Serum Inflammatory Mediators in Pregnancy: Changes After Periodontal Treatment and Association With Pregnancy Outcomes

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Abstract

Background: The purposes of this study were to determine: 1) if periodontal treatment in pregnant women before 21 weeks of gestation alters levels of inflammatory mediators in serum; and 2) if changes in these mediators are associated with birth outcomes.

Methods: A total of 823 pregnant women with periodontitis were randomly assigned to receive scaling and root planing before 21 weeks of gestation or after delivery. Serum obtained between 13 and 16 weeks, 6 days (study baseline) and 29 to 32 weeks of gestation was analyzed for C-reactive protein; prostaglandin E_2; matrix metalloproteinase-9; fibrinogen; endotoxin; interleukin (IL)-1β, −6, and −8, and tumor necrosis factor-α. Cox regression, multiple linear regression, and the t, χ^2, and Fisher exact tests were used to examine associations among the biomarkers, periodontal treatment, and gestational age at delivery and birth weight.

Results: A total of 796 women had baseline serum data, and 620 women had baseline and follow-up serum and birth data. Periodontal treatment did not significantly alter the level of any biomarker (P >0.05). Neither baseline levels nor the change from baseline in any biomarker were significantly associated with preterm birth or infant birth weight (P >0.05). In treatment subjects, the change in endotoxin was negatively associated with the change in probing depth (P <0.05).

Conclusions: Non-surgical mechanical periodontal treatment in pregnant women, delivered before 21 weeks of gestation, did not reduce systemic (serum) markers of inflammation. In pregnant women with periodontitis, levels of these markers at 13 to 17 weeks and 29 to 32 weeks of gestation were not associated with infant birth weight or a risk for preterm birth.

Preterm birth is a growing problem worldwide and has been an intractable one in the United States during the last 2 decades. Nearly 13% of all births in the United States occur before 37 weeks of gestation,[1] and

preterm birth is the leading cause of perinatal morbidity and mortality, with costs estimated at $26 billion a year.[2]

Maternal infection and inflammation have long been thought to contribute to poor pregnancy outcomes. More than 50 years ago, investigators demonstrated that the administration of *Shigella* and *Salmonella* endotoxins were capable of inducing abortions in monkeys.[3] *Porphyromonas gingivalis* lipopolysaccharide and heat-killed whole bacterium are also known to cause fetal resorption and growth restriction in rodents.[4, 5] Although the associations between preterm birth and infections such as chorioamnionitis, bacterial vaginosis, and urinary tract infections are well established, there is also evidence that non-uterine infections, including periodontal disease, may increase a woman's risk for preterm birth.[3, 6]

Collectively, case-control and cohort studies indicate that women with periodontitis are about two to three times more likely than healthy women to experience preterm birth or deliver a low birth weight infant.[7, 8] However, whether periodontitis is causally associated with adverse pregnancy outcomes or is merely a marker for other risk factors or behaviors continues to be debated.[9] Periodontitis and adverse pregnancy outcomes may be linked through a chronic, systemic inflammatory challenge to the mother and fetus in response to pathogens in the mother's oral cavity. Alternatively, these pathogens may affect the uterus more directly through repeated bacteremias with periodontitis-associated microbial species. Cytokines, which are capable of eliciting the acute-phase response, are a component of this inflammatory response and are classified as: 1) proinflammatory, which initiate or enhance a cascade of events (e.g., tumor necrosis factor [TNF]-α and interleukin [IL]-1); 2) IL-6-like (e.g., IL-6 and −11), which propagate many of the systemic manifestations of acute-phase responses; and 3) anti-inflammatory, which downregulate the acute-phase response (e.g., IL-10 and transforming growth factor-β).[10, 11]

Because of the associations among infection, inflammation, and preterm birth, researchers have attempted to identify inflammatory biomarkers that predict preterm labor and delivery. Depending on the timing and source of the sample (maternal serum, amniotic fluid, or vaginal swabs), numerous classical markers of inflammation, including white blood cell counts; C-reactive protein (CRP); IL-1β, −6, and −8; and alkaline phosphatase, have been variably associated with preterm labor and/or delivery.[12, 13] In general, the utility of these markers to predict preterm birth is improved when combined with cervical length measures.

Previously, we reported that scaling and root planing in pregnant women with periodontitis, delivered before 21 weeks of gestation, were not associated with improvements in preterm birth or birth weight.[14] The present article examines the effect of periodontal therapy on selected serum biomarkers and the relationship between these markers and birth outcomes in the same pregnant women.

MATERIALS AND METHODS

The design of the interventional study has been previously described in more detail.[14] Briefly, 823 pregnant women aged 16 to 44 years with periodontitis were enrolled between March 2003 and June 2005 at four clinical centers (University of Kentucky Chandler Medical Center, Lexington, Kentucky; Hennepin County Medical Center; Harlem Hospital; and the University of Mississippi Medical Center). This study was approved by Institutional Review Boards at all participating centers. All study women provided written informed consent for their participation. Women were enrolled between 13 and 16 weeks, 6 days of

gestation and randomly assigned to receive scaling and root planing before 21 weeks of gestation, followed by monthly periodontal maintenance (treatment group), or scaling and root planing after delivery (control group). Women were ineligible if they had multiple fetuses, required antibiotic prophylaxis prior to dental treatment, had a medical condition that precluded elective dental treatment, had extensive tooth decay, or were likely to have <20 remaining teeth after treatment of tooth decay, abscesses, or other non-periodontal pathoses. Serum samples were obtained from women at baseline prior to dental care (13 to 16 weeks, 6 days of gestation) and following study dental care at 29 to 32 weeks. Samples were stored at −80°C in aliquots and shipped in batches on dry ice via overnight courier to a central laboratory. None of the women in the study who were lost to follow-up (n = 7), withdrew consent (n = 2), or had an elective abortion (n = 2) provided a serum sample at 29 to 32 weeks.

Calibrated and masked examiners (nine across four clinical sites) measured probing depth (PD), the distance from the gingival margin to the cemento-enamel junction (GM-CEJ), and bleeding on probing (BOP) at six sites on all teeth excluding third molars. Clinical attachment loss (AL) was calculated from the PD and GM-CEJ measures. BOP was scored as present or absent.

The clinical results of the trial have been reported elsewhere.[14] In brief, control and treatment groups did not differ significantly (P >0.1) at baseline in terms of their mean number of natural teeth (26.8 versus 26.7, respectively) and percentage of tooth sites with PD ≥4 mm (24.8% versus 26.5%, respectively), clinical AL ≥2 mm (41.2% versus 43.6%, respectively), or BOP (69.0% versus 69.6%, respectively). Compared to controls, participants in the treatment group had significantly (P <0.001) greater reductions in the percentage of sites with PD ≥4 mm (11.5% versus 0.5%), clinical AL ≥2 mm (9.7% versus 0.8%), and BOP (22.7% versus 2.1%).

Laboratory Methods

Serum samples were assayed for CRP; prostaglandin E_2 (PGE_2); matrix metalloproteinase-9 (gelatinase B) (MMP-9); fibrinogen; endotoxin; IL-1β, −6, and −8; and TNF-α. All analyses were conducted at a central laboratory at the University of Kentucky. CRP was analyzed using a capture enzyme-linked immunosorbent assay (ELISA) as described elsewhere.[15, 16] Mouse monoclonal antibodies to IL-1β, −6, and-8; PGE_2;[‡‡] and TNF-α[§§] were used for ELISA. MMP-9 was assayed using a commercial ELISA kit[||||] according to the manufacturer's instructions. Endotoxin activity was assessed using a commercial kit[¶¶] as an indicator of local and systemic challenge with this inflammatory stimulant and as a correlate with increases in Gram-negative bacteria in the host. The assay was semiquantitative with a range of endotoxin units (EtxU) in each category as follows: category 1 = <0.0125 EtxU; category 2 = 0.0125 to <1 EtxU; category 3 = 1 to <5 EtxU; category 4 = 5 to <10 EtxU; and category 5 = ≥10 EtxU.

Statistical Analyses

For CRP, PGE_2, MMP-9, and fibrinogen, log (base 10) of the measurements was analyzed. Endotoxin, which was measured using a semiquantitative assay, was analyzed as both a categorical and continuous measure. Here, we present only results from the latter analyses because they have higher power to find associations with other measures. Cox regressions with likelihood ratio tests were used to study the association between gestational age at delivery and each biomarker (at baseline, at 29 to 32 weeks, and its change from baseline to 29 to 32 weeks). Full-term pregnancies were censored at 37 weeks of gestation.

Adjusted analyses of baseline serum data included clinical site, race/ethnicity, age, and body-mass index (BMI; kilograms/meter^2). Adjusted analyses of the follow-up serum data (29 to 32 weeks and changes from baseline) also included assignment of periodontal treatment group. Associations between the biomarkers and birth weight were examined using simple linear regression in unadjusted analyses and multiple linear regression in adjusted analyses.

Associations between changes in the periodontal measures and these biomarkers were tested using linear regression. The change in periodontal status was summarized as the change from baseline in the percentage of sites with PD ≥4 mm and the percentage of sites with BOP. To calculate the change from baseline in the clinical measures, we used data from the 29- to 32-week examination when they were available; data from the 21- to 24-week examination were substituted when 29- to 32-week data were not available. However, only five of the 620 women with both baseline and 29- to 32-week serum data did not have 29- to 32-week clinical periodontal data.

We also tested for the association between changes in periodontal measures and changes in serum biomarkers by comparing women in the upper and lower quartiles of change in the percentage of tooth sites with PD ≥4 mm, where the quartiles were determined separately for the treatment and control groups. In a two-way analysis of variance, the outcome was the change in the serum biomarker, and the independent variables were group (treatment or control), quartile of change in percentage of PD ≥4 mm, and interaction. For these latter analyses, the 25th and 75th percentiles were −19.64 and −3.04 percentage points, respectively, for the treatment group and −5.95 and 5.55 percentage points for the control group. The median change was −10.60 percentage points for the treatment group and −1.19 percentage points for the control group.

Few women had detectable levels of IL-1β, −6, and −8 and TNF-α at either baseline or 29 to 32 weeks of gestation. For these biomarkers, we compared treatment and control groups and explored associations with preterm birth using χ^2 and Fisher exact tests. In all cases, reported *P* values were not adjusted for multiple comparisons. Eighteen women randomized to the periodontal treatment group did not receive their assigned treatment, but none of these women provided a 29- to 32-week serum sample. Thus, intent-to-treat and per-protocol analyses of the available data were identical. Analyses of the 29- to 32-week samples also excluded 19 women (14 control and five treatment subjects) whose pregnancies ended in a spontaneous abortion or stillbirth; only four of these women were still pregnant and provided a serum sample at 29 to 32 weeks of gestation.

RESULTS

Figure 1 describes sample sizes for the analyses. Of the 823 randomized women, 82 delivered a live preterm infant, 19 experienced a spontaneous abortion (loss before 20 weeks of gestation) or stillbirth (loss between 20 and 36 weeks, 6 days), two electively ended their pregnancy, and nine were lost to follow-up or withdrew consent. Baseline serum data were available for 796 of the 823 randomized women; 27 lacked a baseline serum sample or data. Fewer women had follow-up serum data and complete information on the covariates used in the adjusted analyses. The smallest sample for any analysis reported here (n = 568) was for women who delivered a live infant, had complete birth data, baseline and follow-up serum data, and complete information on adjusters.

Figure 1

Samples sizes for analyses of baseline serum data, 29- to 32-week serum data, and changes from baseline. PTB = live preterm birth.

Relationship Between Serum Markers and Periodontal Treatment

Levels of the serum markers did not differ significantly between treatment and control groups at baseline (Table 1). Only 2.0% to 8.1% of women had detectable levels of IL-1β, −6, and −8 and TNF-α at baseline. For these markers, we compared groups in terms of biomarker presence or absence (Table 2) and found no significant differences. Also, changes in biomarker levels from baseline did not differ significantly ($P > 0.05$) between treatment and control groups (Tables 3 and 4). The average CRP level decreased, and average PGE$_2$ and fibrinogen levels increased significantly in both the treatment and control groups. However, average changes were small and did not differ between treatment and control groups (Table 3). For IL-1β, −6, and −8 and TNF-α, we compared groups in terms of the proportion of participants for whom the biomarker was detectable at baseline but not at 29 to 32 weeks. The treatment and control groups did not differ in terms of changes in these biomarkers ($P > 0.05$; Table 4).

Table 1. Baseline Levels[*] of Serum Biomarkers by Group for Markers With a High Frequency of Detection

Biomarker	Treatment Group (n = 402)	Control Group (n = 394)	P Value[†]
CRP (pg/ml)	0.76 (0.46)	0.75 (0.46)	0.81
PGE$_2$ (pg/ml)	2.27 (0.51)	2.28 (0.49)	0.80
MMP-9 (μg/ml)	−0.24 (0.29)	−0.23 (0.28)	0.62
Fibrinogen (pg/ml)	1.23 (0.24)	1.25 (0.25)	0.35
Endotoxin (EtxU)	1.79 (1.09)	1.77 (1.11)	0.77

* Mean (SD) of common log (base 10) for CRP, PGE^2, MMP-9, and fibrinogen; mean (SD) for endotoxin.

† From two-sample t test.

Table 2. Presence[*] of Serum Biomarkers at Baseline by Group for Markers With a Low Frequency of Detection

Biomarker	Treatment Group (n = 402)	Control Group (n = 394)	P Value[†]
IL-1β	23 (5.7)	29 (7.4)	0.39
IL-6	27 (6.7)	28 (7.1)	0.89

Biomarker	Treatment Group (n = 402)	Control Group (n = 394)	P Value[†]
IL-8	16 (4.0)	8 (2.0)	0.15
TNF-α	28 (7.0)	32 (8.1)	0.59

* Number (%) of samples above the lower limit of detection.

† Fisher exact test.

Table 3. Changes (mean change [SD]) From Baseline in Serum Biomarkers by Group for Markers With a High Frequency of Detection

Biomarker	Treatment Group (n = 302)	Control Group (n = 318)	P Value (unadjusted analysis)	P Value (adjusted analysis)[†]
CRP (pg/ml)	−0.06 (0.39)[*]	−0.08 (0.36)[*]	0.60	0.65
PGE_2 (pg/ml)	0.08 (0.56)[*]	0.06 (0.54)[*]	0.71	0.34
MMP-9 (µg/ml)	−0.02 (0.26)	−0.02 (0.31)	0.97	0.93
Fibrinogen (pg/ml)	0.30 (0.29)[*]	0.27 (0.32)[*]	0.35	0.35
Endotoxin (EtxU)	0.05 (1.54)	0.02 (1.54)	0.76	0.67

Positive values indicate an increase from baseline; negative values indicate a decrease from baseline.

* Significantly different from baseline (P <0.05).

† Comparing groups from adjusted analyses, including clinical site, clinic by group interaction, age, BMI, and race/ethnicity (total N = 586; see Fig. 1).

Table 4. Reduction of Detectable Serum Biomarkers by Group[*] for Markers With a Low Frequency of Detection

Biomarker	Treatment Group (n = 302)	Control Group (n = 318)	P Value[†]
IL-1β	10/16 (62.5)	13/23 (56.5)	0.75
IL-6	12/20 (60.0)	16/23 (69.6)	0.54
IL-8	8/9 (88.9)	4/5 (80.0)	1.00

Biomarker	Treatment Group (n = 302)	Control Group (n = 318)	P Value[†]
TNF-α	15/23 (65.2)	14/25 (56.0)	0.57

* Proportion (%) of women with baseline and follow-up biomarker data who had readings above the lower limit of detection at baseline and below the lower limit at 29 to 32 weeks.

† Comparing groups (unadjusted comparison using the Fisher exact test).

Relationship Between Serum Biomarkers and Pregnancy Outcomes

The fraction of live preterm births did not differ significantly between women with undetectable or detectable levels of IL-1β, −6, or −8 or TNF-α at baseline (Table 5). However, the number of women with positive samples was small, and the results should be viewed with appropriate caution. The number of women with detectable levels of these cytokines in 29- to 32-week samples was even smaller (11 for IL-8 to 33 for IL-1β), and these biomarkers were not analyzed further. None of the other biomarkers (at baseline, at 29 to 32 weeks, or change from baseline) were significantly associated with gestational age at delivery in unadjusted (data not shown) or adjusted analyses (Table 6; for all hazard ratios, the 95% confidence interval includes 1.0). For example, a 10-fold increase in baseline CRP was associated with a non-significant 30% decrease in the hazard of delivery, which is a relative risk of 0.70 for preterm delivery (Table 6, upper left data cell). For fibrinogen, the adjusted hazard ratio was 0.72 at baseline and 1.68 at 29 to 32 weeks. Thus, whereas higher fibrinogen levels at baseline were associated with a lowered risk for preterm delivery, at 29 to 32 weeks, higher fibrinogen was associated with an elevated risk. However, none of these hazard ratios differed significantly from 1.0 (P >0.05).

Table 5. Number (%) of Women With a Live Preterm Birth by Serum Biomarker Detection at Baseline (13 to 17 weeks of gestation)

Biomarker	Biomarker Detectable (preterm delivery/total [%])	Biomarker Undetectable (preterm delivery/total [%])	P Value[*]
IL-1β	7/49 (14.3)	70/718 (9.8)	0.32
IL-6	3/52 (5.8)	74/715 (10.4)	0.47
IL-8	3/23 (13.0)	74/744 (10.0)	0.50
TNF-α	5/57 (8.8)	72/710 (10.1)	1.00

* Fisher exact test.

Table 6. Change in Hazard of Preterm Delivery Associated with Changes in Serum Biomarkers

Biomarker*	Adjusted Hazard Ratio for Preterm Delivery (95% confidence interval)[†]		
	Baseline	29 to 32 Weeks	Change From Baseline
CRP	0.70 (0.40, 1.27)	1.09 (0.52, 2.36)	1.36 (0.60, 3.06)
PGE_2	1.08 (0.66, 1.86)	0.78 (0.43, 1.52)	0.64 (0.37, 1.13)
MMP-9	0.59 (0.24, 1.45)	0.58 (0.24, 1.43)	1.05 (0.35, 3.16)
Fibrinogen	0.72 (0.25, 2.03)	1.68 (0.51, 5.53)	1.28 (0.48, 3.53)
Endotoxin	0.90 (0.70, 1.14)	0.86 (0.64, 1.12)	0.95 (0.79, 1.15)

* Hazard ratios and confidence limits refer to a 10-fold (one log base 10 unit) increase in the level of the biomarker, except for endotoxin, where they refer to a one-step change in the endotoxin scale.

† Adjusted for clinic, race/ethnicity, age, and BMI. Hazards for 29- to 32-week data and change from baseline were adjusted for periodontal treatment group.

Figure 2 plots birth weight as a function of the biomarkers at baseline. No marker was significantly associated with birth weight (Table 7; column 2). Similarly, birth weight was not significantly associated with any biomarker at 29 to 32 weeks or with any change from baseline (Table 7, columns 3 and 4).

Table 7. *P* Values* for Association Between Serum Markers and Birth Weight

Marker	Baseline	29 to 32 Weeks	Change From Baseline
CRP	0.76	0.19	0.14
PGE_2	0.62	0.44	0.91
MMP-9	0.72	0.68	0.46
Fibrinogen	0.30	0.08	0.22
Endotoxin	0.87	0.17	0.45

* From multiple linear regression with birth weight as the dependent variable and serum marker (log10), age, gender, and BMI as independent variables. Periodontal treatment group also included as an independent variable in the analysis of the 29- to 32-week data (columns 3 and 4). *P* values in column 2 correspond to data shown in Figure 2.

Figure 2

Birth weight versus baseline serum biomarkers CRP **(A)**, PGE_2 **(B)**; MMP-9 **(C)**, fibrinogen **(D)**, and endotoxin **(E)**. None of the adjusted slopes (red lines; adjusted for clinic, race/ethnicity, age, and BMI) differed significantly from zero (P >0.05; see Table 7, column 2, for P values). • = denotes individual study participants; BL = baseline; EtxU_CAT = endotoxin unit category.

Relationship Between Changes in Periodontal Status and Serum Biomarkers

Changes in CRP, PGE_2, MMP-9, and fibrinogen levels were not associated with changes in periodontal status in either the treatment or control groups (Figs. 3A through 3D; P >0.05; results for BOP are not shown). In the treatment group only, a change in periodontal status was significantly associated with a change in endotoxin levels (P = 0.005 for the change in percentage of sites with PD ≥4 mm [Fig. 3E]; P = 0.03 for the change in percentage of sites with BOP [data not shown]). Surprisingly, endotoxin levels tended to increase over time in women in the treatment group who experienced the greatest improvements in the clinical periodontal measures (see, e.g., the blue line in Fig. 3E). No similar association was seen in the controls (P >0.1). Finally, for both treatment and control groups for changes in all biomarkers, we found no significant difference between those in the lowest (best) and highest (worst) quartiles in terms of change from baseline in periodontal condition.

Figure 3

Changes in the percentage of tooth sites with PD ≥4 mm versus the changes in serum biomarkers CRP **(A)**, PGE_2 **(B)**, MMP-9 **(C)**, fibrinogen **(D)**, and endotoxin **(E)**. A negative value on the horizontal axis indicates a reduction or improvement in the percentage of tooth sites with PDs ≥4 mm. A negative value on the vertical axis indicates a reduction in the biomarker level from baseline to 29 to 32 weeks. X = treatment group participant; • = control; blue line = regression slope for women in the treatment group; red line = controls. See text for P values.

DISCUSSION

We analyzed serum samples from pregnant women who received non-surgical treatment for periodontitis either before 21 weeks of gestation (treatment group) or after delivery (control group). None of the biomarkers differed significantly between groups at baseline. In both groups, CRP decreased and PGE_2 and fibrinogen levels increased slightly but significantly between baseline and follow-up. MMP-9 and endotoxin levels did not change significantly over time in either group.

We also explored associations between the biomarkers and subsequent preterm delivery and birth weight (Tables 5 through 7). None of the biomarkers at baseline were significantly associated with either outcome. Hazard ratios for preterm delivery were computed for a 10-fold increase in CRP, PGE_2, MMP-9, or fibrinogen level at either baseline or follow-up or in their changes over time (Table 6). Although none of the hazard ratios were significant, some may seem substantially different from 1.0. However, of these four biomarkers, the standard deviations of their levels and their changes from baseline ranged from 0.24 to

0.56 logs (Tables 1 and 3). Thus, the computed hazard ratios correspond to differences that are approximately two to four times the relevant standard deviation. For example, a 10-fold difference in MMP-9 at baseline was associated with a non-significant 41% increase in risk for preterm delivery (Table 6, column 2). The standard deviation of baseline common log MMP-9 was 0.29 in the treatment group (a $10^{0.29}$-fold difference) and 0.28 in controls (Table 1). Thus, this non-significant increase in risk is the difference between two women at the extreme tails of the distribution of change in fibrinogen separated by >3 standard deviations.

Finally, associations between changes in the biomarkers and periodontal measures were tested separately in treatment and control subjects. With the exception of endotoxin in the treatment group, none of the associations were statistically significant. Paradoxically, participants in the treatment group with the largest reductions in pocketing and BOP also tended to have the largest increases in serum endotoxin activities over time. Previous studies[17] suggest that periodontitis is associated with endotoxemia, measured directly or indirectly by elevated concentrations of lipopolysaccharide-binding proteins, soluble CD14, and antibodies to lipopolysaccharides from periodontal pathogens. Although it is possible that women with the greatest response to treatment experienced the largest and most prolonged treatment-induced bacteremia, follow-up serum samples were obtained at least 8 weeks after treatment. Although short-term spikes in serum inflammatory markers (e.g., CRP and IL-6) have been reported after scaling and root planing, levels appear to subside rapidly, on average within a week after treatment.[18]

Endotoxin was measured using a semiquantitative assay stratified into five category levels. We analyzed endotoxin as a categoric measure and found no significant associations with periodontal treatment, birth outcomes, or changes in clinical periodontal measures (results not shown). We presented results from analyzing the semiquantitative measure of endotoxin because this gives greater statistical power to find associations. Despite this unconventional but statistically more powerful approach, we found no association between endotoxin and any birth outcome and only a counter-intuitive association with change in clinical periodontal status in the treatment group. To our knowledge, the effect of periodontal treatment specifically on serum endotoxin has not been previously reported.

In the current study, periodontal treatment was not associated with a change in any serum inflammatory marker. These findings are consistent with several studies of non-pregnant populations in the literature, where the effect of treatment on systemic markers of inflammation continues to be debated. For example, Ide et al.[19] found that treatment did not significantly reduce serum/plasma levels of fibrinogen, CRP, TNF-α, or IL-6 or −1β. Similarly, Tonetti et al.[18] and D'Auito et al.[20] showed that scaling and root planing alone did not significantly reduce serum levels of IL-1β or −6 or TNF-α 1 to 6 months after treatment. In contrast, Elter et al.[21] demonstrated significant reductions in CRP and IL-6 1 month after treatment, which included scaling and root planing and surgery. Paraskevas et al.[22] concluded from their meta-analysis that, overall, there was only modest evidence that mechanical periodontal therapy reduces serum CRP.

The current study's failure to find a treatment effect on serum markers may be attributed to the type of treatment provided, which consisted of scaling and root planing followed by monthly tooth polishings. Topical or systemic antimicrobial agents were not used. In contrast, reductions in select serum markers were observed with more extensive treatment protocols involving surgery or the widespread use of locally delivered antimicrobial agents.[21, 23] For example, D'Aiuto et al.[23] found that levels of IL-6 decreased significantly from baseline in patients treated with scaling and root planing and an average of 80

applications of a locally delivered minocycline product but not in patients treated with scaling and root planing alone. Also, as we reported earlier,[14] women in our treatment group had relatively extensive BOP after treatment (mean BOP was reduced in this group to 47.1% from 69.8%). It is possible that we did not achieve some threshold in BOP improvements, above which these serum biomarkers could have been affected.

There also is some evidence that serum inflammatory biomarkers, including CRP and granulocyte-macrophage colony stimulating factor, increase during normal pregnancy and exceed levels found in non-pregnant women.[24, 25] According to Belo et al.,[24] "raised CRP levels [in pregnancy] may result from different stimuli occurring at different phases of pregnancy; the implantation and monocyte/macrophage production of IL-6, the necrotic process associated with placenta ageing and the progressive increments in the levels of estrogens during gestation." Thus, the lack of a significant periodontal treatment effect on these selected biomarkers in our study may have been masked by increases associated with pregnancy. We were unable to test this hypothesis because the study did not have non-pregnant controls.

None of the serum biomarkers were significantly associated with preterm birth or birth weight. These findings are consistent with obstetrics studies[26, 27] that failed to demonstrate the clinical utility of some of the same serum biomarkers to predict preterm delivery in asymptomatic women. The Preterm Prediction Study[28] examined a variety of markers in serum obtained at 24 or 28 weeks of gestation. Alkaline phosphatase, α-fetoprotein, and corticotrophin-releasing hormone were associated with subsequent delivery at <35 weeks of gestation, but CRP and IL-6 were not. Vogel et al.[12] reviewed the utility of biomarkers in serum, amniotic fluid, and vaginal swab samples to predict preterm delivery in asymptomatic women. Although a combination of markers, including cervical fetal fibronectin and serum α-fetoprotein, were found to predict delivery before 37 weeks, many other serum markers, including CRP, had little clinical utility to predict preterm birth.

Many of the reported associations between inflammatory markers and preterm delivery have been based on analyses of amniotic fluid or vaginal or cervical secretions.[29-31] Similarly, associations between serum markers and preterm delivery are frequently detected only in select groups, including those with previous preterm deliveries[13] or those who are symptomatic for preterm delivery at the time of sampling.[32, 33] In the current study, we sampled all women, very few of whom would have been symptomatic at the time of sampling. Finally, whereas some investigators[13] reported correlations between specific biomarker levels in serum and vaginal fluids (cervical or cervicovaginal), others have not. For example, although Vogel et al.[13] found that IL-1β and GM-CSF levels in serum were positively correlated with one another, IL-1β levels in serum and vaginal fluid were not.

Previously, we showed that scaling and root planing plus monthly tooth polishing in pregnant women with periodontitis significantly improved clinical and microbial measures of the disease.[14, 34] Despite this, treatment was not associated with reduced rates of preterm birth or low birth weight.[14] In the current study, we showed this same treatment did not alter systemic markers of inflammation, which themselves were not associated with preterm delivery or birth weight. While it is possible that more aggressive periodontal treatment, including the use of locally delivered antimicrobial agents with or without surgery, might have reduced systemic markers of inflammation, we found no evidence that the magnitude of the periodontal treatment response was associated with changes in these biomarkers or, previously,[14] with preterm birth risk.

CONCLUSIONS

Non-surgical periodontal treatment in pregnant women who delivered before 21 weeks of gestation does not reduce systemic (serum) markers of inflammation. In pregnant women with periodontitis, levels of these markers at 13 to 17 weeks and 29 to 32 weeks of gestation were not associated with infant birth weight or a risk for preterm birth.

ACKNOWLEDGMENTS

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