

Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York

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The aim of this report is to provide early data from an ongoing study examining (i) the relationship between periodontal infections and pre-term low birth weight (PLBW) in a cohort of young, minority, pregnant and post-partum women; and (ii) the effect of periodontal interventions on pregnancy outcome. During the first 2 yr of the study, 213 women were enrolled and examined clinically for dental plaque, calculus, bleeding on probing, and probing depth. Birth outcome data were available for 164 women, including one group ($n=74$) subjected to oral prophylaxis during pregnancy, and a second group ($n=90$) who received no prenatal periodontal treatment. Subgingival plaque samples were available from 145 subjects (4 samples/subject) and were analyzed by checkerboard DNA hybridization with respect to 12 bacterial species. The prevalence of PLBW was 16.5% (27 cases) in this cohort. No differences in clinical periodontal status were observed between PLBW cases and women with normal birth outcome. However, PLBW mothers had significantly higher levels of *Bacteroides forsythus* and *Campylobacter rectus*, and consistently elevated counts for the other species examined. PLBW occurred in 18.9% of the women who did not receive periodontal intervention (17 cases), and in 13.5% (10 cases) of those who received such therapy.

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Pre-term low-birth weight (PLBW) is considered the foremost problem in obstetrical medicine and remains the leading cause of morbidity and mortality among neonates (1). An estimated 11% of pregnancies end in pre-term birth (2) and, in spite of significant improvements in medical care, the pre-term birth rate in the western world appears to be increasing (3). PLBW infants are at higher risk for a number of acute and chronic disorders, including respiratory distress syndrome, cerebral palsy, pathologic heart conditions, epilepsy, and severe learning problems (4). Each year, more than USD 5 billion is spent in the United States for neonatal intensive care, with the majority of this amount absorbed by PLBW babies (5). The costs associated with the care of pre-term infants and neonatal intensive care add over USD 10 billion per year to the aggregate cost of childbirth in the US (6). Babies severely affected by pre-term birth require a lifetime of care costing an average of USD 500,000 per child (6).

Established risk factors for PLBW include young maternal age, low maternal weight gain, low pregravid weight, multiple gestations, gestational diabetes, genitourinary tract infections, drug use, cigarette smoking, and excessive alcohol consumption, while previous pre-term birth is a strong predictive marker of future pre-term labor (7). However, as much as 58% of the variance of the incidence of PLBW remains unexplained (8).

A growing body of evidence suggests an infectious etiology for a large percentage of cases of pre-term birth. Genito-urinary tract infections, such as bacterial vaginosis, and inflammatory mediators resulting from such infections, have been considered a biologically plausible pathway for pre-term labor and premature rupture of membranes. However, women with pre-term labor do not invariably present with a positive amniotic fluid culture (9), suggesting that subclinical infections may account for some of the inflammatory processes associated with pre-term birth. Alternatively, it was hypothesized that PLBW may be indirectly mediated through *distant* infections resulting in translocation of bacteria, bacterial vesicles and lipopolysaccharide (LPS) in the systemic circulation. However, the exact mechanisms for the proposed relationship remain unclear. One line of reasoning suggests that maternal infections may lead to excessive production of pro-inflammatory cytokines and eicosanoids, all of which are established biochemical mediators of parturition (10–12). For example, administration of interleukin (IL)-1 β has been shown to be capable of inducing pre-term labor in animal models (13). Likewise, injection of live bacteria or endotoxin in experimental animals have been shown to result in shortened pregnancy and lower birthweight (14, 15).

The possibility that periodontal infections may constitute remote maternal infections that may adversely

influence the birth outcome was raised for the first time in the late 1980s (16). It is well established that these infections are dominated by Gram-negative anaerobic microbiota (17) and are characterized by high levels of inflammatory mediators in the periodontal tissues (18). It is also known that transient bacteremias commonly occur in subjects with inflamed gingiva (19) and may conceivably reach the placental tissues providing the inflammatory impetus for labor induction (20). An interesting observation in this context was provided in a publication by HILL and co-workers (21), who reported that amniotic fluid cultures from women with vaginosis rarely contained bacteria common to the vaginal tract, but frequently harbored fusobacteria which are common constituents of the periodontal microbiota. Thus, these authors proposed that oral bacteria may reach amniotic fluids and influence maternal fetal tissues via a hematogenous spread. Interestingly, several periodontal pathogens display tissue invasion properties (22, 23), which may constitute an additional mechanism of chorio-amniotic challenge. So far, there is only a single full-length publication available in the literature suggesting a positive association between periodontal infections and pre-term birth. This case-control study by OFFENBACHER and co-workers (24) revealed that mothers giving birth to pre-term, low birthweight babies suffered more extensive and severe periodontal disease than women with normal birth outcomes. However, it is apparent from a number of recent abstracts (25, 26) and baseline reports (27) that this relationship is currently under investigation in several research centers.

Our group has initiated a study addressing various aspects of the association between periodontal infections and adverse birth outcomes in a cohort of young minority women of low socioeconomic status in New York. The aim of this first report was to provide some early clinical and microbiological data obtained after the first 2 yr of the study.

Material and methods

Study sample

The design and methods of this study were approved by the Columbia Presbyterian Medical Center Institutional Review Board. The study cohort was recruited among consecutively enrolled pregnant or post-partum young women attending the School for Pregnant and Parenting Teens in Central Harlem. These women, aged 12–19 yr (mean 16.7; S.D. 1.4), were 60% African-American and 39% Hispanic, all of low socioeconomic status. Students at this school receive oral health services through the Community DentCare Network (28) of the Columbia University School of Dental and Oral Surgery, and medical services through the adjoining Columbia University School of Public Health School-based medical clinic. All of the participants live in Northern Manhattan and receive uniform prenatal care including regular examinations, nutritional and prenatal counseling by an attending physician. After being informed about the scope and methods of the study, participants were required to sign an informed consent form.

Clinical examination and procedures

At baseline, medical charts were reviewed for the following parameters: sociodemographic data, medical and dental history, smoking status, gestational age (if pregnant), and birth outcome (if post-partum). An attempt to determine use of alcohol, illicit drugs and smoking habits was made by means of a questionnaire. Presence of gestational diabetes, genitourinary tract infections, multiple gestation, previous pre-term birth, low maternal weight gain, and low pregravid weight was recorded.

A clinical oral examination was carried out by two calibrated examiners, a dentist (author DML) and a dental hygienist, according to the criteria used by National Health and Nutrition Examination Survey III (NHANES III). This included an examination for the number of permanent and primary teeth present, and recording of the number of decayed, missing, filled teeth and surfaces (DMFT score). The periodontal examination consisted of assessments at buccal and mid-buccal sites of all present teeth of the following parameters:

Dental calculus; recorded dichotomously as present or absent.

Dental plaque; recorded on a scale of 0–3 according to the criteria of the Silness-Löe index.

Probing depth; defined as the distance between the gingival margin and the bottom of the probeable pocket to the nearest whole mm.

Bleeding on probing; recorded dichotomously and deemed positive if it occurred within 15 s after the assessment of probing depth.

Bacterial plaque samples

Four subgingival plaque samples were obtained prior to the clinical examination from each subject, one from each quadrant at a first molar. If missing, a second molar or a second premolar was selected. All samples were obtained from mesial surfaces, accessed from the buccal aspect, by means of sterile Gracey curettes. The curette was inserted into the pocket and subgingival plaque was collected by a single scaling stroke. Caution was exercised to assure that all pressure against the root surface was ceased when the curette tip reached the gingival margin in order to minimize contamination by supragingival plaque. The collected plaque mass was immediately transferred into an Eppendorf tube containing 150 μ l of sterile T-E buffer (10 mM Tris HCl, 1.0 mM EDTA, pH 7.6). The plaque pellet was resuspended by using a sterile pipette, was vortexed vigorously, and 150 μ l of a 0.5 M NaOH solution were added. The samples were thereafter transported to the laboratory of the Division of Periodontics, Columbia University School of Dental and Oral Surgery and kept at +4°C. Processing was completed within 4 wk from sample collection.

Processing of the bacterial plaque samples

Digoxigenin-labeled, whole genomic DNA-probes were prepared by random priming by the use of the High-Prime labeling kit (Boehringer-Mannheim, Mannheim, Germany) from the following 12 microbial species: *Porphyromonas gingivalis* (strain FDC381), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Bacteroides forsythus* (ATCC 43037), *Actinobacillus actinomycetemcomitans* (FDC Y4), *Fusobacterium nucleatum*

(ATCC 10953), *Treponema denticola* (OMGS 3271), *Peptostreptococcus micros* (ATCC 33270), *Campylobacter rectus* (ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Eubacterium nodatum* (OMGS 3356), and *Streptococcus intermedius* (OMGS 3177).

Analysis of plaque samples was performed according to the "checkerboard" DNA-DNA hybridization method (29). The sensitivity and specificity of whole genomic probes constructed as above have been previously described (29, 30), and a comparison between checkerboard hybridizations and culture in the identification of subgingival microbiota has been published (31). In brief, the samples were boiled for 5 min, neutralized, transferred onto nylon membranes by means of a MiniSlot device (Immunetics, Cambridge, MA, USA) and immobilized by ultraviolet (UV)-light and baking at 120°C. After 2 h of prehybridization, the DNA probes were allowed to hybridize overnight with the sample DNA in a Miniblotter device (Immunetics) at 42°C. After a series of stringency washes, hybrids were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubation with an appropriate chemiluminescent substrate (CSPD, Boehringer-Mannheim). Evaluation of the chemiluminescent signal was performed at a LumiImager Workstation (Boehringer-Mannheim) by comparing the obtained signals with the ones generated by pooled standard samples containing 10^6 or 10^5 of each of the 12 species. In the present analysis, bacterial load is expressed in chemiluminescence units relative to the signal obtained by the high standard. As extensively validated during calibration experiments, signal amounting to $\leq 3\%$ of the high standard was considered negative.

Dental/periodontal intervention

After baseline data collection, all enrolled women received oral hygiene instructions and full mouth debridement, including scaling with hand and/or ultrasonic instruments. Fluoridated polishing paste was applied with slow speed rotary instruments. Dental sealants were applied as needed. Whenever appropriate, referrals were made for the treatment of carious and/or pulpally involved teeth.

Data analysis

In all analyses, the individual subject was the computational unit. Thus, mean values for all clinical parameters (calculus, plaque, gingivitis scores, and probing depths) were calculated for each subject. Bacterial scores from each individual sample were averaged to describe each subject's bacterial load by each species.

To facilitate comparisons with respect to birth outcome between women who received periodontal intervention during pregnancy and those who did not, the examined cohort was further divided in subgroups:

- (i) a group of women enrolled during pregnancy who received dental intervention prior to delivery; and
- (ii) a group consisting of women who enrolled post-partum, and, thus, received periodontal intervention after delivery. For the specific purposes of the present study, i.e., the role of periodontal infections on adverse pregnancy outcomes, these women were considered untreated, since their periodontal status remained unaltered during pregnancy.

Furthermore, women were grouped according to pregnancy outcome into a PLBW group, if they either delivered pre-term (gestational age at birth < 37 wk), or if they gave birth to a low weight baby (birth weight $< 2,500$ g). If neither of the above conditions occurred, the birth outcome was defined as normal.

Statistical methods included the Student's *t*-test for comparing means and the Chi-square test for comparing frequencies.

Results

Table 1 describes the study sample with respect to time of enrollment, availability of subgingival plaque samples, and birth outcome. In total, 213 young women were enrolled during the first 2 yr of the study, and all were examined with respect to clinical periodontal conditions. Of these, 107 were pregnant at the time of examination, predominantly in the second trimester, while 106 women were recruited within three months after delivery. Birth outcome data were available from 164 women, while 32 had not yet given birth at the time of analysis. Only a few women ($n=17$, i.e., 8% of the recruited sample) were lost to follow up. Subgingival plaque samples were obtained from 145 women; 86 pregnant and 59 post-partum. Out of the total number of women with known birth outcome, 27 (16.5%) qualified as PLBW, i.e., they either delivered pre-term or gave birth to a low birthweight baby.

A comparison of the clinical periodontal status of pregnant and recently post-partum women, and mothers with PLBW and normal birth outcome is presented in Table 2. The data revealed that pregnant and post-partum women displayed comparable levels of plaque, gingivitis and calculus. However, pregnant women had

Table 1

Distribution of subjects with respect to time of enrollment, availability of subgingival plaque samples and birth outcome

Status	Enrolled during pregnancy		Enrolled post-partum		Total
Clinically examined	107		106		213
Plaque samples available	86		59		145
Birth outcome	PLBW	Normal	PLBW	Normal	
	10	64	17	73	164

PLBW: preterm birth/low birth weight.

Table 2

Clinical characteristics of the subject sample (N=213). Mean values (standard deviations). Comparisons performed by means of the Student's two-tailed t-test for unpaired samples

Variable	Enrolled during pregnancy	Enrolled post-partum	P-value	PLBW	Normal	P-value
Plaque index	1.4 (0.73)	1.3 (0.69)	NS	1.3 (0.72)	1.3 (0.69)	NS
% of sites with bleeding on probing	54 (27)	54 (24)	NS	53 (24)	56 (28)	NS
% of sites with calculus	50 (30)	45 (26)	NS	56 (27)	51 (25)	NS
Probing depth (mm)	2.7 (0.50)	2.4 (0.50)	$P < 0.001$	2.5 (0.50)	2.6 (0.50)	NS

Table 3

Average subgingival microbial load (chemiluminescence equivalent of $\times 10^4$ cells) in subjects enrolled during pregnancy (n=86) or post-partum (n=59), and in women enrolled post-partum with PLBW (n=17) or normal birth outcome (n=42). Comparisons performed by means of the Student's two-tailed t-test for unpaired samples

Bacteria	Enrolled during pregnancy	Enrolled post-partum	P-value	PLBW	Normal	P-value
<i>Porphyromonas gingivalis</i>	20.4 (55.0)	10.3 (18.1)	NS	11.6 (16.4)	10.1 (18.5)	NS
<i>Prevotella intermedia</i>	58.2 (259.5)	33.3 (32.7)	NS	39.9 (29.6)	32.2 (33.4)	NS
<i>Prevotella nigrescens</i>	46.5 (88.4)	43.0 (44.3)	NS	63.0 (61.1)	39.9 (41.1)	$P = 0.17$
<i>Bacteroides forsythus</i>	41.9 (68.1)	42.8 (60.9)	NS	113.4 (112.9)	31.7 (39.9)	$P = 0.0002$
<i>Actinobacillus actinomycetemcomitans</i>	9.6 (19.6)	7.6 (19.6)	NS	13.4 (29.2)	6.6 (12.3)	NS
<i>Fusobacterium nucleatum</i>	44.6 (40.2)	44.8 (40.3)	NS	51.5 (44.3)	43.7 (40.0)	NS
<i>Treponema denticola</i>	16.3 (29.2)	12.5 (22.4)	NS	18.5 (12.6)	11.6 (23.5)	NS
<i>Peptostreptococcus micros</i>	52.4 (27.6)	56.2 (28.0)	NS	65.3 (33.8)	54.8 (27.1)	NS
<i>Campylobacter rectus</i>	14.1 (52.5)	8.1 (17.7)	NS	20.9 (34.5)	6.1 (12.9)	$P = 0.02$
<i>Eikenella corrodens</i>	11.5 (17.6)	11.2 (16.0)	NS	21.0 (31.4)	9.6 (11.8)	$P = 0.06$
<i>Eubacterium nodatum</i>	24.7 (33.4)	22.9 (23.1)	NS	37.2 (41.0)	20.6 (18.6)	$P = 0.06$
<i>Streptococcus intermedius</i>	37.9 (31.6)	46.2 (46.5)	NS	53.4 (26.6)	45.1 (48.9)	NS

Table 4

Distribution of preterm low birth weight cases with respect to delivery of periodontal therapy during pregnancy

	No periodontal therapy during pregnancy	Periodontal therapy during pregnancy	
Normal birth outcome	73	64	137
PLBW	17 (18.9%)	10 (13.5%)	27 (16.5%)
Total	90	74	164

Chi-square = 0.85; DF = 1; P-value = 0.36.

significantly higher mean pocket depth than post-partum women (2.7 mm vs. 2.4 mm; $P < 0.01$, Student's t-test for unpaired samples). No statistically significant differences in any of the recorded clinical periodontal parameters were observed between women with PLBW and those with normal birth outcome.

We further examined the subgingival microbial profiles of pregnant and post-partum women, and post-partum women with PLBW and normal birth outcome (Table 3). The data revealed no statistically significant difference between pregnant women and women enrolled soon after delivery. However, a comparison involving post-partum women with PLBW and their counterparts with normal birth outcome revealed statistically

significantly higher levels of *B. forsythus* and *C. rectus* in women with PLBW, while the differences for *P. nigrescens*, *E. corrodens* and *E. nodatum* approached statistical significance. Interestingly, the average bacterial load for all the remaining tested species were also higher in women with PLBW. It should be noted, however, that an adjustment for multiple comparisons would identify only *B. forsythus* as statistically elevated in PLBW cases.

Finally, Table 4 describes the distribution of PLBW cases with respect to delivery of periodontal therapeutic intervention during pregnancy. It was revealed that, among the 90 women who were not subjected to periodontal therapy, 17 (18.9%) gave birth to PLBW babies. The corresponding incidence among the 74 women who did receive periodontal therapy during pregnancy was 13.5% (10 cases). Although the difference in incidence between the treated and untreated women was not statistically significant, it represents a 28.6% reduction in PLBW in the periodontally treated group.

Discussion

This report provides early results from an ongoing study of the effect of periodontal therapeutic interventions on pre-term delivery and low birth weight, from a cohort of young minority women of low socioeconomic status in

Harlem, New York. Our findings after the initial 2 yr revealed that: (i) women with PLBW harbored significantly increased levels of periodontal pathogens; and (ii) the incidence of PLBW in women who received basic periodontal therapy during pregnancy was substantially reduced, although this reduction did not reach statistical significance.

A number of features inherent to the study cohort deserve some comments. First, the study sample exclusively involved young minority (predominantly African-American) women of low socioeconomic status. It is well established in the literature that all three attributes (young maternal age, black race, and low socioeconomic status) are significant risk factors for PLBW (32, 33). Indeed, as expected, the incidence of PLBW in this particular cohort was higher than the national average in the United States (16.5% versus the reported 11%, (4, 34)). A second feature that deserves attention is the level of periodontal disease in the sample. Although attachment level measurements were not routinely performed in this study, the examiners were instructed to record any clinically unequivocal loss of the supporting periodontal tissues and/or gingival recession, to enable diagnosis of localized or generalized juvenile periodontitis (data not shown). However, despite the relatively frequent occurrence of pocket depths of ≥ 5 mm and occasional loss of attachment at individual tooth sites, only four young women included in the study were diagnosed with localized juvenile periodontitis. Hence, the cohort should be viewed as a sample of women with poor oral health habits, abundant plaque and calculus, overt gingivitis, but low prevalence of destructive periodontal disease.

Given the low prevalence of destructive periodontal disease and the ubiquitous occurrence of gingivitis in the cohort, the absence of differences in clinical periodontal status between PLBW cases and women with normal birth outcome (Table 2) should not be viewed as corroborating the null-hypothesis of no association of periodontal diseases and PLBW. Instead, attention should be focused on the finding that PLBW mothers appeared to harbor a substantially higher load of periodontal pathogens than women with a normal birth outcome (Table 3). This comparison was exclusively limited to women enrolled post-partum for the following reason: According to the study protocol, immediately after the clinical examination, all enrolled women were subjected to scaling, resulting in an alteration of their subgingival microbial profiles. However, a study of the impact of infection by specific periodontal microbiota on pregnancy outcomes should involve only women with undisturbed subgingival plaques during pregnancy, such as the women enrolled immediately after delivery ($n=59$, Table 1). Importantly, the comparison of the subgingival microbiota between all pregnant and all post-partum women, irrespective of birth outcome, revealed no statistically significant differences between the two groups (Table 3). Therefore, it appears justified to assume that the detected post-partum differences in periodontal microbiota between PLBW cases and women with normal birth outcome were also prevalent

during pregnancy, and, thus, that exposure to periodontal pathogens was different in the two groups during pregnancy. In turn, this finding appears to corroborate our hypothesis that a subgingival infection by a biofilm rich in Gram-negative, LPS-producing species may play an etiological role in the pathogenesis of PLBW.

The above suggestion is further supported by the analysis associating birth outcome to delivery of periodontal therapy during pregnancy, presented in Table 4. These preliminary data revealed a reduction in the incidence of PLBW with periodontal therapy, from 18.9% in the untreated group to 13.5% in the group that received scaling prior to delivery. Although this observed difference did not reach statistical significance, it should be emphasized that the performed power calculations required a substantially increased sample size than the 164 women involved in this comparison, in order to detect statistically significant effects, if existing. Interestingly, this observed reduction of 28.6% in the incidence of PLBW compares favorably with the prediction of OFFENBACHER *et al.* (24), according to which the risk for PLBW attributable to periodontal disease was estimated to amount to 18.2%. Although it can not be ruled out that differences in exposure to other, unaccounted factors, and not solely to periodontal therapy, may underlie the difference in incidence between the periodontally treated and untreated women, the two groups were similar with respect to major risk factors for PLBW such as socioeconomic status, maternal age and race. Importantly, the data presented in Table 3, revealing no differences in periodontal microbiota between pregnant and post-partum women, further strengthen the notion that the two groups were similar in background characteristics except for delivery of periodontal therapy.

To our best knowledge, this is the first study reporting on the effects of periodontal interventions on the incidence of adverse birth outcomes. Given the relatively small sample size examined so far, we consider the present results encouraging and in accordance with our initial hypothesis that periodontal therapies are potential candidates for interventional strategies aimed at reducing the incidence of PLBW. So far, genitourinary tract infections such as bacterial vaginosis have been considered to be the leading candidates for such interventions (35). However, a recent large prospective randomized controlled trial (36) failed to demonstrate that treatment of bacterial vaginosis is effective in reducing PLBW incidence. Our ongoing study provides the possibility to examine whether low cost periodontal therapy is, in fact, a valuable alternative in this context.

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