Effect of Nonsurgical Periodontal Therapy on Adiponectin Levels in Type 2 Diabetics with Moderate to Severe Chronic Periodontitis-A Pilot Trial

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Abstract

Background Adiponectin is a novel adipocyte-specific protein, which plays an important role in decreasing insulin resistance and inflammation, and hence can be considered in understanding the underlying mechanisms of both diabetes and periodontitis. As periodontitis and diabetes mellitus share common pathways of pathogenesis, this study was conducted to determine the effect of nonsurgical periodontal therapy on adiponectin levels in type 2 diabetics with moderate to severe chronic periodontitis.

Material and Methods Ten poorly controlled type 2 diabetics (hemoglobin A1c [HbA1c] ≥ 6.5%) with moderate to severe periodontitis (test group) and 10 age, sex, and body mass index matched systemically healthy patients with moderate to severe periodontitis (control group) were recruited. All the subjects underwent nonsurgical periodontal therapy. Gingival crevicular fluid (GCF) and serum were collected at the baseline and 3 months after periodontal treatment for assessment of adiponectin levels.

Results Adiponectin levels increased postperiodontal therapy in both the groups, both in GCF (p = 0.206, 0.12) and serum (p = 0.051, 0.06). HbA1c reduced in the test group posttreatment (p = 0.229).

Conclusion Nonsurgical periodontal therapy affects adiponectin levels locally as well as systemically thereby leading to improvement of glycemic control.
Introduction

Diabetes mellitus has been established as a significant risk predictor for periodontal disease, with diabetes leading to an exaggerated expression of proinflammatory cytokines in patients with periodontal disease. Similarly, periodontitis, a local oral and chronic inflammatory disease has been seen as a risk factor for diabetes mellitus, wherein the systemic immune response to peripathogen-related infection leads onto increased insulin resistance. This immune response toward the periodontal infection can also increase the vulnerability to diabetic complications and further deteriorate the glycemic control. There are few studies in the literature which implicate microbial dysbiosis in periodontitis to poorly controlled glycemic index. As there occurs a bidirectional relationship among the two diseases, periodontal disease is seen as a comorbidity of diabetes. Inflammation is a possible linking mechanism between the two diseases, also AGE/RAGE interactions, vascular abnormalities, altered collagen metabolism, defective neutrophil function, and altered apoptosis are other mechanisms linking the two.

Among the cytokines released in the inflammatory state, adipokines are a group of bioactive molecules which have been recently discovered in context to process of inflammation and are secreted mainly from the adipose tissues. So, adipose tissue apart from functioning as an energy store is emerging as an important factor in the regulation of many pathological processes. The adipokines have been also seen as important candidates that could elucidate the interrelation between the two above-mentioned diseases. Of all the adipokines, adiponectin has been extensively studied because of its anti-inflammatory role in immune response, as an insulin-sensitizing agent and its effects on cell proliferation, differentiation, and regeneration.

Adiponectin molecule, a 30-kDa protein is homologous to collagen and complement 1q family consisting of 244 amino acids. It is known to modulate several metabolic processes through the activation of 5′-adenosine monophosphate-activated protein kinase and peroxisome proliferator-activated receptor-α. These activations can lead to decrease in the inflammation, which is the primary objective for the treatment of both diabetes and periodontitis.

There is sufficient literature to suggest that periodontal therapy improves the glycemic control in diabetics. But there is not much clarity in terms of the underlying mechanism. So, the aim of this study was to analyze the changes in the serum and local levels of adiponectin 3 months following nonsurgical periodontal therapy (NSPT), as an important mechanistic link between the two comorbidities.

Materials and Method

An observational, nonrandomized trial was performed at the Department of Periodontology, Dr. Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh from March 2019 to August 2019. A convenience sample of 20 subjects was taken. Test group comprised of 10 poorly controlled diabetics (hemoglobin A1c [HbA1c] ≥ 6.5%) with moderate to severe periodontitis. Patients were labeled as diabetics if they were diagnosed as diabetics at least 1 year prior to the study, HbA1c ≥ 6.5%, and fasting plasma glucose (FPG) ≥ 126 mg/dL. Control group comprised of 10 systemically healthy patients with moderate to severe periodontitis. Moderate periodontitis was defined as a condition with 3 to 4 mm clinical attachment loss (CAL) and cases with ≥ 5 mm CAL were classified as severe periodontitis. Exclusion criteria included presence of any other systemic diseases, antibiotic therapy within the preceding 3 months, history of smoking, and periodontal treatment within last 6 months. Pregnant or lactating mothers were also excluded from the study. Both the groups were matched with regard to age, sex, and body mass index. Written informed consent was obtained from the subjects. Approval was obtained from the Institute Ethics Committee, Panjab University, Chandigarh (PUIEC). The procedures used in this study adhered to the principles of Declaration of Helsinki.

After the enrollment based on the inclusion and exclusion criteria, demographic data was collected from all subjects. The duration of diabetic condition, body weight, and height of each patient were also documented. The subjects underwent periodontal assessment at baseline and 3 months following NSPT which included plaque index (Silness and Löe 1967), gingival index (Löe and Sillness 1963), Oral Hygiene Index-Simplified (OHI-S) (Greene and Vermillion 1964), Gingival Bleeding Index (GBI) (Ainamo and Bay 1976), probing depth (PD), and CAL. All the measurements were performed by the same calibrated clinician using mouth mirror, dental explorer, and periodontal probes.

Serum Collection

Venous samples were obtained using a regular venipuncture method. After allowing it to clot at room temperature, serum was removed after centrifugation for 10 minutes. Serum samples were analyzed to determine FPG, HbA1c, high-density lipoprotein, and low-density lipoprotein, total cholesterol, and triglyceride. All the clinical and biochemical evaluations were performed at baseline and at 3 months post-NSPT.

Gingival Crevicular Fluid Sampling

Gingival crevicular fluid (GCF) samples were collected from the sites with signs of clinical inflammation and deepest PD at baseline and 3 months from all groups. Note that 2 µL of GCF sample was collected using 1 to 5 µL calibrated volumetric Hirschmann’s microcapillary pipettes inserted into the entrance of gingival crevice. The site was wiped and isolated with cotton before sampling. Any sample contaminated with saliva or blood was discarded and subsequent sample was taken from another location with similar pocket depth. Serum and GCF samples were immediately stored at −80°C until further biochemical analysis.

Nonsurgical Periodontal Intervention

All patients received 3 to 4 visits of NSPT including supra-gingival scaling and root planing (SRP) using manual curettes and ultrasonic devices followed by oral hygiene instructions. Oral hygiene instructions included demonstration of
appropriate brushing technique, counseling to brush twice daily, use of chlorhexidine mouthwash, and use of any interdental aid wherever required. The oral hygiene practices were recorded from each patient before therapy and rerecorded on the recall visit at 3 months.

**Enzyme Immunoassay**
Ray Bio Human Acrp30 enzyme-linked immunosorbent assay kit was used for the detection of adiponectin. The minimum detection limit was 25 pg/mL (Lot#: 1103170101).

**Statistical Analysis**
Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Inc., IBM, version 17.0 for Windows, Chicago, Illinois, United States). Student’s t-test and the Mann–Whitney U test were used where appropriate for group comparisons of biochemical data and clinical periodontal measurements. A p-value of less than 0.5 was considered statistically significant.

**Results**
Mean age of the subjects was 49.89 ± 7.184 years for the test group and 48.56 ± 6.7 years for the control group. Out of the 10 recruited patients, there were 5 males and 5 females in each group. Clinical periodontal parameters in terms of the OHI-S scores were significantly higher in the case group than the control group, at both preintervention and postintervention time periods (p < 0.001; p = 0.003). There was a significant decrease in GBI, OHI-S, PD, and CAL values postintervention in both the groups (► Figs. 1 and 2). The laboratory parameters in both the groups, at baseline and postintervention have been presented in ► Table 1. There was a statistically –nonsignificant improvement in glycemic control in the test group (p-value = 0.23) though mean fasting blood sugar level did not show any change. Adiponectin levels increased postintervention in both the groups in GCF (p = 0.206, 0.12) as well as in serum (p = 0.051, 0.06) (► Table 1).

**Discussion**
There is sufficient literature to propose the role of adiponectin in the various inflammatory conditions including diabetes and periodontitis as stated by some recent systematic reviews and meta-analyses.\(^6,15,16\) Its ability to decrease inflammation and insulin resistance through various interactions at the molecular level improves the overall systemic health.\(^5\) NSPT aims to eliminate subgingival microbial loads responsible for the inflammation in the periodontal tissues. However, there is a lack of evidence to state the impact of local inflammation elimination therapy on the levels of adiponectin per se.

Adiponectin levels in serum and GCF were lower at baseline in diabetics compared with the systemically healthy, though the values were statistically nonsignificant. These findings are similar to those reported by Sun et al and Natalina et al indicating low adiponectin levels in insulin resistant conditions.\(^17,18\) In our case group, the two comorbidities existed together in the test group, thereby having a more profound influence on the adiponectin levels.\(^6\) Also, it has been seen that presence of periodontitis in both the groups may have further lowered the levels of adiponectin in GCF due to reduced expression of receptors of adiponectin, namely adipoR1 and adipoR2.\(^19\)

Three months post-NSPT, adiponectin levels were found to increase in both serum and GCF of both the groups, though the increase was statistically nonsignificant. These increased levels can be due to the effect of tumor necrosis factor (TNF)-α and the upregulation of receptors of adiponectin, namely adipoR1 and adipoR2, following periodontal therapy. It is well known that inflammatory conditions have an influence on the cytokine milieu enhancing the proinflammatory markers like TNF-α. Besides this, inflammation also creates alterations in the secretory functions of adipocytes better known as adipose...
tissue dysfunction. Increased TNF-α levels as seen in inflammatory conditions can trigger the adipocytes to reduce the expression of adiponectin from adipocytes. Hence, a reduction in the inflammation following periodontal treatment might create a negative energy balance inducing the production of high level of adiponectin both locally and systemically. Our findings are in accordance with those of Kardesler et al who also reported a nonsignificant increase in adiponectin levels in serum in uncontrolled diabetic subjects. Our results are also in concordance with those of Ghalwash et al who have also reported an increase in the levels of adiponectin postperiodontal treatment. The reason for nonsignificant association can be due to a small sample size of this pilot trial.

As expected, the baseline clinical parameters in diabetics were higher than those in nondiabetics. This can be explained on the basis of effect of diabetes on the upregulation of the inflammatory processes toward microbial challenge in periodontitis. The clinical parameters (PD, CAL, OHI-S, GBI) significantly decreased in both the groups after 3 months of performing NSPT. Our results confirmed the effect of gold standard NSPT in restoring the diseased periodontal tissues toward health and are in line with those reported by Tanwar et al in their review on changes in periodontal parameters following NSPT. Our findings are also in accordance with those of many authors who reported statistically significant improvement in clinical parameters postintervention.

Lipid profile did not show any significant changes post-intervention in both the groups. This indicates no additional benefit of NSPT on the laboratory markers for fat metabolism. HbA1c was recorded at baseline and 3 months posttherapy in an attempt to estimate the glycemic control of the subjects. HbA1c levels were found to reduce after NSPT in the diabetic group but the reduction was statistically nonsignificant. Our postintervention glycemic index results are in contrast to those reported by Kardesler et al and Ghalwash et al, who found a significant reduction of HbA1c levels at 3 months. The variation in habits as well as lifestyle of the patients may have affected the results. Also, as per a recent systematic review by Sabharwal et al, a 3-month period is insufficient time period to analyze glycemic control as the glycated hemoglobin level considers the mean serum glucose levels during the 120-day life of the red blood cell. Also, a significant reduction in HbA1c might occur only after the complete elimination of inflammation which may also require surgical therapy in addition to NSPT. But in our study, we assessed only the effect of NSPT on glycemic control.

The strength of our study was to assess the effect of NSPT on adiponectin levels both locally as well as systemically at the same point of time. Second, an effort was made to understand the practical impact of NSPT on the molecular level affecting the two most prevalent comorbidities. However, a small sample size and a single follow-up at 3 months were the limitations of our study and larger sample size studies are recommended for better analysis of this association.

### Conclusion

The study shows that effective NSPT has the ability to show improvement in glycemic control of diabetic patients. SRP caused an increase in levels of adiponectin along with a collateral improvement in the periodontal status 3 months post-NSPT. However, prospective clinical trials on large scale with longer follow-up are required to authenticate the positive role of adiponectin in decreasing inflammation and blood sugar levels.

### Authors’ Contributions


### Table 1 Pre- and post-NSPT intragroup comparison of hematological and biochemical parameters

<table>
<thead>
<tr>
<th>Laboratory investigations</th>
<th>Diabetics with periodontitis</th>
<th>Systemically healthy with periodontitis</th>
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<tbody>
<tr>
<td></td>
<td>Before intervention</td>
<td>After intervention</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.64 ± 3.7</td>
<td>26.56 ± 3.6</td>
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<tr>
<td>HbA1c</td>
<td>9.05 ± 1.34</td>
<td>8.809 ± 1.29</td>
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<tr>
<td>FBS (mg/dL)</td>
<td>162.2 ± 47.2</td>
<td>169.1 ± 41.2</td>
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<tr>
<td>TC (mg/dL)</td>
<td>164 ± 25.8</td>
<td>173 ± 39.8</td>
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<tr>
<td>TG (mg/dL)</td>
<td>122.48 ± 42.6</td>
<td>99.8 ± 37.6</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>44.08 ± 3.9</td>
<td>51.22 ± 16.5</td>
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<tr>
<td>LDL (mg/dL)</td>
<td>91.67 ± 21.9</td>
<td>86.678 ± 40.4</td>
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<tr>
<td>GCF adiponectin (ng/mL)</td>
<td>16.8 ± 6.9</td>
<td>18.6 ± 5.2</td>
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<tr>
<td>Serum adiponectin (µg/mL)</td>
<td>39.9 ± 15.9</td>
<td>57.8 ± 32.2</td>
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Abbreviations: BMI, body mass index; FBS, fasting blood sugar; GCF, gingival crevicular fluid; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NSPT, nonsurgical periodontal therapy; TC, total cholesterol; TG, triglyceride.

Note: p < 0.05 was considered as statistically significant.

All authors approved the final submitted version of the manuscript. A.K., G.S., S.S., and N.B. are the “guarantors” for the current study. Each author believes that the manuscript represents honest work.

Conflict of Interest
None declared.

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