Case Series

Effect of Intensive Oral Hygiene Regimen During Pregnancy on Periodontal Health, Cytokine Levels, and Pregnancy Outcomes: A Pilot Study

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Background: Data are limited on the potential effect of intensive oral hygiene regimens and periodontal therapy during pregnancy on periodontal health, gingival crevicular fluid (GCF) and serum cytokines, and pregnancy outcomes.

Methods: A clinical trial was conducted on 120 community-dwelling, 16- to 35-year-old pregnant women at 16 to 24 weeks of gestation. Each participant presented with clinical evidence of generalized, moderate-to-severe gingivitis. Oral hygiene products were provided, together with instructions for an intensive daily regimen of hygiene practices. Non-surgical therapy was provided at baseline. Oral examinations were completed at baseline and again at 4 and 8 weeks. In addition, samples of blood and GCF were collected at baseline and week 8. Mean changes in clinical variables and GCF and serum cytokine levels (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α) between baseline and week 8 were calculated using paired *t* test. Pregnancy outcomes were recorded at parturition.

Results: Results indicated a statistically significant reduction in all clinical variables ($P<0.0001$) and decreased levels of TNF-α ($P=0.0076$) and IL-1β ($P=0.0098$) in GCF during the study period. The rate of preterm births (<37 weeks of gestation) was 6.7% ($P=0.113$) and low birth weight (<2,500 g) was 10.2% ($P=1.00$).

Conclusions: Among the population studied, intensive instructions and non-surgical periodontal therapy provided during 8 weeks at early pregnancy resulted in decreased gingival inflammation and a generalized improvement in periodontal health. Large-scale, randomized, controlled studies are needed to substantiate these findings. *J Periodontol 2014;85:1684-1692.*

KEY WORDS

Cytokines; gingivitis; inflammation; oral hygiene; pregnancy; premature birth.

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Compelling evidence based on epidemiologic, microbial, and intervention studies suggests an association between maternal periodontal inflammation and adverse pregnancy outcomes that include preterm birth (PTB), defined as gestational age (GA) <37 weeks, and low birth weight (LBW) of <2,500 g.[1-4]

Periodontal inflammation is known to produce increased secretion of several proinflammatory cytokines found in gingival crevicular fluid (GCF). Most notably, levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and prostaglandin E_2 (PGE_2) are increased. Furthermore, analyses of serum and amniotic fluid at the time of parturition demonstrate elevated proinflammatory markers that have been associated with preterm delivery.[5-9] Periodontal pathogens and their virulence factors are able to disseminate systemically and induce local and systemic inflammatory responses in the host.[10-12] During pregnancy, these processes can progress to the amniotic cavity, affect placental tissues, and cause disturbances in the maternal–fetal unit. These events can alter fetal development and may lead to premature uterine contractions.[13-15]

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During pregnancy, the prevalence and severity of gingivitis increase throughout the gestational period, and this increase in inflammatory signs is disproportionate to the quantity of plaque accumulation.[16-18] Hormonal changes in pregnancy have been found to be a modifying factor, and bacterial plaque is a necessary primary etiology for gingivitis. In the absence of bacterial challenge, gingival tissues can remain in a healthy state during pregnancy.[19-21] A positive correlation was also observed between an overgrowth of *Porphyromonas gingivalis, Tannerella forsythia*, and *Campylobacter rectus* and an increase in estradiol concentrations.[22,23]

Interventional studies evaluating the effects of periodontal therapy in pregnant women with periodontitis have demonstrated inconsistent outcomes.[24,25] Pregnancy gingivitis is the most common form of periodontal disease in pregnant women, affecting 36% to 100% of pregnant women; however, there are limited data demonstrating the effects of gingivitis as a potential risk factor for PTB/LBW.[26,27] A landmark investigation of Chilean women showed that women with gingivitis who were untreated were at a higher risk of PT/LBW than women who received periodontal treatment (odds ratio [OR] 2.76; 95% confidence interval [CI] 1.29 to 5.88; $P = 0.008$).[28]

The present investigation evolved from a belief in the need for a practical, effective, and cost-efficient approach toward reducing the prevalence of pregnancy-associated gingivitis across large populations. This pilot study seeks to determine if early intervention with an individually tailored, oral hygiene education and counseling regimen, coupled with professional non-surgical periodontal therapy, could improve oral health and lead to fewer PTBs and low-weight neonates. An aim of the study is to observe the impact of the intervention on inflammatory responses as measured by serum and GCF proinflammatory cytokine levels and periodontal inflamed surface area (PISA). The authors hypothesized that alterations in inflammatory load may be significant and that changes noted in systemic inflammatory mediators could help to elucidate the biologic mechanisms responsible for gingivitis–pregnancy interactions and, ultimately, pregnancy outcomes.

MATERIALS AND METHODS

Study Population

The study population consisted of community-dwelling pregnant women recruited from the Center for Women's Reproductive Health at the University of Alabama at Birmingham (UAB), where they presented for their prenatal checkup. Before enrollment, the protocol was reviewed and approved by the UAB Institutional Review Board. Each enrollee participated in an informed consent discussion and signed an Institutional Review Board–approved informed consent form.

Sample Size Estimates

Data on inflammation markers are limited regarding sample size. Sample size calculation was performed with data from an interventional study on pregnant women with pregnancy gingivitis.[28] For a 33% reduction in clinical periodontal parameters and 80% power, a sample size of 107 participants was calculated. To account for approximately 10% loss to follow-up, 120 women were enrolled in this investigation.[28]

Participant Enrollment

A total of 672 pregnant women were screened. From this pool, 120 participants (aged 16 to 35 years) consented and, based on the following inclusion and exclusion criteria, were enrolled in the study. Inclusion criteria were: 1) pregnant women aged 16 to 35 years, with a single fetus at 16 to 24 weeks of gestation at the time of enrollment; 2) minimum of 20 natural teeth; 3) moderate-to-severe gingivitis, defined as gingival index (GI) ≥2 at ≥50% of sites; and 4) able to read and understand written English without the aid of an interpreter and willing to participate in the consenting process. Exclusion criteria: 1) plural gestations or positive history of human immunodeficiency virus infection, AIDS, autoimmune disease, diabetes mellitus (except gestational diabetes); 2) periodontitis, defined as clinical attachment level (CAL) >3 mm at ≥3 sites; 3) concomitant orthodontic treatment; 4) previous spontaneous PTB; or 5) bacterial vaginosis as assessed by Gram staining.

Participants were recruited during a period of 18 months from April 2007 to October 2008, because many of the screened participants presented with periodontitis. Of all the patients recruited, 90 participants completed all study visits. Among those who failed to complete all study visits, one failed to return for any visits after consenting, 16 were lost to follow-up after baseline, and an additional 13 failed to return after visit 2. These participants either lost interest in participating or moved away from the Birmingham area. Pregnancy outcome data were obtained on 118 participants who delivered at the UAB hospital. Of these participants, 89 completed all study visits and 29 were lost to follow up. Final parturition data were obtained in February 2009.

Study Visits

All study visits were scheduled concomitantly with monthly obstetric visits. At the baseline visit (visit 1), the participant's demographic data and initial oral hygiene habits and oral hygiene knowledge were collected via survey. All participants viewed an

educational video that discussed proper oral hygiene techniques and were provided with a DVD for further reference at home. A comprehensive periodontal examination was performed, and plaque index (PI), probing depth (PD), CAL, GI, periodontal epithelial surface area (PESA), and PISA were recorded. GCF and serum samples were collected as described below. All participants received one-on-one intervention. Participants were provided with a power toothbrush,§ dental floss,‖ 0.454% stannous fluoride toothpaste,¶ and 0.07% alcohol-free mouth rinse# dispensed in amounts that were adequate for ≈6 weeks of use as prescribed.

Participants returned for visit 2 at week 4. At this visit, study clinicians performed oral examinations to determine PI and GI and record any adverse events. Counseling was provided to reinforce daily habits and oral hygiene practices with recommended techniques; product kits that included dental floss, toothpaste, and mouth rinse were replenished. The final assessments were performed at week 8. Clinical data and biologic collection mirrored visit 1. In an effort to capture postintervention oral hygiene knowledge, beliefs, and behavior, participants completed a second survey and were provided with a supply of dental care products.

Recording of Periodontal Parameters

The periodontal evaluation included clinical assessment on all fully erupted teeth (excluding third molars) and was performed by a calibrated examiner (MK) who was trained in study protocol and examination procedures before study initiation. Annual retraining sessions were held throughout the study period. Intraexaminer κ scores between measurements were 0.962 and 0.884 for PD and CAL, respectively. CAL, PD, and the presence of bleeding on probing (BOP) were measured at six sites per tooth using a manual periodontal probe.** GI and PI were measured at six sites per tooth as described by Löe.[29] For PI, categories 2 and 3 from the original index were collapsed into a single category.

PESA and PISA calculations were performed using data collected during the periodontal examinations. These calculations account for the varying epithelial surface area of different tooth types throughout the oral cavity based on anatomic differences in radicular form. PESA quantifies the surface area of pocket epithelium, and PISA incorporates the presence of BOP to allow quantification of the overall inflammatory burden posed by periodontal disease. Although PISA is a great tool currently available for assessment of the amount of periodontal inflamed tissue, it might not be very precise. Some of the shortcomings are that PISA quantifies the amount of inflamed periodontal tissue in only two dimensions. Its accuracy may be affected by errors related to observer, instrument, teeth, patients, and their interactions. The individual variations in root surface area and root length are not taken into account when calculating PISA.[30]

PESA/PISA were calculated based on previously published algorithms using PD and BOP as clinical measurements.[30] The investigators opted to use PD based on the patient population and the diagnosis of gingivitis, which might indicate a propensity for gingival edema and/or overgrowth. If CAL had been used as the basis for the PESA/PISA calculation, it could result in an underestimation of inflammatory burden because the gingival margin is located above the cemento-enamel junction.

Collection and Measurement of GCF and Blood Samples

GCF samples were collected at baseline and 8 weeks postintervention to allow for quantification of local inflammatory mediators present in the GCF. GCF samples were collected using a standardized protocol on filter paper†† placed for 30 seconds in the gingival sulcus of two randomly selected sites on non-adjacent teeth in each participant. Strips with visible blood marks or saliva contamination were discarded. The volume of fluid absorbed by each strip was measured using a calibrated, electronic gingival fluid–measuring instrument.‡‡ Both filter paper strips for each patient were pooled in one 1.5-mL screw-top cryovial and stored frozen at –70°C until batch laboratory analysis.

Blood was drawn by venipuncture from each participant by a certified nurse practitioner (Carol Morgan, Department of OBGYN, University of Alabama at Birmingham, Birmingham, AL) into an anticoagulant-free vacuum tube. Samples were aliquoted and centrifuged for 5 minutes, and serum was stored at –70°C until batch laboratory analysis.

Analyses of Samples

GCF cytokine analysis. The stored GCF samples were transferred to 5-mL Eppendorf tubes§§ and eluted using 100 μL phosphate-buffered saline. Samples were allowed to sit at room temperature for 30 minutes and then tube centrifuged for 10 minutes at 16.1 relative centrifugal force. The eluted sample was then used for immunoassay analysis to determine the concentrations of TNF-α, IL-1β, and IL-6; a bead-based multiplex profiling system‖‖ and appropriate

§ Oral B Triumph, Procter & Gamble, Cincinnati, OH.
‖ Crest Glide, Procter & Gamble.
¶ Crest Pro-Health, Procter & Gamble.
# Procter & Gamble.
** UNC-15, Hu-Friedy, Chicago, IL.
†† Periostrips, OraFlow, Plainview, NY.
‡‡ Periotron 8000, OraFlow.
§§ Eppendorf, Hauppauge, NY.
‖‖ Luminex 200, EMD Millipore, Billerica, MA.

compatible software¶¶ were used to generate the raw data report. All study samples were assayed following the manufacturer's guidelines.

Serum cytokine analysis. The serum samples were thawed immediately before assay. Aliquots of each sample were assayed using enzyme-linked immunosorbent assay kits## for TNF-α, IL-1β, and IL-6 according to the manufacturer's recommendations. Serum sample analysis was performed on 72 samples due to a freezer malfunction during the storage time frame.

Treatment Protocol

The intervention was described in detail in Geisinger et al.[31] Briefly, it consisted of individually tailored intensive one-on-one oral hygiene counseling coupled with demonstration and instructions for using oral hygiene products. Non-surgical periodontal therapy, including supragingival and subgingival scaling, was performed by a periodontal resident (Reem Atout, Department of Periodontology, University of Alabama at Birmingham, Birmingham, AL) using ultrasonic instruments and hand scalers and curets. Topical anesthesia was used to improve patient comfort, if necessary.

Pregnancy Outcome Data

To obtain the delivery and neonate outcome data, a masked nurse practitioner (Carol Morgan, Department of OBGYN, University of Alabama at Birmingham, Birmingham, AL) from the department of Obstetrics and Gynecology at UAB reviewed medical records; the reviewer was masked to the patient's periodontal characteristics and response to the intervention. PTB was defined as <37 weeks of gestation and LBW as an infant with birth weight <2,500 g. GA was calculated in this study based on last menstrual period (LMP) confirmed with ultrasound measure at <20 weeks gestation. If LMP and ultrasound measure did not agree within 7 days or if the participant did not have a sure LMP, ultrasound measure was used to determine GA. Prematurity was defined in this study as birth before 37 completed weeks (259 days) of gestation, as these GA benchmarks were used in similar recent studies.[32,33]

Statistical Analyses

To compare demographics of participants who completed the study with those who were lost to follow up, *P* values was estimated from χ^2 for categorical variables and two-tailed *t* test for continuous variables. Mean changes in clinical variables (PI, GI, CAL, PD, PESA, PISA) and GCF and serum cytokine levels (TNF-α, IL-1β, and IL-6) at visits 1 and 3 were calculated using paired *t* test. Mean values of clinical variables (PI, GI, CAL, PD, PESA, PISA) and GCF and serum cytokine levels (TNF-α, IL-1β, and IL-6) among PTB and LBW participants at visit 3 in comparison with normal birth weight (NBW) and full-term birth (FTB) participants were calculated using Wilcoxon rank-sums. Statistical significance was set at $P < 0.05$ so that the data were comparable to similar experiments in previously published reports.[29] Crude and adjusted ORs and associated 95% CIs were calculated for risk of PTB and LBW using baseline level of periodontal disease. The OR was adjusted for current smoking status; none of the other potential confounders showed any modifying effect on the adverse pregnancy outcome in this study. Using plots of sensitivity and specificity against levels of PISA/PESA and cytokines, the threshold at which sensitivity and specificity were maximized was determined by the point at which the lines for sensitivity and specificity crossed, and a receiver operator curve was generated. The area under the curve (AUC; i.e., the *c*-statistic) was computed for each threshold and compared to null *c*-statistic of 0.50 (i.e., no better than chance).

RESULTS

No adverse events related to dental products or the therapy provided were reported. One participant suffered intrauterine fetal demise due to unknown obstetric complications at 20.5 weeks; she did not complete all study visits.

Participants ranged in age from 16 to 35 years; ≈83% were African American, 88% were single, and 13% smoked during the pregnancy. Baseline characteristics of the participants are shown in Table 1. Participants who completed the study visits were more likely to be African American (84.4% versus 78.6%, $P = 0.0547$) and less likely to be current smokers (10.0% versus 25.0%, $P = 0.0429$). There was no significant difference in the baseline level of periodontal disease as measured by extent of PD and CAL ≥4 mm between these two groups. The participants who completed all study visits showed decreases in whole-mouth scores of all the clinical periodontal variables by mean value ± SD of PD (0.34 ± 0.47), PI (0.71 ± 0.50), GI (0.73 ± 0.39), CAL (0.21 ± 0.57), PISA (550.50 ± 533.80), and PESA (228.90 ± 317.40) at a statistical significance of $P < 0.0001$.

Adequate volumes of GCF samples for the assays were collected from 78 participants who completed all study visits. There was a decrease in the mean value of the GCF cytokine levels TNF-α, IL-1β, and IL-6 postintervention, and this change was statistically significant for TNF-α and IL-1β ($P = 0.0076$ and 0.0098, respectively) (Table 2). A freezer

¶¶ Bioplex Software Manager, Hercules, CA.
R&D Systems, Minneapolis, MN.

Table 1.

Comparison of Baseline Characteristics of Study Participants Who Completed the Study and Those Lost to Follow-up

Baseline Characteristics	Completed (n = 90)	Lost to Follow-up (n = 30)*	P
Demographics			
Mean age (years)	23.1 ± 4.3	21.7 ± 4.2	0.1345
Race (%)			
African American	84.4	78.6	0.0547
White	7.8	17.9	
Hispanic	7.8	0.0	
Other	0.0	3.5	
Education (%)			
Elementary	1.1	0.0	0.1422
Junior high	0.0	3.6	
High school	66.7	82.1	
College	30.0	14.3	
Graduate school	2.2	0.0	
Mean body mass index (kg/m^2)	27.9 ± 7.5	28.2 ± 8.2	0.8899
Lifestyle			
Currently smoke (%)	10.0	25.0	0.0429
Currently drink (%)	1.1	0.0	0.5754
Currently use illicit drugs (%)	0.0	0.0	—
Marital status (%)			
Single	84.5	82.1	0.2341
Widowed	0.0	3.6	
Married/Partner	12.2	7.2	
Divorced	1.1	0.0	
Separated	2.2	7.1	
Medical history			
Insurance (%)			
Private	6.7	3.6	0.7512
Medicaid	87.7	92.8	
None	5.6	3.6	
Prior pregnancy (%)	31.1	32.1	0.9181
Mean number of live births	1.6 ± 1.0	1.3 ± 0.7	0.3044
Periodontal variables			
PD ≥4 mm			
Mean number	29.77	30.24	0.9380
Mean %	18.22	18.63	0.9119
CAL ≥4 mm			
Mean number	7.36	6.72	0.8303
Mean %	4.55	4.20	0.8484

* Marital status, education, smoking, and alcohol variables missing for one enrolled participant who was lost to follow-up.

malfunction led to degradation of serum samples; analysis of serum cytokines was completed on serum drawn from 72 participants. In serum, an increased mean value for IL-6 by 0.1538 ± 0.9473 pg/mL was noted; however, as shown in Table 2, none of these changes were statistically significant.

Table 3 shows that the rate of PTB among all participants for whom parturition data were available was 6.7% ($P = 0.113$) and rate of LBW was 10.2% ($P = 1.00$). The PTB rate for participants completing the study visit was 5.6%, and for those who were lost to follow-up, 10.3%. The LBW rate for participants completing all study visits was 9.0%, and for those who were lost to follow up, 13.8%. The adjusted OR and 95% CI indicate that participants who were non-compliant with study visits were 3.05 times more likely to have PTB and 2.21 times more likely to have LBW babies compared with participants who completed all study visits; perhaps because of the small sample size, the difference was not statistically significant (Table 4).

By comparison, the PTB rate found in this study is lower than that of historic controls from a preliminary study conducted at the same institution and using the same recruitment criteria (9.54%).[34] This difference, however, is not statistically significant. The non-compliant group in this study had higher PTB rate (10.3%) compared with the historic controls, which was not statistically significant.

No statistically significant difference between the mean of 8-week GCF cytokine levels was noted among participants with PTBs compared with those with FTB or among participants with LBW neonates compared with NBW babies (Table 5).

Receiver operator curve analysis was performed to test the discriminatory ability of PESA, PISA, and GCF cytokines to predict the probability of occurrence of PTB and LBW. Low-to-moderate discriminatory ability was shown by PESA and GCF cytokines to predict LBW and PTB, although this ability was not statistically significant. Change in PISA from baseline to 8 weeks postintervention with a chosen threshold of 100 mm^2 had a statistically significant discriminatory ability for LBW in this patient population, though the AUC was fairly low (0.6381, $P = 0.0257$) (Table 6).

DISCUSSION

Previous interventional studies focusing on the effect of periodontal treatment to reduce the risk of adverse pregnancy outcomes have been unable to consistently demonstrate a decrease in PTB/LBW rate.[24,25] Whereas those studies focused on the treatment of mild-to-moderate periodontitis, few investigations have assessed the effect of intervention on PTB in pregnant women diagnosed with gingivitis.[28]

Table 2.

Effect of Intervention on Periodontal Variables and Cytokine Levels (mean ± SD)

Variable	Baseline	8 weeks	Difference	*P**
Periodontal				
PD (mm)	2.79 ± 0.52	2.44 ± 0.50	0.34 ± 0.47	<0.0001
GI	1.44 ± 0.43	0.73 ± 0.49	0.71 ± 0.50	<0.0001
PI	1.38 ± 0.42	0.65 ± 0.47	0.73 ± 0.39	<0.0001
CAL (mm)	2.07 ± 0.52	1.86 ± 0.57	0.21 ± 0.57	0.0008
PISA (mm^2)	920.15 ± 557.34	369.63 ± 478.68	550.50 ± 533.80	<0.0001
PESA (mm^2)	1,527.03 ± 380.01	1,298.08 ± 346.42	228.90 ± 317.40	<0.0001
GCF cytokines (pg/mL)				
TNF-α (n = 78)	3.97 ± 6.82	2.74 ± 3.79	1.23 ± 3.96	0.0076
IL-1β (n = 78)	51.34 ± 72.58	33.90 ± 60.18	17.45 ± 58.16	0.0098
IL-6 (n = 78)	3.77 ± 6.12	2.99 ± 2.89	0.78 ± 4.73	0.1762
Serum cytokines (pg/mL)				
TNF-α (n = 72)	3.110	2.335	0.0637 ± 4.9831	0.951
IL-6 (n = 72)	1.653	1.655	−0.1538 ± 0.9473	0.100
IL-1β (n = 72)	0.381	0.374	0.0396 ± 0.5914	0.792

* Estimated from paired *t* test.

Table 3.

PTB and LBW Rates (n [%])

Participants	n	PTB Rate	LBW Rate
All study participants	118	8 (6.7)	12 (10.2)
Participants completing all study visits	89	5 (5.6)	8 (9.0)
Participants lost to follow-up	29	3 (10.3)	4 (13.8)

Table 4.

PTB and LBW in Participants Lost to Follow-Up

Participants Lost to Follow up (n = 29)	PTB	LBW
OR (95% CI)		
Crude	2.83 (0.71 to 11.38)	1.96 (0.60 to 6.41)
Adjusted for current smoking status	3.05 (0.74 to 12.57)	2.21 (0.66 to 7.44)

Additionally, studies observing the effect of therapy on GCF or serum cytokine levels during pregnancy are also very limited.[35-38]

This study evaluates the effect of one-on-one oral health education and counseling; use of audiovisual aids, including an instructional video; and non-surgical therapy on gingival and systemic inflammation in pregnant women diagnosed with pregnancy-associated gingivitis. The intervention successfully reduced the clinical signs of gingival inflammation and the levels of TNF-α and IL-1β in GCF in pregnant patients during an 8-week intervention. Despite known increases in systemic inflammation during the gestational period, levels of serum TNF-α and IL-1β did not demonstrate a statistically significant change from baseline to 8 weeks, and a slight increase in IL-6 levels was noted. The effects of this intervention are very similar to a previous report by Fiorini et al.,[38] in which they noted a decrease in the clinical signs of periodontal inflammation and the levels of IL-8 and IL-1β in GCF, but no major impact on serum inflammatory biomarkers was shown after non-surgical therapy and oral hygiene instructions during pregnancy. Because of the lack of

Table 5.

Comparison of Mean GCF Cytokine Levels (mean ± SD) at Visit 3 Between PTB and FTB and Between LBW and NBW

GCF Cytokine	PTB (n = 8)	FTB (n = 110)	*P**	LBW (n = 12)	NBW (n = 106)	*P**
TNF-α	3.30 ± 0.62	2.65 ± 3.81	0.0889	1.70 ± 1.14	2.80 ± 3.91	0.6154
IL-1β	84.38 ± 164.20	28.89 ± 45.63	1.0000	34.98 ± 41.22	31.90 ± 60.70	0.5498
IL-6	4.37 ± 2.92	2.79 ± 2.84	0.1253	1.50 ± 1.35	3.10 ± 2.96	0.1044

* *P* values estimated from Wilcoxon ranked-sums test.

Table 6.

Discriminatory Abilities of the Periodontal Variables (PISA, PESA) and GCF Cytokine Levels to Predict PTB and LBW

	LBW			PTB		
Variable	Threshold	AUC	*P**	Threshold	AUC	*P**
Periodontal						
PISA	100	0.6381	0.0257	200	0.5781	0.3109
PESA	1,200	0.6098	0.0756	1,200	0.6006	0.1924
GCF cytokine						
TNF-α	2	0.5061	0.9271	3	0.5811	0.3415
IL-1β	10	0.5377	0.6072	10	0.5541	0.5327
IL-6	2	0.5889	0.0970	3	0.6186	0.1872

* *P* value for comparison of AUC to null AUC of 0.50.

a control group in this study, it is difficult to determine if the changes in the local and systemic inflammatory markers demonstrate an alteration from normal changes during gestation. Also, the decrease in the biomarkers of inflammation in GCF may be a representation of fluctuations in their levels with time. Because the level of TNF-α is usually very low in the systemic circulation and IL-1β level alone does not represent the biologic activity of multiple components, including IL-1Ra, soluble receptors, and density of cell surface receptors may be another factor for the lack of effect on the serum inflammatory markers. The complex immunologic events that occur during pregnancy may limit the ability to assess the systemic effect of periodontal therapy. Pregnancy is itself a dynamic state, and pregnant women exhibit a higher production of several cytokines that increases throughout the gestational period. Future investigations should be designed to include a control group who receive delayed intensive oral hygiene intervention postparturition to allow for comparison of the natural course of changes in cytokine levels during pregnancy with changes seen in the intervention group.

It is established that vaginal and other distant infections by Gram-negative bacteria may activate a cell-mediated immune response resulting in the production of cytokines such as IL-1β, IL-6, TNF-α, and prostaglandins able to precipitate preterm labor.[13,39,40] When pregnant female hamsters were injected with the periodontal pathogen *P. gingivalis,* exposure to the bacteria resulted in a ≈20% decrease in fetus size and an increase in local and intra-amniotic inflammatory mediators, particularly TNF-α and PGE_2.[41] Patients with inflamed gingival tissues demonstrate increased levels of proinflammatory markers associated with PTBs.[42,43] IL-1β is a critical mediator of acute innate immune responses to microbial components and acute inflammation that, if persistently present, can result in both hard and soft connective tissue breakdown. Additionally, IL-1β and TNF-α induce synthesis of IL-6, which is associated with chronic innate immunity and therefore represents a second wave of inflammatory mediators and can serve to mediate additional connective tissue breakdown.

Furthermore, ulceration of the subgingival epithelial tissues allows an aperture for inflammatory

mediators and bacteria to enter systemic circulation. Because PISA quantifies the amount of inflamed periodontal pocket epithelium, it is assumed that PISA also quantifies the inflammatory burden posed by periodontal inflammation. Receiver operator curve analysis revealed that a reduction in PISA of 100 mm^2 after oral hygiene intervention was associated with decreased LBW in this patient population. This may indicate that there is a threshold of periodontal inflammatory burden and the intervention is able to reduce PISA below that threshold level in patients with pregnancy gingivitis. This may result in improved systemic inflammation levels and, ultimately, improved maternity outcomes. It is plausible that interventional procedures able to sufficiently reduce ulcerated pocket epithelium will result in a subsequent reduction in bacteremia secondary to periodontal inflammation. This reduction in the opportunity for systemic migration of bacteria and bacterial byproducts may then result in decreased transmission of these factors to the placenta and amniotic environment. Published reports have implicated the putative periodontal pathogen *Fusobacterium nucleatum* in colonization of the immune-protected intrauterine space and have associated it with fetal demise.[44]

The strengths of this study lie in the emphasis on oral health education and patient self-care as an intervention. This investigation used technology to educate pregnant women about their oral and overall health. Oral hygiene reinforcement throughout the study period, accomplished with cellular telephone reminders and combining study visits with the prenatal care visit at the Center for Women's Reproductive Health, helped made this intervention and oral health behavioral change acceptable to participants. A limitation of this study is the lack of a control group enrolled with the same inclusion/exclusion criteria receiving a delayed oral hygiene intervention regimen. Another limitation is the higher-than-anticipated loss to follow-up rate in this study compared to previous studies conducted under similar conditions, which may have underpowered this investigation. Additionally some serum samples were lost because of a freezer malfunction. To enhance recruitment and retention efforts, postparturition incentives and flexible treatment hours could be incorporated in future studies. Also, the analysis of more robust markers of systemic inflammation (C-reactive protein, fibrinogen, PGE_2) was not within the scope of this study.

CONCLUSIONS

A low-cost, low-morbidity oral hygiene intervention may be beneficial and cost effective in overall improvement of maternal oral and systemic health and reduce adverse pregnancy outcome in high-risk populations. However, because this is an underpowered pilot study, caution must be exercised in interpreting the results. Large-scale randomized trials are required to substantiate the results of this study and for effective policy recommendations based on this type of intervention. This data may be used to inform future investigations and allow for sample size calculation for larger trials designed to overcome the limitations of this study and to include analysis of a more complete panel of systemic inflammatory markers, including IL-1Ra, C-reactive protein, and fibrinogen, and microbiologic sampling at gingival and vaginal sites.

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