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Systemic Immune Responses in Pregnancy and Periodontitis: Relationship to Pregnancy Outcomes in the Obstetrics and Periodontal Therapy (OPT) Study

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Abstract

Background—Our previous studies reported on the obstetric, periodontal, and microbiologic outcomes of women participating in the Obstetrics and Periodontal Therapy (OPT) Study. This article describes the systemic antibody responses to selected periodontal bacteria in the same patients.

Methods—Serum samples, obtained from pregnant women at baseline (13 to 16 weeks; 6 days of gestation) and 29 to 32 weeks, were analyzed by enzyme-linked immunosorbent assay for serum immunoglobulin G (IgG) antibody to *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* (previously *T. forsythensis*), and *Treponema denticola*.

Results—At baseline, women who delivered live preterm infants had significantly lower total serum levels of IgG antibody to the panel of periodontal pathogens ($P = 0.0018$), to *P. gingivalis* ($P = 0.0013$), and to *F. nucleatum* ($P = 0.0200$) than women who delivered at term. These differences were not significant at 29 to 32 weeks. Changes in IgG levels between baseline and 29 to 32 weeks were not associated with preterm birth when adjusted for treatment group, clinical center, race, or age. In addition, delivery of low birth weight infants was not associated with levels of antibody at baseline or with antibody changes during pregnancy.

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The authors report no conflicts of interest related to this study.

Conclusions—Live preterm birth is associated with decreased levels of IgG antibody to periodontal pathogens in women with periodontitis when assessed during the second trimester. Changes in IgG antibody during pregnancy are not associated with birth outcomes.

Keywords

Antibody; bacteria; periodontitis; pregnancy; preterm birth

Periodontal tissue destruction occurs as a result of host immunoinflammatory processes triggered by specific bacteria contained within a complex microbial plaque biofilm.¹⁻⁴ Preterm birth (PTB) before 37 weeks of gestation and low birth weight (LBW; <2,500 g) are adverse pregnancy outcomes that demonstrate an increasing incidence in disparate United States populations.⁵⁻⁷ Elevated local or systemic levels of select biomolecules have been linked to PTB/LBW events.⁸⁻¹³ Although a link between chronic oral infections and general health has been postulated for more than a century, the association between periodontitis and PTB/LBW has been particularly noted in pregnant women with extensive or severe periodontal disease.¹⁴⁻¹⁷ Additionally, a significant increase in small-for-gestational age babies, miscarriages, and spontaneous abortions has been identified in women with moderate-severe periodontitis, even after adjusting for age, smoking, and selected other factors.^{14-16,18-20} A recent review²¹ of studies on adverse pregnancy outcomes in women with periodontitis concluded that the majority of studies supported a relationship. However, our data from a recent randomized clinical trial, the Obstetrics and Periodontal Therapy (OPT) Study, demonstrated that non-surgical periodontal treatment during the second trimester of pregnancy did not alter the incidence of PTB or LBW deliveries.²² Eight hundred twenty-three pregnant women with periodontitis were enrolled in the OPT Study; 413 received scaling and root planing before 21 weeks of gestation plus monthly tooth polishing and oral hygiene instruction throughout their pregnancy. Although there were significant improvements in the periodontal health of the women receiving periodontal treatment, this did not result in significant alterations in the rates of PTB, LBW, or fetal growth restriction compared to those women who did not receive periodontal treatment during their pregnancy.²² In addition, a subsequent report²³ from the same study showed that periodontal treatment caused a reduction in the levels of seven subgingival periodontal microorganisms during pregnancy, but these reductions in bacterial challenge were not associated with reductions in PTB or LBW. There is minimal information available on the humoral responses to these same periodontal bacteria during pregnancy and the potential effects of changes in serum immunoglobulin G (IgG) on adverse pregnancy outcomes. Madianos et al.²⁴ found that elevated IgG antibody to certain oral bacteria in the mother's serum was related to a decreased rate of PTB and increased birth weight, suggesting some protective capacity for this antibody. However, other similar studies indicated no relationship of serum antibody with birth outcomes²⁵ or that elevated antibody to *Porphyromonas gingivalis* was associated with an increased risk for LBW deliveries.²⁶ A recent case-control study of generally periodontally healthy women by Lin et al.²⁷ noted increased antibody levels to *P. gingivalis* at baseline in mothers with full-term deliveries. These studies were generally of small groups of subjects or did not provide an intervention. The purpose of the present study was to determine if serum levels of IgG, measured at baseline and during pregnancy, to the same select panel of seven periodontal bacteria

previously studied in this population²³ were related to adverse pregnancy outcomes (PTB and LBW).

MATERIALS AND METHODS

Patient Population

Details of the OPT trial design and its obstetric, periodontal, and safety outcomes were reported elsewhere.²² Briefly, all women had 20 teeth; had periodontitis, defined as the presence of at least four teeth with probing depth ≥ 4 mm and clinical attachment loss ≥ 2 mm; and had bleeding on probing at ≥ 35% of tooth sites. After the completion of written informed consent approved by the Institutional Review Boards of the participating centers, 823 women (16 to 44 years of age) with periodontitis were enrolled at four centers between March 2003 and June 2005: University of Kentucky Chandler Medical Center, Lexington, Kentucky; Hennepin County Medical Center, Minneapolis, Minnesota; Harlem Hospital, New York, New York; and the University of Mississippi Medical Center, Jackson, Mississippi. 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 (test 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 the treatment of tooth decay, abscesses, or other non-periodontal pathoses. Serum samples were obtained from women at baseline (13 to 16 weeks; 6 days of gestation) and at 29 to 32 weeks. Samples were stored at -80°C in aliquots of ~1 ml.

Antigens and Serum Antibody Analysis

Serum IgG antibodies to seven oral bacteria were quantified using an enzyme-linked immunosorbent assay as described previously.²⁸ Briefly, *Aggregatibacter actinomycetemcomitans* JP2 (previously *Actinobacillus actinomycetemcomitans* JP2), *Campylobacter rectus* American Type Culture Collection (ATCC) 33238, *Fusobacterium nucleatum* ATCC 49256, *P. gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 25611, *Tannerella forsythia* (previously *T. forsythensis*) ATCC 49307, and *Treponema denticola* ATCC 35405 were prepared as antigens using formalin-fixed bacteria.²⁹ Each plate also contained serial dilutions of purified human IgG for standard curves used to quantify the antibodies in gravimetric units (µg/ml).

Statistical Analyses

The distribution of patient samples included in the assessments is presented in Table 1. The serum antibody levels to each microorganism, the sum of antibodies to the seven bacterial species, and the sum of antibody levels to *A. actinomycetemcomitans* plus *P. gingivalis*, *T. forsythia*, and *T. denticola* species (red complex microorganisms) were analyzed. Antibody levels were analyzed at baseline (13 to 16 weeks; 6 days of gestation) and at 29 to 32 weeks, as were changes in antibody levels from baseline to 29 to 32 weeks. Because measured antibody levels exhibited marked skewness, all analyses used the logarithm (base 2) of the antibody levels to individual species or the base-2 log of sums of levels over groups of

species. Comparisons between groups of subjects used one-way analysis of variance (ANOVA) for unadjusted tests and analysis of covariance (ANCOVA) for adjusted tests. Adjusted analyses of changes from baseline to 29 to 32 weeks included the woman's periodontal treatment group.

Our prespecified primary serologic outcome was the sum of the seven bacteria-specific antibodies. Thus, we compared this measure between groups at an alpha (type I error rate) of 0.05. We did eight additional comparisons (antibody levels to the seven individual species plus the sum of antibodies to the red complex species); the threshold applied to P values to determine significance was adjusted for multiple comparisons using the Bonferroni step-down (Holm) procedure. In this procedure, the eight P values are sorted from smallest to largest and tested in that order. The i^{th} smallest P value is compared to a threshold of $0.05/(9 - i)$; if the i^{th} smallest P value is declared non-significant, all larger P values are also declared non-significant. The respective thresholds for the P values, ranked from smallest to largest for each set of comparisons, were $0.05/8 = 0.0063$, $0.05/7 = 0.0071$, $0.05/6 = 0.0083$, and so on. Post hoc tests were done in the context of the ANOVA or ANCOVA; reported P values were not adjusted for multiple comparisons.

Because many women did not have a 29- to 32-week serum sample, we also conducted weighted analyses to adjust for the women who did not have antibody data at baseline and at 29 to 32 weeks. Weights were inversely proportional to the probability of having data at 29 to 32 weeks as estimated from a logistic regression with dependent variable "had baseline and 29- to 32-week data versus had baseline data only" and with these independent variables: black (yes/no), Hispanic (yes/no), other non-white race (yes/no), study group (treatment/control), baseline percentage of sites with probing depth ≥ 4 mm, and base-2 logarithm of sum of baseline antibody titers. The weights were scaled so they summed to 620, the number of women who had data at baseline and at 29 to 32 weeks. The weight for person i , who had baseline and 29- to 32-week data, was weight for person $i = 620 \times (1/p_i)/\sum (1/p_i)$, where p_i is the estimated formula for the probability of having 29- to 32-week data (from the logistic regression), substituting in person i values of the independent variables. If p_i is small, $1/p_i$ is large; thus, a woman gets a relatively large weight if she resembles women who were disproportionately likely to not have 29- to 32-week data. The sum $(1/p_i)$ in the denominator allows the weights to add to 620, the number of women who actually had 29- to 32-week data.

RESULTS

Baseline Characteristics of the Study Population

The characteristics of the study population were reported previously, with no significant differences observed between control and treatment groups for age, race/ethnicity, education, previous pregnancies, coexisting medical conditions, or dental/periodontal status.²²

Serum Samples

Table 1 describes the distribution of serum samples by collection time, periodontal treatment group, and birth outcome. At baseline, 786 serum samples were available for analysis; this was reduced to 635 by the 29- to 32-week visit; 620 matched samples were available for within- and between-patient comparisons of changes between baseline and 29 to 32 weeks. For women experiencing a fetal loss prior to 29 to 32 weeks, either through spontaneous abortion or stillbirth, no sample was available for the 29- to 32-week comparison because these subjects were exited from the study at the time of fetal loss. The weighted analysis performed to account for the differences in sample size between baseline and the 29- to 32-week visit showed that the results of the study were similar with and without weighting; therefore, only non-weighted data are presented.

Serum Antibody Responses in Relation to Pregnancy Outcomes

Table 2 summarizes the antibody levels at baseline by pregnancy outcomes. The sum of baseline serum IgG levels to the selected panel of periodontal pathogens was significantly lower in women delivering live preterm infants compared to those delivering at term ($P = 0.0018$). Using the multiple-comparisons adjustment of the P value threshold for antibody levels to the individual bacteria, and considering P values from adjusted analyses, baseline antibodies to *P. gingivalis* and *F. nucleatum* differed significantly among the birth outcome groups. Women with live PTBs had significantly lower antibody levels to *P. gingivalis* ($P = 0.0013$) and *F. nucleatum* ($P = 0.0200$) than women with full-term births. In addition, women with full-term births ($P = 0.0173$) or live PTBs ($P = 0.0011$) had significantly lower baseline antibody levels to *F. nucleatum* compared to women who experienced a spontaneous abortion or stillbirth. Table 3 shows the changes in antibody levels from baseline to 29 to 32 weeks by pregnancy outcomes. Seven of the nine antibody measures decreased over time in full-term mothers, whereas the same fraction increased in preterm mothers. However, full-term and preterm mothers did not show a significant difference for any species. As a result of the changes in antibody levels between baseline and 29 to 32 weeks, the significant differences observed in antibody levels at baseline were not sustained through the 29- to 32-week visit (data not shown; adjusted $P = 0.3958$). Table 4 summarizes the antibody levels at baseline in women by infant birth weight. There were no significant differences between normal and low birth weight groups in antibody levels to any individual or group of species. Table 5 gives changes in antibody levels from baseline to 29 to 32 weeks by infant birth weight. Again, mothers of low and normal birth weight infants did not differ significantly in terms of changes in antibody levels from baseline.

Systemic Antibody Responses in Pregnancy Related to Treatment

Antibody (mg/ml) levels to *F. nucleatum*, *T. denticola*, and *T. forsythia* had decreased by 29 to 32 weeks in both groups, although the change from baseline was only statistically significant for *T. forsythia* (treatment group: mean $\pm$ SE = 2.59 ± 0.04 at baseline and 2.32 ± 0.06 at 29 to 32 weeks, $P = 0.0001$; control group: 2.55 ± 0.04 at baseline and 2.36 ± 0.05 at 29 to 32 weeks; $P = 0.0043$).

DISCUSSION

The hypothesis that periodontitis during pregnancy provides an enhanced risk for adverse pregnancy outcomes has been examined using various clinical research designs. We reported a randomized clinical trial (OPT Study) of the effect of non-surgical periodontal therapy provided during the second trimester of pregnancy on the incidence of PTB; periodontal therapy did not reduce the incidence of preterm deliveries.²² The OPT Study also demonstrated that dental treatment is safe when delivered during the second trimester.³⁰

The clinical manifestations of periodontitis result from a chronic polymicrobial infection of the subgingival sulcus and a resulting chronic immunoinflammatory response to the infection that is observed locally in the periodontal tissues and the systemic circulation.^{1,2} To better understand linkages between periodontitis and systemic diseases, a fundamental requirement is to determine critical microbiologic and/or immunologic fingerprints that are hallmarks of those patients at most risk for systemic complications of oral disease. It is clear that oral bacteria trigger local and systemic adaptive immune responses in healthy adults^{29,31} and children.^{32–34} However, substantial literature has identified significant increases in antibody responses, with specificity for selected oral bacteria, in patients with various forms of periodontitis. The quantity of antibody has been related to the severity/extent of disease, exacerbations of disease progression, and therapeutic interventions.^{28,35–37} The bacterial specificity of the elevated antibody varies among patients and categories of periodontitis.^{29,38–43} Some recent studies^{24,44,45} suggested that the level and isotype of antibody in maternal serum or cord blood, with specificity for individual oral species, is correlated with an increased risk for PTB/LBW deliveries. These findings were interpreted to support the potential oral microbial contribution to maternal–fetal infections and suggested that individual variation in maternal responses may enhance the susceptibility to adverse pregnancy outcomes. Thus, we evaluated the serum antibody responses of pregnant women in the OPT Study and their relationship to pregnancy outcomes.

Women in the OPT Study who delivered preterm live births, irrespective of treatment group, had significantly lower antibody levels to *P. gingivalis* at baseline than women who delivered at term (Table 2). However, we previously reported no significant differences in the quantity or proportions of this microorganism in the subgingival plaque of women delivering at term or preterm.²³ Together, these findings suggest that the risk for PTB may be associated with a less robust antibody response to *P. gingivalis* and a depressed local and/or systemic ability to manage periodontal infections.^{44,46–48} Additionally, women with full-term pregnancies generally showed decreases in antibody levels from baseline to 29 to 32 weeks (Table 3). These findings are consistent with baseline response levels, suggesting that a general improvement in oral health care, coupled with positive responses to the therapeutic intervention outcomes in the treatment group, would decrease the antigenic burden, resulting in greater decreases in the homologous antibody response. However, given that women delivering full-term and preterm infants showed similar clinical responses during the OPT Study, it is unclear why a similar pattern of antibody level changes did not occur in these groups of mothers,²² if the clinical response to therapy was the controlling factor.

We found that women who lost their pregnancies had significantly higher antibody levels to *F. nucleatum* at baseline than women who delivered preterm (Table 2). On average, antibody levels to *F. nucleatum* were 52% higher ($2^{[1.80 - 1.20]} = 1.52$; Table 2) in the fetal loss group compared to the preterm group. *F. nucleatum* has been implicated in bacterial vaginosis and fetal infections.^{24,49–52} Moreover, oral colonization with *F. nucleatum* and the maternal response to this species have been associated with an increased risk for adverse pregnancy outcomes.^{24,51} A rodent study⁵² also demonstrated the capacity of systemic *F. nucleatum* infections to trigger fetal death and growth restriction. These types of antibody changes are not inconsistent with antibody specificities to oral bacteria identified in other studies^{24,49–51} of periodontitis and birth outcomes. Thus, the elevated level of response to *F. nucleatum* is consistent with an enhanced communication of this microorganism to the systemic circulation and the potential increased risk for seeding placental/fetal tissues. Whether the microbial species associated with maternal–fetal infections is the same strain that infects the oral cavity of the mothers and is hematogenously transmitted to the fetus remains to be determined. However, the fact that these particular species have been implicated in a variety of studies suggests that additional research should target these species and the associated host-response variations as potential risk markers for adverse pregnancy outcomes.

CONCLUSIONS

The results of this study indicated that the serum responses to a select panel of periodontal pathogens showed some specific relationships to pregnancy outcomes. Mothers who delivered preterm had significantly lower antibody levels to *P. gingivalis* during the second trimester than women who delivered at term. In contrast, serum antibodies to *F. nucleatum* were elevated in women who suffered a fetal loss. Baseline antibody levels to *C. rectus*, the sums of levels of all seven target microorganisms, and to *A. actinomycetemcomitans* plus the red complex bacterial species also tended to differ among groups according to birth outcomes, but they did not reach statistical significance after accounting for multiple comparisons. Further analyses are needed to examine how the differences in antibody responses between term and preterm deliveries relate to individual variations in the response to therapy.

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Table 1

Sample Sizes by Periodontal Treatment Group and Pregnancy Outcome

Antibody Sample	Treated Before 21 Weeks of Gestation			Treated After Delivery		
	Full-Term	Live Preterm	Fetal Loss*	Full-Term	Live Preterm	Fetal Loss
Baseline	353	39	5	337	38	14
29 to 32 weeks	282	24	1	301	24	3
Baseline and 29 to 32 weeks [†]	278	23	1	291	24	3
						620

* Includes spontaneous abortions (fetal loss before 20 weeks of gestation) and stillbirths (loss between 20 weeks and 36 weeks, 6 days of gestation).

[†] Group used to calculate changes from baseline to 29 to 32 weeks.

Table 2

Antibody Levels ($\log_2$ level [$\mu\text{g/ml}$]; mean $\pm$ SE) at Baseline (13 weeks to 16 weeks, 6 days of gestation) by Pregnancy Outcome

Dependent Variable	Full-Term (n = 690)	Live Preterm (n = 77)	Fetal Loss (n = 19)	P Value for Three-Way Comparison	P Value From Adjusted Analyses*
Sum of bacteria	5.48 $\pm$ 0.03 [†]	5.21 $\pm$ 0.09	5.34 $\pm$ 0.19	0.0197	0.0072
<i>A. actinomycetemcomitans</i>	2.59 $\pm$ 0.04	2.37 $\pm$ 0.13	2.36 $\pm$ 0.27	0.2245	0.3150
<i>P. gingivalis</i>	3.46 $\pm$ 0.05 [‡]	3.00 $\pm$ 0.16	3.29 $\pm$ 0.32	0.0228	0.0049 [§]
<i>F. nucleatum</i>	1.42 $\pm$ 0.03 ^{//}	1.20 $\pm$ 0.09 [¶]	1.80 $\pm$ 0.19	0.0081	0.0028 [§]
<i>P. intermedia</i>	2.33 $\pm$ 0.03	2.07 $\pm$ 0.10	2.02 $\pm$ 0.21	0.0282	0.0586
<i>C. rectus</i>	2.23 $\pm$ 0.03	1.94 $\pm$ 0.10	2.22 $\pm$ 0.20	0.0261	0.0204
<i>T. denticola</i>	1.91 $\pm$ 0.03	1.85 $\pm$ 0.10	2.03 $\pm$ 0.21	0.7096	0.4232
<i>T. forsythia</i>	2.58 $\pm$ 0.03	2.57 $\pm$ 0.10	2.54 $\pm$ 0.20	0.9800	0.9316
Sum of <i>Aa</i> + red complex	4.94 $\pm$ 0.03	4.67 $\pm$ 0.10	4.77 $\pm$ 0.21	0.0343	0.0128

* Analyses included adjustments for clinical center, race (black or other), and age.

[†] Post hoc test: full-term versus live preterm, $P = 0.0018$.

[‡] Post hoc test: full-term versus live preterm, $P = 0.0013$.

[§] Significant under multiple-comparisons adjustment.

^{//} Post hoc test: full-term versus live preterm, $P = 0.0200$; versus fetal loss, $P = 0.0173$.

[¶] Post hoc test: live preterm versus fetal loss, $P = 0.0011$.

Table 3

Changes (mean $\pm$ SE) in Antibody Levels (log₂ level [μ g/ml]) from Baseline to 29 to 32 Weeks by Pregnancy Outcome

Dependent Variable	Full-Term (n = 569)	Live Preterm (n = 47)	Fetal Loss (n = 4)	P Value for Three- Way Comparison	P Value From Adjusted Analyses *
Sum of bacteria	-0.09 $\pm$ 0.03	0.10 $\pm$ 0.11	0.20 $\pm$ 0.37	0.1833	0.0834
<i>A. actinomycetemcomitans</i>	0.02 $\pm$ 0.04	0.11 $\pm$ 0.13	0.27 $\pm$ 0.44	0.7019	0.5888
<i>P. gingivalis</i>	-0.09 $\pm$ 0.05	0.15 $\pm$ 0.16	0.66 $\pm$ 0.56	0.1547	0.0817
<i>F. nucleatum</i>	-0.10 $\pm$ 0.04	-0.01 $\pm$ 0.14	-0.41 $\pm$ 0.49	0.6811	0.6409
<i>P. intermedia</i>	-0.11 $\pm$ 0.04	0.05 $\pm$ 0.13	0.06 $\pm$ 0.44	0.4682	0.3048
<i>C. rectus</i>	0.02 $\pm$ 0.03	0.25 $\pm$ 0.12	-0.33 $\pm$ 0.41	0.1183	0.0756
<i>T. denticola</i>	-0.12 $\pm$ 0.04	0.05 $\pm$ 0.13	0.22 $\pm$ 0.45	0.3546	0.2525
<i>T. forsythia</i>	-0.27 $\pm$ 0.04	-0.15 $\pm$ 0.13	0.63 $\pm$ 0.45	0.1019	0.0921
Sum of Aa + red complex	-0.11 $\pm$ 0.03	0.06 $\pm$ 0.12	0.37 $\pm$ 0.40	0.1725	0.0864

* Analyses included adjustments for treatment group, clinical center, race (black or other), and age.

Table 4

Antibody Levels (log₂ level [μg/ml]; mean ± SE) at Baseline (13 weeks to 16 weeks, 6 days of gestation) by Birth Weight

Dependent Variable	LBW (<2,500 g; n = 79)	Normal Birth Weight (≥ 2,500 g; n = 707)	P Value for Two-Way Comparison	P Value From Adjusted Analyses *
Sum of bacteria	5.35 ± 0.09	5.46 ± 0.03	0.2626	0.3612
<i>A. actinomycetemcomitans</i>	2.50 ± 0.13	2.57 ± 0.04	0.5727	0.7742
<i>P. gingivalis</i>	3.22 ± 0.16	3.44 ± 0.05	0.1841	0.2212
<i>F. nucleatum</i>	1.38 ± 0.09	1.41 ± 0.03	0.7863	0.8877
<i>P. intermedia</i>	2.21 ± 0.10	2.31 ± 0.03	0.3585	0.5690
<i>C. rectus</i>	2.17 ± 0.10	2.21 ± 0.03	0.6959	0.9476
<i>T. denticola</i>	1.92 ± 0.10	1.91 ± 0.03	0.8958	0.9944
<i>T. forsythia</i>	2.60 ± 0.10	2.57 ± 0.03	0.7488	0.6352
Sum of Aa + red complex	4.80 ± 0.10	4.93 ± 0.03	0.2419	0.3026

* Analyses included adjustments for clinical center, race (black or other), and age.

Table 5

Changes (mean $\pm$ SE) in Antibody Levels ($\log_2$ level [$\mu\text{g/ml}$]) From Baseline to 29 to 32 Weeks by Birth Weight

Dependent Variable	LBW (<2,500 g; n = 40)	Normal Birth Weight (2,500 g; n = 580)	P Value for Two-Way Comparison	P Value From Adjusted Analyses*
Sum of bacteria	-0.02 ± 0.12	-0.08 ± 0.03	0.6732	0.6201
<i>A. actinomycetemcomitans</i>	0.01 ± 0.14	0.03 ± 0.04	0.8637	0.8525
<i>P. gingivalis</i>	0.02 ± 0.18	-0.08 ± 0.05	0.6260	0.5408
<i>F. nucleatum</i>	-0.24 ± 0.16	-0.09 ± 0.04	0.3266	0.3026
<i>P. intermedia</i>	-0.11 ± 0.14	-0.10 ± 0.04	0.9246	0.9415
<i>C. rectus</i>	-0.02 ± 0.13	0.04 ± 0.03	0.6620	0.5673
<i>T. denticola</i>	-0.00 ± 0.14	-0.11 ± 0.04	0.4480	0.4333
<i>T. forsythia</i>	-0.19 ± 0.14	-0.26 ± 0.04	0.6521	0.5919
Sum of Aa + red complex	-0.02 ± 0.13	-0.10 ± 0.03	0.5039	0.4340

* Analyses included adjustments for treatment group, clinical center, race (black or other), and age.