

The relationship between periodontal disease and preterm low birthweight: clinical and microbiological results

M. V. Vettore^{1,2}, A. T. Leão³, M. do C. Leal¹, M. Feres⁴, A. Sheiham²

¹Department of Epidemiology and Quantitative Methods in Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ) – RJ, Brazil, ²Department of Epidemiology and Public Health, University College London, London, UK, ³Division of Graduate Periodontics, Federal University of Rio de Janeiro (UFRJ) – RJ, Brazil and ⁴Department of Periodontology, Guarulhos University – SP, Brazil

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Background and Objective: Findings on the effect of periodontal disease on preterm low birthweight are inconclusive. The objective of this study was to compare periodontal clinical measures and the levels and proportions of 39 bacterial species in subgingival biofilm samples in puerperal women with preterm low birthweight and nonpreterm low birthweight.

Material and Methods: A case-control study with 116 postpartum women over 30 years of age was conducted. Four case groups of subjects with preterm and/or low birthweight [preterm ($n = 40$), low birthweight ($n = 35$), preterm and/or low birthweight ($n = 50$) and preterm and low birthweight ($n = 25$)] were compared with normal nonpreterm low-birthweight controls ($n = 66$). Periodontal clinical parameters of dental plaque, calculus, bleeding on probing, periodontal pocket depth and clinical attachment level were recorded. Covariates included socio-demographic and anthropometric characteristics, smoking, alcohol consumption, obstetric history, prenatal care and diseases during pregnancy. Two subgingival biofilm samples per women were analyzed for 39 bacterial species using a checkerboard DNA–DNA hybridization technique.

Results: The mean periodontal pocket depth was significantly higher in nonpreterm low-birthweight controls than in subjects in the preterm low birthweight, preterm and/or low birthweight, and preterm and low-birthweight groups. Clinical attachment level measures were not different between all pairs of cases and control groups. Groups did not differ with respect to the mean proportions of different microbial complexes. The mean counts of *Treponema socranskii* were lower in all case groups compared with the control group.

Conclusion: Maternal periodontal microbiota and clinical characteristics of periodontal disease were not associated with having preterm low-birthweight babies.

Mário V. Vettore, Escola Nacional de Saúde Pública – Fundação Oswaldo Cruz, Rua Leopoldo Bulhões, 1480 sl 809, Manguinhos – Rio de Janeiro – RJ, CEP: 21041–210, Brazil
Tel: +55 21 25982620
Fax: +55 21 22706772
e-mail: mario@ensp.fiocruz.br

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The incidence of preterm low-birthweight babies is increasing in developing countries (1). It remains an important public health problem with

great impacts on neonatal mortality and morbidity (2). Despite the identification of some potential risk factors for preterm low birthweight, a consider-

able proportion of preterm low birthweight is of unknown etiology. It has been suggested that puerperal genitourinary tract infections affect the

normal course of gestation by altering the levels of local cytokines, resulting in growth restriction of the fetus, preterm labor and preterm birth (3,4). Genitourinary tract infections and periodontal disease are both caused by gram-negative anaerobic bacteria and result in local and systemic elevations of prostaglandin E₂ and tumor necrosis factor- α (5,6). The similarity in biological mechanisms between genitourinary infections and periodontal disease involving pro-inflammatory cytokines suggested the influence of periodontal disease on preterm low birthweight and prompted dental researchers to test whether periodontal disease was a new risk factor for preterm low birthweight.

The initiation and progression of destructive periodontal disease is attributed to the presence of elevated levels of inflammatory mediators and pathogenic bacteria within the gingival crevice (7). Although clinical measures of periodontal disease provide some information on the severity of periodontitis, they do not measure disease activity (7,8). Consequently, microbiological testing and biochemical analyses of the host response have been proposed in an effort to monitor the activity of periodontal disease (9).

There is a relationship between periodontal pathogens and preterm low birthweight in animal models. In pregnant hamsters with localized subcutaneous infection with *Porphyromonas gingivalis*, there was an increase of prostaglandin E₂ and tumor necrosis factor- α levels in the pelvic region. Fetal development was affected and fetal weight was significantly decreased in infected compared with noninfected hamsters (10,11). However, there is no consensus about the influence of specific bacterial species from the periodontium on preterm low birthweight in pregnant women.

Periodontal pathogens have been associated with preterm birth (12), and the red-complex species *P. gingivalis*, *Treponema denticola* and *Tannerella forsythia* (13) were found at higher levels in mothers who gave birth to preterm low-birthweight infants (14). On the other hand, other studies did not detect differences in the levels of periodontal pathogens between pre-

term and nonpreterm mothers (15,16), and preterm and low-birthweight and nonpreterm and/or nonlow-birthweight mothers (17).

The lack of consensus on the possible influence of periodontal disease on preterm low birthweight has also been observed in studies that assessed periodontal disease clinically. Despite several studies finding more severe periodontal disease in preterm low-birthweight mothers compared with nonpreterm low-birthweight mothers (18–20), other studies did not find an association between periodontal disease and preterm low birthweight (12,17,21). A recent systematic review highlighted the diversity of findings (22).

Based on the current uncertainty of a relationship between periodontal infection and preterm low birthweight, a case-control study was carried out in order to evaluate the association between certain maternal periodontal pathogens and poor periodontal status, and preterm low birthweight.

Material and methods

In this case-control study, a sample of 116 postpartum women aged ≥ 30 years were randomly selected from a large case-control study of 542 subjects investigating the relationship between clinical parameters of periodontal disease and preterm low birthweight. Figure 1 shows the flow chart for selecting study subjects. The women had attended referral hospital centres for high-risk pregnancies in Rio de Janeiro (RJ, Brazil).

The inclusion criteria to take part in the study were women of at least 30 years of age who had given birth to a live child in the past 3 d. The criterion of ≥ 30 years of age was used because the prevalence of periodontal disease is low in women under 30 years of age. Other inclusion criteria were single birth mothers; the presence of 15 or more natural teeth; the absence of systemic conditions that could affect the progression of periodontal disease, the use of psychotropic drugs or any medicines related to periodontal changes; or the absence of professional tooth cleaning or periodontal treatment during the last 6 mo and of

systemic antibiotic treatment during the last week. Women excluded were those with human immunodeficiency virus infection, chronic hypertension and chronic diabetes mellitus. Women who required prophylactic antibiotics for a periodontal examination were also excluded.

Babies delivered before 37 complete weeks of gestation were considered as preterm. The estimation of gestational age was assessed from the last menstrual period (23). When last menstrual period data were missing, the Capurro score was used (24). The reliability analysis between the last menstrual period and the Capurro score was tested by the intraclass correlation coefficient. The intraclass correlation coefficient of agreement findings was 0.92.

Low-birthweight newborns were infants weighing less than 2500 g at birth. All newborns were weighed immediately after the delivery using calibrated scales. The gestational age estimate and infant weights were obtained from medical records.

The study was approved by the Committee of Ethics and Research of the National School of Public Health – Oswaldo Cruz Foundation (FIOCRUZ) (protocol no. 78/02). Subjects were informed that they were free to withdraw from the study at any time.

Calibration study

A pilot study including 30 patients with at least four sites with periodontal pocket depth > 4.0 mm was conducted to calibrate six examiners and to test the understanding and layout of the questionnaires. Kappa test and intraclass correlation coefficient of agreement findings for periodontal pocket depth were, respectively, ≥ 0.78 and ≥ 0.72 for intra-examiner, and ≥ 0.77 and ≥ 0.72 for interexaminer. All examiners were masked concerning the purpose of the main study.

Main study

The randomly selected 116 women were assigned to four case groups and one control group. The case groups

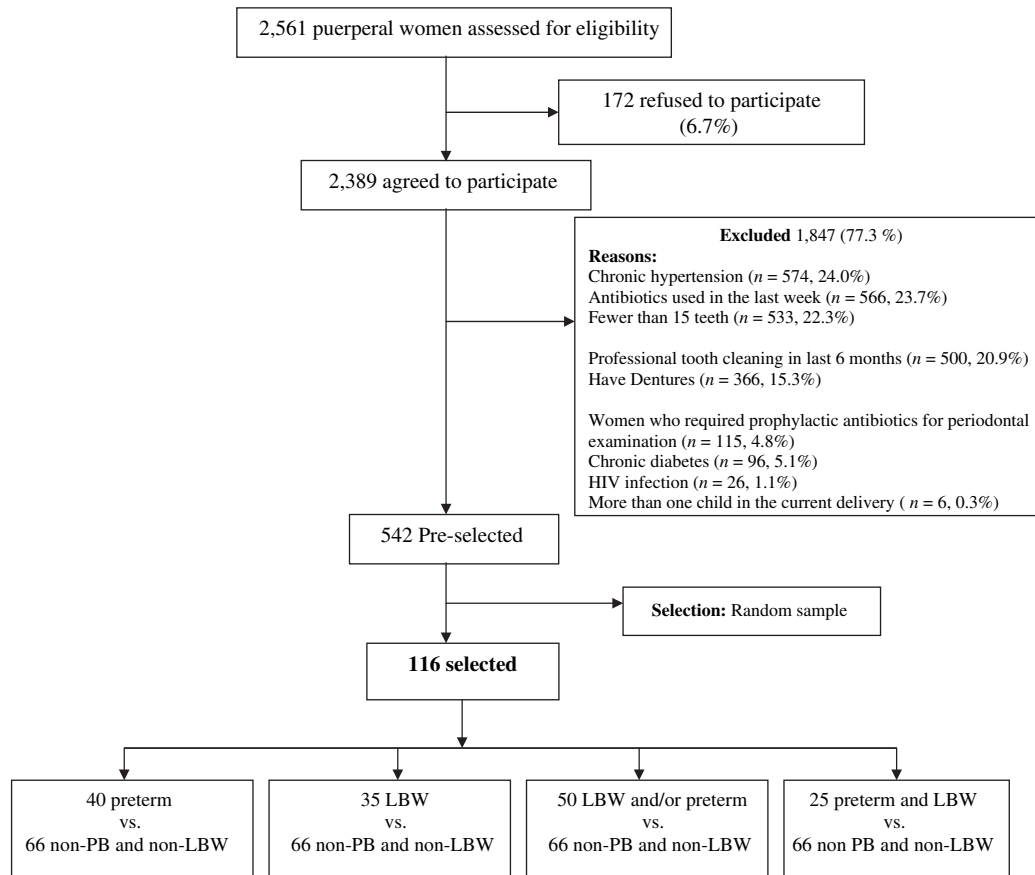


Fig. 1. Flow chart for selecting study objects. HIV, human immunodeficiency virus; LBW, low birthweight; PB, preterm birth.

were as follows: preterm births ($n = 40$); low birthweight ($n = 35$); preterm births and/or low birthweight ($n = 50$); and preterm births and low birthweight ($n = 25$). All case groups were compared with a control group composed of subjects with nonpreterm birth and nonlow birthweight ($n = 66$). All case and control definitions were as used in a previous study (22).

Sample size calculation

Assuming that the standard deviation of periodontal pocket depth from the large case-control study of 542 subjects was equal to 0.5, with 80% power and 5% Type I error probability, a study with a selection ratio case/control of 1 : 2 needed 112 subjects to detect 25% of the differences between groups.

Covariates

Covariate data of anthropometric and socio-demographic characteristics

included age, corporal mass index, ethnicity, marital status, income and level of education.

Cigarette consumption during pregnancy was recorded, and the T-ACE questionnaire for risky drinking assessment in pregnant women was used (25). The modified Kotelchuck index, adapted for a Rio de Janeiro city population, was used to assess prenatal care (26).

Pregnancy information, including gestational age, baby weight at birth, type of birth and gender of neonate, were transcribed from medical records. The occurrence of previous preterm infant, previous low-birthweight infant, hypertension during pregnancy, gestational diabetes and urinary infection were also recorded.

Measurement of periodontal status

Periodontal clinical measurements, including visible plaque index (27),

visible calculus, bleeding on probing index (28), periodontal pocket depth and clinical attachment level, were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth, excluding third molars.

Periodontal pocket depths were registered in millimeters from the free gingival margin to the base of the gingival sulcus or periodontal pocket (27). Clinical attachment level measurements were determined using the cemento–enamel junction as a reference point. Periodontal pocket depth and clinical attachment level measures were recorded to the nearest higher millimetre using the North Carolina periodontal probe, 15 mm in length and 0.35 mm in diameter (Hu-Friedy, Chicago, IL, USA). Oral plain mirrors (Hu-Friedy) and a head light (model 8720; Trilhas & Rumos, Rio de Janeiro, RJ, Brazil) were used to facilitate the periodontal examinations.

Microbiological assessment

Counts of 39 subgingival species were determined in each biofilm sample using the checkerboard DNA–DNA hybridization technique (29). Subgingival biofilm samples were taken from the two deepest periodontal disease sites collected from different teeth per subject before clinical examination. When the mother had no periodontal pockets, biofilm samples were collected from two random sites in different quadrants of the mouth.

An initial screening clinical examination was performed in order to identify the presence of periodontal pockets and also to identify the two deepest sites to be sampled. After biofilm sample collection, the periodontal clinical measurements were recorded.

After removal of supragingival biofilms, subgingival biofilm samples were taken with individual sterile Gracey curettes (Hu-Friedy) and placed in separate Eppendorf tubes containing 0.15 mL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), then 0.15 mL of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer.

Eppendorf tubes containing the biofilm samples were stored at -20°C and transported to the laboratory of Oral Microbiology (Guarulhos University, São Paulo, Brazil).

First, the samples were boiled for 10 min and neutralized using 0.8 mL of 5 M ammonium acetate. The released DNA was then placed in the extended slots of a Minislot-30 apparatus (Immunitics, Cambridge, MA, USA), concentrated onto a 15×15 -cm positively charged nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking at 120°C for 20 min. The membrane was placed in a Miniblotter 45 (Immunitics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 39 subgingival species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes were detected using antibody to digoxigenin conjugated with alkaline phosphatase followed by chemiluminescence detection.

The 39 reference strains employed for the development of DNA probes are shown in Table 1. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Statistical analysis

All statistical analyses were carried out using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The significance level established for all analyses was 5% ($p \leq 0.05$). Comparisons between groups for covariates were tested by the chi-square test and Fisher's Exact test for variables expressed in proportions and by the Mann–Whitney test and the *t*-test for continuous variables.

Clinical parameters, including number and percentage of sites with visible plaque, visible calculus, bleeding on probing as well as the average periodontal pocket depth and clinical attachment level were computed for each subject and then averaged across subjects in the groups.

Differences among clinical parameters were examined in the subset of sites according to their periodontal pocket depth (≥ 4 mm, ≥ 5 mm and ≥ 6 mm), clinical attachment level (≥ 3 mm, ≥ 4 mm, ≥ 5 mm and ≥ 6 mm) and using a combination of both (periodontal pocket depth ≥ 4 mm and clinical attachment level ≥ 3 mm). The statistical significance of differences between the groups was checked by the Mann–Whitney test.

Periodontal pocket depths of ≥ 4 mm in sites with clinical attachment level ≥ 4 mm were summed, giving a continuous measure of periodontal disease load, namely the periodontal inflammatory load. Sites with a periodontal pocket depth of ≤ 3 mm or a clinical attachment level of < 3 mm were not included. Periodontal disease was considered in terms of load of periodontal infection according to percentiles of the periodontal inflammatory load. The subjects of the sample were classified into four levels of periodontal disease, as follows: level 1, 0–11 mm; level 2,

Table 1. Bacterial strains employed for the development of DNA probes*

Species	Strain
Actinomyces species	
<i>Actinomyces gerencseriae</i>	23860
<i>Actinomyces israelii</i>	12102
<i>Actinomyces naeslundii</i>	43146
Purple complex	
<i>Actinomyces odontolyticus</i>	17929
<i>Veillonella parvula</i>	10790
Yellow complex	
<i>Streptococcus gordonii</i>	10558
<i>Streptococcus intermedius</i>	27335
<i>Streptococcus mitis</i>	49456
<i>Streptococcus oralis</i>	35037
<i>Streptococcus sanguinis</i>	10556
Green complex	
<i>Aggregatibacter</i>	43718
<i>actinomycetencomitans a + b</i>	29523
<i>Capnocytophaga gingivalis</i>	33624
<i>Capnocytophaga ochracea</i>	33596
<i>Capnocytophaga sputigena</i>	33612
<i>Eikenella corrodens</i>	23834
Orange complex	
<i>Campylobacter gracilis</i>	33236
<i>Campylobacter rectus</i>	33238
<i>Campylobacter showae</i>	51146
Orange complex	
<i>Eubacterium nodatum</i>	33099
<i>Fusobacterium nucleatum ss nucleatum</i>	25586
<i>Fusobacterium nucleatum ss polymorphum</i>	10953
<i>Fusobacterium nucleatum ss vincentii</i>	49256
<i>Fusobacterium periodonticum</i>	33693
<i>Peptostreptococcus micros</i>	33270
<i>Prevotella intermedia</i>	25611
<i>Prevotella nigrescens</i>	33563
<i>Streptococcus constellatus</i>	27823
Red complex	
<i>Tannerella forsythia</i>	43037
<i>Porphyromonas gingivalis</i>	33277
<i>Treponema denticola</i> _b	B1
<i>Treponema socranskii</i> _b	S1
Other species	
<i>Eubacterium saburreum</i>	33271
<i>Gemella morbillorum</i>	27824
<i>Leptotrichia buccalis</i>	14201
<i>Neisseria mucosa</i>	19696
<i>Prevotella melaninogenica</i>	25845
<i>Propionibacterium acnes</i>	11827
I + II	11828
<i>Selenomonas noxia</i>	43541
<i>Streptococcus anginosus</i>	33397

*All strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA), except *Treponema denticola* B1 and *Treponema socranskii* S1 were obtained from the Forsyth Institute (Boston, MA, USA). Microbial "complexes" were described by Socransky *et al.* (13).

12–55 mm; level 3, 56–113 mm; and level 4, 114 mm and over. The odds ratios were calculated using level 1 as the category of reference.

Microbiological data available for 116 subjects were counts of each of the 39 species from two subgingival biofilm samples for each subject.

In total, 232 subgingival biofilm samples were examined. The mean levels of each species evaluated were computed for each subject and then averaged across subjects within the different groups. The total DNA probe count was also computed at each sample site in each subject and the proportion that individual species comprised of that count was determined and averaged across subjects in every experimental group.

Significance of differences between pairs of groups in mean counts of bacterial species and in mean proportions of different microbial complexes (13) (Table 1) was analyzed using the Mann–Whitney test. When the levels of individual species were compared, adjustments were made for multiple comparisons, as described by Socransky *et al.* (30).

Multiple logistic regression analysis was performed to examine the association between periodontal disease and preterm low birthweight. Periodontal disease was included in the logistic regression analysis using two strategies. First, frequency of periodontal pocket depth ≥ 4 mm and frequency of clinical attachment level ≥ 3 mm were used to indicate the presence of clinical periodontal disease. Second, mean counts of the red complex (13) were used as a microbiological measure of periodontal disease. Variables considered as potential confounders for the outcomes, such as age, marital status, income, schooling, ethnicity and smoking, were used in the multiple logistic regression. For the multiple analysis, the stepwise forward procedure was used for entry of variables.

Results

Covariate variables of the 116 women in the study are presented in Table 2. The gestational ages and birthweights

of the infants were significantly different between all pairs of groups. There were no differences in the anthropometric and socio-demographic characteristics between groups. Inadequate prenatal care was more common in all case groups compared with the control group ($p < 0.05$).

The frequency of previous preterm and previous low birthweight was higher in all case groups compared with the nonpreterm births and nonlow-birthweight groups (Table 2). Hypertension during pregnancy was more common in preterm, and preterm and/or low-birthweight, mothers ($p < 0.05$).

Periodontal clinical parameters

Table 3 shows the comparisons of periodontal clinical parameters between all pairs of cases and control groups. Numbers of teeth, bleeding on probing and visible calculus were similar in all comparisons between groups. Visible dental plaque scores were significantly higher in the control group compared with preterm and preterm and low-birthweight case groups ($p < 0.05$). Mean periodontal pocket depth was significantly higher in the control group than in the case groups (Table 3). Periodontal inflammatory load was not different between all pairs of case and control groups (Table 4).

In the multivariate analysis, the frequency of periodontal pocket depth ≥ 4 mm and the frequency of clinical attachment level ≥ 3 mm remained not associated with preterm low-birthweight outcomes after adjustment for age, marital status, income, schooling, ethnicity and smoking ($p > 0.05$).

Microbiological results

The comparisons of the mean counts of all species evaluated in the subgingival biofilm samples of case and control groups are shown in Figs 2 and 3. The profile of colonization was similar among the groups studied. Overall, the species found to be present at the highest levels were *Actinomyces gerencseriae*, *Actinomyces naeslundii* genospecies 2, *Veillonella parvula*,

Streptococcus gordonii, *Streptococcus sanguinis*, *Campylobacter gracilis* and *Neisseria mucosa*. Few differences in the microbial composition of subgingival biofilm were observed when the pairs of groups were compared. The mean counts of *Streptococcus mitis* were statistically higher in mothers with nonpreterm and nonlow-birthweight babies than in those with preterm newborns. The mean counts of *Treponema socranskii* were lower in all case groups (preterm, low birthweight, preterm and/or low birthweight, and preterm and low birthweight) than in the nonpreterm and nonlow-birthweight control group. The counts of the other species evaluated did not differ significantly between case and control groups.

Figure 4 presents the mean proportions of the microbial complexes described by Socransky *et al.* and Socransky & Haffajee (13,31). The different groups of oral microorganisms are determined by distinct colours (Table 1). Some bacterial species not associated with any complex, and DNA probes for new species, were compiled in the same group, represented by the gray colour. The proportions of the microbial complexes were similar among different groups of subjects. Overall, the blue complex was found in the highest mean proportions, followed by the yellow and orange complexes. There were no statistically significant differences between all pairs of case/control groups with regard to the mean proportion of these microbial complexes ($p > 0.05$).

Logistic regression showed no association between the mean counts of red complex and preterm low-birthweight outcomes in multivariate analysis after adjustment for age, marital status, income, schooling, ethnicity and smoking ($p > 0.05$).

Discussion

The present investigation did not find an association between specific periodontal pathogens and undesirable pregnancy outcomes. In addition, maternal clinical periodontal status was not associated with preterm low-birthweight babies.

Table 2. Covariate variables of the 116 women examined in the study

	Non-PB and non-LBW (n = 66)	Preterm (n = 40)	LBW (n = 35)	p-value*	PB and/or LBW (n = 50)	p-value*	PB and LBW (n = 25)	p-value*
GA, weeks, mean ± SD	39.8 ± 1.6	33.6 ± 2.2	34.2 ± 3.0	< 0.05†	34.4 ± 2.7	< 0.05†	32.8 ± 2.2	< 0.05†
Birthweight (g), mean ± SD	3325.3 ± 490.9	2271.4 ± 692.8	1949.3 ± 479.8	< 0.05†	2250.4 ± 628.2	< 0.05†	1862.5 ± 526.0	< 0.05†
Type of birth, %	NS	NS	NS	NS				
Normal birth	53.0	37.5	46.0	48.6				
Caesarian	47.0	62.5	54.0	51.4				
Neonate gender, % male	56.1	42.5	48.6	NS	46.0	NS	44.0	NS
Age, mean ± SD	34.2 ± 4.5	34.5 ± 3.8	34.0 ± 3.8	NS†	34.6 ± 4.0	NS†	33.6 ± 3.4	NS†
CMI, mean ± SD*	24.1 ± 3.8	24.0 ± 5.6	23.0 ± 5.4	NS‡	23.3 ± 5.5	NS‡	23.9 ± 5.6	NS‡
Ethnicity, %	NS	NS	NS	NS				
White	31.8	25.0	32.0	34.3				
Brown	51.5	50.0	44.0	42.8				
Black	16.7	25.0	24.0	22.9				
Marital status, %	NS	NS	NS	NS				
Married/partner	89.4	82.5	82.0	80.0				
Young	7.6	10.0	10.0	8.6				
Divorced/widow	3.0	7.5	8.0	11.4				
Income (MW), %	NS	NS	NS	NS				
≥ 1/2	50.0	50.0	46.0	40.0				
< 1/2	50.0	50.0	54.0	60.0				
Schooling (years), %	NS	NS	NS	NS				
> 8 years	40.9	50.0	46.0	42.9				
≤ 8 years	59.1	50.0	54.0	57.1				
PN care, % *	< 0.05	< 0.05	< 0.05	< 0.05				
Adequate	21.0	5.4	6.7	9.7				
Intermediate	53.2	37.8	35.6	32.3				
Inadequate	25.8	56.8	57.7	58.0				
Risk-drinking, %	3.0	7.5	11.4	NS§	8.0	NS§	12.0	NS§
Number of cigarettes smoked per day, %	NS	NS	NS	NS				
Did not smoke	91.0	87.5	86.0	80.0				
≤ 5	4.5	7.5	8.0	11.4				
≥ 5	4.5	5.0	6.0	8.6				
Previous preterm, % *	9.3	43.3	46.4	< 0.05	45.0	< 0.05	44.4	< 0.05
Previous LBW, % *	11.1	30.0	42.9	< 0.05	35.0	< 0.05	38.9	< 0.05
Hypertension during pregnancy, % *	18.2	38.5	34.3	< 0.05	34.7	< 0.05	40.0	NS
Gestational diabetes, % *	3.0	10.5	5.7	NS§	8.3	NS§	8.0	NS§
Urinary infection, % *	30.3	23.1	22.9	NS	20.4	NS	28.0	NS

CMI, corporal mass index; GA, gestational age; LBW, low birthweight; MW, minimum wage; PB, preterm birth; PN, prenatal care; SD, standard deviation. *Chi-square test results, except for those obtained using †the Mann-Whitney test, ‡the t-test, or §Fisher's Exact test.

Table 3. Periodontal clinical parameters between case and control groups of women

		Non-PB and Non-LBW (n = 66)	Preterm (n = 40)	p-value*	LBW (n = 35)	p-value*	PB and/ or LBW (n = 50)	p-value*	PB and LBW (n = 25)	p-value*
No. of teeth	mean ± SD	23.2 ± 3.5	23.3 ± 3.5	0.862	23.7 ± 3.6	0.393	23.3 ± 3.5	0.816	23.9 ± 3.6	0.054
VPI	mean ± SD	59.1 ± 37.1	42.1 ± 42.8	0.024	47.4 ± 43.4	0.189	46.3 ± 43.0	0.080	41.2 ± 43.4	0.353
	%	65.1 ± 39.6	45.3 ± 45.8	0.028	49.6 ± 44.3	0.139	49.3 ± 44.9	0.079	43.4 ± 45.4	0.041
BOP	mean ± SD	18.0 ± 22.4	14.3 ± 24.2	0.111	17.7 ± 28.8	0.518	15.6 ± 26.1	0.169	16.4 ± 27.2	0.434
	%	19.9 ± 25.6	15.3 ± 26.1	0.104	18.9 ± 30.0	0.505	16.6 ± 27.1	0.163	17.8 ± 29.7	0.411
Calculus	mean ± SD	4.2 ± 11.6	7.2 ± 21.2	0.311	7.5 ± 21.5	0.681	6.3 ± 19.3	0.314	9.5 ± 24.9	0.767
	%	5.3 ± 15.7	8.4 ± 25.2	0.311	8.5 ± 25.0	0.660	7.3 ± 22.8	0.303	10.9 ± 29.1	0.767
PPD	mean ± SD	2.5 ± 0.5	2.3 ± 0.5	0.025	2.3 ± 0.5	0.032	2.3 ± 0.5	0.027	2.3 ± 0.5	0.027
≥ 4 mm	mean ± SD	16.5 ± 13.8	15.0 ± 16.0	0.292	14.4 ± 15.9	0.227	14.9 ± 15.5	0.284	14.3 ± 16.9	0.218
	%	12.1 ± 10.5	10.6 ± 10.9	0.263	9.6 ± 10.3	0.155	10.3 ± 10.4	0.222	9.7 ± 11.2	0.178
≥ 5 mm	mean ± SD	3.3 ± 5.9	4.9 ± 7.2	0.527	4.3 ± 6.7	0.508	5.0 ± 7.1	0.367	3.8 ± 6.6	0.806
	%	2.5 ± 4.8	3.6 ± 5.3	0.544	2.9 ± 4.6	0.594	3.6 ± 5.1	0.402	2.6 ± 4.7	0.878
≥ 6 mm	mean ± SD	0.2 ± 0.6	0.3 ± 1.4	0.787	0.1 ± 0.6	0.931	0.3 ± 1.4	0.605	0.0 ± 0.0	0.121
	%	0.1 ± 0.4	0.2 ± 0.9	0.829	0.1 ± 0.4	0.931	0.2 ± 0.9	0.576	0.0 ± 0.0	0.121
CAL	mean ± SD	2.6 ± 0.5	2.4 ± 0.6	0.105	2.4 ± 0.6	0.112	2.5 ± 0.6	0.120	2.4 ± 0.6	0.086
≥ 3 mm	mean ± SD	75.8 ± 33.8	63.7 ± 38.1	0.067	66.1 ± 41.9	0.174	65.4 ± 39.3	0.095	63.7 ± 41.1	0.125
	%	54.9 ± 24.00	45.5 ± 25.7	0.068	45.6 ± 27.1	0.099	46.2 ± 25.9	0.072	44.3 ± 27.2	0.094
≥ 4 mm	mean ± SD	20.4 ± 16.6	19.1 ± 17.1	0.542	19.1 ± 18.8	0.469	19.6 ± 17.7	0.585	18.2 ± 18.3	0.388
	%	15.4 ± 13.9	14.0 ± 12.6	0.625	13.3 ± 12.9	0.414	14.1 ± 12.6	0.632	13.0 ± 13.0	0.357
≥ 5 mm	mean ± SD	5.0 ± 9.1	5.7 ± 8.0	0.394	5.1 ± 7.7	0.355	5.9 ± 7.9	0.167	4.4 ± 7.7	0.925
	%	4.0 ± 7.9	4.2 ± 6.0	0.387	3.6 ± 5.3	0.370	4.3 ± 5.7	0.161	3.1 ± 5.5	0.962
≥ 6 mm	mean ± SD	1.0 ± 2.9	0.7 ± 2.4	0.790	0.6 ± 2.1	0.368	0.7 ± 2.2	0.873	0.5 ± 2.4	0.061
	%	0.9 ± 2.8	0.5 ± 1.6	0.783	0.4 ± 1.4	0.354	0.5 ± 1.4	0.888	0.3 ± 1.6	0.059
PPD ≥ 4 mm and CAL ≥ 3 mm	mean ± SD	16.5 ± 13.8	15.0 ± 16.0	0.291	14.3 ± 15.8	0.226	14.9 ± 15.4	0.284	14.2 ± 16.9	0.214
	%	12.1 ± 10.5	10.5 ± 10.9	0.254	9.6 ± 10.2	0.146	10.3 ± 10.4	0.217	9.7 ± 11.1	0.164

*Mann-Whitney test results between the the control group (nonpreterm and nonlow birthweight) and the case groups preterm birth, low birthweight, preterm and/or low birthweight, and preterm birth and low birthweight.

BOP, bleeding on probing; CAL, clinical attachment level; LBW, low birthweight; PB, preterm birth; PPD, periodontal pocket depth; VPI, visible plaque index.

Table 4. Unadjusted odds ratio of relationship between periodontal disease in preterm low-birthweight mothers and controls in different pregnancy outcome groups by percentiles of periodontal inflammatory load (PIL)*

Levels	LBW		Preterm		LBW and preterm		LBW and/or preterm	
	OR	CI	OR	CI	OR	CI	OR	CI
1	1	–	1	–	1	–	1	–
2	0.39	0.11–1.36	0.38	0.10–1.35	0.41	0.10–1.61	0.37	0.11–1.23
3	0.26	0.07–1.00	0.33	0.09–1.28	0.22	0.05–1.07	0.33	0.10–1.16
4	0.37	0.10–1.30	0.30	0.08–1.14	0.30	0.07–1.28	0.36	0.11–1.20

*PIL, sum of all periodontal pocket depth measurements (PPD) ≥ 4 mm of sites with clinical attachment level (CAL) ≥ 4 mm.

†Odds ratio.

Percentiles: $P_{25} = 12$, $P_{50} = 56$, $P_{75} = 114$ of sum of all periodontal pocket depth measurements ≥ 4 mm of sites with clinical attachment level ≥ 4 mm.

Level 1, 0–11 mm of PIL.

Level 2, 12–55 mm of PIL.

Level 3, 56–113 mm of PIL.

Level 4, 114 mm of PIL.

CI, confidence interval; LBW, low birthweight; OR, odds ratio.

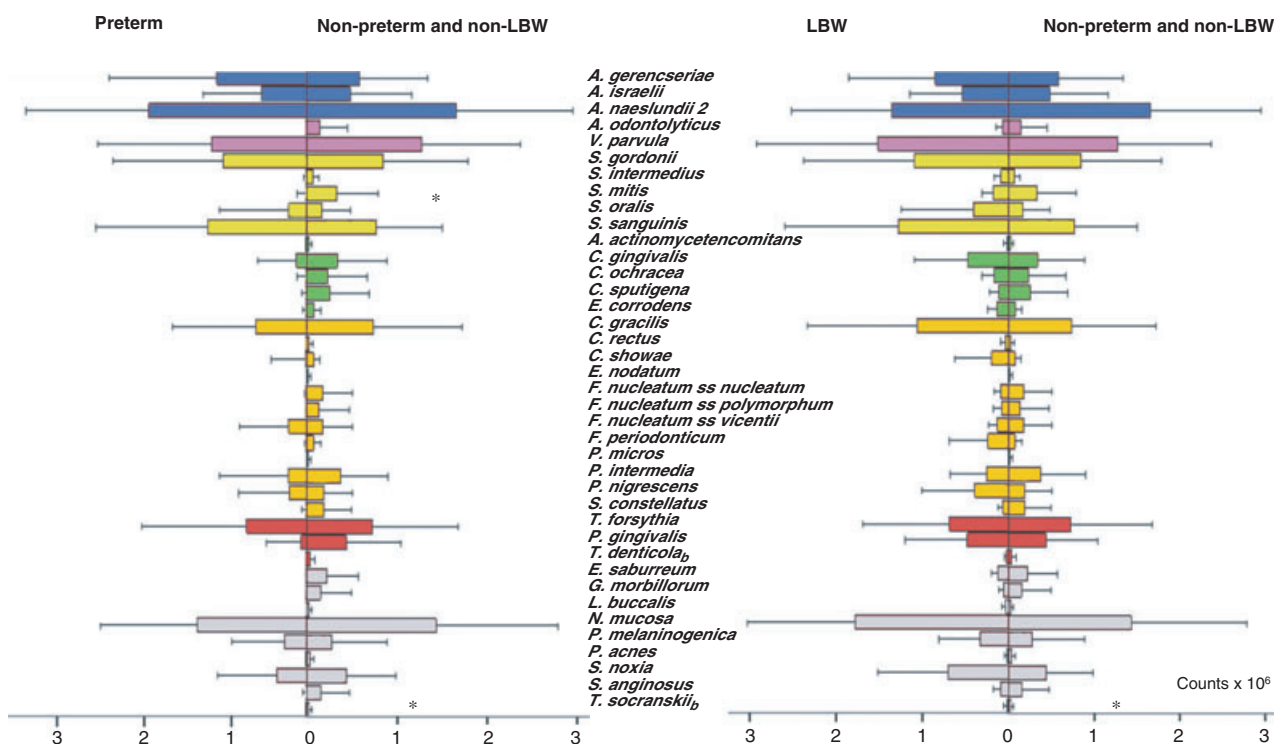


Fig. 2. Bar chart of mean counts ($\times 10^6$, \pm SD) of individual species in subgingival plaque samples between Preterm vs. Non-preterm and non-LBW and LBW vs. Non-preterm and non-LBW post-partum women. Differences between the groups were detected by Mann-Whitney test after adjusting for multiple comparisons. * $p < 0.05$; LBW, low birth weight.

Minor differences were observed in the composition of the subgingival microbiota between the case and control groups. Of the 39 microorganisms evaluated, the levels of only two species differed significantly between the four pairs of groups evaluated. *S. mitis* and *T. socranskii* were present at lower levels in case

than in control groups. It is interesting to note that one of these species, *T. socranskii*, is considered to be associated with periodontal disease. A direct relationship between the frequency of detection of *T. socranskii* and the severity of periodontal disease has been reported (32). In addition, this species was present at higher lev-

els in diseased than in healthy sites in subjects with rapidly progressive periodontitis (9). The counts of *T. socranskii* in the present study were significantly lower in preterm, low-birthweight, preterm and low-birthweight, and preterm and/or low-birthweight groups, compared with the control subjects.

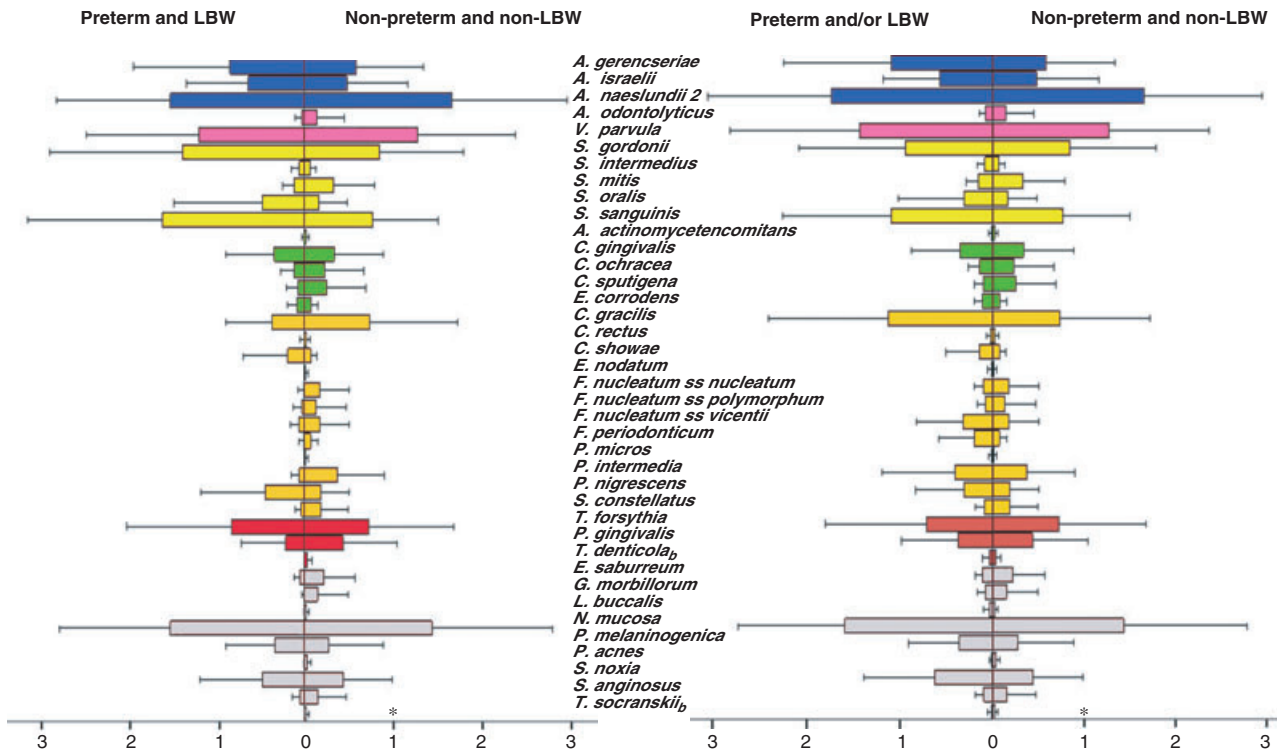


Fig. 3. Bar chart of mean counts ($\times 10^6$, \pm SD) of individual species in subgingival plaque samples between Preterm and LBW vs. Non-preterm and non-LBW and Preterm and/or LBW vs. Non-preterm and non-LBW post-partum women. Differences between the groups were detected by Mann-Whitney test after adjusting for multiple comparisons. * $p < 0.05$; LBW, low birth weight.

We found similar mean proportions of the microbial complexes in all pairs of case and control groups, including those complexes that harbour most of the species considered to be beneficial (blue, purple, green and yellow), and the orange and red complexes, mainly consisting of periodontal pathogens. It is important to note that the red complex species, *P. gingivalis*, *T. denticola* and *T. forsythia*, and to a certain extent some orange complex species, were found more frequently and at higher levels in periodontal disease than in periodontal health (33–38). Therefore, the lack of difference in counts and proportions of microorganisms of the red complex between all pairs of case/control groups in the present study suggests no association between periodontal pathogens from dental biofilms and preterm low birthweight. This finding is in accordance with studies by Jarjoura *et al.* (15) and Noack *et al.* (17) who found no significant differences in the counts of red complex microorganisms between preterm and nonpreterm groups, and

preterm and low birthweight and controls, respectively. The absence of differences in the prevalence of pathogens of the orange and red complexes in the periodontal biofilm of preterm mothers compared with full-term mothers was also reported by Madianos *et al.* (16). However, they reported a significant increase in the prevalence of maternal immunoglobuline M. (IgM) for species of the orange complex, such as *Campylobacter rectus* and *Prevotella intermedia*. Similarly, Dasanayake *et al.* found a relationship between serum IgG levels against *P. gingivalis* and low birthweight (39). As suggested by Jarjoura *et al.* (15), the association between such antibody titers and pregnancy complications remains speculative owing to a broad range of antibody responses to periodontal pathogens observed in patients with various forms of periodontal disease (40).

Although the studies mentioned above (15–17) and the present investigation did not find an association between microbiological parameters

and adverse pregnancy outcomes, other investigations found a positive relationship between certain subgingival species and preterm births (12,14,41,42). The proportions of pathogens in the orange and red clusters were higher among women delivering preterm compared with full-term mothers (41). The average microbial load in periodontal sites of *T. forsythia* has been associated with preterm births (12,42). Offenbacher *et al.* found that preterm low-birthweight mothers had higher levels of *T. forsythia*, *P. gingivalis*, *Actinobacillus actinomycetencomitans* and *T. denticola* (14). The differences between those studies might be explained, in part, by variations in sample size, in the clinical criteria used to define periodontal disease and in the number of samples and bacterial species evaluated (43).

It should be emphasized that one limitation of these studies, including the present study, is the number of plaque samples evaluated. Most of the studies evaluated from two to four samples (12,14,16,17,41,42), and only

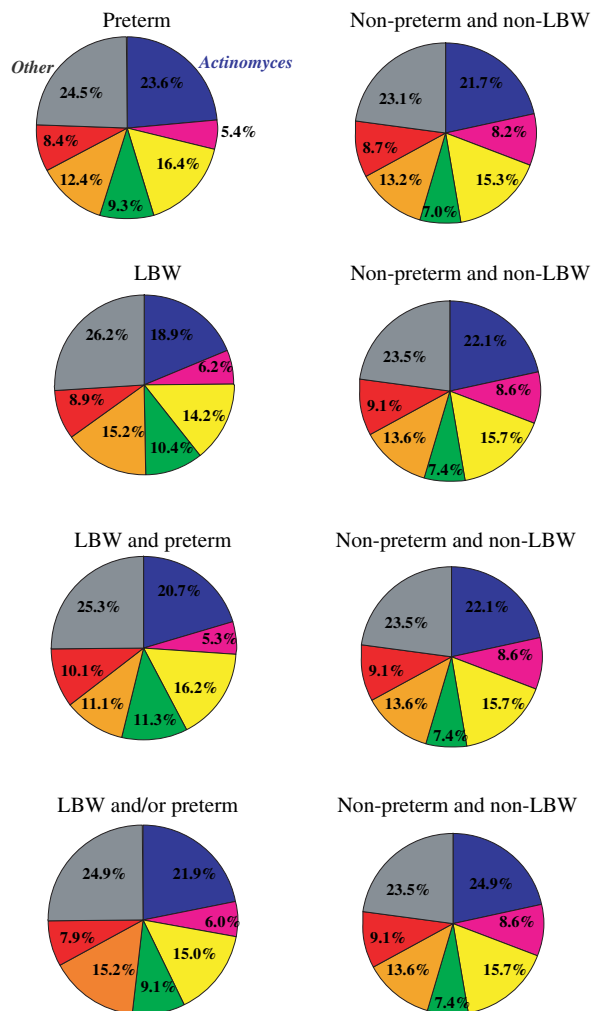


Fig. 4. Pie charts of the mean proportions of each microbial complex in subgingival plaque samples from subjects in the different groups. LBW, low birthweight. The colours in the pie diagrams represent the microbial complexes described by Socransky *et al.* (13) (Table 1). Significance of differences in mean proportions between groups for each complex was tested using the Mann–Whitney test ($p > 0.05$).

one study evaluated eight samples per subject (15). A positive aspect of the present investigation is the number of bacterial species evaluated – 39 microorganisms. To our knowledge, only one previous study (14) has analyzed a large number of subgingival species.

Indeed, the greater the number of sites and microorganisms examined, the more comprehensive the evaluation of the subgingival microbial composition and the association between this microbiota and other diseases. Therefore, studies with larger number of samples and bacterial species would certainly enhance our knowledge on the influence of the oral microbiota in adverse pregnancy outcomes.

In the present study, several periodontal clinical parameters were compared between the case and control groups. With the exception of mean periodontal pocket depth, which was higher in the control group when compared with all case groups, no periodontal measure differed between case and control groups. This result is similar to other studies that examined the relationship between periodontal disease and preterm low birthweight (12,17,21). However, other studies reported higher levels of periodontal disease in preterm low-birthweight mothers (18–20). Possible reasons for the different findings among studies could include methodological differ-

ences, such as controlling or not controlling for confounders, and heterogeneity between studies concerning measurement of periodontal disease and selection of type of adverse pregnancy outcome (22). To overcome the potential bias on these factors, in the present study, possible confounders were excluded through sample selection. In addition, all measures of periodontal disease and adverse pregnancy outcomes used in previous studies were included in the analysis.

The association between periodontal disease and preterm low birthweight has been tested in interventional studies (12,44–47). Similarly, as observed in case-control studies, there is no consensus in their findings. The effect of periodontal treatment in reducing the occurrence of preterm and low birthweight was reported in two clinical trials (44,45), but in three other interventional studies periodontal therapy did not reduce significantly the risk for preterm births (46,47) and preterm or low-birthweight babies (12).

Within the limits of the present study, we conclude that periodontal disease was not associated with preterm, low birthweight, preterm and low birthweight, and preterm and/or low-birthweight babies in a subset of Brazilian women aged 30 years or over.

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