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ORIGINAL ARTICLE

A pilot study of the association between cariogenic oral bacteria and preterm birth*

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OBJECTIVES: We examined the associations between preterm birth and low birth weight and maternal caries history, maternal periodontal status, and salivary levels of mutans streptococci and *Lactobacilli*.

DESIGN: This study was a matched case–control study in women during their pregnancy or up to 8 weeks after delivery.

SUBJECTS AND METHODS: Thirty-four women delivering before 37 weeks gestation were recruited along with 73 term controls matched for age and race/ethnicity. Demographic and obstetric information was collected from questionnaires and medical records and oral examinations along with commercial salivary tests were completed within the study groups.

MAIN OUTCOME MEASURES: The main outcome variables were the preterm birth and low birth weight status. The independent variables measured were the salivary levels of *Lactobacilli* and mutans streptococci and the caries and periodontal status of the subjects.

RESULTS: The odds ratio comparing low levels of bacteria in preterm mothers and controls was statistically significant for *Lactobacilli* (odds ratio (OR) = 3.45, 95% confidence interval (CI) = 1.27 to 10.00) and almost significant for mutans streptococci (OR = 2.63, 95% CI = 0.95 to 8.33). Clinical caries and periodontal disease measures did not differ significantly between groups.

CONCLUSION: Within the limitation of our study, low levels of *Lactobacilli* in saliva were found to be associated with preterm birth.

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Keywords: preterm birth; caries; *Lactobacilli*; mutans streptococci; saliva; periodontal diseases

Introduction

In the United States, 85% of perinatal morbidity and mortality occurs in the 12% of children born before 37 weeks of gestation (Norwitz and Robinson, 2001; Ashton, 2006). Many but not all published studies suggest a link between maternal periodontal disease and risk for preterm birth (Offenbacher *et al*, 1996, 2006; Davenport *et al*, 2002; Rajapakse *et al*, 2005; Michalowicz *et al*, 2006; Radnai *et al*, 2006; Bassani *et al*, 2007). It is believed that periodontopathic bacteria, or inflammatory mediators generated in response to them, reach the uterine cavity through the bloodstream and elicit an inflammatory cascade that leads to spontaneous preterm labor (Madianos *et al*, 2001).

Levels of *Lactobacillus casei* in maternal saliva have been reported to predict infant birth weight and gestational age at delivery (Dasanayake *et al*, 2005). In that study, every 10-fold increase in *L. casei* levels was associated with a 42-g increase in birth weight and a 0.13 week increase in gestational age at delivery. The goal of the present project was to determine whether risk for preterm birth is associated with cariogenic bacteria in maternal saliva, clinical caries experience, and periodontal status. As a secondary aim, we also tested for associations between maternal clinical and microbiological caries measures, periodontal measures, and risk for infant low birth weight.

Materials and methods

Selection of subjects

The study protocol was approved by Institutional Review Boards at the University of Minnesota and Hennepin County Medical Center. All study procedures were undertaken with the understanding and written consent of each subject and according to ethical

principles, including the World Medical Association Declaration of Helsinki. Women were recruited between May 2005 and February 2006 at a hospital-based obstetrics and gynecology clinic. Women were eligible from the first trimester of pregnancy until 8 weeks postpartum and ineligible if they were under 16 years of age, required antibiotics prior to a dental examination, had fewer than 20 natural teeth, were unable to give informed consent, or had a plural pregnancy. Preterm birth cases were those who delivered a live infant earlier than 37 weeks of gestation and controls delivered an infant at 37 weeks of gestation or later. We attempted to recruit one or more matched controls for each case, with matching based on age (divided into 5-year blocks) and race/ethnicity.

Clinical and microbiological assessments

All salivary samples and clinical data were obtained by one of the two examiners (RD, ELG) who were trained and calibrated for the caries, periodontal, and salivary assessments. To ensure the masking of the clinical examiners, a study coordinator scheduled subjects for oral examinations and salivary sampling. At the time of the examination, the clinical examiner was unaware of the woman's pregnancy outcomes and obstetrics or prenatal information. First, saliva samples were obtained by having participants chew on a piece of paraffin wax for 5 min and spit into a plastic cup. Five milliliter of saliva was spread uniformly on both sides of a culture test tube using a sterile pipette, which was a part of a commercially available salivary test (CRT bacteria®; Ivoclar-Vivadent, Amherst, NY, USA). Samples were then incubated at 99° Fahrenheit for 48 h. Levels of mutans streptococci and *Lactobacilli* species were scored as containing either $<10^5$ or $\geq 10^5$ colony forming units per milliliter of saliva (CFU/ml) as recommended by the manufacturer.

Examiners used a mouth mirror, a #23 explorer and a manual periodontal probe to assess each woman's caries and periodontal status. Clinical caries measures were defined as the number of decayed (DS), decayed and filled (DFS), and decayed, missing and filled (DMFS) tooth surfaces on all teeth excluding third molars. Caries were assessed as described elsewhere (Radike, 1972). The periodontal measures included probing depth, clinical attachment loss, plaque and bleeding upon probing. These measures were assessed on the disto-buccal, mid-buccal, and mesio-buccal tooth surfaces in two randomly selected maxillary and mandibular quadrants.

Recording of maternal characteristics

Demographic information and risk factors for preterm delivery were collected by questionnaire. Obstetrics history and delivery data, including date of delivery and birth weight, were abstracted from medical records. The questionnaire had been used extensively with obstetrics patients at the clinical study site. Gestational age at delivery was determined from menstrual history and ultrasonography as described elsewhere (Carey *et al*, 2000).

Statistical analyses

Preterm birth cases and controls were divided into different strata according to age (divided into 5-year blocks) and race/ethnicity (stratification #1). To test for associations between low birth weight and the microbiological and clinical outcomes, it was necessary to combine some of these strata to ensure that each stratum contained at least one case (infant birth weight <2500 g) and one control (infant birth weight ≥ 2500 g) (stratification #2). Therefore, by analyzing the data from these strata, we conducted the equivalent of a matched analysis for both preterm birth and low birth weight outcomes.

Exact stratified Mantel–Haenszel tests were used to test associations between case–control status and levels of mutans streptococci and *Lactobacilli* (high/low) in saliva (R language v. 2.3.1; R Foundation for Statistical Computing, Vienna, Austria). Mixed linear models (SAS version 8; SAS Institute, Cary, NC, USA) were used to test associations of clinical caries measures with case–control status. Repeated measure analyses of variance were used to calculate stratified but otherwise unadjusted differences between cases and controls. Adjusted analyses added these further independent variables to the mixed linear model: the periodontal measures, the previous adverse pregnancy outcomes, the number of previous pregnancies, and self-reported alcohol and tobacco use. Similarly, mixed linear models were also used to examine the association between the periodontal measures and case–control status. We also explored the relationships between gestational age and birth weight, measured as continuous variables, and clinical caries measures using Pearson's correlation. Finally, because we sampled women at various times, we used Pearson's chi-square tests and Fisher's exact tests (two-tail) to determine if sampling time was associated with the presence of the target microorganisms. Specifically, we compared salivary levels between women who were sampled before versus after delivery. We also compared levels among women who were sampled during their first half of pregnancy, during their second half, or after delivery.

Results

One hundred twenty participants completed the study procedures. All had attended at least one prenatal visit. Twelve women were subsequently excluded because they lacked clinical or microbiological data, delivered at another hospital, or delivered a stillborn infant. An additional control subject, an Asian-American, could not be matched to any case and was therefore excluded. Consequently, we analyzed complete data from 107 subjects, including 34 preterm birth cases and 73 full-term controls (Table 1). Twenty-two preterm infants and two term infants weighed <2500 g at birth and were considered to be of low birth weight. Fifty-seven women in the final sample completed the study procedures 1–8 weeks after delivery.

Table 1 summarizes demographics, preterm birth risk factors and obstetrical histories for preterm birth cases

Table 1 Demographics and obstetrical history, by preterm birth status

Variable	*Cases (GA <37 weeks)* (n = *34*)	*Controls (GA >37 weeks)* (n = *73*)	P-*value*[a]
Mean age (years, ± s.d.)	26.3 ± 5.9	25.6 ± 5.5	0.92
Race/ethnicity (*n*, %)			
Black[b]	11 (32)	29 (40)	0.33
Caucasian Non-Hispanic	3 (9)	8 (11)	
Caucasian Hispanic	18 (53)	34 (47)	
Native American	2 (6)	2 (3)	
Tobacco use during pregnancy (*n*, %)			
Yes	4 (12)	11 (15)	1.00
No	30 (88)	62 (85)	
Alcohol use during pregnancy (*n*, %)			
Yes	0 (0)	5 (7)	0.15
No	33 (100)	67 (93)	
Unknown	1	1	
Previous pregnancy (*n*, %)			
Yes	22 (65)	62 (85)	0.09
No	12 (35)	11 (15)	
No. of previous pregnancies (mean ± s.d.)	1.8 ± 1.9	2.0 ± 1.5	0.46
Number of previous preterm births (*n*, %)			
0	30 (88)	68 (93)	0.09
1	2 (6)	5 (7)	
2	2 (6)	0 (0)	
Number of previous spontaneous abortions or stillbirths (*n*, %)			
0	24 (71)	51 (70)	0.33
1	5 (15)	18 (25)	
2	4 (12)	2 (3)	
≥3	1 (3)	2 (3)	
Number of previous induced abortions (*n*, %)			
0	31 (91)	61 (84)	0.08
1	1 (3)	9 (12)	
≥2	2 (6)	3 (4)	
Previous preterm, spontaneous or induced abortion, stillbirth (*n*, %)			
Yes	14 (41)	35 (48)	0.66
No	20 (59)	38 (52)	

GA, gestational age.
[a]Calculated using exact stratified Mantel–Haenszel test except for total number of previous pregnancies, which was calculated using a mixed linear model, and for mean age and race/ethnicity, which were calculated using Chi-Square test.
[b]Includes African-American, Afro-Caribbean, and African.

and controls. Five percent of participants reported using alcohol and 14% reported smoking cigarettes during their pregnancy. A higher proportion of cases than controls had previously delivered a preterm infant (12% *vs* 7%), although this difference was not statistically significant. Cases and controls had similar numbers of previous induced abortions, spontaneous abortions and stillbirths.

Significantly more preterm birth cases than controls had low levels of *Lactobacilli* ($P = 0.009$, Table 2). There was no significant difference between cases and controls in terms of mutans streptococci, although again more cases tended to have lower levels when compared with the controls ($P = 0.053$). Preterm birth cases and controls, however, did not differ significantly in mean clinical caries or periodontal disease measures (Table 3).

We also compared DFS and DMFS scores between preterm birth cases and controls in analyses that included, individually and in combination, adjustments for periodontal measures, number of previous pregnancies, previous adverse pregnancy outcomes, and alcohol

Table 2 Association between salivary levels and pregnancy outcomes

Outcome	*Salivary measures*	*Cases* n *(%)*	*Controls* n *(%)*	*Odds ratio (95% CI)*[a]	P-*value*[b]
Preterm birth		34	73		
	Mutans streptococci $\geq 10^5$ CFU/ml	7 (21)	29 (40)	2.63 (0.95, 8.33)	0.053
	Lactobacilli species $\geq 10^5$ CFU/ml	9 (26)	42 (58)	3.45 (1.27, 10.00)	0.009
Low birth weight		24	83		
	Mutans streptococci $\geq 10^5$ CFU/ml	5 (21)	31 (37)	2.33 (0.78, 8.33)	0.15
	Lactobacilli species $\geq 10^5$ CFU/ml	7 (29)	44 (53)	2.63 (0.93, 8.33)	0.062

[a]Odds of being a preterm case associated with having bacteria levels $< 10^5$ CFU/ml.
[b]Calculated using exact stratified Mantel-Haenszel method.

Table 3 Caries and periodontal measures by gestational age at delivery

Variable	*Cases (GA < 37 weeks) (n = 34)*	*Controls (GA ≥ 37 weeks) (n = 73)*	P-*value*[a]	*95% CI for the difference: Control – Case*
	Mean ± s.e.[b]			
Decayed surfaces	5.5 ± 1.6	6.0 ± 1.3	0.80	−3.2 to 4.12
Decayed and filled surfaces	11.9 ± 2.1	14.5 ± 1.7	0.29	−2.2 to 7.3
Decayed, missing and filled surfaces	17.9 ± 2.8	20.1 ± 2.3	0.50	−4.2 to 8.7
Mean probing depth (mm)	2.77 ± 0.08	2.75 ± 0.07	0.90	−0.20 to 0.18
Mean attachment level (mm)	0.56 ± 0.09	0.47 ± 0.07	0.36	−0.29 to 0.11
Mean percent of sites with bleeding on probing	51.8 ± 4.1	51.8 ± 3.4	1.00	−9.3 to 9.3

GA, gestational age.
[a]Calculated using mixed linear models, with strata as the random effect.
[b]Estimated using mixed linear models, with strata as the random effect.

and tobacco use during pregnancy. Scores did not differ significantly between groups in any adjusted analysis (all P values >0.2; results not shown).

When cases and controls were defined in terms of infant birth weight, normal birth weight mothers tended to have more high *Lactobacilli* and mutans streptococci counts than mothers who delivered a low birth weight infant, although the differences did not reach statistical significance ($P = 0.062$ for *Lactobacilli*, $P = 0.15$ for mutans streptococci, Table 2). Low birth weight cases and controls also did not differ significantly in terms of mean clinical caries or periodontal disease measures (Table 4). Correlations between gestational age at delivery and DMFS scores and between birth weight and DMFS scores were exceedingly low and statistically insignificant (r = 0.03 and 0.00, respectively, $P > 0.5$; results not shown).

We found no significant association between sampling time and salivary levels of either mutans streptococci or *Lactobacilli* (results not shown). Eighteen of 50 (36.0%) women sampled before delivery and 18 of 57 (31.6%) sampled after delivery had elevated levels of mutans streptococci ($P = 0.63$). The respective figures for *Lactobacilli* were 28 of 50 (56%) and 23 of 57 (40.4%) ($P = 0.11$). Women who were sampled during the first half of their pregnancy tended to have more elevated counts of both target species when compared with women sampled during the second half or after pregnancy, although again the differences were not statistically significant ($P = 0.09$ for mutans streptococci, $P = 0.10$ for *Lactobacilli*). In addition, only nine women were sampled during their first half of pregnancy.

Women with high mutans streptococci levels had significantly more decayed tooth surfaces than women with low mutans streptococci levels ($P = 0.006$, Table 5). Women with high mutans streptococci levels also tended to have higher DFS and DMFS scores, although these differences were not statistically significant ($P > 0.1$).

Discussion

Our results indicate that in a pilot study that included a predominantly Black and Hispanic population, elevated levels of *Lactobacilli* in maternal saliva were inversely associated with preterm birth. Mutans streptococci levels, clinical caries and periodontal measures, however, were not significantly associated with either preterm birth or low birth weight.

Many environmental and genetic factors have been associated with preterm birth (Goffinet, 2005; Leitich,

Table 4 Caries and periodontal measures by birth weight

Variable	*Cases (BW < 2500 g) (n = 24)*	*Controls (BW ≥2500 g) (n = 83)*	P-*value*[a]	*95% CI for the difference: control–case*
	Mean ± s.e.[b]			
Decayed surfaces	6.0 ± 1.1	6.3 ± 1.8	0.86	−3.6 to 4.4
Decayed and filled surfaces	13.3 ± 2.4	13.7 ± 1.5	0.94	−4.8 to 5.8
Decayed, missing and filled surfaces	19.4 ± 3.3	19.1 ± 2.1	0.87	−7.5 to 7.0
Mean probing depth (mm)	2.72 ± 0.09	2.80 ± 0.06	0.49	−0.14 to 0.28
Mean Attachment level (mm)	0.54 ± 0.10	0.51 ± 0.06	0.74	−0.26 to 0.18
Mean percent of sites with bleeding on probing	50.9 ± 4.6	52.8 ± 2.9	0.71	−8.2 to 12.0

BW, birth weight.
[a]Calculated using mixed linear models, with strata as the random effect.
[b]Estimated using mixed linear models, with strata as the random effect.

Table 5 Caries measures by mutans streptococci levels

Caries measures	*Mutans streptococci salivary levels*[a]	*Mean ± s.d.*	P-*value*[b]
Decayed surfaces	High	8.9 ± 11.9	0.006
	Low	4.2 ± 5.5	
Decayed and filled surfaces	High	13.9 ± 13.2	0.22
	Low	11.0 ± 10.3	
Decayed, missing and filled surfaces	High	17.7 ± 18.4	0.35
	Low	14.6 ± 14.6	

[a]High, $\geq 10^5$ CFU/ml, $n = 35$; Low, $< 10^5$ CFU/ml, $n = 72$.
[b]From *t*-test.

2005). Consequently, it is possible that reported associations between dental disease and preterm birth may be confounded by multiple factors, including socioeconomic status and smoking (Xiong *et al*, 2006). Given our relatively small sample, we were not able to consider the many potential confounders or effect modifiers, both measured and unmeasured, that may have contributed to the observed associations. Several features of our study, however, suggest our results are robust. First, women were recruited from a single obstetrics clinic that serves mostly minority and underserved populations; as evidence, all but two participants had their labor and delivery costs covered by public assistance programs. Second, the prevalence of smoking and alcohol use during pregnancy was relatively low in this population and similar in cases and controls. Lastly, in the analyses of clinical caries measures, we conducted analyses that included adjustments for age, race/ethnicity, periodontal measures, previous pregnancy outcomes and alcohol and tobacco use (results not shown). The inferences from these analyses did not differ with and without these adjustments.

Our findings are consistent with the results of another study in the dental literature in which the authors found that levels of *Lactobacillus casei* in maternal saliva could help to predict infant birth weight and gestational age at delivery (Dasanayake *et al*, 2005). In addition, our findings are consistent with reports in the obstetrics literature in which elevated levels of vaginal *Lactobacilli* have been inversely associated with preterm birth and low birth weight (Donders *et al*, 1993; Hillier *et al*, 1995; Usui *et al*, 2002). High levels of *Lactobacilli* in the vaginal canal may create or reflect an environment that inhibits growth of bacterial species associated with poor pregnancy outcomes. For example, many *Lactobacilli* species in the vagina can secrete H_2O_2 (Wilks *et al*, 2004), which is toxic to bacteria that are involved in vaginosis, an important risk factor for preterm birth. These findings suggest that levels of vaginal *Lactobacilli* may be employed to predict risk for poor pregnancy outcomes. While we did not test this, it would be useful to determine if levels of *Lactobacilli* in saliva and the vagina are strongly correlated.

We found a trend towards an inverse association between salivary levels of mutans streptococci and preterm birth. In addition, we found a similar trend for an inverse association between mutans streptococci and *Lactobacilli* salivary levels and low birth weight. The lack of statistical significance for the associations with low birth weight may, in part, reflect that this study was powered to detect associations with preterm birth, not low birth weight. Thus, larger studies will be needed to estimate these associations with greater precision. Another limitation of our study is that we used saliva samples and a chairside bacteriological test to detect and quantify *Lactobacilli* and mutans streptococci. Chairside tests for mutans streptococci are useful in predicting risk for clinical caries (Alaluusua *et al*, 1990; Twetman *et al*, 1994; Yoshihara *et al*, 2001), although results can differ significantly between chairside tests and cultivable methods (Hildebrandt and Bretz, 2006). While our estimates for mutans streptococci and *Lactobacilli* species may have been different if we had used a different chairside test or cultivable or real time PCR detection method, there is less reason to suspect that the relative differences between groups (cases and controls) would vary among these assessment methods. In addition, similar salivary tests to the CRT® tests were shown to be influenced by different external factors such as eating and tooth brushing (el-Nadeef and Bratthall, 1991; Soderling *et al*, 1991), although others found that only eating could significantly affect the salivary scores (Schlagenhauf *et al*, 1995). However, a recent report found that the CRT® test shows similar results to those of the conventional laboratory test in an elderly population (Sanchez-Garcia *et al*, 2008). Despite the limitations of our microbial assessment method, it is noteworthy that we found the same inverse relationship between *Lactobacilli* and preterm birth as reported by others (Dasanayake *et al*, 2005).

No significant difference in DS, DFS, or DMFS scores was found between cases and controls, regardless of whether we defined cases in terms of gestational age at delivery or birth weight. This finding is consistent with the previous reports that found maternal clinical caries measures were not associated with preterm birth or low birth weight (Dasanayake, 1998; Buduneli *et al*, 2005). Because these caries scores are hierarchical (i.e., filled surfaces are counted in two of the three, decayed surfaces in all three), the comparisons between groups and resulting P values are not independent of one another. While such test dependence could mitigate the importance of multiple positive or negative findings, none of the index scores differed significantly between groups ($P > 0.2$).

We found that the severity of periodontal disease, measured from selected teeth, did not differ significantly between preterm birth or low birth weight cases and their respective controls. Because our primary intent was to explore relationships between caries, cariogenic bacteria and preterm birth, we used a partial mouth periodontal assessment to summarize a woman's periodontal status for use as a covariate in the caries-related analyses. Nonetheless, while this partial-mouth examination protocol underestimates the ''true'' prevalence of disease (Susin *et al*, 2005), mean scores from this subset

of teeth correlate strongly with full-mouth means (average r of 0.949, (Kingman *et al*, 1988)). It is possible that by excluding the third molars, we might have underestimate the inflammatory burden as periodontal pockets can be present in about 25% of young adults with asymptomatic third molars (Blakey *et al*, 2002). In addition, we calculated our initial sample size with the salivary levels of bacteria as the primary independent variable and it is possible that our sample size might not have the power to detect a potential association between periodontal disease and preterm birth. On the other hand, a recent multicenter clinical trial with a higher power in a population with similar demographics failed to find such an association (Michalowicz *et al*, 2006). While most studies have reported an association between periodontitis and risk for preterm birth (Xiong *et al*, 2006), our findings regarding the periodontal disease measures are consistent with several others that did not find such an association (Noack *et al*, 2005; Wood *et al*, 2006; Bassani *et al*, 2007; Vettore *et al*, 2008).

Another limitation of our study was that we enrolled women from early in their second trimester of pregnancy to up to 8 weeks post-partum. While we originally planned to assess all women within 48 h of delivery, logistical problems in the hospital's labor and delivery ward precluded us from following this protocol. This variable sampling scheme could have added unnecessary variability to the clinical and microbial outcome measures. In comparing microbial findings from women sampled at various time points, however, we found no evidence that the fraction of women with positive microbial test results differed significantly across the enrollment eligibility period. Furthermore, periodontal measures do not change significantly from the end of the first trimester of pregnancy onward in the absence of treatment (Michalowicz *et al*, 2006).

In our study, 34% of women overall and 40% of full-term controls had high levels ($\geq 10^5$ CFU/ml) of mutans streptococci. These rates are lower than reported by most but not all related studies in the literature. For example, in three studies on pregnant women (Acton *et al*, 1999; Soderling *et al*, 2000; Thorild *et al*, 2004), the prevalence rates of high mutans streptococci levels ranged from 42% to 58%. By contrast, other authors (Brambilla *et al*, 1998) found that only 65 of 310 (21%) pregnant women had high mutans streptococci salivary counts. While yet others have reported high mutans streptococci counts in women (e.g., (Barkeling *et al*, 2002; Linke *et al*, 2003)), these studies were not limited to pregnant women and are probably not comparable with our study population.

In summary, we found that low levels of *Lactobacilli* in maternal saliva are significantly associated with preterm birth through exploratory analyses. Larger, prospective studies are needed to further explore these relationships. Some investigators have hypothesized that oral administration of *Lactobacilli* might help modulate inflammation and suppress the emergence of pathogens from the rectum into the vagina (Reid and Bocking, 2003). Future research is warranted to explore the relationships between oral and vaginal microbiota and to determine if and how host responses to these microbes affect pregnancy outcomes.

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Author contributions

R Durand and EL Gunselman designed the study and collected the data included in this manuscript. JS Hodges wrote the statistical analysis section and edited the manuscript. AJ DiAngelis provided logistical support at Hennepin Medical Center and edited the manuscript. BS Michalowicz provided logistical support at University of Minnesota and Hennepin Medical Center and edited the manuscript.

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