Original Research
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Supragingival biofilm control and systemic inflammation in patients with type 2 diabetes mellitus

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Abstract: The objective of this study was to evaluate the effect of strict supragingival biofilm control on serum inflammatory markers and on periodontal clinical parameters in type 2 diabetes mellitus (T2DM) patients with chronic severe periodontitis. Twenty-four individuals with T2DM and periodontitis were randomly allocated to two treatment groups. The supragingival therapy group (ST, n = 12) received supragingival scaling, whereas the intensive therapy group (IT, n = 12) underwent supra- and subgingival scaling, as well as root planing. Patients from both groups received professional oral hygiene instructions every month. Data regarding visible plaque index (VPI), gingival bleeding index (GBI), bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment level (CAL), serum levels of interleukin (IL)-6, IL-17A, IL-8, tumor necrosis factor α (TNF-α), monocyte chemoattractant protein (MCP)-1 enzyme-linked immunosorbent assay (ELISA), and glycated hemoglobin (HbA1c) levels were obtained at baseline and at 6 months post-therapy. Both therapies resulted in the improvement of almost all clinical periodontal parameters ($p < 0.05$). There were no differences in TNF-α, IL-8, IL-17A and HbA1c levels in either group ($p > 0.05$), between the two periods. However, MCP-1 levels were significantly reduced in both the ST ($p = 0.034$) and the IT ($p = 0.016$) groups, whereas the serum IL-6 levels were significantly reduced only in the IT group ($p = 0.001$). Strict control of supragingival biofilm has a limited effect on systemic inflammatory markers, and a moderate effect on periodontal clinical parameters.

Keywords: Diabetes Mellitus; Periodontitis; Cytokines.

Introduction

Periodontitis is a destructive form of periodontal disease (PD), affecting approximately 50% of all adults and more than 60% of over 65-year-olds. Severe periodontitis is known to affect approximately 10%-15% of the population.[1,2] Periodontal destruction is mediated by the host's immune-inflammatory response[3] elicited by pathogen-dominated biofilms. This response leads to the production of specific antibodies and inflammatory mediators within the local tissues, as well as a systemically elicited response.[4]

Cytokines participate actively in the initiation and maintenance of immune responses to oral bacteria.[5] An imbalance between pro- and anti-inflammatory cytokines leads to the destruction of periodontal tissue and to clinical signs of periodontal disease.[6] The local production of cytokines in response to periodontal bacteria is related to serum concentrations of pro-inflammatory cytokines.[7] Non-surgical periodontal therapy promotes the reduction of bacterial challenge,[8,9,10] and may also affect the inflammatory burden.[11]

Diabetes and PD are highly prevalent in the population, and share a common etiopathogenesis.[12] Type 2 diabetes mellitus (T2DM) is a recognized risk factor for PD, affecting its prevalence, incidence, and severity;[13,14] the relationship between the two disorders is bidirectional[12]. PD may represent an additional factor contributing to the total inflammatory burden in individuals with T2DM. Furthermore, periodontal treatment has been shown to improve metabolic control in T2DM.[15,16]

The role of periodontal treatment in serum inflammatory markers is somewhat conflicting, possibly because of differences in periodontal therapy protocols (time of follow-up and use of local/systemic antimicrobials). Some studies have shown considerable changes in the serum levels of IL-6 and TNF- α,[17,18] whereas others have disclosed no significant changes in TNF- α,[19] IL-6,[20] and IL-8.[21] To the best of our knowledge, there is no information regarding the effect of strict supragingival biofilm control on inflammatory burden. Thus, the purpose of this study was to test the null hypothesis that supragingival scaling does not have beneficial effects on the serum levels of inflammatory markers or on periodontal clinical parameters in patients with chronic generalized periodontitis and T2DM, compared with the effects of intensive periodontal therapy (supra- and subgingival scaling, as well as root planing).

Methodology

Study population

Patients selected for the study were recruited between February 2011 and December 2013 in complete accordance with the Helsinki Declaration of 1975, as revised in 2000. Ethical approval was received by the Ethics Committee of the *Universidade de São Paulo* – SP, São Paulo, Brazil (FR 284313-127/2009 protocol). All patients with T2DM were diagnosed according to the World Health Organization classification,[22] and remained under the supervision of an endocrinologist. The participants were treated with a dietary intervention and/or oral hypoglycemic agents.

The inclusion criteria were as follows: individuals ≥ 35 years of age, confirmed diagnosis of T2DM for a period of over 3 years, generalized severe chronic periodontitis (number of probing pocket depth [PPD] sites ≥ 30%, clinical attachment level [CAL] > 4 mm, and bleeding on probing), and ≥ 15 teeth. Pregnant women, smokers, patients with body mass index (BMI) > 35 kg/m^2, or those who had received periodontal therapy, systemic antibiotic, or oral antiseptic therapy 6 months prior to the study were excluded.

Sample size calculation was undertaken by assuming a reduction of 2 mm in mean pocket depth, with 0.6 mm standard deviation in the IT group, and 1 mm mean pocket depth reduction, with 0.6 mm standard deviation in the ST group (90% statistical power and 5% significance level). The required sample size for each group was determined as 11, and 12 patients were recruited to account for potential dropouts and missing data.

Periodontal Clinical Data

The presence of supragingival biofilm was recorded as visible plaque index (VPI),[23] whereas marginal gingival bleeding was recorded as gingival bleeding index (GBI).[23] Bleeding on probing (BOP), PPD, and CAL were also evaluated. The North Carolina manual probe was used in this study, (Hu-Friedy®, Chicago, USA); VPI, GBI, and BOP were recorded as (0) absent, or (1) present. Clinical examinations were performed by two blinded and calibrated examiners (H.P.C.A. and A.M. F.) on six sites per tooth (excluding the third molars), at baseline and at 6 months after periodontal therapy. Reproducibility during the study was assessed in 10% of the participants, with intraclass correlation coefficients ranging from 0.90 to 0.94 for periodontal PPD, and 0.85 to 0.89 for CAL.

Experimental Design and Periodontal Therapy

This study comprised 24 individuals randomly assigned by a computer random number generator (Excel), and allocated by sequentially numbered sealed opaque envelopes. All individuals were given oral hygiene instructions. The supragingival therapy group (ST, n = 12) received supragingival scaling using an ultrasonic device and periodontal curettes (Hu-Friedy®, Chicago, USA). A Single appointment lasted ~ 60 minutes. The intensive therapy group (IT, n = 12) received supra- and subgingival scaling and root planing, (in sites with PPD ≥ 4 mm) using an ultrasonic device and periodontal curettes. The procedures for the IT group were performed under local anesthesia (3% prilocaine with felypressin), in two appointments lasting ~ 120 minutes each. Supportive therapy for biofilm control consisted of professional instructions on oral hygiene given to each patient every month. Periodontal therapy was carried out by an experienced periodontist (G.H.G.). No changes in medication or diet were made during the study period.

Inflammatory Markers and HbA1c Measurements

Samples of approximately 5 mL of blood were collected by venipuncture and placed in untreated Vacutainer blood collection tubes (Becton Dickinson Co., São Paulo, Brazil). An experienced nurse technician (J.P.R.) performed the blood collections. Glycated hemoglobin (HbA1c) was analyzed by high-performance liquid chromatography (DiaSTAT Hemoglobin A1c Analyzer System, BioRad Laboratories, Hercules, USA), at the clinical laboratory located in the *Hospital Universitário* – HU (University Hospital), at the USP. The serum was obtained by centrifugation at 2,000 g for 10 minutes, aliquoted, and stored at -80 °C until further analysis.

Quantitative measurements of IL-6, IL-8, IL-17, TNF-α and MCP-1 were assayed by enzyme-linked immunosorbent assay (ELISA) (Peprotech Inc., Rockyhill, USA), according to the manufacturer's instructions, whereas the optical density was determined using a Micro Plate reader model 680 (BioRad Laboratories Inc., Hercules, USA). Serum from each patient was tested in triplicates.

Statistical analysis

Data from the ELISA were analyzed using Prism software (Prism 5 Project, Graphpad Software Inc., La Jolla, USA). Shapiro-Wilk normality test was used and the data did not follow Gaussian distribution. The data were analyzed by non-parametric statistical methods. Periodontal clinical data were analyzed using SPSS software (SPSS for Windows, version 17.0, SPSS Inc., Chicago, USA), considering the individual as a study unit. Intergroup comparison was determined using the Mann-Whitney test. The intragroup comparison was determined using the Wilcoxon signed rank test. The tests were based on the median values with variability measures (25% and 75% quartiles). The results were considered statistically significant at $p < 0.05$. The GBI, VPI, and BOP data were obtained by calculating percentages of positive sites per patient, and thereafter, median values were calculated for the groups. For PPD and CAL, measured in millimeters, the median value per patient was obtained first, followed by the median value for the group.

Results

All patients selected for analysis in the present study completed 6 months of the clinical trial. The ST group was comprised of 56.3% women (mean age, 54.4 ± 5.8 years; BMI, 25.6 ± 4.4), whereas the IT group was comprised of 52.0 % women (mean age, 52.0 ± 3.3 years; BMI, 26.9 ± 3.8). Both groups were similar in age, gender, and BMI at baseline ($p > 0.05$).

No significant differences in periodontal parameters were observed between the two groups at baseline (Table 1). Both therapies resulted in statistically significant improvements in most clinical parameters after 6 months of follow-up, except for CAL in the ST group. However, the effects on BOP and PPD were more pronounced in the IT group, with a significant gain in CAL observed in this group only (Table 1). Changes in periodontal clinical parameters are represented in Table 2. The effectiveness of periodontal treatment on PPD (4–6 mm; ≥ 7 mm) and CAL (4–6 mm; ≥ 7 mm) was observed in both groups. Changes in PPD (4–6 mm) ($p = 0.001$) and CAL (4–6 mm) ($p = 0.001$) were found to be significantly different between the two groups in this study (Table 2).

Table 1. Medians values (75th/25th percentiles) for clinical periodontal parameters at baseline and 6 months post-therapy for the supragingival therapy (ST; n = 12) and the intensive periodontal therapy (IT; n = 12) groups.

Clinical parameters	Groups		p-value
	ST (n = 12)	IT (n = 12)	
VPI (%)			
Baseline	87.80 (63.60–97.02)	72.60 (59.90–97.27)	0.57
6 months	43.20 (12.50–72.60)	26.60 (7.42–33.60)	0.25
p-value	0.03*	0.005*	
GBI (%)			
Baseline	56.30 (40.00–81.20)	68.20 (31.75–88.55)	0.15
6 months	17.30 (13.20–49.60)	12.20 (4.20–20.27)	0.12
p-value	0.004*	0.007*	
PPD (mm)			
Baseline	4.47 (3.03–4.66)	4.75 (3.12–4.96)	0.10
6 months	3.30 (2.80–3.375)	2.32(2.05–2.78)	0.01#
p-value	0.04*	0.005*	
CAL (mm)			
Baseline	4.77 (3.94–5.64)	4.29 (3.67–5.13)	0.07
6 months	4.26 (3.46–4.99)	3.31 (2.94–4.18)	0.05#
p-value	0.06	0.006*	
BOP (%)			
Baseline	73.20 (55.40–83.90)	60.70 (47.40–69.07)	0.06
6 months	42.09 (23.80–49.40)	10.70 (6.07–20.20)	0.001#
p-value	0.03*	0.005*	

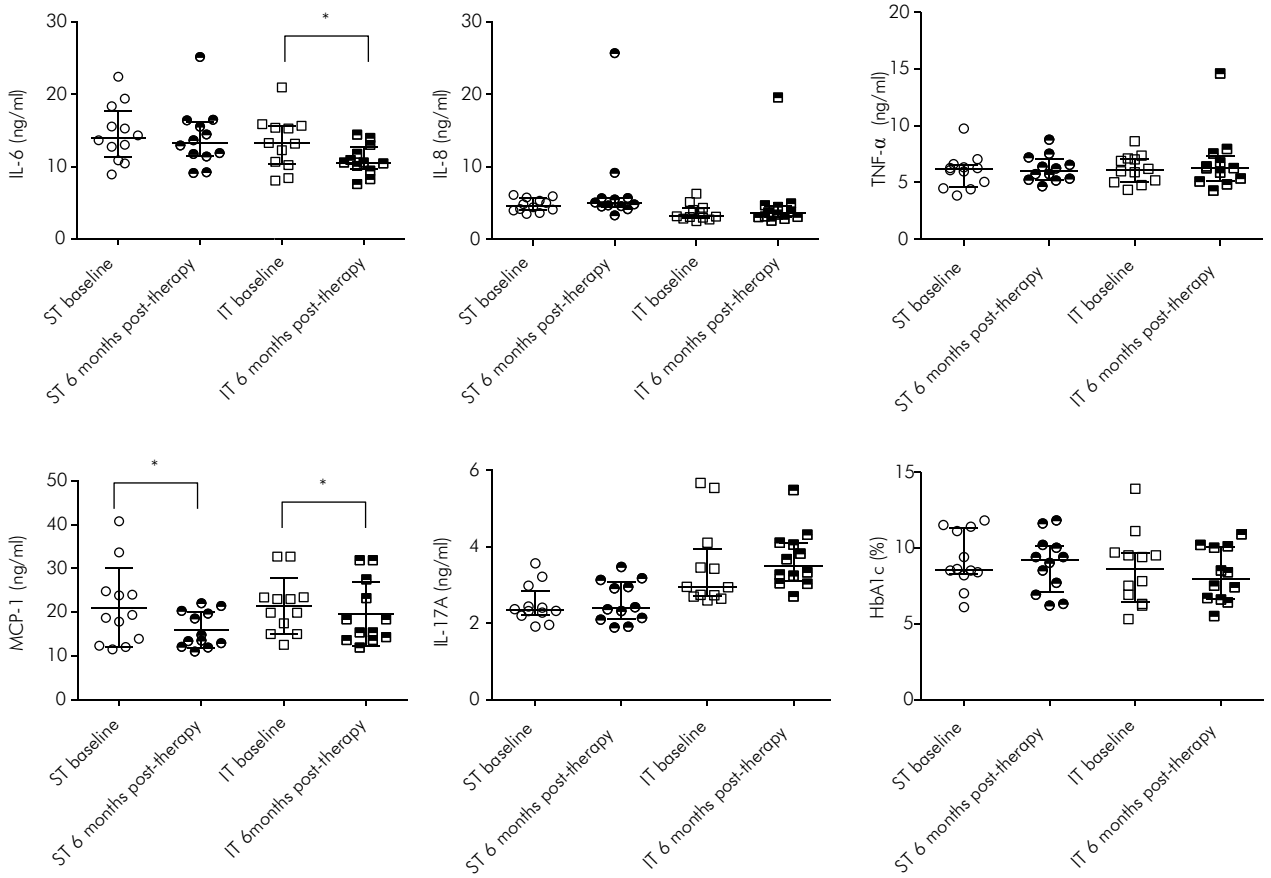
*p-values represent the intragroup (vertical) comparison between baseline and 6-month period (Wilcoxon's test; $p < 0.05$). #p-values represent the intergroup (horizontal) comparison between baseline and 6-month period (Mann–Whitney U-test; $p < 0.05$). VPI: visible plaque index; GBI: gingival bleeding index; PPD: probing pocket depth; CAL: clinical attachment level; BOP: bleeding on probing.

Table 2. Changes in the periodontal clinical parameters PPD (4–6 mm; ≥7 mm) and CAL (4–6 mm; ≥7 mm) from baseline to 6 months post-therapy.

Clinical parameters	Mean Reduction (SEM) [95%CI]		
	ST (n = 12)	IT (n = 12)	p-value
PPD 4–6 mm (%)£	10.1 (2.9) [4.8–14.10]	23.3 (2.0) [13.5–41.6]	0.001*
PPD ≥ 7 mm (%) £	3.8 (0.9) [1.2–9.5]	5.8 (1.2) [0.6–11.9]	0.298
CAL 4–6 mm (%)£	7.2 (1.6) [1.2–16.7]	14.6 (3.1) [3.0–33.9]	0.001*
CAL ≥ 7 mm (%)£	3.2 (1.4) [0.8–14.1]	4.8 (1.1) [0.6–15.5]	0.09

£ Significant reductions in all periodontal parameters from baseline to 6-month period (Wilcoxon signed rank test; $p < 0.05$).
*Significant difference between groups (Mann–Whitney test; $p < 0.05$). PPD: Probing pocket depth; CAL: clinical attachment level.

The levels of inflammatory markers and HbA1c, at baseline and at the end of the experimental period, are shown in Figure 1. Both groups of patients showed similar levels of serum inflammatory markers at baseline ($p > 0.05$). No significant differences for serum levels of IL-8, IL-17A and TNF-α were recorded between the two groups ($p > 0.05$), 6 months after periodontal therapy. However, there was a statistically significant reduction in IL-6 serum levels in the IT group, after 6 months of therapy ($p < 0.001$), but not in the ST group ($p = 0.33$). Furthermore, MCP-1 levels were reduced in both groups (ST, $p = 0.03$; IT, $p = 0.01$). There were no significant differences in serum HbA1c levels between the groups at baseline and at 6 months post-therapy ($p > 0.05$) (Figure 1).



The diagram represents median values: inferior quartile (25%) and superior quartile (75%).*Statistical difference between the two groups (ST and IT) at baseline and 6 months post-therapy ($p < 0.05$; Wilcoxon signed rank test).

Figure 1. Distribution according to the serum levels of IL-6, MCP-1, IL-8, IL-17, TNF-α, and HbA1c at baseline and 6 months post-therapy in the supragingival therapy (ST) and intensive periodontal therapy (IT) groups.

Discussion

The results of the current study demonstrate that supragingival biofilm control reduces the local symptoms of inflammation, with a limited systemic effect on serum biomarkers in T2DM individuals. This is in concurrence with the findings from previous clinical studies that have reported similar effects of periodontal therapy on IL-6 levels.[17,24] To our knowledge, there are no previously published studies available regarding the effect of strict supragingival biofilm control on serum levels of IL-6, IL-8, IL-17, TNF-α, and MCP-1 in T2DM patients. Our follow-up data showed that both therapies were comparable in promoting clinical improvement of the periodontal parameters; however, the best effect was seen in the IT group.

Minimal periodontal treatment in the form of supragingival scaling, prophylaxis, and oral hygiene instructions was given to the patients in the ST group. Although not considered the ideal form of therapy, it promoted clinical improvement of most of the periodontal parameters in this study. It has been known for decades that professional supragingival biofilm control has an effect on the subgingival microbiota, and, consequently, on periodontal clinical parameters.[25] This effect is attributed to the reduced inflammation of adjacent periodontal tissues, leading to a restriction in the availability of nutrients necessary for bacterial multiplication. It is important to highlight that previous studies have not been performed on diabetic individuals. Moreover, in the present study, no microbiological or local cytokine level analyses were performed, and thereby are recommended for further studies on T2DM individuals.

In order to determine more specific differences between the two groups, periodontal sites were divided

into two categories; intermediate (4–6 mm) and deep (≥ 7 mm), for both PPD and CAL parameters. The shallow sites (≤ 3 mm) revealed no considerable changes in PPD and CAL after treatment (data not shown). Clinical attachment gain was observed during analysis of the median values, and was significantly different in the IT group only (Table 2). Stratification and analysis of the changes in CAL (4–6; ≥ 7 mm) revealed a gain in clinical attachment in both groups, with additional benefits observed in the IT group, indicating the importance of root debridement in treating diseased sites. We could speculate that the reduction of inflammation in the adjacent periodontal tissue was the major cause for the changes in PPD and CAL measurements.

The mechanisms by which periodontitis influences T2DM have not been precisely defined. Increasing evidence suggests that severe chronic periodontitis induces subclinical bacteremia, leading to a low-grade systemic inflammation that may affect metabolic control.[26,27] Some of the inflammatory markers linked to T2DM complications and insulin resistance are IL-6, IL-12, MCP-1, and IL-8. Moreover, IL-6 has also been associated with vascular complications,[28] and atherogenesis may be regulated by chemotactic factors, such as MCP-1, which has been linked to both insulin resistance and hyperglycemia. Our study demonstrated that both therapies were able to reduce serum MCP-1 levels, as also achieved by some recently developed drugs.[29] This reduction may be partly explained by the partial improvement of periodontal parameters and local inflammation. On the other hand, this partial periodontal improvement was not able to reduce IL-6 serum levels in the ST group.

Conclusions

Supragingival therapy has a modest effect on periodontal clinical parameters, and a limited effect on systemic inflammatory markers, compared with the effect of intensive periodontal therapy. Therefore, intensive periodontal therapy should be prioritized to control the inflammatory burden on T2DM individuals.

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