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Effect of nonsurgical periodontal therapy on serum and gingival crevicular fluid cytokine levels during pregnancy and postpartum

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Background and Objective: A low-grade systemic inflammatory status originating from periodontal infection has been proposed to explain the association between periodontal disease and systemic conditions, including adverse obstetric outcomes. The aim of this study was to evaluate the effect of periodontal therapy during pregnancy on the gingival crevicular fluid and serum levels of six cytokines associated with periodontal disease and preterm birth.

Material and Methods: A subsample of 60 women (18–35 years of age) up to 20 gestational weeks, previously enrolled in a larger randomized clinical trial, was recruited for the present study. Participants were randomly allocated to receive either comprehensive nonsurgical periodontal therapy before 24 gestational weeks ($n = 30$, test group) or only one appointment for supragingival calculus removal ($n = 30$, control group). Clinical data, and samples of blood and gingival crevicular fluid, were collected at baseline, at 26–28 gestational weeks and 30 d after delivery. The levels of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70 and tumor necrosis factor-α were analyzed by flow cytometry.

Results: After treatment, a major reduction in periodontal inflammation was observed in the test group, with bleeding on probing decreasing from 49.62% of sites to 11.66% of sites ($p < 0.001$). Periodontal therapy significantly reduced the levels of IL-1β and IL-8 in gingival crevicular fluid ($p < 0.001$). However, no significant effect of therapy was observed on serum cytokine levels. After delivery, the levels of IL-1β in the gingival crevicular fluid of the test group were significantly lower than were those in the control group ($p < 0.001$), but there were no significant differences between test and control groups regarding serum cytokine levels.

Conclusion: Although periodontal therapy during pregnancy successfully reduced periodontal inflammation and gingival crevicular fluid cytokine levels, it did not have a significant impact on serum biomarkers.

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Periodontitis has been associated with several systemic conditions, including cardiovascular disease (1), diabetes (2) and adverse obstetric outcomes (3). The biologic plausibility for such relationships is based on the inflammatory response that is initiated and perpetuated by cytokines released in response to periodontal infection. This inflammatory burden may have repercussions beyond the oral cavity, leading to a low-grade systemic inflammatory status that may affect the course of gestation (4).

Early observational studies reported a strong association between destructive periodontal disease and adverse obstetric outcomes (5,6). Although the initial findings were confirmed by epidemiological studies (7,8) and by some randomized clinical trials (9–11), larger interventional studies could not confirm a beneficial effect of periodontal therapy on preterm low birth weight (12–15). This lack of effectiveness in reducing preterm birth rates could be explained, at least in part, by the conflicting evidence on the impact of periodontal treatment on systemic proinflammatory mediators. Although some clinical studies have shown a reduction in the concentration of serum cytokines after periodontal therapy (16,17), this systemic effect was not confirmed in other investigations using samples from nonpregnant women (18,19).

To the best of our knowledge, no studies have concomitantly evaluated the effect of periodontal therapy on the levels of cytokines in gingival crevicular fluid and serum during pregnancy and after delivery. Our hypothesis was that although periodontal therapy has a local effect on cytokine levels, this local effect does not have a significant impact on systemic cytokine levels. The aim of this study was to evaluate the effect of nonsurgical periodontal therapy on the cytokine levels in gingival crevicular fluid and serum.

Material and methods

Study design and sample

The present study used a subsample of pregnant women who were previously enrolled in a randomized controlled clinical trial designed to assess the effect of periodontal treatment performed during pregnancy on the reduction of PTLBW rates (20). We selected the first 60 women who had complete clinical and immunological data according to the study design. Recruitment for the study was performed from April 2007 to June 2009. Women of 18–35 years of age with a gestational age up to 20 wk were randomly allocated to the test or the control groups using a block-stratified strategy according to smoking status. A randomization table was computer generated, and allocation to treatment was concealed in an opaque, sealed and serially numbered envelope that was opened after completion of the baseline examination. Gestational age was established by a gynecologist using information from sequential physical examinations, data from menstrual cycles and ultrasound. Women with multiple fetuses, with conditions that needed antibiotic prophylaxis for dental treatment or who were receiving orthodontic therapy, were not included. Clinical data and immunological samples were collected at baseline (before 20 wk of gestation), after treatment (26–28 wk of gestation) and 30 d postpartum. The study sample comprised 60 women: 30 in the control group and 30 in the test group. Gingival crevicular fluid and blood samples for three patients were lost during processing, and thus the samples of 57 patients were available for analysis. The Ethical Committee of the Maternal Hospital Presidente Vargas (Porto Alegre, Brazil) approved the study protocol, and each participant signed an informed consent form.

Maternal data

A structured questionnaire comprising demographics, socio-economic status and medical and dental histories was used. In brief, anthropometric data, previous and current pregnancy conditions, hospitalization during pregnancy, medication, personal and family history of diseases, smoking, alcohol consumption and oral-hygiene habits were assessed. During the study, the occurrence of gestational events, such as vaginosis, urinary infections, pre-eclampsia, gestational diabetes, medication and hospitalization were recorded using hospital records. The questionnaire used was previously tested, and trained interviewers collected data. The reproducibility of this information was tested in 10% of the sample by repeat administration of key questions from the questionnaire, 1 wk after administration of the initial questionnaire ($\kappa = 0.79$).

Periodontal clinical examination

Periodontal clinical examination was performed by three calibrated examiners and the information obtained was recorded in preset forms by trained assistants. Full-mouth periodontal examination was carried out on six sites per tooth, excluding third molars, using a manual periodontal probe (North Carolina Probe 15; Neumar, São Paulo, Brazil). Plaque index (21), gingival index (22), supragingival calculus, cavities, overhanging restorations, bleeding on probing, periodontal probing depth and clinical attachment level were recorded. Reproducibility during the study was assessed in 10% of the participants, and the intraclass correlation coefficients ranged from 0.95 to 0.96 for periodontal probing depth and from 0.84 to 0.93 for clinical attachment level. Participants were examined at baseline, between 26 and 28 gestational weeks and 30 d after delivery.

Collection of gingival crevicular fluid and blood samples

Four sites per subject were randomly selected, from among the deepest periodontal probing depths, for the collection of gingival crevicular fluid. Teeth were isolated with cotton rolls and gently air-dried. Supragingival plaque was carefully removed with curettes, and absorbent paper strips (Periopaper; Oraflow, Plainview, NY, USA) were inserted for 30 s into the periodontal pocket. Strips with blood marks or saliva were discarded. The volume of fluid absorbed by each strip was measured using a calibrated meter (Periotron 8000; Oraflow). The paper strips

were immediately transferred to an Eppendorf tube (Eppendorf do Brasil, São Paulo, Brazil) and stored frozen until analysis. Blood samples were collected from patients in the morning (after 8 h of fasting) by trained assistants. Five milliliters of blood was withdrawn from each subject by venipuncture into an anticoagulant-free vacuum tube. Samples were immediately centrifuged at 3000 *g* for 5 min, and serum was kept frozen until assayed.

Cytokine assessment

The concentrations of gingival crevicular fluid and serum cytokines were determined by flow cytometry (BD™ Cytometric Bead Array, San Jose, California, USA). The gingival crevicular fluid was eluted from the frozen Periopaper strips as follows. In brief, the Periopaper strips were placed in an Eppendorf tube containing 200 μL of phosphate-buffered saline and 2 μL of phenylmethanesulfonyl fluoride (20 mM) and incubated for 30 min. Then, the paper strips were transferred to a second Eppendorf tube, containing the same reagents, and incubated for a further 30 min. The contents of the two Eppendorf tubes were homogenized and 50 μL of this solution was used for the analysis of gingival crevicular fluid. The Human Inflammatory Cytokine Kit (BD Biosciences, San Diego, CA, USA) used allows discrimination between the following cytokines: interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70 and tumor necrosis factor- α (TNF-α). Sample processing and data analysis were performed according to the manufacturer's specification. Briefly, gingival crevicular fluid and serum samples were incubated with the six cytokine capture beads and phycoerythrin-conjugated detection antibodies for 3 h at room temperature, protected from light. Then, the samples were washed and cytokine levels were assessed using a FACSCalibur flow cytometer (BD Bioscience, San Jose, California, USA). The results were generated in graphical and tabular formats using the BD CBA Analysis Software (BD Bioscience, San Jose, California, USA).

Intervention

Periodontal treatment was performed by two periodontists (TF and JMR) at the dental unit of the Maternal Hospital Presidente Vargas. The test group received comprehensive nonsurgical periodontal therapy before the 24th gestational week. Treatment included excavation and sealing of cavities, removal of overhanging restorations, extraction of hopeless teeth, supragingival calculus removal and subgingival scaling and root planing under local anesthesia. Oral hygiene instructions were given at each appointment. No limits were imposed on the number of dental visits needed to accomplish periodontal therapy. After treatment completion, patients were seen at least once a month, according to individual needs, in order to maintain optimal plaque control. The control group received the standard dental treatment rendered to all patients at the Hospital, comprising a 1-h session of supragingival calculus removal and oral hygiene instruction. The same comprehensive periodontal therapy provided to the test group was offered to the control group after delivery. Patients in both experimental groups received pain relief treatment whenever necessary. No significant differences between test and control groups regarding the occurrence of gestational events were observed (data not shown). Only minor events related to the periodontal treatment were noted; dentin hypersensitivity was significantly more frequent in the test group.

Statistical analysis

Data analysis was performed using STATA 11.1 for Mac (Stata, College Station, TX, USA). Mean and standard deviation for all clinical parameters were calculated and reported. Differences between groups were assessed using independent *t*-tests. Sample distribution according to demographic, socio-economic and behavioral data was assessed using chi-square and Fisher's exact tests. Between-group differences on the distribution of obstetric data were assessed using Fisher's exact test, and differences in gestation period and birth weight were compared using the independent *t*-test. Cytokine levels in serum and gingival crevicular fluid were presented as median and 25% and 75% percentiles. Differences between groups were assessed using the Mann–Whitney *U*-test, and differences among experimental periods were assessed using the Wilcoxon signed-rank test. The Holm–Bonferroni method was used to adjust for multiple comparisons. Statistical significance was set at 5%.

Results

Distribution of participants according to demographics, socio-economic status and behavioral variables is presented in Table 1. Gingival crevicular fluid and blood samples for three patients were lost during processing, so data for 57 women were available for analysis. Most women were: younger than 30 years; white; high school educated; of medium–low socio-economic status; never smokers; of normal weight; nonprimiparous; and did not have a previous history of miscarriage or preterm birth. No significant differences were observed between groups for these characteristics. With regards to previous and current obstetric data, no differences for pregnancy occurrences and outcomes were observed between groups (Table 2).

At baseline, participants showed widespread inflammation but limited periodontal destruction, and no significant differences were found between the groups (Table 3). In the test group, periodontal therapy yielded a significant reduction in plaque, gingival bleeding and calculus, and this improvement remained after delivery (Table 3). After treatment, a major reduction in periodontal inflammation was observed in the test group, with bleeding on probing decreasing from 49.62% to 11.66% of sites ($p < 0.001$) and periodontal probing depth of ≥ 4 mm decreasing from 9.79% to 2.32% of sites ($p < 0.001$). These parameters remained stable after delivery. On the other hand, the control group had a significantly higher percentage of sites with plaque, supragingival calculus, gingival bleeding, bleeding on probing, periodontal probing depth ≥ 3 mm and periodontal

Table 1. Demographic, socio-economic and behavioral data at baseline in each group

Characteristic	Test group (n = 27)	Control group (n = 30)	p-value
Age			
18–24 years	9 (33.3)	9 (30)	0.84
25–30 years	12 (44.4)	12 (40)	
31–35 years	6 (22.3)	9 (30)	
Race			
White	19 (70.4)	23 (76.7)	0.76
Non-White	8 (29.6)	7 (23.3)	
Education			
Elementary	8 (29.6)	10 (33.3)	0.91
High school	18 (66.7)	18 (60)	
College	1 (3.7)	2 (6.7)	
Socio-economic status			
Low	4 (14.8)	6 (20)	0.70
Medium–low	15 (55.6)	16 (53.3)	
Medium–high	5 (18.5)	7 (23.3)	
High	3 (11.1)	1 (3.4)	
Smoking			
Never	21(77.8)	20 (66.7)	0.39
Current/former	6 (22.2)	10 (33.3)	
Body mass index before gestation[a]			
< 18.5 kg/m^2	1 (3.7)	3 (10)	0.35
18.6–24.99 kg/m^2	9 (33.3)	15 (50)	
25–29.99 kg/m^2	9 (33.3)	5 (16.7)	
≥ 30 kg/m^2	6 (22.2)	6 (20)	

Results are given as n (%).
[a]Data for three individuals were not available.

Table 2. Obstetric data regarding previous and current pregnancies

Obstetric characteristic	Test group (n = 27)	Control group (n = 30)	p-value
Previous pregnancy			
Primiparous women	11 (40.7)	15 (50)	0.60
Previous miscarriage	2 (7.4)	4 (13.6)	0.90
Previous preterm	3 (11.1)	4 (13.6)	0.89
Current pregnancy			
Genitourinary infection	4 (14.8)	4 (13.3)	0.99
Diabetes	2 (7.4)	2 (6.7)	0.67
Pre-eclampsia	1 (3.7)	1 (3.3)	0.99
Other disease and conditions[a]	0 (0)	3 (10)	0.23
Antibiotics	7 (25.9)	5 (16.7)	0.52
Alcohol consumption	0 (0)	0 (0)	–
Pregnancy outcomes			
Stillbirth	0 (0)	0 (0)	–
Neonatal death	0 (0)	0 (0)	–
Preterm birth	3 (11.1)	2 (6.7)	0.66
Gestation period (days)	2737 ± 12.5	27,693 ± 8.5	0.26
Newborn birth weight (g)	3292 ± 457	3327 ± 503	0.79

Results are given as n (%) or as mean ± standard deviation.
[a]Depression and polyhydramnios.

probing depth ≥ 4 mm than did the test group post-treatment and after delivery. Only borderline significant differences between groups were observed for clinical attachment level after periodontal treatment and delivery.

Table 4 presents the cytokine levels in gingival crevicular fluid according to experimental groups and experimental

Table 3. Clinical parameters according to the experimental period

	Baseline			After treatment			Postpartum		
Clinical parameter (% sites)	Test group	Control group	p-value	Test group	Control group	p-value	Test group	Control group	p-value
Visible plaque	56.04 ± 23.01	49.71 ± 23.01	0.31	6.88 ± 10.21	33.51 ± 24.17	< 0.001	8.69 ± 12.32	21.71 ± 20.16	0.005
Gingival bleeding	33.26 ± 15.06	33.84 ± 17.05	0.89	9.06 ± 8.18	23.45 ± 12.57	< 0.001	11.30 ± 6.87	23.87 ± 13.00	< 0.001
Calculus	22.71 ± 13.43	17.36 ± 10.62	0.10	0.38 ± 1.08	12.67 ± 11.96	< 0.001	0.76 ± 1.48	14.14 ± 11.92	< 0.001
Bleeding on probing	49.62 ± 20.74	45.65 ± 17.52	0.44	11.66 ± 8.46	37.15 ± 18.87	< 0.001	12.29 ± 8.04	30.32 ± 17.17	< 0.001
Periodontal probing depth ≥ 3 mm	50.21 ± 12.85	47.76 ± 13.39	0.48	27.82 ± 15.38	52.03 ± 13.98	< 0.001	38.79 ± 17.26	52.81 ± 13.16	0.001
Periodontal probing depth ≥ 4 mm	9.79 ± 7.52	9.11 ± 7.14	0.72	2.32 ± 4.00	13.56 10.15	< 0.001	3.58 ± 3.77	12.62 ± 11.31	< 0.001
Clinical attachment level ≥ 1 mm	13.50 ± 17.66	14.59 ± 18.55	0.82	11.13 ± 10.59	19.08 ± 21.32	0.07	12.88 ± 12.53	20.05 ± 18.14	0.08
Clinical attachment level ≥ 2 mm	5.39 ± 8.42	8.41 ± 13.13	0.31	3.28 ± 4.26	6.42 ± 10.14	0.12	2.74 ± 3.98	6.11 ± 9.38	0.08

Results are given as mean ± standard deviation. Test group, n = 27; control group, n = 30.

Table 4. gingival crevicular fluid cytokines levels in pg/mL according to treatment group and experimental period

	Baseline			After treatment			Postpartum		
Cytokine	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*
IL-1β	114.90 (67.00/239.80)^{A}	134.15 (54.05/248.63)^{a}	0.96	57.90 (15.90/94.10)^{B}	136.05 (60.30/247.80)^{a}	0.003	55.80 (40.40/76.10)^{B}	129.90 (67.60/248.90)^{a}	< 0.001
IL-6	5.40 (3.00/9.20)^{A}	3.8 (2.50/7.23)^{a}	0.20	4.40 (2.40/6.70)^{A}	4.30 (2.38/9.10)^{a}	0.76	3.30 (2.10/7.70)^{A}	4.30 (2.38/6.78)^{a}	0.59
IL-8	322.50 (249.7/498.40)^{A}	296.00 (209.18/500.8)^{a}	0.51	238.30 (64.00/383.20)^{B}	269.25 (161.98/620.9)^{a}	0.28	256.90 (144.20/550.10)^{AB}	322.60 (207.3/514.65)^{a}	0.29
IL-10	1.00 (0.90/1.50)^{A}	1.00 (0.90/1.18)^{a}	0.68	1.00 (0.90/1.10)^{A}	1.00 (0.90/1.45)^{a}	0.90	1.00 (0.90/1.00)^{A}	1.00 (0.90/1.03)^{a}	0.48
IL-12p70	1.60 (1.20/2.40)^{A}	1.30 (1.28/2.55)^{a}	0.83	1.30 (1.30/2.10)^{A}	1.30 (1.20/2.40)^{a}	0.974	1.70 (1.20/2.10)^{A}	1.40 (1.20/2.53)^{a}	0.94
TNF-α	1.70 (1.10/3.70)^{A}	1.15 (1.00/2.23)^{a}	0.28	1.20 (1.10/3.00)^{A}	1.80 (1.10/2.63)^{a}	0.85	1.30 (1.10/3.00)^{A}	1.20 (1.10/2.83)^{a}	0.73

Test group, $n = 27$; control group, $n = 30$.

Comparisons for the test group throughout the study: medians followed by different capital letters represent statistically significant differences ($p < 0.05$) between experimental periods. Comparisons for the control group throughout the study: medians followed by different lower-case letters represent statistically significant differences ($p < 0.05$) between experimental periods. IL, interleukin; TNF-α, tumor necrosis factor-α.

*p-value for comparison between groups in each experimental period.

periods. The amounts of IL-1β and IL-8 were consistently higher in gingival crevicular fluid than in serum. No significant differences were observed for IL-6, IL-10, IL-12p70 and TNF-α between groups or among experimental periods. Periodontal therapy significantly reduced the levels of IL-1β in gingival crevicular fluid and this reduction remained statistically significant after delivery. The test group had significantly lower levels of IL-1β in gingival crevicular fluid than did the control group after treatment and delivery. Regarding IL-8, there were no significant differences between groups throughout the study. When within-group analyses through the experimental periods were performed, only the test group had a significantly decreased level of IL-8 in the gingival crevicular fluid after treatment, but this difference did not remain significant after delivery.

Serum cytokine levels are presented in Table 5. No significant differences were observed between groups or among experimental periods in the serum levels of IL-1β, IL-10, IL-12p70 and TNF-α. Inconsistent trends were observed for the levels of IL-6 and IL-8 in serum, with the levels of both cytokines increasing over time, reaching statistical significance in controls after delivery. In the test group, the level of IL-6 did not change significantly during the study, whereas the level of IL-8 postpartum was significantly higher than the level post-treatment, but no differences were observed between baseline and postpartum.

Discussion

The present analysis was carried out to assess the effect of nonsurgical periodontal treatment on systemic and local inflammatory biomarkers during pregnancy and postpartum. To the best of our knowledge, this is the first study to evaluate concomitantly the effect of periodontal therapy on serum and gingival crevicular fluid levels of six cytokines that have been associated with periodontal disease and preterm birth. Although periodontal therapy during pregnancy successfully reduced the clinical signs of periodontal inflammation and the levels of IL-8

Table 5. Serum cytokine levels in pg/mL according to treatment group and experimental period

	Baseline			After treatment			Postpartum		
Cytokine	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*	Test group median (25%/75%)	Control group median (25%/75%)	*p*-value*
IL-1β	1.70 (1.40/2.00)^{A}	1.70 (1.37/2.20)^{a}	0.76	1.70 (1.30/2.10)^{A}	2.00 (1.47/2.22)^{a}	0.15	1.50 (1.40/1.90)^{A}	1.85 (1.37/2.42)^{a}	0.15
IL-6	4.15 (3.40/5.70)^{A}	3.55 (2.80/4.47)^{a}	0.07	5.20 (4.20/7.20)^{A}	4.15 (3.35/4.90)^{ab}	0.006	4.45 (3.62/6.35)^{A}	4.40 (3.40/5.50)^{b}	0.17
IL-8	10.90 (8.35/15.10)^{A}	7.85 (6.65/10.65)^{a}	0.09	9.85 (8.20/12.02)^{AB}	8.20 (6.90/11.47)^{ab}	0.14	14.70 (10.80/19.30)^{AC}	11.45 (9.47/15.80)^{b}	0.47
IL-10	1.00 (1.00/1.90)^{A}	1.00 (0.97/1.62)^{a}	0.07	1.00 (1.00/1.40)^{A}	1.10 (1.00/1.70)^{a}	0.42	1.00 (1.00/1.70)^{A}	1.00 (0.97/1.50)^{a}	0.93
IL-12p70	1.30 (1.20/1.70)^{A}	1.30 (1.20/2.42)^{a}	0.42	1.30 (1.20/1.70)^{A}	1.50 (1.30/2.12)^{a}	0.08	1.30 (1.20/2.00)^{A}	1.30 (1.20/1.92)^{a}	0.66
TNF-α	1.10 (0.97/1.10)^{A}	1.05 (0.90/1.12)^{a}	0.77	1.10 (1.00/1.10)^{A}	1.00 (0.97/1.32)^{a}	0.93	1.00 (0.90/1.10)^{A}	1.01 (1.00/1.10)^{a}	0.14

Test group, $n = 27$; control group, $n = 30$.
Comparisons for test group throughout the study: medians followed by different capital letters represent statistically significant differences ($p < 0.05$) between experimental periods. Comparisons for control group throughout the study: medians followed by different lower-case letters represent statistically significant differences ($p < 0.05$) between experimental periods.
IL, interleukin; TNF-α, tumor necrosis factor-α.
*p-value for comparison between groups in each experimental period.

and IL-1β in gingival crevicular fluid, it did not seem to have a major impact on serum inflammatory biomarkers.

The systemic impact of local periodontal inflammation has been hypothesized as a possible explanation for the association observed between periodontal disease and systemic disorders (4,23). Corroborating this hypothesis, periodontitis patients had a higher total number of leukocytes and higher plasma levels of C-reactive protein than did healthy controls (24). Compared with healthy individuals, periodontitis patients also presented higher serum levels of IL-1β (25), IL-6 (26,27), TNF-α and IL-10 (28) and IL-12 (29). These findings have been used to support the notion that periodontitis may perpetuate a low-grade systemic inflammation status.

Results concerning the impact of periodontal therapy on systemic inflammation in samples from pregnant women are scarce. Recently, a multicenter study of 823 women evaluated the effect of periodontal treatment on serum levels of C-reactive protein, prostaglandin E2, MMP-9, fibrinogen, endotoxin, IL-1β, IL-6, IL-8 and TNF-α (30). No significant differences were observed between groups for any of the biomarkers evaluated. In contrast to our results, a high level of periodontal inflammation was still observed after periodontal therapy (13). An early pilot study by Offenbacher *et al.* (10), on 53 patients, evaluated the effect of periodontal therapy during pregnancy on the levels of serum and gingival crevicular fluid biomarkers after delivery. Similarly to our findings, a significant impact of periodontal therapy on the levels of IL-1β in gingival crevicular fluid was observed, whereas no differences were observed between experimental groups for IL-6.

Conflicting evidence of the systemic impact of periodontal therapy in samples from nonpregnant women have been observed in interventional studies. D'Aiuto *et al.* (17,31) showed that nonsurgical periodontal treatment significantly reduced the serum levels of IL-6. O'Connell *et al.* (16) also showed reduced levels of systemic inflammatory biomarkers, including IL-6 and IL-12p70, in diabetic patients

after periodontal treatment. Full-mouth extraction of periodontally compromised teeth significantly decreased the serum levels of C-reactive protein, plasminogen activator inhibitor-1, fibrinogen and white cell counts in patients with advanced periodontitis (32). However, other clinical studies did not observe significant differences in the levels of IL-1β, IL-6 and TNF-α in serum after periodontal treatment (18,19). Collectively, these inconsistent results in studies of samples from pregnant and nonpregnant women question the hypothesis that periodontal disease may lead to a significant increase in subclinical markers of systemic inflammation.

The present study used a subsample of pregnant women, previously enrolled in a randomized clinical trial, to investigate the effect of periodontal treatment and strict plaque control during pregnancy on preterm/low birth-weight rates (20). Although the therapy significantly reduced periodontal inflammation, it did not affect the occurrence of adverse obstetric outcomes. The incidence of preterm births was not significantly different between test and control groups (11.72% and 9.09%, respectively), and was similar to the incidence observed in the Porto Alegre population (10.7%) (33). Additional analysis of the baseline cytokine levels in this sample did not reveal a strong correlation between gingival crevicular fluid and serum (34). The incidence of adverse gestational events in the subsample of the present study was somewhat similar to the incidence observed in the study of Weidlich *et al*. (20), where the whole sample was analyzed. The test group presented three preterm births and one low-birthweight birth, whereas the control group presented two preterm births. However, the evaluation of adverse gestational events was not the aim of this study and, because of the limited sample size and number of events, any comparison with other studies is not feasible.

One of the strengths of the present study that should be mentioned was the efficacy of periodontal therapy, which reduced bleeding on probing to 11% of sites. Although it is stated that "when RCTs are being designed to evaluate the effect of periodontal therapy on general health outcomes, it is critically important that a clinically acceptable targeted endpoint for successful periodontal therapy be included in the study design", and reductions of bleeding on probing to < 15% have been proposed (35), few clinical trials performed in pregnant women have reached this threshold of reduction on periodontal inflammation. Moreover, the longitudinal evaluation of a panel of six cytokines in gingival crevicular fluid and serum provides a better understanding of the impact of periodontal therapy on local and systemic inflammation. The study design also provided the opportunity to evaluate not only the effect of periodontal therapy during pregnancy but also the effect of pregnancy *per se*, because periodontal inflammation was not reduced in the control group during pregnancy. One of the possible limitations of the present study was the small percentage of women with severe periodontal destruction. However, the disease pattern observed in this sample is consistent with that in women of childbearing age in this same population (36,37) and the inclusion only of women with severe destructive periodontitis could limit the external validity of our findings. It is also important to acknowledge that the complex immunological events that occur during pregnancy may limit the ability to assess the systemic effect of periodontal therapy, because studies have shown that pregnant women exhibit a higher production of several cytokines depending on gestational period and pregnancy outcome.

In conclusion, periodontal therapy was effective in reducing the cytokine levels in the gingival crevicular fluid of pregnant women. However, it did not have an impact on systemic biomarkers of inflammation. These findings may explain, at least in part, the lack of effectiveness of periodontal therapy in decreasing the incidence of preterm birth.

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