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Association between periodontal status and pre-term and/or low-birth weight in Spain: clinical and microbiological parameters

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Background and Objective: Studies performed over the last 15 years have suggested that periodontal diseases may be associated with adverse pregnancy outcomes. However, this association has not been found in all populations. The aim of this study was to evaluate whether periodontal status and the presence of specific periodontal pathogens may influence the incidence of adverse pregnancy outcomes.

Material and Methods: Pregnant women were clinically examined before 26th week of gestation, and divided in two groups: non-periodontitis and periodontitis. Microbial samples were obtained in the periodontitis group and processed by anaerobic culturing. After delivery, data on the pregnancy outcome were taken; mother's socio-demographic and risk factors were obtained at inclusion. Simple and multiple regression analysis were performed.

Results: One hundred and seventy women were included in the study (116 non-periodontitis and 54 with periodontitis). The incidence of preterm (PTB) and low-birth weight (LBW) was 2.94% and 3.53%, respectively. Periodontal status did not show any association with adverse pregnancy outcomes. The presence of *Eikenella corrodens* was significantly related to PTB ($p = 0.022$) and the presence *Capnocytophaga* spp. was related to LBW ($p = 0.008$). The multivariate analyses showed a significant association between PTB and newborn weight and counts of *E. corrodens*. Maternal health and counts of *E. corrodens* were significantly associated with PTB or LBW.

Conclusion: The clinical periodontal condition was not associated with adverse pregnancy outcomes in a Spanish Caucasian population with medium-high educational level. The presence and counts of *E. corrodens* and the presence of *Capnocytophaga* spp. showed a significant association with PTB and LBW, respectively, in the bivariate and/or multivariate model.

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Preterm birth (PTB) is defined when newborn delivery occurs before the 37th gestational week. This condition has been associated with important health problems in the newborn, being the main cause of infant mortality (75% of perinatal mortality). Its prevalence is high in developing countries, but also in developed countries, ranging from 12–13% (USA) to 5–9% (Europe) (1). In Spain, with 95% of the pregnant women receiving prenatal care, the reported prevalence is 7.9% (2).

The cause of prematurity is multifactorial and several risk factors have been identified from prospective studies: smoking, increased age of the mother, non-white ethnicity, low socio-economic status, malnutrition, multiple gestation, previous adverse pregnancy outcome and current or previous genital-urinary infections (1,3,4). There are, however, approximately 25% of PTB births where these risk factors are not present (5) and in these instances the presence of a subclinical or chronic infection, such as periodontitis, has been attributed as a possible causative factor (6). Although the exact mechanism of this possible association is still unclear, different plausible explanations have been proposed (1), such as exposure to systemic bacterial products as lipopolysaccharide from gram-negative bacteria or the dumping of inflammatory mediators in plasma, such as prostaglandin E2 or tumour necrosis factor alpha. These mediators are normally elevated during labour, but if they rise abnormally due to the chronic infection, they can reach the levels necessary to initiate labour and result in a PTB. Several case–control or cohort studies have evaluated the relationship between PTB or low-birth weight (LBW) and periodontal disease, with conflicting results. Numerous reviews have evaluated these associations (7–9), some including meta-analysis (10,11), and most have found an association between periodontitis and adverse pregnancy outcomes, although 'a high and unexplained degree of heterogeneity between studies' was also noted (12). These studies, therefore, could not establish clear conclusions, mainly due to methodological differences, which included diverse periodontal disease and adverse pregnancy outcomes definitions, lack of appropriate controls for recognized risk factors (age, ethnicity, socio-economic status, smoking, multiple gestations, etc.) and statistical heterogeneity (9,13). In fact, differences in the diagnostic criteria of the exposure (periodontitis) in the same database have shown significant differences in the magnitude of the outcome (PTB) (14–16). The Consensus report from the 6th European Workshop on Periodontology pointed that, in certain study populations, periodontal disease has been associated with adverse pregnancy outcomes, but not in others (17).

Although the most plausible explanation of this possible association is based on the infective link either through the microorganisms themselves or by evoking a systemic inflammatory reaction, few studies have assessed microbiological outcome variables as a measure of the exposure. These studies have also ensued in conflicting results (18–26). This heterogeneity warrants the need for further investigations assessing both periodontal and microbiological exposure parameters in well-defined Caucasian populations with homogeneous social and economic status. Our working hypothesis is that women in a well-defined social and ethnic population in presence of periodontitis and high number of periodontal pathogens will have a higher risk of PTB or LBW. It is therefore, the aim of the present investigation to evaluate whether the periodontal status may influence the incidence of adverse pregnancy outcomes such as PTB and/or LBW, in a homogeneous Spanish population; and further to assess whether the presence of specific periodontal pathogens could influence this association.

Material and methods

Study population

The population of pregnant women was selected among those attending different community clinics belonging to the municipal health services of the city of Madrid, if they fulfilled the following criteria:

1 Age: 20–40 years.
2 Gestational age: before the 26th week (27).
3 Women with at least 10 teeth in the mouth.

Subjects were excluded if:

1 Multiple carriage.
2 Need of antibiotic prophylaxis for evaluation.
3 Diabetes (before pregnancy).
4 Systemic antibiotic treatment in the previous 4 wk.
5 Patients under active or supportive periodontal therapy.

Study design

This study was designed as a prospective cohort study, with a visit during pregnancy where the exposure parameters, both periodontal and microbiological were recorded and a visit after delivery where the pregnancy outcomes were registered. During the screening visit, pregnant women were evaluated for compliance with the mentioned criteria, informed about the purpose and processes of the study, and accepted to participate after signing an informed consent previously approved by the institutional review board of the University Complutense of Madrid. Once enrolled, participating subjects underwent a clinical examination registering both periodontal variables and risk factors for pregnancy adverse results. According to results of the screening, women were classified in two groups: periodontitis and non-periodontitis. Periodontitis women underwent a new clinical examination and collection of subgingival samples for microbiological evaluation.

Clinical variables

The periodontal examination consisted on full-mouth recordings at six sites per tooth with registration of the following parameters, by two

experienced and calibrated (percentage of agreement, according to kappa score, 86%) examiners:

1 Plaque index (PlI): percentage of surfaces with visible plaque as detected with the probe.
2 Bleeding on probing (BoP): percentage of surfaces bleeding after gentle probing.
3 Probing pocket depth (PPD) measured with an electronic, pressure-controlled periodontal probe (Florida® probe Corporation, Gainesville, FL, USA). The number of pockets > 3 mm was calculated.
4 Gingival recession (Rec), in mm. Clinical attachment level (CAL) was calculated as the sum of PPD and Rec at each site.

Microbiological samples

Four sites (those with the deepest sulcus and inflammation) were selected, and two consecutive paper points were inserted and kept in place for 10 s. All paper points were pooled in a vial with Reduced Transport Fluid (RTF) and transferred to the laboratory within 2 h (28).

The obtained samples were dispersed (30 s of vortex), serially diluted and processed for culture by inoculation on two different media: blood agar medium (no 2; Oxoid Ltd., Basingstoke, UK), with horse serum at 5%, and with haemin (5 mg/L) and menadion (1 mg/L); and Dentaid-1 medium (29).

The blood agar plates were studied after 7 and 14 d of anaerobic incubation (80% N_2; 10% H_2; 10% CO_2) at 37°C; and the plates on Dentaid-1 medium after 3–5 d at 37°C in air with 5% CO_2.

Total microbial counts were calculated on the blood agar plates. Also in these plates, *Porphyromonas gingivalis, Prevotella intermedia/nigrescens, Tannerella forsythia, Parvimonas micra, Campylobacter rectus, Fusobacterium nucleatum, Eikenella corrodens, Capnocytophaga* spp. were identified based mainly on their colony morphology and further confirmed with different specific chemical tests. Counts for every bacterial species were obtained and the percentage relative to the total flora calculated. *Aggregatibacter actinomycetemcomitans* were identified on Dentaid-1 medium plates, based on colony morphology and catalase reaction.

Demographic and medical data

The following gynaecological and medical records, together with data on possible risk factors for PTB and/or LBW which were retrieved from health questionnaires: gestational age, race, maternal weight before pregnancy, maternal height, previous deliveries (number of the present), previous PTB or LBW, maternal diseases, metabolic or genetic alterations, socio-economic and educational status (defined as low, for primary education level, medium for high-school education and high for university), tobacco (cigarettes/day), alcohol or other drugs consumption, genital-urinary infections.

Study cohorts

After the first clinical assessment, participating subjects were stratified in two groups, according to pre-defined criteria of periodontal exposure (30):

1 Healthy, gingivitis or initial/localised periodontitis: all women, with the exception of those with a diagnosis of generalized moderate to severe periodontitis. For study purposes, this cohort was denominated as 'non-periodontitis' group.
2 Generalized moderate to severe periodontitis: at least 15 sites (31) or more with 3 mm or more CAL loss (PPD > 3 mm, without evident gingival overgrowth). This cohort was denominated as 'periodontitis' group.

Patients with gingivitis or initial/localized periodontitis were informed of their situation including a recommendation for receiving a professional prophylaxis.

Patients with generalized moderate to severe periodontitis were included in a randomized intervention study where one group received basic periodontal therapy during pregnancy and the other after delivery. For the present study, only patients treated after delivery were considered.

Evaluation of pregnancy outcomes

Once the delivery occurred, data on the number of weeks of pregnancy and the weight and sex of the newborn were obtained.

Statistical analyses

Based on previous studies (32), convenience sample of around 200 women was selected in order to have approximately 60 women with periodontitis.

Descriptive statistics were used to depict the demographic data categorized by periodontal status. The association between periodontal status (periodontitis and non-periodontitis) and microbiological variables (presence or absence of pathogens) with different adverse pregnancy outcomes [PTB (< 37 wk), LBW (< 2500 *g*), PTLBW (PTB and LBW) and PTB/LBW (PTB or LBW)] was tested using Chi-square test or Fisher's exact test. Non-adjusted odds ratio (ORs) and 95% Confidence Intervals (CI) were also calculated to assess the magnitude of the association.

In order to build up the regression models, the relationship between each explanatory variable and the adverse pregnancy outcomes was analysed. After assessing their normal distribution, Student *t*-test was used to compare continuous variables and Chi-square test for the categorical variables. Those variables showing a trend for an association ($p < 0.20$) in the bivariate analysis were included in the four logistic regression models (dependent variables being the adverse pregnancy outcomes). Since microbiological data were available only from a subset of patients ($n = 39$), three different regressions were performed for each dependent variable: one excluding the microbiological variables ($n = 170$), one combining all the variables ($n = 54$) and one considering only the microbiological variables ($n = 39$). Adjusted ORs and 95% CI were obtained for all variables associated

with the adverse pregnancy outcomes and p values lower than 0.10 were reported.

Results

After the screening visit, 170 women (mean age 31.9, ranging 20–40) were included in the study. Selected study subjects were examined between the 8th to 26th wk of pregnancy (mean 18.5) (Table 1). Maternal health was assessed in the questionnaire and overall revealed a normal health status, since only 14 women reported some problems (hypertension, hypothyroidism, sickle cell anaemia, celiac disease, hepatitis…). Clinical data were available for 163 subjects, microbiological data for 39 women with periodontitis, and pregnancy outcomes for 170 women. Sixteen women (9.47%) were smokers, and 116 (68%) were diagnosed as healthy, gingivitis or initial/localised periodontitis, and 54 (32%) as generalised moderate to severe periodontitis.

Table 2 depicts the periodontal status of the study population. Selected pregnant women presented a mean number of teeth of 26.5, with a mean PPD of 2.68 mm and a mean CAL of 2.76 mm. The mean BoP was 53.8% and the mean PlI 66.2% and 16.1% of PPD were 4 mm or more.

The microbiological findings from 39 pregnant women diagnosed with periodontitis are presented in Table 3, showing high frequencies of detection of *F. nucleatum* (97.4%), *P. intermedia/nigrescens* (94.9%), *P. gingivalis* (76.9%) and *P. micra* (56.4%) with high proportions of microbiota for *P. gingivalis* (18.9%), *P. intermedia/nigrescens* (3.9%) or *F. nucleatum* (5.5%).

Out of the 170 women, 5 (2.94%) had PTB delivery, and 6 (3.53%) had a LBW delivery and 2 (1.18%) had both PTB and LBW delivery (Table 1). Regarding the gender of the babies, 90 were boys and 80 were girls. The level of studies was moderate or high for 91% of the mothers (Table 4).

Bivariate analysis

The periodontal status of our sample did not show any association with any of the four dependent variables analysed in this study (Table 5).

The results of the association between the presence or absence of periodontal pathogens and the four dependent variables are also shown in Table 5. The presence of *E. corrodens* was significantly related to PTB outcome ($p = 0.022$) with a significant OR of 12.400 (95% CI: 0.940–163.581) indicating that the presence of *E. corrodens* increases the risk of PTB in women with periodontitis. The detection of *Capnocytophaga* spp. was significantly related to LWB outcome ($p = 0.008$), with and OR of 17.143 (95% CI: 1.027–154.317), indicating that the presence of *Capnocytophaga* spp. increases the risk of LWB in women with periodontitis.

Multivariate analysis

The explanatory variables showing a trend for an association ($p < 0.20$) in the bivariate analysis that were included in the four logistic regression models are listed in Table 6. Table 7 shows the results of the logistic regression analyses looking for variables with possible influence in adverse pregnancy outcomes. After adjusting for confounding variables, a significant association was found between PTB and newborn weight. When the microbiological variables

Table 1. Demographic and other features of the mothers, pregnancy and partum, for the all population and per group: mother characteristics, associated factors and pregnancy results

	All patients			Non-periodontitis			Periodontitis		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Gestational age at birth (w)	170	39.5	1.5	116	39.5	1.3	54	39.3	1.9
Infant weight (*g*)	170	3181.4	426.8	116	3190.8	437.9	54	3161.3	405.1
Screening (w)	168	18.5	3.9	114	18.5	3.7	54	18.7	4.1
Mother age (year)	170	31.9	4.0	116	31.6	4.1	54	32.6	3.7
Mother weight (kg)	162	61.5	9.7	111	60.6	9.4	51	63.3	10.2
Mother height (m)	141	1.6	0.1	98	1.6	0.1	43	1.6	0.1
Mother BMI	141	23.5	3.4	98	23.1	3.3	43	24.3	3.6
Mother weight at birth (g)	76	3522.1	583.9	52	3465.0	573.9	24	3645.8	598.5
	n	No	Yes	*n*	No	Yes	*n*	No	Yes
Low BMI	141	135	6	98	93	5	43	42	1
Smoking	169	153	16	116	110	6	53	43	10
Medical problems	168	154	14	115	107	8	53	47	6
Preterm	170	165	5	116	113	3	54	52	2
Low-weight	170	164	6	116	111	5	54	53	1
PTB and LBW	170	168	2	116	115	1	54	53	1
Diabetes	170	165	5	116	115	1	54	50	4
Induced/provoked	170	159	11	116	108	8	54	51	3
Other health problems	170	156	14	116	105	11	54	51	3

SD, standard deviation; BMI, body mass index; PTB, pre-term birth; LBW, low-weight birth; w, weeks; g, grams; kg, kilograms; y, years; m, meters.

Table 2. Clinical status of all patients, non-periodontitis women and periodontitis women, expressed as mean and standard deviation (SD)

	All patients			Non-periodontitis			Periodontitis		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
n teeth	163	26.5	2.1	109	26.5	2.3	54	26.6	1.7
% PPD 1–3 mm	163	83.58%	14.17%	109	91.10%	6.51%	54	68.40%	13.27%
% PPD 4–6 mm	163	16.15%	13.77%	109	8.89%	6.51%	54	30.82%	12.87%
Mean PPD	163	2.68	0.41	109	2.46	0.23	54	3.11	0.34
%CAL 1–3 mm	162	81.67%	15.18%	109	89.85%	6.71%	53	64.86%	13.83%
% CAL 4–6 mm	162	17.95%	14.59%	109	10.11%	6.69%	53	34.08%	13.11%
Mean CAL	162	2.76	0.44	109	2.53	0.23	53	3.23	0.36
Mean PlI	162	66.18%	19.91%	109	60.22%	18.51%	53	78.43%	16.99%
Mean BoP	162	53.86%	22.85%	109	46.10%	20.75%	53	69.82%	18.35%

PPD, probing pocket depth; CAL, clinical attachment level; PlI, plaque index; BoP, bleeding on probing.

Table 3. Log of total counts, counts and proportions, as mean and standard deviation (SD), and frequency of detection of different bacterial species in subgingival samples of 39 pregnant women with periodontitis

	Counts in CFU		Proportions		
	Mean	SD	Mean (%)	SD (%)	Frequency of detection (%)
A. actinomycetemcomitans	8775	33,367	0.3	1.3	17.95
P. gingivalis	2,321,677	6,339,816	18.9	26.5	76.92
P. intermedia	194,142	344,448	3.9	6.1	94.87
T. forsythia	273,815	1,322,659	1.5	3.2	30.77
P. micra	47,215	65,835	1.4	2.0	56.41
F. nucleatum	257,806	283,211	5.5	5.0	97.44
C. rectus	7785	30,202	0.2	0.5	12.82
Capnocytophaga spp.	8969	29,613	0.2	0.6	23.08
E. corrodens	3892	11,895	0.1	0.3	17.95
	Log of counts				
Total anaerobic counts	6.55	0.48			

CFU, colony forming units.

Table 4. Demographic and other features of the mothers, pregnancy and partum, for the all population and per group: levels of studies, previous deliveries and mother ethnicity

	All patients	Non-periodontitis	Periodontitis
Level of studies (*n*)			
n	167	115	52
Low	15	9	6
Medium	71	45	26
High	81	61	20
Level of studies (%)			
Low	9.0	7.8	11.5
Medium	42.5	39.1	50.0
High	48.5	53.0	38.5
n previous deliveries			
n	168	115	53
One	130	92	38
Two or more	38	23	15
Mother ethnicity			
n	169	115	54
Caucasian	158	108	50
Other	11	7	4

were incorporated to the analysis, newborn weight and the counts of *E. corrodens* were incorporated to the regression equation, with the counts of *E. corrodens* showing a tendency towards significant association also when the regression was built up with just the microbiological variables (adjusted OR: 1.293; 95% CI: 1.099–12.976). A significant association was also found between LBW and weeks of pregnancy (protective factor). When the microbiological variables were considered, weeks of pregnancy and presence of *Capnocytophaga* spp. were incorporated into the equation with the presence of *Capnocytophaga* spp. showing a tendency towards a significant relation (adjusted OR: 1.818; 95% CI: 0.997–86.091). PTB/LBW was significantly associated with maternal health (adjusted OR: 7.278; 95% CI: 1.436–21.520); when patients with microbiological data were included, counts of *E. corrodens* showed a tendency towards a significant association. PTLBW was significantly associated with low body mass index of the mother (adjusted OR: 8.000; 95% CI: 1.751–85.168).

Discussion

In this prospective cohort study, 170 pregnant women, with a homogeneous ethnic, social and educational background, were investigated to assess whether those diagnosed of periodontitis, both in terms of clinical and microbiological exposure variables,

Table 5. Association of periodontal status and microbiological variables with adverse pregnancy outcomes, evaluated in 2 × 2 contingency tables, and expressed as p value (Chi-Square or Fisher's Exact Test), Odds Ratio (OR) and 95% Confidence Interval (CI)

Row	Column	*p* value	OR	CI lower	CI upper
Preterm	perio status	0.605	1.325	0.455	3.855
	freqAa	0.399	0.806	0.686	0.946
	freqPg	0.661	0.571	0.046	7.143
	freqPi	0.603	0.917	0.831	1.012
	freqTf	0.920	1.136	0.093	13.886
	freqPm	0.709	1.600	0.133	19.276
	freqFn	0.675	0.944	0.873	1.022
	freqCr	0.489	0.861	0.755	0.982
	freqCapno	0.661	1.750	0.140	21.876
	freqEc	0.022	12.400	0.940	163.581
Low-weight	perio status	0.386	0.561	0.150	2.101
	freqAa	0.497	0.811	0.694	0.947
	freqPg	0.354	0.276	0.015	4.915
	freqPi	0.675	0.919	0.835	1.011
	freqTf	0.333	0.676	0.540	0.845
	freqPm	0.851	0.762	0.044	13.133
	freqFn	0.736	0.946	0.876	1.022
	freqCr	0.578	0.865	0.761	0.982
	freqCapno	0.008	17.143	1.027	154.317
	freqEc	0.497	0.811	0.694	0.947
Preterm and low-weight	perio status	0.566	0.528	0.058	4.843
	freqAa	0.636	0.816	0.701	0.949
	freqPg	0.064	0.211	0.114	0.390
	freqPi	0.770	0.921	0.839	1.011
	freqTf	0.499	0.684	0.551	0.849
	freqPm	0.249	0.421	0.290	0.611
	freqFn	0.814	0.947	0.879	1.021
	freqCr	0.698	0.868	0.767	0.983
	freqCapno	0.064	7.500	0.634	68.954
	freqEc	0.636	0.816	0.701	0.949
Preterm or low-weight	perio status	0.978	1.010	0.408	2.517
	freqAa	0.323	0.800	0.678	0.944
	freqPg	0.923	0.889	0.081	9.767
	freqPi	0.542	0.914	0.826	1.012
	freqTf	0.792	0.727	0.068	7.803
	freqPm	0.429	2.526	0.239	26.726
	freqFn	0.624	0.943	0.869	1.023
	freqCr	0.418	0.857	0.749	0.981
	freqCapno	0.177	4.000	0.476	33.585
	freqEc	0.078	6.000	0.681	52.900

perio status, periodontitis vs. non-periodontitis; Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Pi, *P. intermedia/nigrescens*; Tf, *T. forsythia*; Pm, *P. micra*; Fn, *F. nucleatum*; Cr, *C. rectus*; Capno, C*apnocytophaga* spp.; Ec, *E. corrodens*.

were associated with adverse pregnancy outcomes. The results showed that there was a lack of association between the clinical periodontal status and pregnancy outcomes. These results are in contrast with numerous case–control studies reporting a significant association (14,27,32–39). Case–control studies, however, lack the evaluation of the sequence exposure (periodontitis) and may reflect a biased evaluation of the periodontal condition since this condition usually worsens during pregnancy (31,40,41), especially if only PPD and BoP are used to evaluate the periodontal condition (34,35). In this study we assessed the periodontal condition before the 26th gestational week, what may partially explain the discrepancy in the findings (case–control studies usually do the periodontal evaluation after delivery).

Similarly, results from prospective studies reported in the literature are controversial since there are studies unable to report a significant association between the periodontal condition during pregnancy and PTB (42–45) while, others reported it with PTB (24,30,46–48) or LBW (42,46,47,49) or with both PTB and LBW (47,50). One possible explanation for these discrepancies may be the characteristics of the studied populations, both in terms of the expected outcome (prevalence of adverse pregnancy outcomes) and the level of exposure (extent and severity of the periodontal condition).

It is well known that populations with low socioeconomic and health care levels have prevalences of PTB and/or LBW higher than 10% (51). Precisely, the studies where a stronger association were reported were those that included a high percentage of Afro-American women with a low socio-economic background (30,46). Similarly, the studies by Rajapakse *et al.* (50) and Lopez *et al.* (47), included populations with low educational level and poor access to prenatal and health care. In contrast, in our investigation more than 90% of the women were Caucasian and with a medium-high education level, resulting in a prevalence of PTB of 2.94%, significantly below the frequencies reported in the previously mentioned studies (Table 4).

Two previous prospective studies studying these associations have been carried out in Spain. Agueda *et al.* (48) reported a significant association between the presence of periodontal disease and PTB, with an adjusted OR of 1.77. Again in this study the population assayed was different, since it included only a 78% of Caucasian women and mainly of low social, economic and educational level, reporting a prevalence of PTB of 6.6%, higher than in our study, but more similar to the epidemiological figures reported for the general population in Spain (7.9% in 2001–2005) (2). Moreu *et al.* (49), in a prospective study, although not demonstrating a significant association between periodontal disease and PTB, reported a significant association between sites with PPD > 3 mm and LBW.

Table 6. Explanatory variables showing an association with a $p < 0.20$ in the bivariate analysis that were included in the four logistic regression models

Pre-term	Low-weight	Pre-term and low-weight	Pre-term or low-weight
Newborn weight	Weeks	Mother height	Age
Maternal weight	Mother height	Proportion Capno	Mother height
PPD 4–6	BMI	Low BMI	Mother BW
Counts Fn	Mother BW	Presence Pg	*n* teeth
Counts Ec	BoP	Presence Capno	Counts Ec
Proportion Ec	Proportion Fn		Previous PTB
Previous PTB	Maternal health		Maternal health
Smoking	Medical problem		Medical problem
Presence Ec	Smoking		Mother education
	Cesarea		Smoking
	Presence Capno		Presence Capno
			Presence Ec

BW, birth weight; BoP, bleeding on probing; PTB, pre-term; PPD, probing pocket depth; BMI, body mass index; Pm, *P. micra*; Pg, *P. gingivalis*; Cr, *C. rectus*; Fn, *F. nucleatum*; Ec, *E. corrodens*; Capno, C*apnocytophaga* spp.

Table 7. Multivariate analysis: adjusted Odd Ratios (OR) and 95% Confidence Intervals (CI) for the variables influencing preterm birth, low-weight- birth, preterm and low-birth weight and preterm or low-birth weight

		95% CI		
	Adjusted OR	lower	upper	*p* Value
Outcome: pre-term				
All (n = 163)				
Newborn weight	0.998	0.996	0.999	0.002
Patients with microbiology (n = 39)				
Newborn weight	0.997	0.995	0.999	0.057
Counts *E. corrodens*	1.435	1.354	16.882	0.061
Only microbiology (n = 39)				
Counts *E. corrodens*	1.293	1.099	12.976	0.054
Outcome: low-weight				
All (n = 163)				
Weeks	0.533	0.356	0.799	0.002
Patients with microbiology (n = 39)				
Weeks	0.415	0.132	0.901	0.028
Presence *Capnocytophaga* spp.	1.818	0.997	86.091	0.061
Only microbiology (n = 39)				
None				
Outcome: pre-term or low-weight				
All (n = 163)				
Medical problem	7.278	1.436	21.520	0.014
Patients with microbiology (n = 39)				
Counts *E. corrodens*	1.188	1.661	3.477	0.053
Only microbiology (n = 39)				
Counts *E. corrodens*	1.188	1.661	3.477	0.053
Outcome: pre-term and low-weight				
All (n = 163)				
Low Body Mass Index	8.000	1.751	85.168	0.048
Patients with microbiology (n = 39)				
None				
Only microbiology (n = 39)				
None				

Another explanation for the heterogeneity in the results from different studies may be related to the lack of consensus in periodontitis case definitions and therefore, different criteria to diagnose periodontitis, influence the association between periodontal diseases and adverse pregnancy outcomes (15). In the present study, the criteria defining cases, as generalised moderate and severe periodontitis (having at least 15 sites with CAL of 3 mm or more, and PPD > 3 mm, without gingival overgrowth), were more stringent if compared with other studies (34,35,49,50) and were based on attachment loss, rather than on the presence of pockets. These criteria were selected since increased gingival inflammation and presence pseudopockets is frequent in pregnancy, and according to the study by Manau *et al*. (15) 'the significance of the association between periodontitis and adverse pregnancy outcomes was only reached in few cases, most of them when measurements were based on clinical attachment loss', although as reported by Gomes-Filho *et al*. (14) 'the more strict the periodontal criteria are, the lower are chances to find an association'.

This study has assessed the exposure not only by measuring the history of periodontal destruction, but also by evaluating the infectious risk through the study of the subgingival biofilm in 39 of the women diagnosed of periodontitis. Few studies have explored associations between specific oral bacteria and adverse pregnancy outcomes. Using DNA–DNA checkerboard hybridization analysis, Madianos *et al*. (18) reported no differences in the presence of red and orange complex pathogens in women with PTB, compared to women who delivered at term, and similar results were reported by Noack *et al*. (19) and Novak *et al*. (20), using quantitative polymerase chain reaction. In contrast, other studies found significant higher levels of *A. actinomycetemcomitans* (21), *P. gingivalis* (21), *T. forsythia* (21–23,25) and *Treponema denticola* (21,25), in subgingival samples of woman who delivered PTB infants, compared with mothers who delivered them at full-term. In our study, using anaerobic culturing, both the bivariate and the multivariate analysis found a significant (bivariate) or close to significant (multivariate) association between the presence and counts of *E. corrodens* with PTB and the presence of *Capnocytophaga* spp.

with LBW. The importance of *E. corrodens* in pregnancy adverse events has been previously highlighted (52) in a study evaluating preeclampsia and it is considered among the periodontal pathogens with an initial level of association with periodontitis. These results cannot be directly compared with the other studies also assessing microbiological exposure date, since the differences in the microbiological methods preclude any attempt to explain the results together. In addition, the study design of the previous studies includes only on sample time, and it has been observed that the subgingival microbiota in pregnant women may change during pregnancy (53).

The obtained results, however, clearly show that pregnant women with periodontitis harboured a very complex and pathogenic subgingival microbiota, including high prevalences of *F. nucleatum, P. intermedia/nigrescens, P. gingivalis* and *P. micra*, and high proportions of *P. gingivalis*, *P. intermedia/nigrescens* and *F. nucleatum*. Although other studies have reported similar microbiological findings in the subgingival microbiota in pregnant women (53), few have shown that these pathogenic microbiota may represent a challenge for the host, by increasing the risk of adverse pregnancy outcomes. These results, however, should be interpreted with caution due to inherent limitations of the study, such as the small sample size, the low rate of adverse pregnancy outcomes, the evaluation of microbiological variables only in the cohort of periodontitis women and the inclusion of initial/localised periodontitis patients in the non-periodontitis cohort.

Within the limitations of the present study, we can conclude that the clinical periodontal condition was not associated with adverse pregnancy outcomes in a Spanish Caucasian population with medium-high educational level. Conversely, the study of the periodontal infectious exposure, namely the presence and counts of *E. corrodens* were significantly associated with PTB and the presence of *Capnocytophaga* spp. was significantly associated with LBW. Further studies focusing on the infectious nature of the periodontal exposure are needed to clarify this issue.

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