study. Saito et al. concluded that obesity is associated with deep periodontal pockets. Wood et al. analyzed NHANES III data and demonstrated that BMI was positively correlated with the severity of periodontal attachment loss. Morita et al. in their longitudinal study have demonstrated a dose-response relationship between BMI and the development of periodontal disease in a population of Japanese individuals. There was a significant association between BMI and the development of periodontal pockets of ≥4 mm.

Studies have indicated that maintaining a normal weight by regular physical activity is associated with lower periodontitis prevalence. The decrease in adiponectin levels with obesity most likely arises from increasing adipose tissue macrophage infiltration resulting in inflammation. The pro-inflammatory cytokine TNF-α and IL-6 decrease adiponectin expression. Conversely, adiponectin has an anti-inflammatory effect inhibiting activation of NFκβ by TNF-α. Interventions to decrease inflammation associated with obesity may elevate adiponectin concentrations.

In obesity, plasma adiponectin levels are lower despite that adipose tissue is the only tissue of its synthesis, suggesting negative feedback on its production. Consequently, body weight reduction would result in at least transient disinhibition, therefore an elevation of plasma adiponectin. A recent demonstration by microarray that the expression of adipogenic genes was suppressed in the development of obesity and DM in mice argues for the existence of a feedback inhibitory pathway. Also, the expression of ADIPOQ, the mouse homolog of adiponectin, and adiponectin were down-regulated, respectively, in ob/ob obese mice and obese human subjects. This is also consistent with the existence of a negative feedback pathway.

This negative feedback mechanism might explain the increase in the adiponectin levels in all the three groups
after weight reduction in our study. This increase in adiponectin levels had an inverse relation to the PPD in all the three groups and CAL in the group.

Madsen et al. in their study noted that long-term weight loss must exceed 10% for achieving significant improvements in adiponectin. Our study noted significant increases in adiponectin levels and reductions in TNF-α after achieving ≥10% reduction in weight. Results of our study and that of Madsen et al indicate that compliance and commitments to long-term weight reduction programs and maintaining body weight within normal limits may be important to avoid low adiponectin-related insults to periodontium.

Goncalves et al. in their study note that obesity-related modulation of adipokines may be independent of periodontal therapy such as scaling and root planning (SRP). Furthermore, in their study, SRP did not affect the circulating levels of adipokines in patients with or without obesity. Thus, correction of obesity-induced modulations of adipokines and pro-inflammatory states may offer more efficacy and consistency to periodontal health as compared to treatments for inflamed periodontium itself. This can be related to our study results were in subjects requiring immediate interventions for gingivitis and periodontitis were excluded, and the subjects were advised on oral hygiene techniques to manage their condition. Changes in the periodontal parameters obtained in our study without any periodontal intervention may be attributed to the changes in the adiponectin levels (anti-inflammatory) and reduction in the TNF levels α (pro-inflammatory) also.

Among the many inflammatory and immune mediators that are established in a GCF, the cytokine TNF α plays a significant role in the pathogenesis and development of periodontitis, and an increased level of TNF α has been found in the GCF of patients with periodontitis. Obesity contributes to a proinflammatory environment by producing pro-inflammatory cytokines and the levels of TNF α and IL-8 in GCF is directly linked to the extent of obesity in relation to BMI. The cytokine TNF α negatively affects the host immunity in periodontal tissue, causing obesity to function as a risk factor for periodontal disease.

According to Lundin et al., the adipose tissue secrete proinflammatory cytokines which may be the molecules linking the pathogenesis of these diseases. The association between BMI and tumor necrosis factor-α (TNF-α) in GCF suggests that TNF-α in this fluid is derived from adipose tissue in obese subjects. Therefore the dysregulation of cytokine secretion by adipose tissues or macrophages may be critical in disease pathogenesis of chronic periodontitis.

Hence decrease in the levels TNF-α and increase in the level of adiponectin following weight reduction may effectively reduce the risk for chronic periodontitis among obese individuals. This is in concurrence with the study by Michael et al. who stated that there is a consensus that weight loss is associated with decreased TNF and C reactive protein (CRP) and increased adiponectin.

Thus results of the current study extend the current evidence base for addressing obesity and the utility of weight-reduction in promoting periodontal health. India has a high burden of obesity and the results of the current study indicate that simple lifestyle interventions based on proper diet, exercise and oral hygiene could have a marked impact in preventing and reversing periodontal diseases.

LIMITATIONS

Despite our best efforts, there are certain shortcomings in the conduct of the study. The sample size is relatively small. The difference of opinion exists among the researchers with the use of BMI among the Asian population, and anyways we have still considered BMI as the measure in our study along with W/H ratio as there are no other standard measures. Since our subjects were highly motivated, our results may be partially due to the Hawthorne effect.

FUTURE DIRECTIONS

Further research with interventional and longitudinal studies with multicenter trials, would be helpful in better understanding of the role of adiponectin in the pathogenesis of periodontitis.

REFERENCES