Effects of Periodontal Therapy on Glycemic Control and Inflammatory Markers

Patricia A.A. O'Connell,* Mario Taba Jr.,* Auro Nomizo,† Maria C. Foss Freitas,‡ Flavia A. Suaid,* Sergio A. Uyemura,† Glauce L. Trevisan,* Arthur B. Novaes Jr.,* Sergio L.S. Souza,* Daniela B. Palioto,* and Marcio F.M. Grisi*

Background: Periodontitis, a complication of diabetes mellitus (DM), can induce or perpetuate systemic conditions. This double-masked, placebo-controlled study evaluated the effects of periodontal therapy (scaling and root planing [SRP]) on the serum levels of glycated hemoglobin (HbA1c) and on inflammatory biomarkers.

Methods: Thirty subjects with type 2 DM and periodontitis were treated with SRP + placebo (SRP; N = 15) or with SRP + doxycycline (SRP+Doxy; N = 15), 100 mg/day, for 14 days. Clinical and laboratory data were recorded at baseline and at 3 months after treatment.

Results: After 3 months, the reduction in probing depth was 0.8 mm for the SRP group (P <0.01) and 1.1 mm for the SRP+Doxy group (P <0.01) followed by a 0.9% (SRP; P = 0.17) and 1.5% (SRP+Doxy; P <0.01) reduction in HbA1c levels. A significant reduction in interleukin (IL)-6; interferon-inducible protein 10; soluble fas ligand; granulocyte colony-stimulating factor; RANTES; and IL-12 p70 serum levels were also verified (N = 30). To our knowledge, this is the first report on the effects of periodontal therapy on multiple systemic inflammatory markers in DM.

Conclusions: Periodontal therapy may influence the systemic conditions of patients with type 2 DM, but no statistical difference was observed with the adjunctive systemic doxycycline therapy. Moreover, it is possible that the observed improvement in glycemic control and in the reduction of inflammatory markers could also be due to diet, which was not controlled in our study. Therefore, a confirmatory study with a larger sample size and controlled diet is necessary. *J Periodontol 2008;79:774-783.*

KEY WORDS

Biomarkers; diabetes; inflammation; periodontitis.

* Department of Oral Surgery and Periodontology, School of Dentistry, University of São Paulo–Ribeirão Preto, Ribeirão Preto, SP, Brazil.
† Department of Clinical Analysis, Toxicology and Bromatology, School of Pharmaceutical Sciences, University of São Paulo–Ribeirão Preto.
‡ Department of Internal Medicine, Division of Endocrinology and Metabolism, School of Medicine, University of São Paulo–Ribeirão Preto.

Diabetes mellitus (DM) and periodontitis are common chronic diseases in adults. Both diseases are highly prevalent in the world population. Approximately 21 million children and adults in the United States (7% of the population) have diabetes,[1] and this incidence is increasing annually. By the year 2025, it is estimated that 300 million people will have diabetes and that more than one in three people >30 years of age will have periodontitis. It is also estimated that ≥35.7 million people in the United States have periodontitis.[2]

DM is one of a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Based on these conditions, DM can be classified into two main types: type 1 DM is caused by destruction of the pancreatic β cells that are known to produce insulin; type 2 DM results from defects in insulin molecules or from defective cell receptors for insulin. This defect indicates impaired insulin function (insulin resistance) rather than deficiency or lack of production.[3] The chronic hyperglycemic condition of diabetes is associated with long-term damage to, dysfunction, or failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Periodontitis is also considered to be one of these complications. It is generally accepted that periodontal disease is more

doi: 10.1902/jop.2008.070250

prevalent and more severe in patients with diabetes.[4,5] The mechanisms responsible for these outcomes in patients with diabetes are mainly related to their increased risk for infections, impairment of the synthesis of collagen and glycosaminoglycan by gingival fibroblasts, and increased crevicular fluid collagenolytic activity.[4,6,7]

The relationship between these diseases represents a well-recognized example of a systemic disease predisposing to oral infection; if this infection actually occurs, it, in turn, exacerbates the systemic disease.[3] Thus, the chronic Gram-negative infection of periodontal origin may be considered a potential focus of infection that aggravates metabolic control in patients who have diabetes.[5] In this context, the virulence of the subgingival bacteria present in the periodontium gains a greater significance.

Strong evidence indicates that pathogenic bacteria or their products stimulate cells such as fibroblasts, keratinocytes, and macrophages, which are present in periodontal tissue, to release a number of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α); prostaglandin E_2; interleukin (IL)-1β, -6,[8] and -12;[9] granulocyte colony-stimulating factor (G-CSF);[10] and chemokines, such as IL-8; regulated on activation, normal T-cell expressed, and secreted (RANTES); interferon-inducible protein (IP)-10; and macrophage inflammatory protein (MIP)-1α, that are relevant to inflammatory processes in periodontal diseases.[9,11,12] The elevation in cytokine and chemokine expression by cells within the gingival connective tissue in chronic periodontitis lesions may result in increased levels of these mediators in the blood circulation where they can induce or perpetuate systemic effects.[13]

The elevated serum levels of these important mediators have deleterious effects on glucose and lipid metabolism.[14] TNF-α was reported to interfere with lipid metabolism and to be an insulin antagonist.[15,16] Chemokines play an important role in the development of obesity-related disorders, such as type 2 diabetes. IL-6 and -1 receptor antagonist (ra) were also reported to antagonize insulin action and to induce the release of acute-phase proteins.[14,15,17] Furthermore, it was shown that diabetes upregulates the production of inflammatory cytokines and chemokines,[18] leading to increased inflammation, tissue damage, and apoptosis in patients who have periodontitis.[3,13,19]

Intervention trials[3,17,20] have assessed the potential effects of periodontal therapy on glycemic control in subjects who had diabetes. However, they did not produce enough evidence to establish the fact that periodontal therapy is influential in improving glycemic control in these patients. Conversely, several studies[21-24] of subjects with diabetes and severe periodontitis showed improvement in glycemic control following scaling and root planing (SRP). Additionally, the treatment of periodontal disease by incorporating systemic doxycycline or local minocycline resulted in a significant reduction in periodontal infection and inflammation; this was followed by a short-term reduction in the levels of glycated hemoglobin (HbA1c).[21,23]

Taking into account the accumulated evidence and the necessity of further investigations into the relationship between diabetes and periodontal disease, the purpose of this study was to evaluate the changes in the serum levels of HbA1c and inflammatory biomarkers after non-surgical periodontal therapy.

MATERIALS AND METHODS

Study Population

This clinical study involved the joint collaboration of the Department of Oral Surgery and Periodontology, the Department of Internal Medicine, Division of Endocrinology and Metabolism, and the Department of Clinical Analysis, University of São Paulo-Ribeirão Preto. The study was reviewed and approved by the Institutional Human Research Committee, University of São Paulo-Ribeirão Preto.

Approximately 2,400 medical records were reviewed between 2004 and 2005 to select subjects for this study. Subjects who met the necessary criteria for inclusion in the study were asked to sign a consent form. After signing this form, they were examined by an experienced periodontist (PAAO) who based her selection of those to be included in the study on their general health status and periodontal condition. Criteria for inclusion were: type 2 DM diagnosed for >5 years and HbA1c >8%, at least one site with probing depth (PD) ≥5 mm, and two teeth with attachment loss ≥6 mm. Exclusion criteria were: the use of antibiotics or periodontal treatment in the previous 6 months, smoking within the past 5 years, pregnancy or lactation, major diabetic complications, and concomitant medical therapy. The subjects who qualified for the study were rescheduled for laboratory, periodontal, and clinical examinations and radiographs. Thirty-five subjects who presented all of the inclusion criteria agreed to participate in the study (Fig. 1).

Clinical Data Collection

An experienced examiner (PAAO) used a computerized periodontal probe§ to perform the periodontal measurements, which were recorded for six sites per tooth at baseline and at 3 months after periodontal treatment. The parameters recorded were PD, relative clinical attachment level (CAL), bleeding on probing (BOP), suppuration (SUP), and the presence or absence of biofilm at four sites per tooth (plaque index [PI]).[22]

§ Florida Probe, Florida Probe, Gainesville, FL.

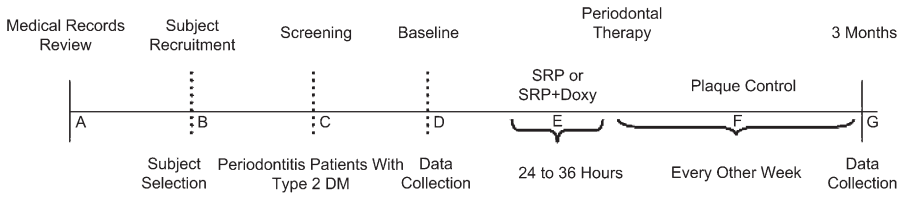

Figure 1.
Study design and subject selection. ***A)*** *A total of 2,400 medical records were reviewed between 2004 and 2005: 2,040 patients had type 2 DM, 240 subjects had type 1 DM, and 120 subjects had other kinds of diabetes.* ***B)*** *A total of 800 subjects who fulfilled the entry medical criteria were called to be examined by an experienced periodontist.* ***C)*** *A total of 481 of 800 subjects had dentures, 153 subjects did not match the periodontal criteria, and 131 subjects did not agree to participate in the study. Thirty-five subjects presented all of the clinical periodontal conditions and agreed to participate in the study.* ***D)*** *Baseline: clinical data collection and measurement of inflammatory markers and HbA1c.* ***E)*** *Periodontal therapy: SRP + placebo or SRP+Doxy. Medicine or placebo was administered for 2 weeks.* ***F)*** *Plaque control: Subjects received prophylaxis and oral hygiene instruction biweekly.* ***G)*** *Final examination: clinical data collection and measurement of inflammatory markers and HbA1c.*

Inflammatory Markers and HbA1c Measurements

Fifteen milliliters of blood (collected in three tubes) was obtained by venipuncture from each participant at baseline and at 3 months after therapy. Serum or plasma was collected by centrifugation, aliquoted, stored, and processed at the end of the study by masked staff.

For the metabolic assessment, peripheral blood samples were analyzed for HbA1c, and fasting plasma glucose (FPG) was determined in triplicate‖ in conjunction with commercial enzymatic kits.¶ The FPG was expressed in milligrams per deciliter, and it was measured using the glucose oxidase method. HbA1c was expressed as a percentage, and it was measured by high-pressure liquid chromatography.#

The inflammatory markers were identified simultaneously using flow cytometry multiplex assays (CMA).** The following mediators were analyzed: IL-1β, -2, -4, -5, -6, -7, -8, -9, -10, and -12 (p70); IP-10; MIP-1α and -1β; monocyte chemoattractant protein (MCP)-1; eotaxin; RANTES; monokine induced by interferon-gamma (MIG); basic fibroblast growth factor (bFGF); G-CSF; granulocyte macrophage colony-stimulating factor (GM-CSF); lymphotoxin-alpha (LT-α); TNF-α; interferon-gamma (IFN-γ); and soluble fas ligand (sFasL). The CMA was used according to the manufacturer's protocol. Briefly, standard mediators were solubilized in assay diluent. Ten microliters of the diluted sample (standards or test) were added to a 50-μl cocktail of capture beads and detector antibodies, and the mixture was incubated for 4 hours at room temperature in the dark. Excess unbound detector antibody was washed off before data acquisition. Two-color flow cytometric analyses were performed using a flow cytometer.†† A total of 2,000 events were acquired following the supplied protocol. Analysis was performed using the appropriate CMA analysis software.‡‡

Periodontal Treatment and Adjunctive Antibiotic Therapy

Subjects were randomly assigned to two groups: full-mouth SRP (SRP; N = 15) or SRP in combination with doxycycline,§§ 100 mg/day, for 2 weeks after an initial dose of 200 mg (SRP+Doxy; N = 15). The SRP group received placebo instead of doxycycline. The antibiotic therapy started on the day before the periodontal treatment. The SRP sessions were performed by the same operator in two to four sessions within 24 to 36 hours[25] using hand instruments‖‖ and an ultrasonic device¶¶ while the subjects were under local anesthesia. Teeth presenting periapic radiolucencies and severe periodontal destruction that deemed them to be unsalvageable were extracted. Oral hygiene was reviewed twice monthly, followed by prophylaxis, for 3 months.

Statistical Analysis

Mean CAL, PD, BOP, PI, SUP, missing teeth, HbA1c, FPG, and serum biomarkers were calculated for each subject as well as for each group, and comparisons between the two groups were made using a two-sample Student *t* test or the Mann-Whitney U test. The paired Student *t* test or Wilcoxon rank-sum test was used to compare the baseline values with those after 3 months. The χ^2 test was used for the frequency of subjects in the different PD categories. The data were tested for normality before applying the adequate parametric or non-parametric tests. The results were considered statistically significant when the *P* value was <0.05. Bonferroni correction was applied in the analysis of serum biomarkers, and the α level was adjusted downward.

RESULTS

Five of the initial 35 subjects were excluded from the study. Two subjects were eliminated because they did not finish the treatment phase, two subjects were eliminated because they had to use an anticoagulant agent, and one subject died. The final enrolled sample

‖ VP Super System Autoanalyzer, Abbott, Irving, TX.
¶ Labtest, Lagoa Santa, MG, Brazil.
# Labtest Sistemas para Diagnóstico, Lagoa Santa, MG, Brazil.
** Cytometric Bead Array, BD Bioscience, San Jose, CA.
†† FACCanto, Becton Dickinson, Sunnyvale, CA.
‡‡ FCap Array, Becton Dickinson.
§§ Vibramicina, Pfizer, São Paulo, SP, Brazil.
‖‖ Gracey curets, Hu-Friedy Instruments, Chicago, IL.
¶¶ Cavitron, Dentsply, York, PA.

consisted of 30 subjects with poorly controlled diabetes with an elevated to severe mean HbA1c serum level of 11.2%. Sixteen subjects (53.3%) were women, and 14 (46.7%) were men. Their mean age was 52.9 years, and they had an average of 21.1 teeth. The SRP group consisted of nine women and six men whose mean age was 53.5 ± 13.6 years, and the SRP+Doxy group consisted of seven women and eight men whose mean age was 52.3 ± 6.3 years (Table 1). At baseline, both groups had similar mean values for age, gender, plaque, and gingival bleeding, as well as for the severity of their periodontal disease, which was measured by PD and CAL. Both groups also had similar average values for the metabolic parameters (Table 1).

Periodontal Parameters

The periodontal treatment protocol (SRP or SRP+Doxy) resulted in a statistically significant improvement in almost all of the analyzed variables after 3 months. The overall (N = 30) results showed a mean PD reduction of 0.9 mm and a mean CAL gain of 0.7 mm. The plaque score decreased 23%, BOP decreased 38%, and no SUP was observed after 3 months. The PD and CAL reductions for the SRP group were 0.8 and 0.5 mm, respectively, and they were 1.1 and 0.9 mm, respectively, for the SRP+Doxy group. Thus, there were no statistically significant differences between the groups after 3 months. The generalized initial periodontal inflammation is noted because of the high prevalence of BOP: 49.1% for SRP and 51.3% for SRP+Doxy. After 3 months, the reductions were not different between the groups: SRP showed a reduction of 34.9%, and SRP+Doxy showed a reduction of 42.4%. The oral hygiene status was evaluated at baseline and twice a month for 3 months. Both groups showed significant reductions in PI scores; SRP showed a reduction of 22.2%, and SRP+Doxy showed a reduction of 23.8%, with no statistically significant difference between them. All individuals had periodontal pockets of up to 3 mm at baseline and after 3 months; however, the frequency of subjects who initially manifested deeper pockets was reduced after the periodontal treatment. The SRP+Doxy group had statistically fewer subjects with PD >4 mm after therapy (Fig. 2).

Metabolic Parameters

The normal healthy range for HbA1c levels is 4.5% to 6%. In our study, the mean HbA1c value was 10.7% for the SRP group and 11.8% for the SRP+Doxy group. Diabetes was poorly controlled in both groups. After 3 months, the periodontal therapy had caused a significant reduction in the average (N = 30) HbA1c levels (Fig. 3). The difference in HbA1c levels after 3 months was 0.9% for SRP and 1.5% for SRP+Doxy. These reductions represent a 7% improvement for SRP (*P* =

Table 1.

Characteristics of Study Population and Monitored Parameters (mean ± SD) at Baseline and 3 Months After Periodontal Therapy

Variable	SRP	SRP+Doxy	*P* Value*
Male (n)	6	8	
Female (n)	9	7	
Mean age (years)	53.5 ± 13.6	52.3 ± 6.3	0.746
PD (mm)			
Baseline	2.9 ± 0.8	3.0 ± 0.5	0.115
3 months	2.1 ± 0.3	1.9 ± 0.3	0.048
P value	0.0001	<0.0001	
Sites with PD ≤3 mm (n)			
Baseline	95.1 ± 31.1	87.5 ± 41.0	0.294
3 months	121.7 ± 34.5	105.8 ± 48.0	0.278
P value	0.001	0.238	
Sites with PD 4 to 5 mm (n)			
Baseline	26.7 ± 20.3	29.2 ± 13.9	0.693
3 months	8.0 ± 7.3	7.4 ± 7.7	0.879
P value	0.004	0.0001	
Sites with PD ≥6 mm (n)			
Baseline	12.6 ± 14.1	7.5 ± 5.2	0.232
3 months	3.7 ± 2.6	2.5 ± 0.7	0.576
P value	0.056	0.006	
CAL (mm)			
Baseline	10.2 ± 1.3	10.7 ± 1.6	
3 months	9.7 ± 1.4	9.8 ± 1.6	0.561
P value	0.0054	<0.0001	1.000
PI (%)			
Baseline	59.7 ± 18.8	69.9 ± 17.3	0.171
3 months	37.5 ± 19.0	46.1 ± 14.2	0.152
P value	<0.0001	<0.0003	
BOP (%)			
Baseline	49.1 ± 18.5	51.3 ± 14.9	0.787
3 months	14.2 ± 12.5	8.9 ± 4.8	0.319
P value	<0.0001	<0.0001	
SUP (%)			
Baseline	0.7 ± 1.4	1.6 ± 2.4	0.197
3 months	0 ± 0	0 ± 0	0.983
P value	0.2500	0.0078	
Remaining teeth (n)			
Baseline	21.9 ± 6.5	20.2 ± 6.7	0.481
3 months	21.7 ± 6.4	18.3 ± 7.0	0.125
P value	0.250	0.001	

Table 1. (continued)

Characteristics of Study Population and Monitored Parameters (mean ± SD) at Baseline and 3 Months After Periodontal Therapy

Variable	SRP	SRP+Doxy	*P* Value*
HbA1c (%)			
Baseline	10.7 ± 2.0	11.8 ± 1.6	0.062
3 months	9.8 ± 2.0	10.3 ± 2.3	0.590
P value	0.168	0.0068	
FPG (mg/dl)			
Baseline	219.1 ± 107.6	237.5 ± 84.3	0.340
3 months	197.4 ± 93.2	236.6 ± 95.1	0.171
P value	0.454	0.934	

**P* values represent intra- (vertical) and intergroup (horizontal) comparisons.

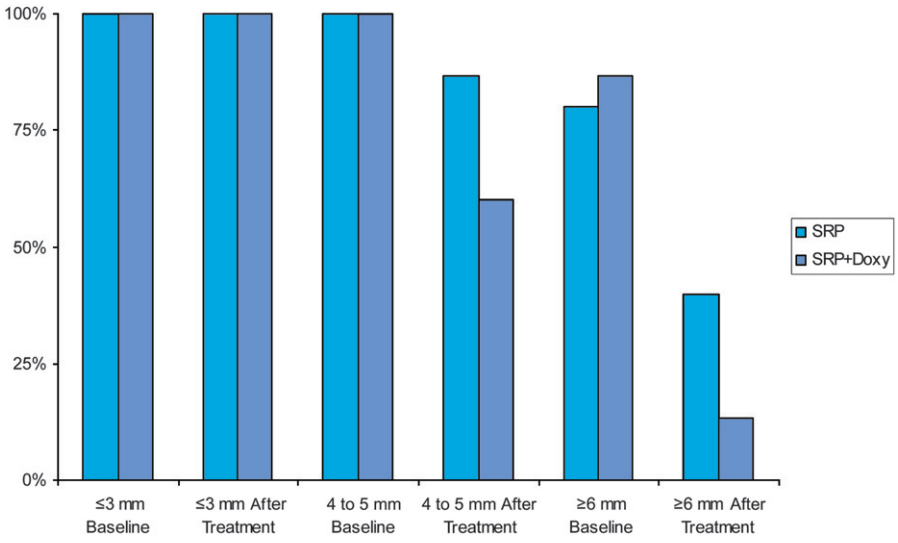

Figure 2.
Percentage of individuals at baseline and 3 months after treatment who had PDs in the respective categories. All subjects (100%) had sites with PD ≤3 mm. The percentage of subjects showing deeper PD values was reduced after therapy. SRP × SRP+Doxy (χ^2 = 16.76; P = 0.01).

0.17) and a 13% improvement for SRP+Doxy (*P* <0.01). Although SRP+Doxy demonstrated a greater reduction in HbA1c levels, the comparison between the groups did not show a statistical difference as measured by analysis of covariance (Fig. 4).

FPG levels were reduced slightly after therapy (from 228.3 to 217 mg/dl), but that reduction was not statistically significant. The differences in FPG levels were 21.7 mg/dl for SRP and 0.9 mg/dl for SRP+Doxy.

Immunologic Parameters

The periodontal treatment protocol led to improvement in 16 of the 24 cytokines that were analyzed after 3 months (Table 2). The overall (N = 30) mean reductions in IL-6, IP-10, sFasL, G-CSF, RANTES, and IL-12 (p70) were 47.6%, 13.2%, 15%, 27.4%, 6.5%, and 36%, respectively. These changes in cytokine and chemokine serum levels were statistically significant (*P* <0.05). However, when the Bonferroni correction was used, the only significant reductions were for G-CSF (*P* = 0.0002) and IL-12 (p70) (*P* = 0.0019). No differences were observed between the groups for any of the monitored serum mediators.

DISCUSSION

Previous clinical studies[3,26] showed that periodontitis and its treatment have the potential to alter glycemic control. These studies indicated that the successful management of periodontal infection is associated with a reduction in the local symptoms and markers of the disease.[21,23] Our study suggests that periodontal therapy improves glycemic control and reduces systemic proinflammatory mediators. However, these results may be influenced by dietary factors, which were not monitored during this investigation. In this regard, it is important to highlight the fact that alterations in systemic mediators may be caused by periodontal infection and factors such as glucose metabolism and other systemic conditions.

Considering that patients with diabetes have a higher risk for infection that is due to vascular alterations and poor healing responses, one-stage periodontal therapy within 24 to 36 hours was adopted to minimize the risk for reinfections[25] and to reduce the repeated trauma and edema that are responsible for the continued maintenance of high levels of proinflammatory cytokines. After treatment, there were improvements in all of the monitored clinical parameters. These improvements were reflected at the systemic level by alterations in inflammatory serum markers and, as verified in previous studies,[6,21,23] a reduction in glycated hemoglobin.

A previous study[22] by our group showed a significant reduction (25%) in PD after SRP. In this study, we observed a 25.2% reduction in PD after SRP; however, the association of SRP with systemic doxycycline provided a better result (37.6% reduction in PD). This superior result can be explained by the antimicrobial and additional anti-inflammatory effects of

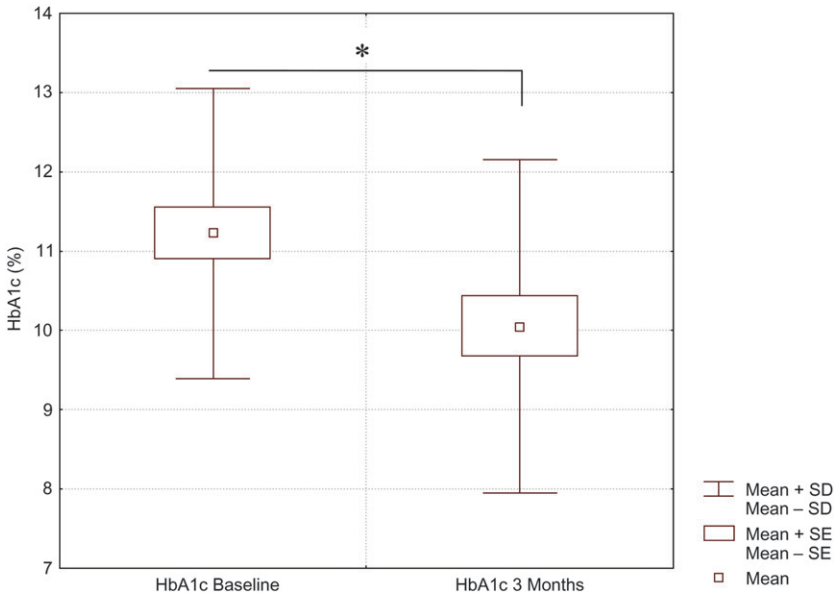

Figure 3.
*Effect of periodontal therapy on HbA1c levels after 3 months. A significant reduction in the HbA1c levels was seen after the periodontal therapy when considering the total sample (N = 30). *Statistically significant mean change from 11.2% to 10% accounted for a nearly 11% reduction in the HbA1c level (P = 0.0005).*

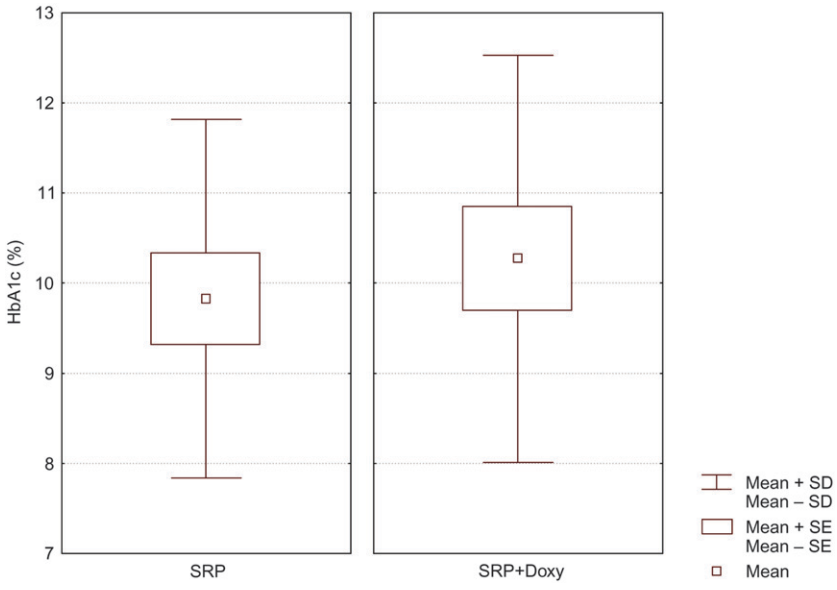

Figure 4.
Effect of periodontal therapy on HbA1c levels after 3 months for each group. Despite the fact that HbA1c baseline levels were different between the groups, a similar final HbA1c level was observed. No statistically significant differences were observed between the groups after 3 months (P = 0.529).

the drug.[23,27] The mean CAL change of 0.7 mm corroborates a previous study.[28]

Significant changes in biofilm and BOP were evident throughout the study. The overall mean improvement was 36.2% for PI and 77% for BOP. The reductions in PI represented improvements of 39.7% and 32.7% for SRP and SRP+Doxy, respectively. For BOP, the improvements were 72.5% and 80%, respectively. Other studies showed similar results. PI reductions were 30% to 34%,[23] 19% to 25%,[22] and 81%.[28] The reductions in BOP were 63% to 65%,[22] and 60%.[28]

The hypothesis that metabolic control can be improved by the successful treatment of periodontitis associated with systemic antibiotics as adjunctive therapy[21-23] was confirmed by this study. In addition to the periodontal improvement, a significant reduction in HbA1c levels was obtained, which represented a mean 10.7% improvement compared to baseline values. The SRP+Doxy group showed a significant reduction of 1.5%, which corresponded to a 13% improvement, whereas the SRP group showed a reduction of 0.9%, which corresponded to only a 7% improvement. These results are corroborated by previous studies,[21-23] but the superior result for the SRP+Doxy group may have been influenced by the baseline HbA1c levels and the individual dietary patterns.[29]

One study[23] verified a 10% HbA1c reduction after 3 months of non-surgical periodontal treatment combined with systemic doxycycline, whereas there was no significant change in the control group. Another investigation[21] that administrated 10 mg local minocycline showed a significant reduction in HbA1c levels after 1 month. The average reduction was 0.8%, which corresponded to a 10.5% reduction compared to baseline. In addition to the methodologic differences, it is important to address the level of metabolic control of the subjects at baseline. The subjects with poorly controlled diabetes in this study seemed to demonstrate the greatest differences between the initial and final levels of HbA1c. Therefore, a larger study with a balanced group is necessary to support this observation.

The use of doxycycline combined with mechanical periodontal treatment in subjects with diabetes is justified as follows. First, it is a broad-spectrum antibiotic that is effective against most periodontal pathogens,

Table 2.

Concentration of Serum Inflammatory Markers (pg/ml; mean ± SEM) at Baseline and 3 Months After Periodontal Therapy (N = 30)

Marker	Baseline	3 Months	*P* Value	Trend
IL-1β	0.3 ± 0.3	0.3 ± 0.3	1.000	=
IL-2	5.9 ± 2.8	5.0 ± 2.7	0.820	↓
IL-4	0.6 ± 0.2	0.5 ± 0.3	0.695	↓
IL-5	1.6 ± 0.3	1.1 ± 0.2	0.103	↓
IL-6	2.1 ± 0.3	1.1 ± 0.2	0.005*	↓
IL-7	1.2 ± 0.5	1.1 ± 0.4	0.946	↓
IL-8	9.0 ± 1.1	10.6 ± 2.5	0.621	↑
IL-9	0.7 ± 0.5	0.9 ± 0.6	0.875	↑
IL-10	1.0 ± 0.4	0.7 ± 0.2	0.804	↓
IL-12 (p70)	2.5 ± 0.2	1.6 ± 0.3	0.001†	↓
IP-10	90.8 ± 8.0	78.8 ± 6.8	0.014*	↓
MIP-1α	156.6 ± 101.9	157.2 ± 108.8	0.845	↑
MIP-1β	104.9 ± 14.3	94.4 ± 12.2	0.224	↓
MCP-1	122.2 ± 17.7	132.3 ± 18.3	0.210	↑
Eotaxin	86.1 ± 6.6	89.7 ± 7.2	0.396	↑
RANTES	18,026.0 ± 537.4	16,845.1 ± 545.0	0.029*	↓
MIG	267.3 ± 54.3	172.2 ± 26.8	0.338	↓
TNF-α	0.3 ± 0.3	0.3 ± 0.2	1.000	=
LT-α	0.4 ± 0.3	0.3 ± 0.2	0.250	↓
IFN-γ	2.8 ± 1.3	3.3 ± 2.0	1.000	↑
bFGF	0.4 ± 0.4	0.0 ± 0.0	1.000	↓
G-CSF	7.3 ± 0.5	5.3 ± 0.4	0.0002†	↓
GM-CSF	0.4 ± 0.2	0.1 ± 0.1	0.500	↓
sFasL	16.6 ± 1.7	14.1 ± 1.5	0.003*	↓

A marker is a substance used as an indicator of a biologic state.
= = stable; ↓ = downtrend; ↑ = uptrend.
* Significant difference ($P < 0.05$).
† Bonferroni correction ($P < 0.0021$).

and it reaches higher concentrations in the gingival fluid than in the serum,[30] providing an important adjunct for the reduction of periodontal pathogens. Second, it is a potent modulator of the host response in the subject with diabetes, as well as being a metalloproteinase inhibitor.[27] It also inhibits non-enzymatic glycation of extracellular proteins, and it may have a similar effect on the glycation of hemoglobin.[23] When amoxicillin with clavulanic acid was used as an adjunct to periodontal therapy, no additional effect on the HbA1c levels was observed.[22]

Neither group manifested a significant alteration in fasting glucose levels as was observed in previous studies.[21-23] However, the FPG level is susceptible to great oscillations; therefore, it is not considered a good indicator of glycemic control.

Sixteen of the 24 investigated serum mediators were higher at baseline than after the periodontal treatment. However, the serum mediator levels after SRP did not manifest differently from those after SRP+Doxy. Serum samples showed high individual variability in their cytokine profiles; no compelling association between their concentrations and the clinical parameters of periodontitis was possible. Conversely, some inflammatory mediators were related to periodontal infection and to diabetes as well, especially those that manifested statistically significant differences, such as IL-6 and -12 (p70), IP-10, sFasL, RANTES, and G-CSF.

In this study, therapy reduced the levels of chemokines, such as RANTES and IP-10 by 6.5% and 13%, respectively. Significantly elevated levels of these mediators were also found in inflamed periodontal tissues and/or in gingival crevicular fluid. This correlated with the presence of inflammatory cells infiltrate and, therefore, corroborated a positive correlation.[11,31]

IL-12 (p70), a proinflammatory cytokine induced by several pathogens or by pathogen-associated molecular patterns, including lipopolysaccharide,[32] experienced a 32% reduction after periodontal therapy. Increased levels of IL-12 and IL-12 (p40) protein/mRNA expression were found in inflamed periodontal tissues in experimental models, in subjects,[12,33] and in the biologic fluids from patients with periodontitis.[34] It also was reported that elevated glucose levels and diabetes increased IL-12 gene expression in mouse macrophages.[35] These findings suggest that diabetes and periodontal diseases may act synergistically to elevate IL-12 levels and may explain why subjects presented elevated serum levels of IL-12 before treatment.

Similarly, the levels of G-CSF that are produced by gingival keratinocytes after stimulation with bacteria or bacterial products[10] were reduced significantly (by 27%). Also, G-CSF is a critical factor for the neutrophil differentiation of bone marrow cells, neutrophil survival, and inflammatory neutrophil differentiation to effector cells in inflamed tissues.[36] Therefore, it is reasonable to suggest that reduced G-CSF levels after therapy may be a consequence of PI reduction and may be relevant to clinical improvements in PD, CAL, and BOP.

For the first time, to the best of our knowledge, our results provide evidence that sFasL is involved in periodontal diseases and that therapy reduced its level significantly (by 15%). sFasL is an alternative form of the transmembrane FasL molecule that triggers apoptosis on Fas-expressing cells. The presence of inflammatory cells, such as neutrophils, causes gingival tissue destruction, and the interaction of FasL and Fas expressed on osteoclast precursors did not induce apoptosis but signaled them to differentiate into mature osteoclasts.[35] Therefore, the upregulated sFasL levels in patients with diabetes and periodontitis may be correlated with the clinical severity in the periodontal tissues, and their reduction is associated with the attenuation of the clinical condition in treated patients. Also, this observation about sFasL levels can provide insights that are of diagnostic value for the evaluation of the severity of periodontal diseases and for monitoring the efficacy of therapy.

The role of IL-6 in the pathogenesis of periodontitis has been supported by studies[14,17,37,38] showing that IL-6 mRNA expression is significantly upregulated in inflamed gingival tissues. Also, IL-6 levels are elevated in the gingival crevicular fluid and serum of patients with chronic periodontitis[14] or diabetes.[4] IL-6 in periodontitis is mainly produced by non-hematopoietic cells,[37] and the upregulated condition is associated with tissue damage and disease recurrence.[38]

Also, increased circulating levels of IL-6 showed a significant correlation with HbA1c and the progression of diabetic nephropathy.[18] In a more general sense, an augmented acute-phase response mediated by IL-6 may explain many of the clinical and biochemical features of type 2 DM and its complications.[18] The periodontal therapy in this study significantly reduced IL-6 serum levels (by 48%) following the significant improvements in the clinical (PD, CAL, and BOP) and metabolic (HbA1c) measurements. However, other studies[39,40] did not report the same benefits.

The fact that some inflammatory mediators decreased and that clinical and metabolic improvements were demonstrated following periodontal therapy is a strong indicator of systemic health amelioration. It is hypothesized that non-surgical periodontal therapy leads to a significant reduction in the pathogenic bacteria load in biofilm. Then, it reduces the release of proinflammatory mediators, such as IL-6 and -12 (p70), IP-10, G-CSF, and RANTES, in the infected tissue and consequently moderates inflammatory cell chemotaxis and activation. The reduced levels of FasL may be an indication of reduced apoptosis and inflammation in the gingival tissues. The reduced levels of inflammatory cells and IL-6 lead to attenuated periodontal tissue damage. The reduced levels of IL-6 lead to improvements in diabetes by controlling HbA1c levels. In turn, the controlled diabetic condition attenuates the inflammatory process in the periodontal tissues by regulating chemokines and IL-12 (p70) production. Effective control of the inflammatory process by the reduction of pathogenic bacteria and of the diabetic condition provide a favorable periodontal tissue environment that allows tissue repair such as the clinical improvements observed in this study.

CONCLUSIONS

Anti-infective periodontal therapy and adjunctive systemic doxycycline may influence the systemic conditions of patients with type 2 DM. However, it is possible that the observed improvements in glycemic control and in the reduction of inflammatory markers might be due to diet, which was not controlled in our study. Therefore, a confirmatory study with a larger sample size and controlled diet is necessary.

ACKNOWLEDGMENTS

The sequence-determines-credit approach was applied for the sequence of authors. The study was financially supported by The State of São Paulo Research Foundation, São Paulo, SP, Brazil (grant 04/09844-8 to Dr. Taba) and the National Council for Scientific and Technological Development, Brasilia, DF, Brazil (grant 470638/2006 to Drs. Novaes and Taba). Dr. O'Connell was supported by a scholarship from the Coordination for the Improvement of Graduated Personnel. The authors thank Pfizer, Brazilian Division, São Paulo, SP, Brazil, for the donation of medicine. The authors also acknowledge the kind assistance of Analuiza S. Costa, Antônio Zanardo Filho, and Fabiana R. de Morais, laboratory technicians, Immunology Laboratory, School of Pharmaceutical Sciences, University of São Paulo-Ribeirão Preto. The authors report no conflicts of interest related to this study.

REFERENCES

1. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. *Diabetes Care* 2006;29: 1263-1268.
2. Oliver RC, Brown LJ, Löe H. Periodontal diseases in the United States population. *J Periodontol* 1998;69: 269-278.

3. Mealey BL, Oates TW. Diabetes mellitus and periodontal diseases. *J Periodontol* 2006;77:1289-1303.
4. Nishimura F, Takahashi K, Kurihara M, Takashiba S, Murayama Y. Periodontal disease as a complication of diabetes mellitus. *Ann Periodontol* 1998;3:20-29.
5. Soskolne WA. Epidemiological and clinical aspects of periodontal diseases in diabetics. *Ann Periodontol* 1998;3:3-12.
6. Stewart JE, Wager KA, Friedlander AH, Zadeh HH. The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *J Clin Periodontol* 2001;28:306-310.
7. Lalla E, Lamster IB, Stern DM, Schmidt AM. Receptor for advanced glycation end products, inflammation, and accelerated periodontal disease in diabetes: Mechanisms and insights into therapeutic modalities. *Ann Periodontol* 2001;6:113-118.
8. Taba M Jr., Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005;49:551-571.
9. Fokkema SJ, Loos BG, van der Velden U. Monocyte-derived RANTES is intrinsically elevated in periodontal disease while MCP-1 levels are related to inflammation and are inversely correlated with IL-12 levels. *Clin Exp Immunol* 2003;131:477-483.
10. Sugiyama A, Uehara A, Iki K, et al. Activation of human gingival epithelial cells by cell-surface components of black-pigmented bacteria: Augmentation of production of interleukin-8, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor and expression of intercellular adhesion molecule 1. *J Med Microbiol* 2002;51:27-33.
11. Kabashima H, Yoneda M, Nagata K, Hirofuji T, Maeda K. The presence of chemokine (MCP-1, MIP-1alpha, MIP-1beta, IP-10, RANTES)-positive cells and chemokine receptor (CCR5, CXCR3)-positive cells in inflamed human gingival tissues. *Cytokine* 2002;20:70-77.
12. Garlet GP, Avila-Campos MJ, Milanezi CM, Ferreira BR, Silva JS. *Actinobacillus actinomycetemcomitans*-induced periodontal disease in mice: Patterns of cytokine, chemokine, and chemokine receptor expression and leukocyte migration. *Microbes Infect* 2005;7:738-747.
13. Graves DT, Liu R, Alikhani M, Al-Mashat H, Trackman PC. Diabetes-enhanced inflammation and apoptosis – Impact on periodontal pathology. *J Dent Res* 2006;85:15-21.
14. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *J Dent Res* 2005;84:269-273.
15. Abbatecola AM, Ferrucci L, Grella R, et al. Diverse effect of inflammatory markers on insulin resistance and insulin-resistance syndrome in the elderly. *J Am Geriatr Soc* 2004;52:399-404.
16. Engebretson S, Chertog R, Nichols A, Hey-Hadavi J, Celenti R, Grbic J. Plasma levels of tumour necrosis factor-alpha in patients with chronic periodontitis and type 2 diabetes. *J Clin Periodontol* 2007;34:18-24.
17. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: An epidemiologic perspective. *Ann Periodontol* 2001;6:99-112.
18. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002;106:2067-2072.
19. Gamonal J, Bascones A, Acevedo A, Blanco E, Silva A. Apoptosis in chronic adult periodontitis analyzed by in situ DNA breaks, electron microscopy, and immunohistochemistry. *J Periodontol* 2001;72:517-525.
20. Janket SJ, Wightman A, Baird AE, Van Dyke TE, Jones JA. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *J Dent Res* 2005;84:1154-1159.
21. Iwamoto Y, Nishimura F, Nakagawa M, et al. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. *J Periodontol* 2001;72:774-778.
22. Rodrigues DC, Taba MJ, Novaes AB, Souza SL, Grisi MF. Effect of non-surgical periodontal therapy on glycemic control in patients with type 2 diabetes mellitus. *J Periodontol* 2003;74:1361-1367.
23. Grossi SG, Skrepcinski FB, DeCaro T, et al. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol* 1997;68:713-719.
24. Schara R, Medvescek M, Skaleric U. Periodontal disease and diabetes metabolic control: A full-mouth disinfection approach. *J Int Acad Periodontol* 2006;8:61-66.
25. Quirynen M, Mongardini C, Pauwels M, Bollen CM, Van Eldere J, van Steenberghe D. One stage full- versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. II. Long-term impact on microbial load. *J Periodontol* 1999;70:646-656.
26. Nishimura F, Murayama Y. Periodontal inflammation and insulin resistance – Lessons from obesity. *J Dent Res* 2001;80:1690-1694.
27. Sorsa T, Ingman T, Suomalainen K, et al. Cellular source and tetracycline-inhibition of gingival crevicular fluid collagenase of patients with labile diabetes mellitus. *J Clin Periodontol* 1992;19:146-149.
28. Kiran M, Arpak N, Unsal E, Erdogan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *J Clin Periodontol* 2005;32:266-272.
29. McNaughton SA, Mishra GD, Stephen AM, Wadsworth ME. Dietary patterns throughout adult life are associated with body mass index, waist circumference, blood pressure, and red cell folate. *J Nutr* 2007;137:99-105.
30. Lavda M, Clausnitzer CE, Walters JD. Distribution of systemic ciprofloxacin and doxycycline to gingiva and gingival crevicular fluid. *J Periodontol* 2004;75:1663-1667.
31. Sakai A, Ohshima M, Sugano N, Otsuka K, Ito K. Profiling the cytokines in gingival crevicular fluid using a cytokine antibody array. *J Periodontol* 2006;77:856-864.
32. Lee SM, Suen Y, Qian J, Knoppel E, Cairo MS. The regulation and biological activity of interleukin 12. *Leuk Lymphoma* 1998;29:427-438.
33. Yamazaki K, Nakajima T, Kubota Y, Gemmell E, Seymour GJ, Hara K. Cytokine messenger RNA expression in chronic inflammatory periodontal disease. *Oral Microbiol Immunol* 1997;12:281-287.
34. Tsai IS, Tsai CC, Ho YP, Ho KY, Wu YM, Hung CC. Interleukin-12 and interleukin-16 in periodontal disease. *Cytokine* 2005;31:34-40.
35. Park HJ, Park OJ, Shin J. Receptor activator of NF-kappaB ligand enhances the activity of macrophages as antigen presenting cells. *Exp Mol Med* 2005;37:524-532.

36. Christopher MJ, Link DC. Regulation of neutrophil homeostasis. *Curr Opin Hematol* 2007;14:3-8.
37. Takahashi K, Takigawa M, Takashiba S, et al. Role of cytokine in the induction of adhesion molecules on cultured human gingival fibroblasts. *J Periodontol* 1994;65: 230-235.
38. Lee HJ, Kang IK, Chung CP, Choi SM. The subgingival microflora and gingival crevicular fluid cytokines in refractory periodontitis. *J Clin Periodontol* 1995;22: 885-890.
39. Talbert J, Elter J, Jared HL, Offenbacher S, Southerland J, Wilder RS. The effect of periodontal therapy on TNF-alpha, IL-6 and metabolic control in type 2 diabetics. *J Dent Hyg* 2006;80:7.
40. Ide M, McPartlin D, Coward PY, Crook M, Lumb P, Wilson RF. Effect of treatment of chronic periodontitis on levels of serum markers of acute-phase inflammatory and vascular responses. *J Clin Periodontol* 2003; 30:334-340.

Correspondence: Dr. Mario Taba Jr., Department of Oral Surgery and Periodontology, University of São Paulo–Ribeirão Preto School of Dentistry, Avenida do Café – s/n, CEP 14040-904, Ribeirão Preto, SP, Brazil. E-mail: mtaba@forp.usp.br.

Submitted May 2, 2007; accepted for publication November 21, 2007.