Short-Term Effects of Non-Surgical Periodontal Treatment on the Gingival Crevicular Fluid Cytokine Profiles in Sites With Induced Periodontal Defects: A Study on Dogs With and Without Streptozotocin-Induced Diabetes

Fawad Javed,* Nasser M. Al-Daghri,† Hom-Lay Wang,‡ Cun Yu Wang,§ and Khalid Al-Hezaimi*†

Background: The aim of this study is to assess the short-term effects of non-surgical periodontal therapy (NSPT) on the gingival crevicular fluid (GCF) cytokine profile in sites with standardized periodontal bony defects in beagle dogs with and without diabetes.

Methods: Four beagle dogs with streptozotocin (STZ)-induced diabetes and four healthy dogs were included. Fasting blood glucose levels were measured using a glucometer. In all animals, a 3-walled bony defect was created on the mesial surface of the second premolar and first molar in all quadrants. After 12 weeks, all animals underwent weekly NSPT for 3 weeks. Baseline and post-NSPT GCF samples were collected, and levels of interleukin (IL)-1, IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α were measured using enzyme-linked immunosorbent assay. Statistical analyses were performed using a software program, and $P$ values $<0.05$ were considered statistically significant.

Results: Mean fasting blood glucose levels were significantly higher in dogs with induced diabetes than those without diabetes ($P<0.01$). At baseline, mean IL-6 ($P<0.01$) and IL-8 ($P<0.05$) levels were higher in dogs with diabetes than those without diabetes. A significant reduction in levels of IL-1, IL-1β, IL-6, IL-8, and TNF-α was noted in dogs without diabetes 1 week after NSPT. However, this significant reduction ($P<0.05$) only appeared 2 weeks after NSPT in dogs with diabetes.

Conclusions: NSPT reduces GCF levels of proinflammatory cytokines in dogs with and without STZ-induced diabetes; however, chronic hyperglycemia seems to retard the effect of NSPT on GCF cytokine concentration. J Periodontol 2014;85:1589-1595.

KEY WORDS
Alveolar bone loss; cytokines; diabetes mellitus; periodontal debridement.
It is well established that systemic conditions influence periodontal health. Studies reported that periodontal inflammatory conditions are worse in patients with poorly controlled diabetes compared with those with well-controlled diabetes and individuals without diabetes. Traditionally, severity of periodontal disease is measured via clinical and radiographic parameters (such as plaque index [PI], bleeding on probing [BOP], probing depth [PD], and marginal bone loss); however, laboratory-based techniques may also yield valuable information in this context. The gingival sulcus contains an osmotically mediated inflammatory exudate, the gingival crevicular fluid (GCF), which increases in volume under periodontal inflammatory conditions (because of capillary permeability). Biochemical assessment of GCF is a non-invasive technique for assessing the host response in individuals with periodontitis. It is known that patients with periodontitis display higher concentrations of proinflammatory proteins, such as cytokines, in their GCF compared with individuals without periodontitis. Studies reported that patients with periodontitis, with and without diabetes, display higher concentrations of various cytokines, including interleukin (IL)-1β, IL-6, matrix metalloproteinases 8 and 9, and tumor necrosis factor (TNF)-α in the GCF. Increased expression of such proinflammatory cytokines in the GCF has been associated with the production of reactive oxygen species (ROS) produced as a result of oxidative stress under periodontal inflammatory conditions. In addition, an increased formation and accumulation of advanced glycation end products (AGEs) in the periodontal tissues under hyperglycemia has also been proposed to increase the burden of proinflammatory cytokines in the serum and GCF. In this context, analysis of proinflammatory cytokines in GCF provides valuable information regarding the pathophysiologic processes associated with periodontitis.

Non-surgical periodontal therapy (NSPT) (with and without adjunctive remedies) is a classic nonsurgical therapy for treating periodontitis. It was reported that NSPT is effective in reducing the levels of proinflammatory cytokines in GCF from patients with periodontitis with and without type 2 diabetes. In a recent literature review, Javed et al. reported that the GCF cytokine profile in patients with and without diabetes is governed by the intensity of periodontal inflammation, and the role of diabetes in this regard is rather secondary. Therefore, it was hypothesized that regular plaque control regimens in animals with standardized periodontal bony defects (representing a clinical scenario of adult chronic periodontitis [CP]) with and without diabetes would demonstrate a gradual yet significant reduction in the levels of proinflammatory cytokines in GCF.

The aim of the present experiment is to assess the short-term effects of NSPT on the GCF cytokine profile in sites with standardized periodontal bony defects in beagle dogs with and without streptozotocin (STZ)-induced diabetes.

**MATERIALS AND METHODS**

**Ethical Guidelines**
The study was approved by the research ethics review committee of the Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration, 3D Imaging and Biomechanical Laboratory, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

**Study Animals**
Eight male beagle dogs (mean age: 12 ± 0.4 months; weight: 13 ± 1.2 kg) were used in this study. All animals were kept in individual cages throughout the study period.

All surgical and non-surgical procedures were performed under general anesthesia using ketamine (10 mg/kg body weight) with buccal infiltration of xylocaine.

**Induction of Experimental Diabetes**
Four dogs were placed on a fasting regimen for 24 hours before the induction of diabetes via intravenous injection of STZ. Blood was collected for baseline levels of fasting plasma glucose. Because there is a risk of fatal hypoglycemia as a result of massive pancreatic insulin release, dogs were treated with 10% glucose solution after 4 hours of diabetes induction. After 5 days, the dogs showed fasting hyperglycemia (≥10 mmol/L).

**Induction of Periodontal Bony Defects**
A 3-walled periodontal bony defect (3 × 5 mm) involving the interdental bone was created on the mesial walls of the second premolars (P2) and first molars (M1) in all quadrants (Figs. 1 and 2). Intramuscular amoxicillin** (25 mg/kg body weight) was administered at the day of surgery. Plaque control regimens (NSPT and chlorhexidine rinses) were started 12 weeks after defect induction. All animals underwent weekly NSPT under general anesthesia using an ultrasonic scaler†† for 3 weeks.

**Collection of GCF Samples and Enzyme-Linked Immunosorbent Assay**
GCF samples were collected at baseline (12 weeks after defect induction) and after weeks 1, 2, and 3.
of NSPT. GCF samples were collected from all quadrants using a no. 30 sterile paper point. The sterile paper point was inserted in the mesial gingival crevice of P2 and M1 of each quadrant and held in place for 60 seconds. After sample collection, paper points were transferred to sterile microcentrifuge tubes. The adsorbed volume of GCF in each paper point was weighed using an analytic digital balance, after which the samples were immediately frozen at –80°C until analysis. To determine the amount of GCF, the adsorbed volume in each single paper point with microcentrifuge tubes was weighed on the analytical balance. The volume of GCF was determined by measuring the difference between the weights of microcentrifuge tubes containing a no. 30 paper point with and without adsorbed GCF. The GCF density volume was considered as 1, and the difference in weight showed the GCF volume.22

Paper points were subjected to centrifugation at 13,000 rpm for 15 minutes in a cold room (4°C). Each paper point was eluted by 500 µL sterilized elution buffer (0.05% polysorbate 20 in phosphate-buffered saline [pH 7.4]) for 24 hours. Inflammatory markers (IL-6, IL-8, IL-1, IL-1β, and TNF-α) were measured in the eluted solution by enzyme-linked immunosorbent assay (ELISA) using adipocytokines canine kits. This analysis was quantitative depending on the standard curve of each test for the standard curve of each item.

Periodontal Parameters

Periodontal parameters (PI, BOP, and PD) were measured preoperatively and after 1, 2, and 3 weeks of NSPT. PD was measured using a graded probe. Periodontal parameters were measured on six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal/lingual, midpalatal/lingual, and disto-palatal/lingual) around teeth.

Statistical Analyses

Statistical analysis was performed using a software program. Differences in clinical parameters and GCF cytokine concentrations in healthy dogs and dogs with diabetes with experimental periodontitis were assessed using one-way analysis of variance. For multiple comparisons, Bonferroni post hoc test was performed. P values <0.05 were considered statistically significant.

RESULTS

Fasting Blood Glucose Levels

The mean fasting blood glucose levels were significantly higher in dogs with STZ-induced diabetes (453 ± 35.2 mg/dL) compared with those without STZ-induced diabetes (78.3 ± 5.4 mg/dL) (P <0.01).

Baseline GCF Cytokine Levels in Dogs With and Without Diabetes

At baseline, the mean ± SD IL-6 levels were significantly higher in dogs with STZ-induced diabetes (4,506.1 ± 3,188.8 pg/mL) compared with those without STZ-induced diabetes (1,592 ± 2,209.8 pg/mL) (P <0.01). The mean IL-8 levels were significantly higher in dogs with STZ-induced diabetes (96.7 ± 85.3 pg/mL) compared with those without diabetes (21.6 ± 16.7 pg/mL) (P <0.05). There was no statistically significant difference in the mean concentrations of IL-1, IL-1β, and TNF-α among dogs with and without diabetes (Table 1).

Baseline and Postoperative Cytokine Levels in the GCF of Dogs With and Without Diabetes

In healthy dogs, IL-1, IL-6, IL-8, TNF-α, and IL-1β levels were significantly higher at baseline compared

-‡‡ Eppendorf, Sigma-Aldrich, St. Louis, MO.
-§§ ABT 120-5DM, Kern and Sohn, Balingen, Germany.
- ii Tween 20, Thermo Scientific, Thermo Fisher Scientific, Waltham, MA.
-¶¶ Ascent Multiskan, Thermo Scientific, Waltham, MA.
- ## Hu-Friedy.
-*** SPSS v.18, IBM, Chicago, IL.
with those measured at weeks 1 (P < 0.01), 2 (P < 0.05), and 3 (P < 0.01) after NSPT. There was no statistically significant difference in the cytokine concentrations (IL-1, IL-1β, IL-6, IL-8, and TNF-α) among weeks 1, 2, and 3 after NSPT (Table 2).

In dogs with STZ-induced diabetes, there was no statistically significant difference in the mean concentrations of IL-1, IL-1β, IL-6, IL-8, and TNF-α at baseline and those measured at week 1 after NSPT (Table 3). IL-1, IL-6, IL-8, TNF-α, and IL-1β levels were significantly higher at baseline compared with those measured at weeks 2 and 3 (P < 0.05) after NSPT. IL-1, IL-6, IL-8, TNF-α, and IL-1β levels were significantly higher after week 1 of NSPT than those measured at weeks 2 (P < 0.05) and 3 (P < 0.05) after NSPT. There was no statistically significant difference in the mean IL-1, IL-6, IL-8, TNF-α, and IL-1β levels measured at weeks 2 and 3 after NSPT (Table 3).

### Table 1.

Preoperative (baseline) Cytokine Levels (mean ± SD, in pg/mL) in Dogs With Diabetes and Healthy Dogs

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Dogs With STZ-Induced Diabetes</th>
<th>Dogs Without STZ-Induced Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>4,506.1 ± 3,188.8*</td>
<td>1,592 ± 2,209.8*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>64.6 ± 52.9</td>
<td>41.4 ± 33.5</td>
</tr>
<tr>
<td>IL-8</td>
<td>96.7 ± 85.3†</td>
<td>21.6 ± 16.7†</td>
</tr>
<tr>
<td>IL-1β</td>
<td>165.5 ± 182.7</td>
<td>119.5 ± 65.8</td>
</tr>
<tr>
<td>IL-1</td>
<td>76.2 ± 63.9</td>
<td>64.3 ± 45.2</td>
</tr>
</tbody>
</table>

* P < 0.01.
† P < 0.05.

### Table 2.

Cytokine Levels (mean ± SD, in pg/mL) in the GCF of Healthy Dogs at Baseline and at Weeks 1, 2, and 3 After NSPT

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Preoperative</th>
<th>1 Week After NSPT</th>
<th>2 Weeks After NSPT</th>
<th>3 Weeks After NSPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>1,592 ± 2,209.8††</td>
<td>1,076.8 ± 728.7*</td>
<td>1,012.3 ± 1,209.3†</td>
<td>1,008.2 ± 1,182.2†</td>
</tr>
<tr>
<td>TNF-α</td>
<td>41.4 ± 33.5††</td>
<td>21.2 ± 12.1*</td>
<td>12.5 ± 9.1†</td>
<td>10.08 ± 13.6†</td>
</tr>
<tr>
<td>IL-8</td>
<td>21.6 ± 16.7††</td>
<td>11.0 ± 7.4*</td>
<td>8.6 ± 7.6†</td>
<td>6.2 ± 9.9†</td>
</tr>
<tr>
<td>IL-1β</td>
<td>119.5 ± 65.8††</td>
<td>37.0 ± 33.8*</td>
<td>11.1 ± 13.4†</td>
<td>7.05 ± 8.5†</td>
</tr>
<tr>
<td>IL-1</td>
<td>76.2 ± 63.9††</td>
<td>21.7 ± 11.4*</td>
<td>17.6 ± 2.6†</td>
<td>7.43 ± 6.9†</td>
</tr>
</tbody>
</table>

* Baseline compared with week 1 after NSPT, P < 0.01.
† Baseline compared with 2 weeks after NSPT, P < 0.05.
‡ Baseline compared with 3 weeks after NSPT, P < 0.01.

### Table 3.

Cytokine Concentrations (mean ± SD, in pg/mL) in the GCF of Dogs With STZ-Induced Diabetes at Baseline and Weeks 1, 2, and 3 After NSPT

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Preoperative</th>
<th>1 Week After NSPT</th>
<th>2 Weeks After NSPT</th>
<th>3 Weeks After NSPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>4,506.1 ± 3,188.8††</td>
<td>4,022.42 ± 1,276.3†§</td>
<td>1,368.96 ± 1,085.7†</td>
<td>1,131.8 ± 314.7†§</td>
</tr>
<tr>
<td>TNF-α</td>
<td>64.6 ± 52.9††</td>
<td>31.8 ± 10.2†§</td>
<td>7.8 ± 5.6†</td>
<td>7.6 ± 6.9†§</td>
</tr>
<tr>
<td>IL-8</td>
<td>96.7 ± 85.3††</td>
<td>84.2 ± 77.3†§</td>
<td>14.6 ± 7.3†</td>
<td>8.7 ± 4.2†§</td>
</tr>
<tr>
<td>IL-1β</td>
<td>165.5 ± 182.7††</td>
<td>114.1 ± 109†§</td>
<td>20.9 ± 15.3†</td>
<td>7.7 ± 5.7†§</td>
</tr>
<tr>
<td>IL-1</td>
<td>64.3 ± 45.2††</td>
<td>31.9 ± 14.8†§</td>
<td>21.1 ± 25.6†</td>
<td>7.7 ± 1†§</td>
</tr>
</tbody>
</table>

* Baseline compared with week 2 after NSPT, P < 0.05.
† Week 1 after NSPT compared with week 2 after NSPT, P < 0.05.
‡ Baseline compared with week 3 after NSPT, P < 0.05.
§ Week 1 after NSPT compared with week 3 after NSPT, P < 0.05.
**Periodontal Parameters in Dogs With and Without Diabetes**

The preoperative periodontal parameters were comparable among dogs with and without STZ-induced diabetes. In dogs without STZ-induced diabetes, significant reductions in PI (P <0.001), and BOP (P <0.001), occurred 1 week after NSPT. In dogs with STZ-induced diabetes, significant reductions in PI (P <0.01), BOP (P <0.01), and PD ≥3 mm (P <0.01) occurred 2 weeks after NSPT. After 3 weeks of NSPT, periodontal parameters in dogs with and without STZ-induced diabetes were comparable (Table 4).

**DISCUSSION**

A critical factor that adds value to the present results is standardization of the methodology. In the present study, male beagle dogs with comparable ages are used. In addition, a clinical scenario of adult CP was depicted via induction of standardized periodontal osseous defects. Moreover, optimal consistency was also ensured in the methodologies adopted in clinical examination and laboratory-based investigations (ELISA).

It is generally accepted that the severity of periodontal inflammation modulates the GCF cytokine concentration in individuals with and without diabetes.9,10,24 One explanation for this is that the increased formation and accumulation of AGEs in periodontal tissues in a chronic hyperglycemic state increases the intensity of the immunoinflammatory response to periodontal pathogens because inflammatory cells, such as monocytes and macrophages, have receptors for AGEs.25,26 Interaction between AGEs and their receptors stimulates the production of proinflammatory proteins.9,26 Moreover, it was claimed that ROS (a potential mechanism for accelerated tissue injury) produced as a result of oxidative stress under periodontal inflammatory conditions causes a state of hyperinflammation, thereby enhancing the production of proinflammatory cytokines.16 Furthermore, studies10,12 also reported comparable IL-1β levels in patients with CP with and without type 2 diabetes. These results suggest that chronic hyperglycemia and CP are two inflammatory conditions that contribute to enhancing the production and accumulation of proinflammatory cytokines in the GCF.

In the present study, GCF concentrations of IL-6 and IL-8 were significantly higher in dogs with STZ-induced diabetes compared with dogs without STZ-induced diabetes. This may be explained by the possibility that elevated levels of AGEs in the serum and gingival tissues of dogs with STZ-induced diabetes may have caused a state of enhanced oxidative stress, thereby increasing the concentrations of IL-6 and IL-8 in the GCF. The present results support the study by Engebretson et al.,13 in which the authors reported GCF IL-8 levels to be significantly higher in patients with periodontitis with type 2 diabetes compared with medically healthy patients with periodontitis. Therefore, it may be hypothesized that, in patients with periodontitis with well-controlled diabetes, IL-8 levels are similar to those in medically healthy individuals with periodontitis. Additional studies are warranted in this regard.

NSPT with and without adjunctive remedies (such as antibiotics and phototherapy) is an effective means of plaque control and treating periodontal inflammatory conditions.19,20,27 In the present study, baseline periodontal parameters and GCF concentrations of IL-1, IL-1β, IL-6, IL-8, and TNF-α are significantly

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dogs Without STZ-Induced Diabetes</th>
<th>Dogs With STZ-Induced Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After Week 1 of NSPT</td>
</tr>
<tr>
<td>PI (% of sites)</td>
<td>100*†‡</td>
<td>40.6*</td>
</tr>
<tr>
<td>BOP (% of sites)</td>
<td>100*†‡</td>
<td>53.2*</td>
</tr>
<tr>
<td>PD ≥3 mm (% of sites)</td>
<td>70.5¶‡</td>
<td>45.2</td>
</tr>
</tbody>
</table>

* Baseline compared with week 1 after NSPT, P<0.001.
† Baseline compared with week 2 after NSPT, P<0.001.
‡ Baseline compared with week 3 after NSPT, P<0.01.
§ Baseline compared with week 2 after NSPT, P<0.05.
¶ Baseline compared with week 2 after NSPT, P<0.01.
Scaling and Root Planing, Cytokines, and Gingival Crevicular Fluid

REFERENCES


Correspondence: Dr. Khalid Al-Hezaimi, Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration, 3D Imaging and Biomechanical Laboratory, College of Applied Medical Sciences and Department of Periodontics and Community Dentistry, King Saud University, Riyadh, Saudi Arabia. E-mail: hezaimik16@gmail.com.

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