Microbiological changes after periodontal therapy in diabetic patients with inadequate metabolic control

Abstract: The present study investigated the effect of non-surgical periodontal treatment (SRP) on the composition of the subgingival microbiota of chronic periodontitis (CP) in individuals with type 2 diabetes (DM2) with inadequate metabolic control and in systemically healthy (SH) individuals. Forty individuals (20 DM2 and 20 SH) with CP underwent full-mouth periodontal examination. Subgingival plaque was sampled from 4 deep sites of each individual and tested for mean prevalence and counts of 45 bacterial taxa by the checkerboard method. Clinical and microbiological assessments were performed before and 3 months after SRP. At baseline, those in the DM2 group presented a significantly higher percentage of sites with visible plaque and bleeding on probing compared with those in the SH group (p < 0.01). Those in the DM2 group presented significantly higher levels of C. rectus and P. gingivalis, and lower prevalence of P. micra and S. anginosus, compared with those in the SH group (p ≤ 0.001). At the 3-month visit, both groups showed a significant improvement in all clinical parameters (p < 0.01). Those in the DM2 group showed significantly higher prevalence and/or levels of A. gerencseriae, A. naeslundii I, A. oris, A. odontolyticus, C. spu-tigena, F. periodonticum, and G. morbillorum compared with those in the SH group (p < 0.001). However, those in the DM2 group showed a significant reduction in the levels of P. intermedia, P. gingivalis, T. forsythia, and T. denticola (p ≤ 0.001) over time. Those in the SRP group showed improved periodontal status and reduced levels of putative periodontal pathogens at 3 months’ evaluation compared with those in the DM2 group with inadequate metabolic control.

Keywords: Diabetes Mellitus; Periodontal Debridement; Bacteria.

Introduction

Chronic hyperglycemia in diabetes mellitus (DM) is associated with an increased risk of development of systemic complications over the years, including microangiopathy, neuropathy, nephropathy, microvascular disease, and delayed wound-healing. Moreover, patients with DM have worse periodontal conditions compared with their non-diabetic counterparts. Persons with poorly controlled DM also present more severe periodontal disease than do those with well-controlled DM. Several mechanisms have been proposed to explain the periodontal etiopathogenesis in patients with DM. Significant differences may be present in...
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However, there are conflicting findings which have shown that poorly controlled insulin-dependent individuals with DM may have elevated levels of microbiota, lower prevalence of pathogenic species, or even a microbiota similar to that of non-DM individuals. Differences among studies may be explained by the metabolic control used. Scaling and root planing (SRP) therapy, in combination (or not) with antimicrobial agents, might be effective in reducing periodontal pathogens in persons with DM, although others have reported that SRP alone may not be capable of eliminating or reducing Tannerella forsythia and Porphyromonas gingivalis in those individuals. In general, a reduced level of those pathogens is crucial for a good response to therapy. Analysis of data in the literature shows that it is unclear whether the subgingival microbiota in persons with DM is significantly affected by SRP. Therefore, the aim of the present study was to investigate the effect of SRP on the composition of the subgingival microbiota in individuals with CP and DM type 2 with inadequate metabolic control (DM2), and in systemically healthy patients (SH).

Methodology

Study Population

The study population has been described in a previous report. The participants were recruited at the Faculdade de Odontologia de Araraquara of the Universidade Estadual Paulista (FOAR/Unesp), Brazil. Informed consent was obtained from all individuals. Twenty participants (12 females; mean age, 45.80 ± 6.01 years) had DM2, inadequate glycemic control (glycated hemoglobin A1c-HbA1c ≥ 7%), and mean DM duration of 9.85 ± 7.10 years; and 20 SH participants (10 females; mean age, 43.65 ± 6.01 years) had no DM, as confirmed by a fasting plasma glucose test. The patients did not receive any instruction on glycemia control, to avoid a bias due to interference in the effect of the periodontal treatment. Also, there was no intervention by doctors regarding medication. The sample size was calculated based on a pilot study. It was estimated that, with a minimum of 20 participants per group, significant differences in the clinical data would be detected between the study periods with 80% statistical power and a 95% confidence interval (unpublished data). The sample size calculation for microbiological analysis was based on a large microbiological database of over 400 individuals in our population evaluated over 8 years. That sample size calculation was performed considering a difference of 1 x 10^4 ± 0.9 x 10^4 cells in the reduction of mean counts of the main outcome variable, “red complex” (Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia), between groups after therapy. A number of 14 individuals was estimated for each group with an error of 5% and a power of 80%. The study protocol was approved by the Ethics in Human Research Committee of the FOAR-UNESP (no. 85/04). All participants had a diagnosis of chronic periodontitis, at least 15 teeth (except third molars), and moderate to severe CP—i.e., at least 4 teeth with one or more sites with probing depth (PD) ≥ 5 mm, clinical attachment level (CAL) ≥ 4 mm, and bleeding on probing (BOP). Exclusion criteria included pregnancy, use of contraceptives or other female hormones, smoking or cessation of smoking for ≤ 5 years, use of local or systemic antimicrobial agents 6 months and anti-inflammatory drugs 3 months prior to the study, and periodontal treatment in the preceding year. Participants with DM2 were under the supervision of an endocrinologist, with no alteration in DM treatment in the year prior to the study. Glycemic control was evaluated by the %HbA1c with high-performance liquid chromatography (DiaSTAT Hemoglobin A1c Analyzer System, BioRad Lab., Hercules, USA) at baseline, immediately before and 3 months after SRP.

Clinical assessment and therapy

Periodontal measurements were performed by one calibrated examiner (kappa = 0.90; D.G.), who was blinded for group assignment, using a manual periodontal probe (North Carolina Probe, Hu-Friedy, Chicago, USA) at 6 sites per tooth in all teeth, except third molars, at baseline and 3 months after SRP. Measurements included PD and CAL, BOP, suppuration on probing (SUP), and visible plaque (VPI) and gingival marginal bleeding (GBI) indices. The SRP was performed by a single experienced periodon-
tist (F.O.B.C.) who was not aware of the group distribution, as follows: oral hygiene instructions and 4 weekly sessions of SRP (1 hour each) under local anesthesia, and professional plaque control twice a month for 3 months. All participants returned on the scheduled days for professional plaque control.

**Microbiological assessment**

The prevalence and levels of 45 bacterial taxa were determined by the checkerboard DNA-DNA hybridization method. After removal of supragingival biofilm, subgingival biofilm samples were taken from 4 deep sites (characterized as PD ≥ 5 mm, CAL ≥ 4 mm, and BOP) in different non-adjacent teeth at baseline and at the 3-month visit after SRP, with sterilized Gracey curettes (Trinity Periodontia, São Paulo, Brazil) and placed in individual tubes. Bacterial cells were subjected to lysis by the addition of 0.5 M NaOH and boiling, and denatured DNA was fixed on a nylon membrane (GE Healthcare Life Science, São Paulo, Brazil) by means of the Minislot 30 device (Immunetics, Cambridge, USA). The membrane was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device, and hybridized against digoxigenin-labeled (Roche Applied Science, São Paulo, Brazil) whole-genomic DNA probes for the selected species. After hybridization, the membranes were washed at high stringency, bound probes were detected with phosphatase-conjugated antibody to digoxigenin (Roche Applied Science), and fluorescence was captured by an imaging system (Storm™860, GE Healthcare Life Science, São Paulo, Brazil). Signals were evaluated visually by comparison with the standards at 10^5 and 10^6 bacterial cells for the test species on the same membrane. They were recorded as: 0, not detected; 1, < 10^5 cells; 2, approximately 10^5; 3, 10^5-10^6 cells; 4, approximately 10^6; and 5, >10^6 cells.

**Statistical analysis**

The participant was the unit of analysis in a statistical program (IBM SPSS Statistics Version 19, IBM, Armonk, USA). Clinical parameters and the microbiological data were calculated for each participant and then across the group. Microbial data were expressed as mean % of colonized sites (prevalence), and mean counts (mean of bacterial cells) were log₁₀-transformed for each species. The mean counts were determined by transforming the scores 0 to 5 in bacterial counts. Clinically significant differences between groups were determined by the Mann-Whitney test. Significant differences in microbiological parameters between groups at baseline were determined by the Mann-Whitney test. Differences in microbiological changes in diseased sites within and between groups over time were evaluated by General Linear Models (GLM) for repeated measures. Any difference between groups detected in the period of reference was controlled in the multivariate model by GLM evaluation. Significant differences within groups over time were calculated by the Wilcoxon signed-rank test. For microbial analysis, adjustments were made for multiple comparisons as described by Socransky et al. In brief, an overall p = 0.05 = 1-(1-k)^45 was computed, where k was the desired individual p value. Thus, a p value ≤ 0.001 was considered to be statistically significant at p < 0.05.

**Results**

There were no significant differences regarding gender and age between groups (data not shown). The glycemic control in those with DM2 showed no significant reduction in HbA1c mean values from baseline (9.43 ± 1.80) to the 3-month visit (9.03 ± 1.94; p = 0.79, Wilcoxon test; data not shown). At baseline, those with DM2 presented significantly higher mean percentages of VPI and BOP compared with those in the SH group (p < 0.001, Mann-Whitney test; Table 1). At the 3-month visit, both groups showed a significant improvement in all clinical parameters (p < 0.01, GLM test). Those in the SH group showed a significant reduction in VPI compared with those with DM2, while the latter showed a greater reduction in mean CAL than the former over time (p < 0.05, GLM test). For further information regarding clinical data, please refer to Gonçalves et al.

At baseline, those in the SH group harbored a greater number of species with high prevalence (> 50% of the sampled sites) than those with DM2, including various species of streptococci, *Fusobacterium nucleatum*, *Prevotella intermedia*, members of the red complex (*P. gingivalis*, *Treponema denticola*, *T.
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Table 1. Mean (± SD) of the periodontal clinical parameters for the control (SH) and diabetes (DM2) groups at baseline and 3 months after SRP.

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>SH (N = 20)</th>
<th>DM2 (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3 months</td>
</tr>
<tr>
<td>VPI (%)</td>
<td>65.3 ± 14.9</td>
<td>8.1 ± 12.8†</td>
</tr>
<tr>
<td>GBI (%)</td>
<td>38.4 ± 16.5</td>
<td>2.9 ± 2.3†</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>76.1 ± 16.1</td>
<td>15.8 ± 10.7†</td>
</tr>
<tr>
<td>SUP (%)</td>
<td>7.5 ± 7.1</td>
<td>0.2 ± 0.5†</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>3.6 ± 0.6</td>
<td>2.4 ± 0.3†</td>
</tr>
<tr>
<td>CAL (mm)†</td>
<td>4.3 ± 1.0</td>
<td>3.9 ± 0.8†</td>
</tr>
<tr>
<td>PD ≥ 5 mm (%)</td>
<td>27.9 ± 11.2</td>
<td>4.2 ± 4.2†</td>
</tr>
<tr>
<td>CAL ≥ 5 mm [%]</td>
<td>42.3 ± 18.5</td>
<td>36.4 ± 21.3†</td>
</tr>
</tbody>
</table>

*p < 0.05, between SH and DM2 at baseline (Mann-Whitney test); †p < 0.001, between baseline and 3 months after SRP (GLM); ‡p < 0.05, between groups over time (GLM). VPI, visible plaque; GBI, gingival marginal bleeding; SUP, suppuration on probing; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

*P. intermedia, P. gingivalis, T. denticola, and T. forsythia, and Neisseria mucosa (Figure 1). However, only Parvimonas micra and Streptococcus anginosus presented significantly higher prevalence in those in the SH group compared with those in the DM2 group (p ≤ 0.001, Mann-Whitney test). At the 3-month visit, those with DM2 showed significantly higher prevalence of Actinomyces naeslundii I, Actinomyces oris, Actinomyces odontolyticus, and Gemella morbillorum than those in the SH group (p ≤ 0.001). Changes over time within groups showed that those in the SH group had a significant decrease in species of the orange (F. nucleatum polymorphum, F. nucleatum nucleatum, P. intermedia, and Prevotella nigrescens) and red (P. gingivalis, T. forsythia, T. denticola) complexes, Aggregatibacter actinomycetemcomitans a, and N. mucosa (adjusted p ≤ 0.001, Wilcoxon test), whereas those with DM2 had a significant decrease only in the members of the red complex and G. morbillorum (p ≤ 0.001). In general, both groups showed a reduction in bacterial load (Figure 2). At baseline, those with DM2 presented significantly higher levels of Campylobacter rectus and P. gingivalis and lower levels of P. micra and S. anginosus compared with SH individuals (adjusted p ≤ 0.001, Mann-Whitney test). After therapy, those with DM2 harbored significantly higher levels of Actinomyces gerencseriae, A. odontolyticus, A. naeslundii I, A. oris, Capnocytophaga sputigena, Fusobacterium periodonticum, and G. morbillorum (adjusted p ≤ 0.001) than SH individuals. Significant reductions in mean levels of several periodontal pathogens were seen in SH individuals over time (adjusted p ≤ 0.001, Wilcoxon test). Conversely, only P. intermedia, P. gingivalis, T. forsythia, and T. denticola showed a significant decrease in mean levels in those with DM2 after therapy (adjusted p ≤ 0.001). In addition, there was a significant increase from baseline to the 3-month visit in the levels of Staphylococcus aureus in those with DM2 (adjusted p ≤ 0.001).

**Discussion**

Patients with DM and poor glycemic control present more severe gingival inflammation and periodontal destruction compared with individuals without DM and/or those with well-controlled DM.21,28 In the current study, those with DM2 and inadequate metabolic control presented significantly higher extensions of VPI and BOP than did SH individuals at baseline. Although those with DM2 presented greater values for other inflammation parameters and periodontal destruction, no significant differences between groups were observed. SRP resulted in significant improvements in all clinical parameters in both groups, corroborating results from previous studies.6,8,14,28 Significant differences between groups were seen for percentages of VPI and mean CAL. Despite a rigorous periodontal maintenance program and a considerable clinical improvement after treatment, those with DM2 still presented higher percentages of sites with VPI than did SH individuals.

Periodontal disease may affect those with DM directly through chronic inflammatory alterations.29 The presence of inflammation plays an important role in the development of systemic complications associated with DM.
role in insulin sensitivity and glucose dynamics. Clinical studies have demonstrated that improvement in metabolic control correlates with improvement in periodontal health. However, in the current study, while the improvement in the periodontal condition observed in those with DM had some

*Significant differences between groups at baseline (adjusted p ≤ 0.001, Mann-Whitney test).
†Significant differences between groups at 3 months post-therapy (adjusted p ≤ 0.001, Mann-Whitney test).
‡Significant differences within the groups over time (adjusted p ≤ 0.001, Wilcoxon test).

Figure 1. Mean prevalence of subgingival species evaluated in biofilm samples from individuals with type 2 diabetes (DM2) and systemically healthy (SH) individuals with chronic periodontitis, at baseline and 3 months after SRP. The species were ordered according to the bacterial complexes. 25 Aa: Aggregatibacter actinomycetemcomitans.

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The study, so the obtained effect might simply be a result of the periodontal treatment. Analysis of microbiological data at baseline showed that both groups presented high mean counts and prevalence of periodontal pathogens, such as members of the red and orange complexes. Impact on glycemic control, it did not reach statistical significance. Moreover, glycemic control is influenced by other variables, such as diet, weight control, physical exercises, and use of medication to control glycemic level. So that bias would be avoided, none of those variables underwent any alteration during the study, so the obtained effect might simply be a result of the periodontal treatment.

Figure 2. Mean counts (in log10 ± SEM) of subgingival species evaluated in biofilm samples from individuals with type 2 diabetes (DM2) and systemically healthy (SH) individuals with chronic periodontitis at baseline (left panel) and 3 months (right panel) after SRP. Aa: Aggregatibacter actinomycetemcomitans.
However, those with DM2 presented significantly higher bacterial levels of *C. rectus* and *P. gingivalis* compared with SH individuals. Mandell et al. also observed increased levels and prevalence of *C. rectus*, as well as other periodontal pathogens, at diseased sites in those with poorly controlled insulin-independent DM. Other species, such as *P. gingivalis*, *A. actinomycetemcomitans*, *Campylobacter spp.*, *Capnocytophaga spp.*, and *Eubacterium nodatum*, can be detected at high frequency in those with DM. Conversely, Cruz et al. observed a low frequency of sites with *T. forsythia* in those with DM. In accordance with other studies, Field et al. demonstrated that the amounts of *A. actinomycetemcomitans*, *F. nucleatum*, and *P. gingivalis* did not differ between individuals with periodontitis, with and without DM. Hintao et al. did not detect any significant difference in 17 subgingival species using the checkerboard method in participants with and without DM.

Analysis of baseline data showed that those with DM2 had a ‘worse’ microbiological profile when compared with those in the SH group; nevertheless, this was not a key factor preventing a good clinical response to SRP. Overall, the microbiological findings regarding changes in bacterial prevalence and levels over time demonstrated that a greater number of species were reduced in SH individuals than in those with DM2, although many of those species are considered beneficial. Significant differences between groups were observed only for *Actinomyces spp.*, *C. sputigena*, *F. periodonticum*, and *G. morbillorum*. Even though more species diminished in SH individuals after therapy, pathogenic species had a significant decrease in prevalence and/or levels, while host-compatible species such as *Actinomyces spp.* and *G. morbillorum* were increased in those with DM2. That is in agreement with results from studies showing that SRP can lead to a shift from pathogenic to beneficial species in the subgingival environment. The levels of the species *S. aureus*, which can be associated with periodontitis, increased significantly in those with DM2 after SRP. Again, longitudinal analysis showed that individuals with DM2 presented a different microbiological profile compared with SH individuals after therapy. As mentioned previously, differences in microbiological profile in those with DM2 can be due to the impaired inflammatory response, which can lead to a favorable environment for proteolytic species. Conversely, a differentiated prognosis should always be considered, not only because of the inflammatory characteristics of DM2, but also due to their microbiological features. As a result, close attention should be given to individuals with DM2 during the maintenance phase. Moreover, species considered to be host-compatible, such as *Actinomyces spp.* and *Streptococcus spp.*, showed no significant variations from baseline to the 3-month visit. The majority of *Streptococcus spp.* and a few species of the genus *Actinomyces* presented a modest increase in counts and prevalence after treatment in both groups. These results are in agreement with those from other studies demonstrating that mechanical treatment can result in a shift from pathogenic to beneficial species in the subgingival environment. The results found in the group with DM2 regarding this matter are important, because they show that individuals with DM2 are also able to present an increase in the levels of those species that will help in the maintenance of a balanced subgingival environment. Mechanical periodontal therapy alone may not be capable of eliminating *T. forsythia* and *P. gingivalis* in individuals with DM. Other studies reported a reduction in the prevalence of these pathogens when SRP was used in combination with antimicrobials. In the present study, those species were not eliminated but had significantly reduced levels, which enabled a good clinical response to be obtained, at least at 3 months after treatment in both groups. In contrast, other studies observed a modest and non-significant reduction in the levels of these species after full-mouth subgingival debridement in individuals with DM. However, these authors used only one or two sessions in contrast to four sessions of SRP and a rigorous periodontal maintenance program as in the present study.

**Conclusion**

SRP associated with a rigorous maintenance program improved the periodontal status and reduced the levels of putative periodontal pathogens at 3 months’ evaluation in individuals with DM2 and inadequate metabolic control compared with SH individuals.
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