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Partial- and full-mouth scaling and root planing in type 2 diabetic subjects: a 12-mo follow-up of clinical parameters and levels of cytokines and osteoclastogenesis-related factors

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Background and Objective: The aim of this study was to evaluate the effects of full-mouth scaling and root planing (FMSRP) and partial-mouth scaling and root planing (PMSRP), up to 12 mo after treatment, on clinical parameters, and levels of cytokines and osteoclastogenesis-related factors in type 2 diabetic subjects with chronic periodontitis.

Material and Methods: Thirty-four subjects received FMSRP ($n = 17$) or PMSRP ($n = 17$) within 24 h or in multiple sessions, respectively. Clinical parameters and local levels of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-17, IL-23, IL-4, receptor activator of NF-β ligand and osteoprotegerin were assessed at baseline, and 3, 6 and 12 mo after therapies.

Results: Clinical parameters improved after both therapies ($p < 0.05$), and no between-group differences were observed at any time-point ($p > 0.05$). Overall, there were no considerable differences in the local levels of the biomarkers studied between groups ($p > 0.05$). The IL-23 concentration and total amount of IFN-γ increased in the FMSRP group and decreased in the PMSRP group from baseline to 3 mo and from baseline to 6 mo, respectively ($p < 0.05$).

Conclusion: Both PMSRP and FMSRP promoted benefits in clinical parameters and showed a similar modulation of cytokines and osteoclastogenesis-related factors at 12 mo in type 2 diabetic subjects.

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Previous studies have shown that scaling and root planing (SRP), alone or in combination with adjunctive therapies, may promote clinical periodontal improvements in diabetic subjects (1–3). Multiple sessions of partial-mouth scaling and root planing (PMSRP), performed at weekly intervals, is the standard protocol to treat periodontitis. However, considering the possible translocation of bacteria from one site to another in the oral cavity, it has been suggested that treated periodontal sites could be reinfected by pathogens from untreated sites when applying PMSRP (4–6). Therefore, some studies have suggested that full-mouth scaling and root planing (FMSRP), performed over a short period of time, could avoid this bacterial reinfection that may challenge periodontal treatment outcomes (7,8).

Periodontal breakdown is mediated by the host through the local production of inflammatory mediators in response to pathogens and their products (9). Several proinflammatory [e.g. tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-17 and IL-23] and anti-inflammatory (e.g. IL-4) markers and osteoclastogenesis-related factors [e.g. receptor activator of NF-β (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG)] have been identified in the gingival crevicular fluid as a result of cellular responses around healthy and diseased periodontal tissues (10–14). Therefore, analysis of biological markers in the gingival crevicular fluid has been proposed as a noninvasive means of studying the impact of systemic disorders on the pathophysiology of periodontal diseases and the host response to periodontal therapies (15).

Considering the bacterial-recolonization hypothesis after employing PMSRP, FMSRP could be a better therapeutic alternative for diabetic subjects in whom there may be an increased risk of infection. Although some studies have assessed the effect of FMSRP in diabetic subjects (2,16), to date, only one study (from our research group) has compared FMSRP with PMSRP in the treatment of periodontitis in type 2 diabetic subjects (3). We demonstrated that FMSRP and PMSRP were similarly effective in improving clinical parameters in diabetic subjects with chronic periodontitis at 3 and 6 mo after therapy (3). Therefore, this study aimed to compare the effects of FMSRP and PMSRP on clinical parameters and on local levels of cytokines and osteoclastogenesis-related factors [soluble RANKL (sRANKL), OPG, TNF-α, IFN-γ, IL-4, IL-17 and IL-23] in type 2 diabetic subjects with chronic periodontitis over a 12-mo study period. We hypothesized that FMSRP and PMSRP could promote similar 12-mo clinical and osteoimmunological outcomes when used to treat chronic periodontitis in subjects with type 2 diabetes.

Material and methods

Sample size calculation

The sample size to assure adequate power for clinical parameter differences was calculated considering differences of at least 1 mm for clinical attachment level and a standard deviation of 1 mm between groups in initially deep pockets (≥ 7 mm). Based on these calculations, it was decided that 17 subjects per group would provide 80% power at a significance level of 5%. The number of sites for gingival crevicular fluid sampling was based on studies that found differences in the levels of cytokines in the gingival crevicular fluid when comparing clinical periodontal status and the effect of SRP (17,18).

Subject population

Thirty-four subjects (42–67 years of age) with type 2 diabetes mellitus (DM) and chronic periodontitis were selected from the population referred to Guarulhos University, from December 2007 until March 2009. All eligible subjects were informed of the nature, potential risks and benefits of their participation in the study and signed informed consent to participate. The study protocol was previously approved by the Guarulhos University's Ethics Committee in Clinical Research.

Inclusion/exclusion criteria

Data concerning the duration of DM and medications were retrieved from the medical records of the subjects. All subjects had type 2 DM for at least the past 5 years and generalized chronic periodontitis (19). They were receiving supplementation with insulin, an appropriate dietary regimen and/or oral hypoglycemic agents. All subjects were > 30 years of age, had at least 15 teeth (excluding third molars and teeth indicated to exodontias) and had more than 30% of sites with a probing depth and clinical attachment level of ≥ 4 mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking and smoking within the past 5 years, periodontal and/or antibiotic therapy in the previous 6 mo, regular use of mouthrinses containing antimicrobials in the preceding 2 mo, other systemic condition that could affect the progression of periodontal disease, long-term treatment with anti-inflammatory and immunosuppressive medications, periapical pathology, orthodontic appliances and multiple systemic complications of DM.

Experimental design and treatment protocols

In this prospective, parallel, blinded, randomized and controlled clinical trial, 34 subjects with type 2 DM and chronic periodontitis were submitted to an initial treatment phase, including the removal of supragingival plaque and calculus, exodontia and provisional restorations. The subjects were instructed to use a soft toothbrush, dental floss and interdental toothbrushes, as necessary, and the same brand of toothpaste for the duration of the study (Colgate Total®; Colgate-Palmolive Ind. e Com, São Paulo, SP, Brazil).

The subjects were randomly assigned, by tossing a coin [performed by the same assessor (P.M.D.)] to one of the following groups: FMSRP (the test group, $n = 17$, in which SRP was completed in two appointments, lasting approximately 120 min each, under local anesthesia using periodontal curettes and an ultrasonic device, within

a maximum time-period of 24 h on two consecutive days); or PMSRP (the control group, $n = 17$, in which SRP was completed in four appointments, lasting approximately 60 min each, performed under local anesthesia using periodontal curettes and an ultrasonic device within a maximum time-period of 21 d). No group received antibiotics or local therapy with antimicrobials.

Supportive therapy sessions were performed at 3, 6 and 9 mo post-therapy and lasted for approximately 60 min each. The supportive therapy included professional plaque control using an abrasive sodium carbonate air-powder system, subgingival debridement (as necessary) and reinstruction of oral hygiene. The subjects were monitored by assessment of the following clinical parameters (at baseline, and 3, 6 and 12 mo after therapy): fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), levels of cytokines and osteoclastogenesis-related factors.

Plasma and clinical monitoring

A single laboratory performed all blood analyses (Clinical Analysis Laboratory, Guarulhos University, Guarulhos, São Paulo, Brazil). FPG, measured using the glucose oxidase method, was expressed in mg/dL. HbA1c, measured using high-performance liquid chromatography, was expressed as a percentage.

Clinical examinations were performed by one examiner (V.R.S.), calibrated according to the method described by Araujo *et al.* (20). The intra-examiner variability was 0.20 mm for probing depth and 0.23 mm for clinical attachment level. Clinical parameters registered dichotomously [e.g. bleeding on probing (BoP)] were calculated using the Kappa-Light test and the intra-examiner agreement was > 0.85. The examiner and the biostatician (F.V.R.) were blinded to the treatment allocation of the subjects.

The following parameters were assessed at six sites of all teeth, excluding third molars, using a manual periodontal probe (North Carolina; Hu-Friedy, Chicago, IL, USA): plaque index (PI, presence/absence) (21); BoP (presence/absence); suppuration (SUP, presence/absence); probing depth (mm); and clinical attachment level (mm).

Gingival crevicular fluid sampling and ELISA

Two noncontiguous sites per subject, with probing depth and clinical attachment level of ≥ 5 mm, BoP and no furcation involvement, were chosen for sampling. At baseline, gingival crevicular fluid was collected 1 wk after clinical examination to avoid changes in its nature. Gingival crevicular fluid samples were subsequently taken from the same selected sites at 3, 6 and 12 mo after therapies. Samples were collected and stored as previously described by Santos *et al.* (22). After removal of the supragingival biofilm with sterile cotton pellets, the sites were isolated with cotton rolls and gently dried with an air syringe to eliminate the possibility of contamination with saliva. Gingival crevicular fluid was collected by inserting standard paper strips (Periopaper; Oraflow Inc., Smithtown, NY, USA) to a depth of approximately 2 mm into the sulcus/pocket for 30 s. Strips visually contaminated with blood were discarded. The gingival crevicular fluid sample volume was measured in a calibrated appliance (Periotron 8000; Proflow Inc., Amityville, NY, USA), after which the readings were converted to an actual volume (μL) by reference to the standard curve. The strips from the two selected sites were immediately placed into separate microcentrifuge tubes containing 250 μL of phosphate-buffered saline plus protease inhibitor cocktail. The samples were stored at −20°C until required for subsequent assays. The concentrations of TNF-α, IL-4, IFN-γ, IL-17, IL-23, sRANKL and OPG were determined in the gingival crevicular fluid samples using ELISA (HS Quantikine; R&D Systems Inc., Minneapolis, MN, USA for TNF-α, IL-4, IFN-γ, IL-17, IL-23; and Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria for sRANKL and OPG), according to the manufacturer's recommendations. The tubes were vortexed for 30 s and centrifuged for 5 min at 1500 *g* in order to elute. The negative control for the assays was phosphate-buffered saline plus protease inhibitor cocktail, without gingival crevicular fluid. The minimum detectable concentration ranges (sensitivity) for TNF-α, IL-4 and IL-23 were 0.038–0.191 pg/mL, 0.03–0.22 pg/mL and 2.7–16.3 pg/mL, respectively. The minimum detectable concentrations of IFN-γ and IL-17 were < 8.0 pg/mL and < 15 pg/mL, respectively. The limit of detection for sRANKL and OPG was 1.6 and 2.8 pg/mL, respectively. In relation to specificity, the manufacturers reported no significant cross-reactivity or interference for the ELISA kits used in this study. The results were reported as the total amount (pg) of each protein per site in 30 s of sampling. Calculations of the protein concentrations in each site (pg/μL) were established by dividing the total amount of each cytokine by the gingival crevicular fluid volume.

Statistical analysis

The primary outcome variable was the change in mean clinical attachment level after therapy in sites with an initial probing depth of ≥ 7 mm. Secondary outcome variables were full-mouth PI, BoP, SUP, probing depth, clinical attachment level and percentage of sites with a probing depth of ≥ 5 mm, mean clinical attachment level, probing depth changes in the full mouth and in sites with an initial probing depth of 4–6 mm, HbA1c and FPG levels and measurements of cytokine- and osteoclastogenesis-related factors. Data were examined for normality using the Kolmogorov–Smirnov test. The data that did not achieve normality were analyzed using nonparametric methods. The percentage of sites with visible plaque, BoP, probing depth ≥ 5 mm, probing depth ≥ 7 mm and SUP were determined for each subject, in addition to the mean probing depth, clinical attachment level, and the levels of HbA1c and FPG. The changes in probing depth and clinical attachment level from baseline to 12 mo after therapy were examined in the full mouth and in

subsets with initial probing depths of 4–6 mm (intermediate pockets) and ≥ 7 mm (deep pockets). The study unit for cytokine levels was the site rather than the subject because periodontitis is a site-specific disease. Clinical parameters were averaged across subjects. Subsequently, clinical and glycemic parameters and cytokine levels and changes were averaged in each group. Between-group differences in clinical and glycemic parameters, age of subject and duration of DM were compared using the Student's *t*-test. Cytokine levels and changes were compared between groups using the Mann–Whitney *U*-test. Repeated-measures analysis of variance (ANOVA) and the Friedman test were used for comparison of clinical/glycemic parameters and cytokine levels, respectively, at different time-points within each group. A pairwise comparison was performed using the Tukey test after ANOVA. The chi-square test was used to detect between-group differences in the distributions of gender, treatment regimen for DM and subjects with an HbA1c of > 8%. The level of significance was set at 5%. Adjustments were made for multiple comparisons when the levels of the seven biomarkers (sRANKL, OPG, TNF-α, IFN-γ, IL-4, IL-17 and IL-23) were evaluated. In brief, an overall p of $0.05 = 1 - (1 - k)^7$ was computed, where k was the desired individual p value. Therefore, for comparison of biomarker levels, p-values of < 0.007 were considered statistically significant at $p < 0.05$.

Table 1. Demographic characteristics of the study population at baseline

Characteristics	FMSRP (n = 17)	PMSRP (n = 17)
Age (years)	51.9 ± 7.8 (range, 42–66)	53.1 ± 8.1 (range, 42–67)
Gender		
Male	9	7
Female	8	10
Duration of DM (years)	6.3 ± 0.9	6.2 ± 0.6
Glycemic status		
HbA1c ≤ 8%	5	6
HbA1c > 8%	12	11
HbA1c ≤ 6.5%	1	2
HbA1c > 6.5%	16	15
Category of treatment regimen		
Diet	3	3
Diet + insulin	1	2
Diet + oral hypoglycemic agents (metformin or glybenclamin)	12	11
Diet + oral hypoglycemic agents (metformin or glybenclamin) + insulin	1	1

Results are given as mean ± standard deviation, with or without the range, or as *n*. There were no differences between groups regarding age and duration of diabetes mellitus (DM) (Student's *t*-test; $p > 0.05$) and regarding gender, treatment regimen and glycemic status (chi-square test; $p > 0.05$).
FMSRP, full-mouth scaling and root planing; HbA1c, glycated hemoglobin; PMSRP, partial-mouth scaling and root planing; SD, standard deviation.

Results

There were no subject or site dropouts during the study period. No adverse effects, such as fever and indisposition after treatment, were reported by any subject, and no changes in the category of treatment regimen for DM occurred during the study.

Clinical and glycemic results

No significant differences were observed between PMSRP and FMSRP groups for age, duration of DM, gender, glycemic status and treatment regimen distributions ($p > 0.05$; Table 1). Table 2 presents the mean values of clinical and glycemic parameters before and after therapy. There were no between-group differences for any clinical and glycemic parameters at any time-point ($p > 0.05$). Both therapies led to a significant decrease in the mean percentage of sites with probing depth ≥ 5 mm, probing depth ≥ 7 mm, plaque accumulation and BoP, and to an overall decrease in the clinical attachment level and gingival crevicular fluid volume ($p < 0.05$). The PMSRP group presented significantly lower values of mean probing depth at 3, 6 and 12 mo post-therapy when compared with baseline ($p < 0.05$). Although the FMSRP group also presented reductions in the mean probing depth at 3 and 6 mo compared with baseline ($p < 0.05$), at 12 mo post-treatment, this parameter showed a trend towards returning to the baseline mean value. Both groups showed similar reductions in probing depth and clinical attachment level, in full-mouth and probing depth categories ($p > 0.05$). The mean reductions in probing depth of intermediate pockets (probing depth = 4–6 mm) from baseline to 12 mo post-therapy were 1.5 ± 0.3 mm and 1.3 ± 0.3 mm for PMSRP and FMSRP, respectively. The mean reductions in probing depth of deep pockets (probing depth ≥ 7 mm) from baseline to 12 mo post-therapy were 2.5 ± 0.5 mm and 2.2 ± 0.4 mm for PMSRP and FMSRP, respectively.

Cytokine results

There were no significant between-group differences in the levels of any biomarker at any time-period ($p > 0.05$; Table 3). The concentration of sRANKL was higher in the PMSRP group at 12 mo and in the FMSRP group at 3 and 12 mo, compared with baseline ($p < 0.05$). The concentration of OPG increased in both groups at 12 mo post-therapy compared with the other time-points ($p < 0.05$). There was a significant increase in the concentration of OPG at all time-points after PMSRP ($p < 0.05$). The RANKL/OPG ratio decreased in both groups at 12 mo post-therapy compared with the other time-points ($p < 0.05$). The concentration of IFN-γ showed an increase in the PMSRP group at 12 mo post-therapy compared with baseline ($p < 0.05$). IL-4 showed a significant increase in the PMSRP group at 12 mo

Table 2. Values of clinical and glycemic parameters for both groups at baseline, and at 3, 6 and 12 mo after therapy

	FMSRP (n = 17 subjects)				PMSRP (n = 17 subjects)			
Parameters	Baseline	3 mo	6 mo	12 mo	Baseline	3 mo	6 mo	12 mo
PI (%)	70.9 ± 31.8a	29.2 ± 23.8b	29.2 ± 18.8b	26.5 ± 19.1b	75.4 ± 28.3a	29.3 ± 16.0b	27.0 ± 15.7b	27.2 ± 20.4b
BoP (%)	47.3 ± 26.0a	8.5 ± 7.1b	6.7 ± 10.4b	5.0 ± 5.2b	53.9 ± 36.3a	10.9 ± 10.8b	10.2 ± 12.2b	9.9 ± 11.7b
SUP (%)	1.7 ± 2.9	1.4 ± 3.2	0.3 ± 0.9	0.3 ± 1.2	3.0 ± 4.8	2.6 ± 4.2	1.0 ± 2.0	1.2 ± 1.9
PD (mm)	3.3 ± 0.9a	2.7 ± 0.9b	2.7 ± 0.5b	2.8 ± 0.5ab	3.6 ± 0.6a	2.6 ± 0.8b	2.7 ± 0.8b	2.7 ± 0.7b
CAL (mm)	3.8 ± 0.6a	3.1 ± 0.7b	3.1 ± 0.6b	3.2 ± 0.6b	4.2 ± 0.9a	3.5 ± 0.7b	3.4 ± 1.2b	3.4 ± 1.0b
GCF (μL)	0.60 ± 0.18a	0.23 ± 0.11b	0.21 ± 0.14b	0.20 ± 0.10b	0.58 ± 0.18a	0.20 ± 0.17b	0.21 ± 0.13b	0.21 ± 0.10b
Sites PD ≥ 5mm (%)	20.4 ± 8.7a	6.3 ± 3.7b	5.5 ± 4.8b	6.3 ± 4.8b	25.2 ± 11.6a	9.6 ± 7.1b	9.5 ± 8.0b	8.2 ± 6.9b
Sites PD ≥ 7mm (%)	6.4 ± 4.8a	1.0 ± 0.8b	1.1 ± 0.9b	1.2 ± 1.2b	6.9 ± 4.4a	1.3 ± 1.0b	1.2 ± 0.9b	1.0 ± 1.3b
HbA1c (%)	9.4 ± 2.5	10.1 ± 2.5	9.7 ± 2.0	10.2 ± 2.5	8.9 ± 2.2	9.3 ± 1.9	10.2 ± 2.9	10.6 ± 2.9
FPG (mg/dL)	170.8 ± 70.0	205.4 ± 87.7	199.2 ± 91.1	190.2 ± 82.8	179.4 ± 55.7	172.8 ± 56.0	198.4 ± 67.7	200.2 ± 70.0

Results are given as mean ± standard deviation.
For each parameter, different letters (a, b) indicate statistically significant differences among the four experimental periods within each therapeutic group (Repeated-measures analysis of variance and Tukey's test; $p < 0.05$). There were no differences between treatment groups at each time-point, determined using the Student's t-test ($p > 0.05$).
BoP, bleeding on probing; CAL, clinical attachment level; FMSRP, full-mouth scaling and root planing; FPG, fasting plasma glucose; GCF, gingival crevicular fluid; HbA1c, glycated hemoglobin; PD, probing depth; PI, plaque index; PMSRP, partial-mouth scaling and root planing; SUP, suppuration.

post-therapy compared with the other time-points ($p < 0.05$). IL-17 decreased in both groups at 12 mo after therapy when compared with baseline, and with 3- and 6-mo time-points ($p < 0.05$). There were no changes in the levels of TNF-α and IL-23 at any time-point ($p > 0.05$; Table 3).

In general, the changes in cytokine levels did not differ considerably between the study groups. Only the concentration of IL-23 (Fig. 1) and the total amount of IFN-γ (Fig. 2) showed any change, increasing in FMSRP and decreasing in PMSRP from baseline to 3 mo and from baseline to 6 mo, respectively.

Discussion

This is the first study to evaluate the effects of FMSRP and PMSRP, over a 12-mo time period after treatment, on clinical outcomes and local levels of cytokines and osteoclastogenesis-related factors in type 2 diabetic subjects with chronic periodontitis. Overall, both therapies promoted improvements in clinical parameters at all study time-points, when compared with baseline. These findings suggest that SRP, within 24 h or in multiple sessions, sustained comparable successful clinical outcomes over a 12-mo period in diabetic subjects. Therefore, the hypothesis that FMSRP and PMSRP could promote comparable 12-mo clinical and osteoimmunological outcomes for the treatment of chronic periodontitis in type 2 diabetic subjects was accepted. In addition, both treatment modalities were similarly able to affect the osteoimmunological profile in periodontal sites at 12 mo, indicating that the control of infection by SRP modulated the levels of the biomarkers evaluated.

In this study, FMSRP and PMSRP promoted similar major clinical improvements during the initial healing phase. Subsequently, the clinical benefits achieved at 3 mo were maintained until 6 and 12 mo after therapy. A previous study from our research group has already demonstrated comparable clinical improvements after FMSRP and PMSRP in type 2 diabetic subjects at 3 and 6 mo (3). In the present study, no additional clinical benefits of FMSRP or PMSRP were observed at 12 mo. These clinical results are in accordance with studies in nondiabetic subjects that showed short-term and mid-term improvements in periodontal parameters following FMSRP and PMSRP (8,23,24). Few studies have evaluated the effects of full-mouth therapies in nondiabetic individuals over a time-period of longer than 8 mo (25,26), and no study has assessed the 12-mo impact of these therapeutic approaches in diabetic patients. Tomasi *et al.* (25) found no difference in the recurrence of diseased sites 12 mo after treatment with full-mouth ultrasonic debridement in one h session or after treatment with SRP in quadrants. Knöfler *et al.* (26) demonstrated that FMSRP and PMSRP, associated with chlorhexidine, have the same clinical benefits at 12 mo post-therapy. However, different treatment protocols and the absence of a diabetic status in both studies make comparisons with our results difficult.

Many investigations have evaluated the impact of periodontal therapies on the glycemic control of diabetic subjects (27–30). In this study, the levels of HbA1c and FPG did not change significantly at any time-point following both periodontal therapies. Also, no differences were observed between groups for these glycemic parameters during the study. The actual effect of periodontitis on glycemic levels remains inconclusive. Janket *et al.* (27) proposed that SRP promotes periodontal clinical benefits without a significant reduction in the glycemic control of diabetic subjects. On the other hand, recent meta-analyses have

Table 3. Levels of cytokines and osteoclastogenesis-related factors for full-mouth scaling and root planing (FMSRP) and partial-mouth scaling and root planing (PMSRP) groups at baseline, and at 3, 6 and 12 mo after therapy

		FMSRP (n = 34 sites)				PMSRP (n = 34 sites)		
Mediators	Baseline	3 mo	6 mo	12 mo	Baseline	3 mo	6 mo	12 mo
sRANKL (pg/site)	44.3 (18.9–82.6)	39.5 (20–66.7)	38.9 (11.5–86.3)	42.8 (24.3–91.1)	42.3 (16.3–137.6)	36.9 (17.8–219.9)	40.2 (18.9–120.1)	40.8 (17.3–65.3)
sRANKL (pg/μL)	226.15a (44.6–1183.3)	427.1b (24.8–5718.1)	445.8ab (50.2–5311.6)	1320.3b (0.0–4476.5)	245.3a (25.8–1710.2)	806.8ab (17.8–3221.6)	449.9ab (24.2–5172.9)	797.2b (0.0–3159.5)
OPG (pg/site)	5.4a (0.0–12.7)	4.8a (0.0–25.1)	5.5a (0.0–11.6)	7.7b (0.0–30.6)	6.2a (0.0–20.9)	6.6a (2.9–26.0)	6.7a (1.5–15.4)	10.7b (1.6–27.7)
OPG (pg/μL)	21.5 (0.0–355.6)	78.3 (0.0–1020.4)	59.0 (0.0–1065.8)	66.6 (0.0–339.4)	30.0a (0.0–291.7)	122.8b (2.5–928.9)	70.8b (7.5–632.6)	75.4b (4.4–903.6)
sRANKL/OPG	19.2a (3.0–128.8)	18.9a (1.8–63.8)	16.5a (2.0–81.8)	4.8b (1.6–81.0)	20.2a (4.1–150.8)	18.2a (3.7–37.7)	19.54a (4.1–19.4)	4.3b (1.5–22.9)
TNF-α (pg/site)	0.0 (0.0–3.3)	0.0 (0.0–3.7)	0.0 (0.0–4.6)	0.0 (0.0–3.1)	0.0 (0.0–0.5)	0.0 (0.0–0.5)	0.0 (0.0–0.4)	0.0 (0.0–0.4)
TNF-α (pg/μL)	0.0 (0.0–0.4)	0.0 (0.0–0.4)	0.0 (0.0–0.3)	0.0 (0.0–0.9)	0.0 (0.0–4.8)	0.0 (0.0–1.7)	0.0 (0.0–3.4)	0.0 (0.0–1.9)
IFN-γ (pg/site)	3.7 (0.0–12.5)	6.1 (0.0–16.9)	7.2 (0.0–31.3)	7.3 (0.0–31.3)	5.2 (0.0–19.3)	5.2 (0.0–14.2)	4.3 (0.0–22.7)	8.4 (0.0–13.4)
IFN-γ (pg/μL)	17.8 (0.0–224.8)	45.8 (0.0–264.5)	43.8 (0.0–314.3)	50.8 (0.0–329.9)	18.2a (0.0–452.7)	26.6ab (0.0–789.9)	21.3ab (0.0–223.6)	43.5b (0.0–533.2)
IL-4 (pg/site)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.2)	0.0 (0.0–0.4)	0.0a (0.0–0.2)	0.0a (0.0–0.1)	0.0a (0.0–0.1)	0.03b (0.0–2.2)
IL-4 (pg/μL)	0.0 (0.0–0.9)	0.0 (0.0–3.0)	0.0 (0.0–3.1)	0.06 (0.0–4.2)	0.0a (0.0–3.6)	0.0a (0.0–3.5)	0.0a (0.0–1.2)	0.14b (0.0–12.4)
IL-17 (pg/site)	11.0a (0.0–195.5)	15.5a (0.0–56.8)	15.5a (0.0–153)	0b (0.0–42.4)	13.6a (0.0–88.0)	13.0a (0.0–183)	14.8a (0.0–63.0)	0.0b (0.0–32.4)
IL-17 (pg/μL)	46.9a (0.0–529.5)	80.3b (0.0–567.5)	144.1b (0.0–2266.7)	0.0c (0.0–392.5)	61.5a (0.0–880.0)	82.9a (0.0–1830.0)	70.5a (0.0–346.0)	0.0b (0.0–249.5)
IL-23 (pg/site)	0.0 (0.0–34.9)	0.0 (0.0–67.7)	0.1 (0.0–46.5)	0.0 (0.0–77.1)	0.0 (0.0–26.1)	0.0 (0.0–31.3)	0.0 (0.0–30.7)	0.0 (0.0–63.7)
IL-23 (pg/μL)	0.0 (0.0–365.9)	0.0 (0.0–277.0)	1.0 (0.0–278.0)	0.0 (0.0–326.2)	0.0 (0.0–261.0)	0.0 (0.0–51.2)	0.0 (0.0–307.0)	0.0 (0.0–490.3)

Results are given as median (range) of the total amount (pg/site) and of the concentration (pg/μL).

For each biomarker, different letters (a, b, c) indicate statistically significant differences among the four experimental periods within each therapeutic group (Friedman test; $p < 0.05$). No differences between treatment groups at each time-point were identified using the Mann–Whitney U-test ($p > 0.05$).

IFN-γ, interferon-γ; IL, interleukin; OPG, osteoprotegerin; sRANKL, soluble RANKL; TNF-α, tumor necrosis factor-α.

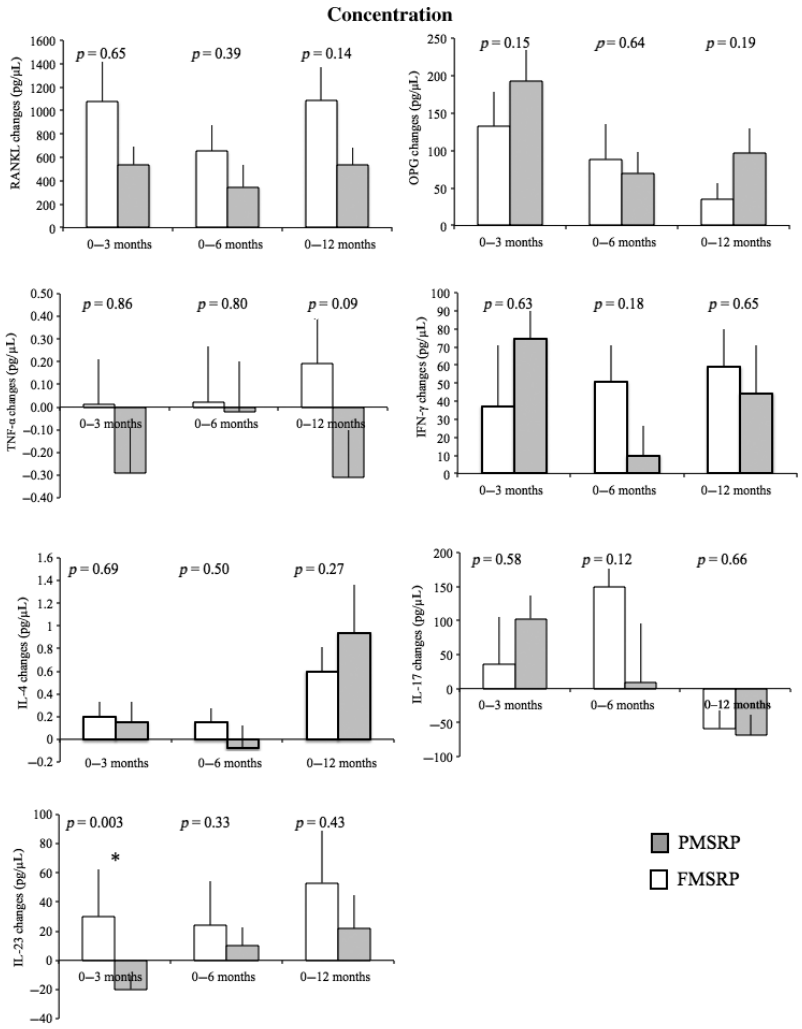

Fig. 1. Bar charts of the mean changes and standard error of the mean (SEM) of the concentrations of cytokines and bone related-factors in full-mouth scaling and root planing (FMSRP) and partial-mouth scaling and root planing (PMSRP) groups from baseline to 3, 6 and 12 mo after therapy. *Differences between treatment groups, determined using the Mann–Whitney *U*-test ($p < 0.05$). IFN-γ, interferon-γ; IL, interleukin; OPG, osteoprotegerin; TNF-α, tumor necrosis factor-α.

demonstrated positive effects of periodontal therapies in the glycemic control of diabetic subjects (28–30). Contradictory results of studies on the effects of periodontal therapies on glycemic control may be attributed to differences in periodontal interventional protocols (with and without antibiotic therapies), types of DM, insufficient sample size (27), baseline HbA1c levels, severity of periodontitis and methods for determining HbA1c values (27,28). In addition, the socioeconomic profile and lifestyle, including diet, physical activity, medical care and compliance to medications, which differ widely among study populations, could also affect the glycemic condition. Worthy of note is that the subjects included in the current investigation were predominantly from families of a low socioeconomic status who often lack financial, social and educational support and have inadequate or limited access to medical care, which is essential for satisfactory glycemic control.

We also investigated whether FMSRP and PMSRP could modulate the local levels of sRANKL, OPG, TNF-α, IFN-γ, IL-4, IL-17 and IL-23 over a 12-mo time-period. These mediators can stimulate and/or inhibit each other as a part of a complex network of molecules that play a role in regulating the immune system and bone metabolism. Periodontal pathogens trigger several antimicrobial innate immune responses through the induction of proinflammatory cytokines, such as TNF-α (31). Subsequently, the innate immune response initiates the development of continuing adaptive immunity through B and T lymphocytes. $CD4^+$ T cells may differentiate into T helper (Th) 1, Th2 or Th17 phenotypes, each of which has distinct cytokine profiles and functions (32). Th1 and Th17 cells mediate destructive inflammatory responses, while the protective Th2 cells oppose the Th1-cell response. The proinflammatory cytokines IFN-γ and IL-17 correspond to Th1 and Th17 responses, respectively, while IL-4 is an anti-inflammatory Th2 cytokine. IL-23 plays a role in stabilizing the Th17

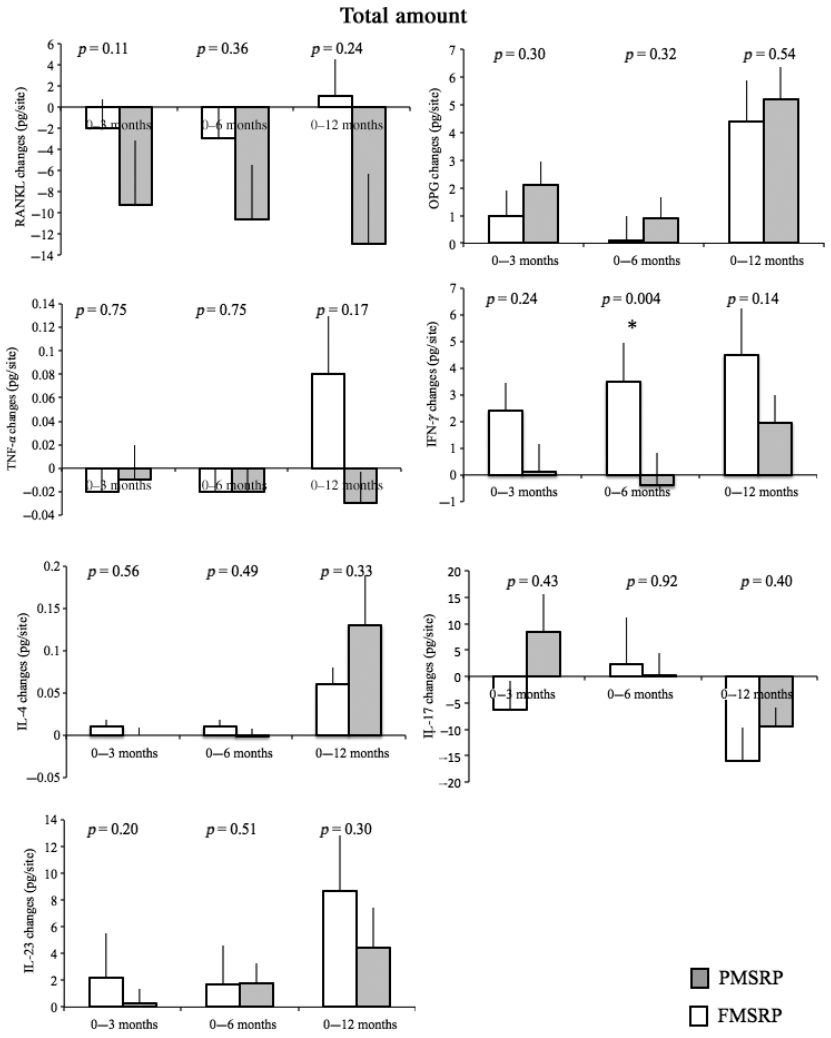

Fig. 2. Bar charts of the mean changes and standard error of the mean (SEM) of the total amounts of cytokines and bone related-factors in full-mouth scaling and root planing (FMSRP) and partial-mouth scaling and root planing (PMSRP) groups from baseline to 3, 6 and 12 mo. *Differences between treatment groups, determined using the Mann–Whitney *U*-test ($p < 0.05$). IFN-γ, interferon-γ; IL, interleukin; OPG, osteoprotegerin; TNF-α, tumor necrosis factor-α.

lineage and expanding Th17 responses (32). In this study, overall, both therapies affected the local levels of some biomarkers, although considerable immunological differences were not observed between groups. One could argue that the biomarker changes could be attributed to the influence of the poor glycemic control, which is related to a systemic inflammatory burden, and not to the resolution of periodontal inflammation. However, it is important to emphasize that both groups presented a comparable number of subjects with poor and good glycemic control at baseline. In addition, the mean HbA1c levels did not change significantly over time, and both groups presented similar levels of glycemic control at all time-points.

RANKL and OPG are positive and negative regulators, respectively, of osteoclastogenesis and bone resorption (33). High levels of RANKL and low levels of OPG have been related to periodontitis (34). In this study, the concentration, but not the total amount, of sRANKL increased in both groups at 12 mo after therapy. However, contradictions between changes in the concentration and total amount of proteins in the gingival crevicular fluid after therapy may be explained by a decrease in the gingival crevicular fluid volume caused by a reduction of inflammation (11). Both therapies induced an increase in the total amount of OPG and a decrease in the RANKL/OPG ratio at 12 mo, which suggests a role for OPG in controlling periodontal bone resorption during this period. IL-17 is a proinflammatory cytokine, currently described in periodontitis, that stimulates the production of other proinflammatory mediators (35,36) and osteoclastogenesis-related factors (37,38). The levels of IL-17 were significantly higher in gingival crevicular fluid, periodontal tissues and culture supernatants of gingival cells from periodontitis subjects (17,39). In this study, simultaneously with the increase in the levels of OPG, IL-17 was reduced significantly

at 12 mo after FMSRP and PMSRP. Together, these findings may indicate stabilization of the healing process and the achievement of a healthy condition in periodontal tissues at 12 mo after SRP. IL-23 is involved in the establishment of chronic inflammation and in the survival/expansion of the T-helper cell subset producing IL-17 (40,41). High levels of IL-23 have been found in periodontitis lesions (39,42). In this study, the concentration of IL-23 increased in FMSRP, while it decreased in PMSRP from baseline to 3 mo (Fig. 1). However, these immunological differences in the initial healing phase between therapies did not provide a notable impact on clinical outcomes over time, and therefore, at this stage, we were not able to determine the actual consequence of this short-term observation.

IFN-γ and IL-4 have proinflammatory and anti-inflammatory biological functions, respectively, and therefore may antagonize each other (43,44). Low levels of IL-4 and high levels of IFN-γ have been associated with periodontal diseases (18,45–47). In this study, there was an increase in the concentration, but not in the total amount, of IFN-γ after PMSRP, which could be attributed to the reduced gingival crevicular fluid volume after SRP, as described above (11). There was also a decrease in the total amount of IFN-γ after PMSRP and an increase in IFN-γ after FMSRP, from baseline to 6 mo (Fig. 2). However, we did not observe major clinical consequences of this immunological finding. Interestingly, the levels of IL-4 increased only for PMSRP at 12 mo (Table 3). As IL-4 was associated with the remission or improvement of periodontal disease (45,48), these data could suggest a favorable immunological response at 12 mo following PMSRP.

In periodontitis, the excessive production of TNF-α, which plays a proinflammatory role (49), has been related to the loss of attachment and bone (50). In diabetic subjects, the majority of the available studies have focused on the circulating levels of TNF-α before and after periodontal therapy, with the aim of explaining the mutual relationship between DM and periodontal diseases (51,52). In this study, there were no differences in the level of TNF-α after either FMSRP or PMSRP at any time-point. Although a previous study (1) showed a significant reduction in the gingival crevicular fluid concentration of TNF-α at 3 and 6 mo post-PMSRP in type 2 diabetic subjects, our results are in agreement with other reports in type 2 diabetic and nondiabetic subjects, in which no significant differences were found in the levels of TNF-α in the gingival crevicular fluid following SRP (10,51).

In conclusion, both PMSRP and FMSRP, for the treatment of chronic periodontitis in type 2 diabetic subjects, may improve clinical parameters and modulate local levels of cytokines and osteoclastogenesis-related factors at 12 mo of follow-up.

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