Clinical and Laboratory Evaluations of Non-Surgical Periodontal Treatment in Subjects With Diabetes Mellitus

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**Background:** The aim of this study was to evaluate the clinical and laboratory changes 3 months after full-mouth scaling and root planing in subjects with and without diabetes mellitus.

**Methods:** This study was performed using 10 subjects with type 2 diabetes mellitus who required insulin therapy (DM) and 10 healthy adult control subjects (NDM) with generalized chronic periodontal disease. Both groups were treated with full-mouth scaling and root planing and given oral hygiene instructions. Clinical parameters, including plaque index (PI), gingival index (GI), probing depth (PD), gingival recession (GR), and clinical attachment level (CAL), were measured at four sites per tooth. Subgingival plaque samples were obtained from sites with the deepest PD (≥5 mm) and with furcations in each subject. Samples were also tested for the presence of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Porphyromonas gingivalis*, and *Tannerella forsythia* (previously *T. forsythensis*) by polymerase chain reaction. Glycemic control (glycosylated hemoglobin [HbA1c] and fasting glucose levels) and clinical and microbiologic assessments were recorded at baseline and 3 months after periodontal treatment.

**Results:** Data revealed statistical changes (P ≤ 0.05; analysis of variance [ANOVA]) in clinical variables (PI, GI, PD, GR, and CAL) between baseline and 3 months in both groups. Conversely, no improvement in the fasting glucose level or glycosylated hemoglobin (P ≤ 0.05; ANOVA) was found after treatment. Besides some reduction in the bacterial frequency 3 months after treatment, no statistically significant difference was found between the groups.

**Conclusion:** Clinical and laboratory responses were similar in DM and NDM groups 3 months after full-mouth scaling and root planing. *J Periodontol* 2008;79:1150-1157.

**KEY WORDS**
Diabetes mellitus; glycosylated hemoglobin; microbiology; periodontal disease.

Diabetes mellitus is a metabolic disorder that is characterized by alterations in glucose tolerance and a deficiency in the metabolism of carbohydrates and lipids, leading to classic triad symptoms that include polyuria, polydypsia, polyphagia, and weight loss. It is categorized as type 1 diabetes, caused by an absolute deficiency of insulin secretion, or type 2 diabetes, caused by a combination of resistance to insulin action and a compensatory insulin secretory response. Type 2 diabetes results from defects in the insulin molecule or from altered cell receptors for insulin and represents impaired insulin function (insulin resistance) rather than deficiency. However, insulin production may be diminished as the disease advances, and insulin supplementation may become necessary.† Diabetes mellitus has been regarded as an important risk factor for gingivitis and periodontitis.‡

Multivariate risk analysis demonstrated diabetes as a risk factor for periodontal disease, with subjects having a 2.8- to 3.4-fold higher chance of presenting periodontitis compared to non-diabetic subjects after adjusting for the effects of confounding variables, such as age, gender, and oral hygiene measures. In another study, Seppäälä et al.§ suggested that the prevalence of gingivitis in children with type 1 diabetes is greater than in children without diabetes with similar plaque

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levels. Bacić et al.5 and Katz et al.6 used the Community Periodontal Index of Treatment Needs and concluded that patients with diabetes have a greater prevalence of periodontal disease and greater need for periodontal treatment than those without diabetes.

There are mechanisms describing the relationship between diabetes and periodontal disease, and these have focused on changes at local and systemic levels. Changes at the local level have been reported and include vascular changes in periodontal tissues (gingival microangiopathy, granulocyte hypofunction, and increased tissue liability resulting from reduced collagen production/enhanced gingival collagenase activity) and changes in oral microbiota.7,8 Systemic changes have been reported, where diabetes was shown to promote alterations in immune cell phenotype and elevation of serum proinflammatory cytokines/lipid levels.9 The functions of immune cells, including neutrophils, monocytes, and macrophages, are altered in diabetes. Neutrophil adherence, chemotaxis, and phagocytosis are often impaired, leading to the inhibition of bacterial killing in the periodontal pocket and significant increases in periodontal destruction. Based on data from a variety of studies, patients with diabetes can exhibit an exaggerated inflammatory host response, deficient healing, and lower resistance, all of which explain the increased severity of periodontal disease and tissue destruction.7,8,10,11

Microbiologic investigations revealed that the bacterial microbiota at periodontal disease sites are similar in subjects with and without diabetes. Sbordone et al.12 reported similar microbiota in subjects with and without type 2 diabetes, where the prevalence of Prevotella intermedia, Campylobacter rectus, Porphyromonas gingivalis (Pg), and Aggregatibacter actinomycetemcomitans (Aa; previously Actinobacillus actinomycetemcomitans) was similar for both groups. Conversely, another study13 demonstrated that Capnocytophaga sp. are more prevalent in the microbiota of subjects with diabetes.

Faria-Almeida et al.14 applied conventional therapy to subjects with and without type 2 diabetes mellitus and found improvements in clinical parameters and in blood glucose. New approaches to periodontal therapy have been suggested, such as the full-mouth scaling and root planing (SRP) protocol that could reduce the number of patient visits, making treatment more efficient. The aim of this study was to evaluate if subjects with and without diabetes and chronic periodontal disease would present different clinical and laboratory responses to full-mouth root planing therapy after 3 months.

**MATERIALS AND METHODS**

Twenty subjects (30 to 70 years old) of the Graduate Clinic of the Piracicaba Dental School, State University of Campinas (UNICAMP), with a clinical diagnosis of generalized chronic periodontitis defined by probing depth (PD) ≥ 5 mm in ≥ 10 teeth, radiographic bone loss ranging from 30% to 50%, and ≥ 20 teeth were included. Exclusion criteria were the use of antibiotics during the previous 6 months, pregnancy, smoking, systemic diseases, or the use of immunosuppressive medication, phenytoin, cyclosporin, calcium channel blockers, or any medication that could interfere with the periodontium or the response to periodontal therapy.

The subjects were divided into two groups of 10: an experimental group diagnosed with type 2 diabetes mellitus (DM group; insulin supplementation confirmed by an endocrinologist) and healthy control subjects without diabetes (NDM group). DM subjects reported the number of years since their diagnosis and insulin initiation and their daily consumption of insulin. All participants provided written informed consent. The study protocol was approved (protocol number 119/2006) by the Ethical Committee of Research, Piracicaba Dental School, UNICAMP. The subjects were evaluated for 3 months, from October to December 2006.

Blood samples were taken at baseline and at the 3-month recall visit. Glycated hemoglobin§ and fasting glucose levels1 were determined for all subjects. Clinical parameters were measured at baseline and 3 months after therapy. PD, gingival recession (GR), clinical attachment level (CAL), plaque index (PI),15 and gingival index (GI)15 were measured by a single examiner with a periodontal probe¶ at four sites per tooth at all teeth, excluding third molars.

After clinical measurements, the supragingival biofilm was removed with sterile gauze. Subgingival samples were collected from the sites with the deepest PD (≥ 5 mm) and with furcations in each subject using a sterile periodontal curet. Pooled biofilms from each site were separated into two Eppendorf tubes containing reduced transport fluid and were stored at −20°C. The samples were collected at baseline and 3 months post-therapy and were analyzed microbiologically by polymerase chain reaction (PCR).

After removing baseline samples of biofilm, full-mouth SRP was performed in all diseased sites under local anesthesia in one session ∼ 2 hours. Oral hygiene instructions for home care procedures (tooth-brushing technique, interdental cleaning, and use of tongue scrapers) were given by one experienced periodontist. The maintenance therapy included professional plaque control at 2-week intervals during the 3 months of the study.

§ Labtest diagnostic test, Labtest, Lagoa Santa, MG, Brazil.
¶ Glucose GOD-ANA enzymatic, Labtest.
¶ PCP-UNC15, Hu-Friedy, Chicago, IL.
Table 1. 
PCR Primers Used

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
<th>Base Position (amplicon length in base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa</td>
<td>5’AAA CCC ATC TCT GAC TTC TTC TTC 3’ 5’ATG CCA ACT TGA CGT TA AT 3’</td>
<td>557</td>
</tr>
<tr>
<td>Tf</td>
<td>5’GGC TAT GTA ACC TGC CCG CA 3’ 5’TGC TTC AGT GTC AGT TAT ACC T3’</td>
<td>641</td>
</tr>
<tr>
<td>Pg</td>
<td>5’AAT CGT AAC GGG CGA CAC AC 3’ 5’GGG TTG CTC CTT CAT CAT AC 3’</td>
<td>593</td>
</tr>
</tbody>
</table>

Microbiologic Assessment
The primers evaluated and used in this study are listed in Table 1. Tests were performed to verify primer specificity. For this purpose, PCRs with specific primers for Aa, Pg, and Tannerella forsythia (Tf; previously T. forsythensis) were processed.

The PCR conditions were published by Klein and Gonçalves. For PCR, 36.25 µl sterile distilled water was added to the clinical samples. The samples were dispensed by vortex for 1 minute and then boiled for 10 minutes. The PCR was processed using 5.0 µl sample added to 45 µl reaction buffer containing 1.5 mM MgCl2 (Taq DNA polymerase), 200 µM deoxynucleotide triphosphate (deoxynucleotide triphosphate mixture), 2 µM of each primer, and 2 U Taq DNA polymerase. In addition to the samples, positive and negative controls were used in each experiment. PCR amplification was performed under the following conditions: Tf = an initial denaturation step at 95°C for 2 minutes, followed by 36 cycles of denaturation and extension at 95°C for 30 seconds, annealing at 60°C for 1 minute, an extension at 72°C for 1 minute, and a final elongation step at 72°C for 2 minutes; Pg = an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 70°C for 1 minute, an extension at 72°C for 1 minute, and a final elongation step at 72°C for 2 minutes; Aa = an initial denaturation step at 95°C for 4°C for 5 minutes, followed by 36 cycles of denaturation and extension at 95°C for 30 seconds, annealing at 60°C for 1 minute, an extension at 72°C for 1 minute, and a final elongation step at 72°C for 2 minutes.

The PCR products were analyzed by electrophoresis in 1.5% agarose gel using Tris-borate-EDTA buffer. A 100–base pair or 1-kilobase DNA ladder was included in each gel. The DNA was stained with ethidium bromide and visualized under ultraviolet illumination.

Statistical Analysis
Statistical tests were performed using software.** Pl and GI were analyzed as the mean percentage of sites per individual at three time intervals. Repeated-measures analysis of variance (ANOVA) was performed to compare clinical parameters (PI, GI, PD, GR, and CAL) between the two time intervals and between DM and NDM groups. All variables were distributed normally, and only GI required log adjustment. Glycosylated hemoglobin was analyzed by the paired Student t test. Fasting glucose was analyzed by the Wilcoxon test to evaluate differences between the NDM and DM groups. The Mann-Whitney test was used to analyze differences between the two time intervals. The percentages of sites with bacteria were compared between the DM and NDM groups using the Fisher exact test. The McNemar test was used to test changes in bacterial presence between the two time intervals. Statistical significance was defined at the 5% level.

RESULTS
Periodontal Results
The subjects characteristics are described in Table 2. No significant differences were observed for age between the DM and NDM groups (Student t test; P = 0.7570). The mean number of sites in the whole mouth, number of sites with periodontal disease, and sites with furcations were seen in both groups. The mean time since the initial diagnosis of diabetes (diabetes duration in years), duration of insulin consumption in years, and insulin consumption were verified in the diabetes group.

The presence of plaque was evaluated on all tooth surfaces (Table 3). PI was determined for the whole mouth (%) at different time intervals (baseline and 30 and 90 days; P = 0.0001; ANOVA), and no significant difference was found between the DM and NDM groups (P = 0.5881; ANOVA, power analysis: group = 0.106, time > 0.999, group x time = 0.260). Both groups demonstrated reduced plaque after 3 months and an improvement in oral hygiene. GI was evaluated on all tooth surfaces examined (Table 3). GI was determined for the whole mouth (%) at different time intervals (baseline and 30 and 90 days; P = 0.0001; ANOVA, log adjustment), and no significant difference was found between the DM and NDM groups (P = 0.5939; ANOVA, log adjustment, power analysis: group = 0.065, time > 0.999, group x time = 0.375). Both groups demonstrated decreased bleeding after 3 months. No difference in the prevalence of gingivitis was seen between the two groups. Based on PI and GI changes, the response to the oral hygiene procedures was similar for the two groups.
found between the two groups (NDM group, but no significant difference was found between the two groups (P = 0.0530; ANOVA, power analysis: group = 0.155, time >0.999, group × time = 0.284). The CAL gains were 1.21 ± 0.24 mm and 1.12 ± 0.15 mm for DM and NDM groups, respectively.

**Blood Analysis**

Blood glucose control was expressed as the fasting glucose level and glycosylated hemoglobin. The fasting glucose level (Fig. 1) showed no significant difference at baseline (P = 0.1988; Mann-Whitney test), but levels were significantly different at 3 months (P = 0.0191; Mann-Whitney test). The fasting glucose level increased after 3 months in the DM group. No statistical difference was found between the DM groups (P = 0.9594; Wilcoxon test) and the NDM group (P = 0.0926; Wilcoxon test) during the study. The glycosylated hemoglobin (HbA1c) concentrations (%) (NDM group at baseline = 5.88 ± 0.16; 3 months = 5.82 ± 2.60; DM group at baseline = 9.23 ± 2.60; 3 months = 9.4 ± 2.53) did not change significantly during the study (P = 0.7115; Student paired t test; Fig. 2).

**Microbiologic Results**

Figure 3 shows the percentages of sites (with PD ≥5 mm) with Aa, Pg, and Tf at baseline and 3 months after full-mouth SRP; a significant difference between the DM and NDM groups at baseline was observed only for Tf (P = 0.033; P = 1.000 for Aa and Pg). No significant reduction in the presence of these microorganisms was found at 3 months after full-mouth root planing (Aa and Pg: P = 1.000; Tf: P = 0.650).

Figure 4 shows the percentages of sites with furcations with Aa, Pg, and Tf at baseline and 3 months after full-mouth SRP. The Fisher exact test showed a
significant difference at baseline between the DM and NDM groups only for Tf ($P = 0.011; P = 1.000$ for Aa and Pg). No significant differences were found at 3 months after full-mouth root planing (Aa, Pg, and Tf: $P = 1.000$). Tf was reduced at 3 months after full-mouth root planing.

The McNemar test was used to compare the changes from the presence to the absence of microorganisms in sites with PD $\geq 5$ mm and furcations between baseline and 3 months. These changes were statistically significant only for Tf ($P = 0.0313$) for the NDM group. Tf was reduced at 3 months after full-mouth root planing only in the NDM group. The DM group did not exhibit any changes in the presence of microorganisms.

**DISCUSSION**

In the present study, two groups (DM and NDM) with generalized chronic periodontal disease were selected and treated by full-mouth SRP to determine whether there were clinical and laboratory differences between them following periodontal treatment. The results did not reveal a statistically significant difference between the groups.

Reductions in PI and GI were obtained in both groups after 3 months of follow-up, which shows the effectiveness of the oral hygiene program adopted in the study. This is in accordance with previous reports showing no differences in plaque levels between subjects with and without diabetes. In contrast, Bridges et al. found a higher level of plaque and gingival bleeding in subjects with diabetes, whereas Kawamura et al.23 did not find any association between plaque accumulation and the presence of periodontal disease because patients maintained a high level of oral hygiene. Considering gingival bleeding, Aren et al.24 found a greater prevalence of gingival inflammation in the group with diabetes compared to the group without diabetes.

Regarding the clinical parameters evaluated, CAL, PD, and CAL gain, no differences were observed between the DM and NDM groups when evaluated after periodontal therapy performed in a single session using full-mouth SRP. The intragroup evaluation showed CAL gains, reductions in PD, and increases in GR in both groups. However, Faria-Almeida et al.14 reported statistically significant differences ($P <0.0207$) for PD between the control and diabetic groups between baseline and 3 months. The authors results obtained the same sample size like the present study because the very strict inclusion and exclusion criteria were applied to minimize confounding factors. The
The present study was based on a statistical analysis of clinical parameter relevance that calculated small differences between means of groups of 4% for PI (power analysis: PI 4% = 0.850), 5% for GI (power analysis: GI 5% = 0.863), 2 mm for PD (power analysis: PD = 0.978), 1 mm for GR (power analysis: GR = 0.874), and 1 mm for CAL (power analysis: CAL = 0.99), considering the alpha error level for power analyses (α = 0.05). The power analyses were calculated based on means ± SD of control groups, and the minimum power was 0.85 (β = 0.15) for all clinical parameters.

Regarding the use of full-mouth SRP in a single session in subjects with diabetes, it was suggested that this procedure was beneficial to the subjects because it reduced the number of scaling sessions; i.e., the bacterial plaque and calculus removal occurs in just one appointment.²⁵ In the present study, the protocol proposed by Quirynen et al.²⁶ including antimicrobial control (chlorhexidine), was not used. The protocol of the study included scaling and planing associated with a rigorous oral hygiene control as suggested by Ternoven and Karjalainen²⁰ and Moore et al.²⁷

Different approaches to periodontal therapy in subjects with diabetes have been reported. Conventional therapy was used by Christgau et al.²⁸ and Faria-Almeida et al.¹⁴ in subjects with and without type 2 diabetes. These investigators found no differences in clinical responses between the two groups. Similarly, Westfelt et al.²⁹ did not find any differences in clinical response following conventional therapy complemented by surgical therapy in subjects with and without type 1 or 2 diabetes after 5 years of follow-up.

The metabolic parameters selected in this study were fasting glucose level and glycosylated hemoglobin. The fasting glucose level was verified during anamneses at baseline and was used to confirm the absence or presence of diabetes in both groups before therapy and 3 months after therapy, whereas the HbA1c glycosylated hemoglobin test was used to monitor the stable glucose/hemoglobin binding during the 90 days after therapy in both groups. The level of fasting glucose and HbA1c in subjects with diabetes did not demonstrate any alteration following periodontal treatment.

According to Ternoven and Karjalainen,²⁰ the influence of the diabetic condition on periodontal tissue health may be significant if metabolic control, as measured by HbA1c, is constantly >10%, which may cause other complications. They found a worse response to periodontal therapy in subjects with poor metabolic control compared to controls without diabetes. In addition, Faria-Almeida et al.¹⁴ and Aren et al.²⁴ reported that a reduction in glycemia occurred in subjects with diabetes after periodontal treatment. In contrast, no reduction in glycemia was observed in the present study. In fact, a slight increase in glycemia occurred after 3 months, probably because the subjects selected for the study did not demonstrate good metabolic control, although the clinical appointments were carried out during the day when glycemic glucose levels are lower. Thus, the periodontal treatment alone was not capable of improving metabolic control, suggesting that other variables may be involved in the control of diabetes.

The use of full-mouth therapy in this study resulted in a significant reduction in infection and periodontal inflammation, which is in accordance with Rodrigues et al.³⁰ and Schara et al.³¹ Rodrigues et al.³⁰ reported that full-mouth root planing can be advantageous to subjects with diabetes with a high risk for infections, vascular alterations, and deficient healing response because this approach may help to minimize possible reinfections of the treated areas. However, a question can be raised about the association of full-mouth root planing with antibiotics. Rodrigues et al.³⁰ compared the use of amoxicillin/clavulanic acid, 875 mg, plus full-mouth root planing to full-mouth root planing alone in subjects with type 2 diabetes; they concluded that the use of an antibiotic did not improve metabolic control. The investigators reported a statistically significant reduction in HbA1c for the group that received full-mouth root planing alone. Schara et al.³¹ showed no improvement in metabolic control after full-mouth root planing in subjects with type 1 diabetes after 6 months. It is important to recognize that more studies with larger sample sizes are needed to evaluate the use of full-mouth root planing therapy alone or in association with antibiotics for the treatment of subjects with diabetes.

Grossi et al.³² reported that chronic periodontal Gram-negative infections may also lead to insulin resistance and inadequate glycemic control. Periodontal treatment can be performed to decrease the bacterial effect and inflammation, restoring sensitivity to insulin over time and resulting in a better metabolic control. According to Grossi et al.³² the improvement in metabolic control observed in studies that combine mechanical periodontal treatment and antibiotics may support this hypothesis.

Miller et al.³³ suggested that the improvement in periodontal health may be accompanied by an improvement in the metabolic control of subjects with diabetes and may indicate potential systemic benefits of periodontal treatment in subjects with periodontitis and poorly controlled diabetes. In contrast, Christgau et al.²⁸ evaluated individuals with good and moderate diabetes control and with periodontitis treated by SRP without systemic antibiotic therapy, but they did not find any alteration in glycemic control associated with clinical improvement.

Sastrowijoto et al.³⁴ evaluated the presence and number of Aa, Bacteroides intermedius (B. intermedius),
and *Capnocytophaga* sp. in healthy and periodontally diseased sites in subjects with controlled and non-controlled insulin-dependent diabetes. These investigators did not observe any difference between the subgingival microbiota in subjects with type 1 diabetes.

In this study, three species of microorganism were studied: *Aa*, *Pg*, and *Tf*. A statistically significant difference in microorganism reduction was observed only for *Tf* in the DM and NDM groups after 3 months. The present findings are in agreement with those of Christgau et al., who evaluated subjects with insulin-dependent diabetes, subjects with non-insulin-dependent diabetes, and subjects without diabetes, all with periodontal disease, and determined the presence of *Aa*, *Pg*, *P. intermedia*, and *Fusobacterium nucleatum* before and after 4 months after conventional periodontal treatment. The investigators did not observe the predominance of any of the species evaluated and reported that the presence of periodontopathogens was similar in both groups. These findings also support those of Sbordone et al., who detected a similar composition of subgingival microbiota in subjects with or without diabetes.

The findings of the present study should be considered with caution because of the relatively small sample size and because, to the best of our knowledge, no other published study has carried out a similar microbiologic evaluation in subjects with diabetes submitted to SRP therapy in a single session.

CONCLUSION

Non-surgical periodontal treatment using full-mouth root planing did not provide a significant difference in clinical and laboratory responses between DM and NDM groups after 3 months of follow-up.

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REFERENCES


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