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Periodontal infections and pre-term low birth weight: a case-control study

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Abstract

Objective: Pre-term delivery of low-birth-weight infants [pre-term low birth weight (PLBW)] remains a significant public health issue and a major cause of neonatal death and long-term health problems. There is a growing consensus that infections remote from fetal–placental unit may influence PLBW infants. Recent studies have suggested that maternal periodontal disease may be an independent risk factor for PLBW. The purpose of the present study was to evaluate the possible link between periodontal infections and PLBW by means of clinical and microbiological data in post-partum women with low socioeconomic level.

Methods: Clinical periodontal recordings comprising dental plaque, bleeding on probing, probing pocket depth and gingival recession were performed (six sites/tooth) in a total number of 181 women (53 cases and 128 controls) within 3 days post-partum. Subgingival plaque samples from mesio–or disto–buccal aspect of randomly selected one first molar and one incisor tooth have been obtained by paperpoints and were analysed by checkerboard DNA–DNA hybridization with respect to 12 bacterial species. In all analyses, the individual subject was the computational unit. Thus, mean values for all clinical parameters were calculated and bacterial scores from each individual sample were averaged. Statistical methods included Student's *t*-test, Fisher's exact test/χ^2 test, and multiple logistic regression analysis.

Results: The cases have gained significantly less weight during the pregnancy than did the controls ($p<0.05$). There were no statistically significant differences between the cases and controls with regard to the dental and periodontal parameters and the values of clinical periodontal recordings were found to be very similar ($p>0.05$). Mean and median scores (bacterial loads) of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Actinobacillus actinomycetemcomitans*, and *Streptococcus intermedius* in the subgingival plaque sampling sites were significantly higher in the controls than in the cases ($p<0.05$). The occurrence rates of *P. intermedia, Fusobacterium nucleatum, Peptostreptococcus micros, Campylobacter rectus, Eikenella corrodens, Selenomonas noxia* and *S. intermedius* were higher in the cases compared with the controls, but the differences were not statistically significant ($p>0.05$). According to the model created by the multiple logistic regression analysis, *P. micros* and *C. rectus* were found to significantly increase the risk of PLBW ($p<0.01$ and $p<0.05$ respectively), while *P. nigrescens* and *A. actinomycetemcomitans* decreased this risk ($p<0.01$).

Conclusion: The present findings indicated that when subgingival bacteria were evaluated together, *P. micros* and *C. rectus* may have a role in increasing the risk for PLBW, although no single bacteria exhibited any relation with the risk of PLBW. Further studies are required to better clarify the possible relationship between periodontal diseases and PLBW.

Key words: bacteria; DNA probes; infant; low birth weight; periodontal diseases/adverse effects; pregnancy; pre-term birth; risk factors

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Babies born prematurely are at a significant risk of developing serious and lasting health problems. Pre-term delivery is the major source of neonatal mortality and of nearly one-half of all serious long-term neurological morbidity (McCormick 1985). An estimated 11% of pregnancies end in pre-term birth (Goldenberg & Rouse 1998), and this rate in the western world appears to be increasing (Ventura et al. 1999). Pre-term or premature birth is usually defined as a gestational age of less than 37 weeks, while low-birth weight indicates a birth-weight of less than 2500 g (WHO 1984). Despite the improvement in the survival rates of pre-term low birth weight (PLBW) neonates in the last two decades, PLBW infants are still much more likely to die during the neonatal period (McCormick & Wise 1993).

Among the known risk factors for PLBW are young maternal age, low maternal weight gain, low pre-gravid weight, multiple gestations, gestational diabetes, genitourinary tract infections, drug use, cigarette smoking, and excessive alcohol consumption, while previous pre-term delivery is a strong predictive marker of future pre-term labour (David & Collins 1997). However, a significant proportion of PLBW is of unknown aetiology. Recently, it has been postulated that distant infections like periodontal diseases may be associated with PLBW through similar mechanisms as other maternal infections (Offenbacher et al. 1998).

Periodontal diseases are a group of infectious diseases caused by predominantly Gram-negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area and cause local and systemic elevations of pro-inflammatory prostaglandins and cytokines (Page 1991, Page & Kornman 1997). Furthermore, there is ample evidence that periodontal bacteria frequently enter the circulation (Beck et al. 1996). Initially the link between periodontal diseases and various systemic diseases was thought to be unidirectional. Currently, there is increasing evidence that the relationship between these entities may be bidirectional. For many years it is known that there is an increase in gingival inflammation during pregnancy (Maier & Orban 1949, Löe & Silness 1963). Immunosuppression in the second trimester has been suggested to underlie this clinical feature (Lopatin et al. 1980). On the other hand, the infected periodontium can also be regarded as a reservoir for both microbial products and inflammatory mediators like prostaglandins, interleukins (ILs), and other cytokines. Local prostaglandin E_2 (PGE_2) and both local and systemic tumour necrosis factor alpha (TNFα)-levels have been shown to be increased in periodontitis (Moss et al. 1995).

Periodontal diseases share many common risk factors with PLBW such as age, smoking, low socioeconomic-level and systemic health status. It has been suggested that maternal infections leading to alterations in the normal cytokine and hormone-regulated gestation, may result in pre-term labour, premature rupture of membranes, and pre-term birth (Hillier et al. 1988, Romero et al. 1994). Hence, maternal periodontal infection has been proposed to influence pre-term delivery through mechanisms involving inflammatory mediators or direct bacterial assault on the amnion.

Clarification of risk factors for PLBW should aid in the development of strategies to reduce the prevalence of these obstetric complications. The hypothesis linking subclinical infection and PLBW suggests that microbes themselves or microbial toxins such as endotoxins enter the uterine cavity during pregnancy by the ascending route from the lower genital tract or by the blood borne route from a non-genital focus. Periodontal infections may mediate PLBW through one or more of the following mechanisms; (1) contamination of the fetoplacental unit by periodontal pathogens, (2) effects of lipopolysaccharide (LPS) from the periodontal reservoir on the fetoplacental unit, and (3) effects of the inflammatory mediators (ILs, prostaglandins, and TNF) from the periodontal reservoir on the fetoplacental unit. Therefore, it may be hypothesized that a subgingival infection by a biofilm rich in Gram-negative, LPS-producing species may play an aetiological role in the pathogenesis of PLBW. The present study was planned to evaluate the possible link between periodontal infections and PLBW by means of clinical and microbiological data in a case-control study of post-partum women with low socioeconomic level.

Materials and Methods

Study population

The study population was drawn from women between the ages of 18–35 years who gave birth in a special maternity hospital in İzmir, Turkey between November 2002 and April 2003. The hospital was visited on a 2 days/week regular basis. Exclusion criteria included systemic conditions like severe anaemia, diabetes, cardiovascular disorders, hepatic deficiency, and high blood pressure associated with premature birth or intrauterine growth delay. Only mothers with a singleton gestation were included in the study. Furthermore, women who had a medically indicated pre-term delivery that followed pregnancies complicated by maternal obstetric disorders like pre-eclampsia or eclampsia, gestational diabetes or placenta previa or mothers whose infants were stillborn were excluded from this case-control study. The hospital birth register was scrutinized each day to identify all cases, defined as those mothers who delivered an infant weighing under 2500 g and/or born before 37 weeks' gestation. A random sample of up to three control mothers (those who delivered an infant with a birth weight more than 2500 g and born after 38 weeks' gestation) was selected daily from the birth register at the same time as the cases. Informed consent of the women was obtained before their enrolment in the study and all data were collected within 72 h of delivery. A total number of 53 cases were found during data collection period and 128 controls were included in the study.

Identification of risk factors

The women participated in the present study were administered a structured questionnaire before subgingival plaque sampling and clinical dental examination to ascertain risk factors for PLBW and periodontal disease (Williams et al. 2000). Smoking habits (smoker or non-smoker), level of education, dietary habits, total number of births, previous PLBW deliveries, PLBW deliveries in her close relatives (grandmothers, mother, sisters), maternal general health during pregnancy, infections during pregnancy (including genito–urinary tract infections) and their treatment, number of pre-natal controls, weight gain during pregnancy, type of delivery and finally dental complaints and treatment were all recorded in the questionnaire. Estimation of gestational age was based on the last menstrual period. When required, details in the questionnaire like

infection history and medications were validated from the Maternity Notes.

Subgingival plaque sampling

Subgingival plaque samples from two sites per subject were obtained subsequent to dichotomous recording of microbial plaque. All plaque samples were collected from randomly selected one molar and one incisor tooth of each subject. Sampling sites were accessed from buccal aspects of the mesial or distal surfaces at the interproximal sites. Prior to subgingival plaque sampling, supragingival plaque was removed gently by sterile curettes and the surfaces were dried and isolated by cotton rolls. Two sterile paperpoints were inserted deep into the gingival sulcus/pocket and left in place for 10 s. The paper-points were then removed, placed in sterile Eppendorf tubes and transported to the laboratory of Oral Microbiology, Göteborg University, Göteborg, Sweden.

Clinical examination

Subsequent to subgingival plaque sampling, intra-oral examination was carried out on the maternity ward with the subject lying flat on her bed, head to the foot end of the bed to facilitate a reproducible examination position for the clinician. Clinical examination of all participating subjects was carried out using mouth mirrors, dental and periodontal probes. Number of teeth present, number of decayed teeth and number of teeth with restorations (fillings or crowns) were recorded prior to the periodontal data collection. The clinical periodontal recordings in each woman included bleeding on probing (BOP) and measurement of probing pocket depth (PPD) at six sites per tooth at all teeth excluding third molars. BOP was determined positive if it occurred within 15 s of probing. PPD measurements were recorded to the nearest millimetres using a manual periodontal probe. All measurements were carried out by two pre-calibrated examiners.

Processing of bacterial plaque samples

Digoxigenin-labelled, whole genomic probes were prepared by random priming by the use of the High-Prime labelling kit (Roche Diagnostics Scandinavia AB, Bromma, Sweden) from the following 12 microbial strains: *Porphyromonas gingivalis* (strain FDC381), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Tannerella forsythensis* (formerly *Bacteroides forsythus*, ATCC 43037), *Actinobacillus actinomycetemcomitans* (FDC Y4), *Fusobacterium nucleatum* (ATCC 10953), *Treponema denticola* (OMGS 3271), *Peptostreptococcus micros* (ATCC 33270), *Campylobacter rectus* (ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Selenomonas noxia* (OMGS 3118), *Streptococcus intermedius* (OMGS 3177).

Analysis of subgingival plaque samples were performed according to the ''checkerboard'' DNA–DNA hybridization method (Socransky et al. 1994). The sensitivity and specificity of whole genomic probes constructed as above have been described previously (Gunaratnam et al. 1992, Socransky et al. 1994). Furthermore, a comparison between checkerboard hybridization and culture in the identification of subgingival microbiota has been already reported (Papapanou et al. 1997). Briefly, the samples were boiled for 5 min., neutralized, transferred onto nylon membranes by means of a Minislot device (Immunetics®, Cambridge, MA, USA) at 42°C. After a series of stringency washes, hybrids were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubated with an appropriate chemiluminescent subsrate (CSPD, Roche Diagnostics). Evaluation of the chemiluminescence signal was performed at a LumiImager Workstation (Roche Diagnostics) by comparing the signals obtained with those generated by pooled standard samples containing 10^6 or 10^5 of each of the species on each membrane. The chemiluminescence units obtained were ultimately transformed into a scale of 0–5, where 0 indicated no signal, 1 indicated a signal lower than that of the low standard (i.e. $<10^5$), 2 a signal equal to the one of the low standard ($=10^5$ bacteria), 3 a signal higher than the one of the low standard but lower than that of the high standard ($>10^5$ but $<10^6$), 4 a signal equal to the one of the high standard ($=10^6$ bacteria), and 5 a signal higher than the one of the high standard ($>10^6$ bacteria).

Statistical analyses

In all analysis, the individual was the computational unit. Thus, mean values for all clinical parameters (plaque, PPD, BOP) were calculated for each subject. Bacterial scores from two individual samples were averaged to describe each subject's bacterial load by each species and clinical periodontal recordings of these two sampling sites were evaluated statistically. Women were grouped according to the pregnancy outcome into a PLBW group, if they delivered pre-term (gestational age at birth <37 weeks), and/or if they gave birth to a low-weight baby (birth-weight <2500 g). If none of the above conditions occurred, the birth outcome was defined as normal. Statistical methods included the Student's *t*-test for comparing means and the Fisher's exact test or χ^2 test for comparing frequencies. Moreover, multiple logistic regression analysis was carried out according to the forward selection method to determine the effects of demographic, clinical and microbiological parameters. For each of the investigated species score 1 was selected as cutoff level to contrast colonized *versus* non-colonized women. Furthermore, score 4 was selected as the second cutoff level to discriminate heavily colonized patients from less heavily colonized ones.

Results

Clinical characteristics

There were 53 women in the case (PLBW) group and 128 women in the control (normal birth) group. Table 1 presents the demographic details of the study population including main risk factors. Most of the women in both groups had only primary level of education. The percentage of women with tertiary level of education was lower in the cases than in the controls, but the difference was not statistically significant ($p>0.05$). Most of the women in both groups were non-smokers. The percentage of heavy smokers was higher in the cases than in the controls, but the difference was not statistically significant ($p>0.05$). More women in the case group had a history of PLBW as well as more relatives with a history of PLBW, although the differences between the cases and the controls were not significant ($p>0.05$). More women in the case group had infection and treatment history during their pregnancy period. Fewer women in the case group had regular pre-natal controls. The only statistically signifi-

Table 1. Demographic details of the study population including main risk factors

	Group 1 controls (normal birth) ($n = 128$)	Group 2 cases (PLBW) ($n = 53$)	p value
Age	25.00 ± 4.57[†]	24.88 ± 5.73	0.898
Level of education			0.063
None	12.6	17.0	
Primary	66.1	71.7	
Secondary	7.9	7.5	
Tertiary	13.4	3.8	
Smoking habits			0.133
None	64.8	64.2	
< 10/day	25.8	17.0	
⩾ 10/day	9.4	18.9	
Total number of births			
Previous PLBW	7.8	17.0	0.067
PLBW in relatives	5.5	13.2	0.122
Infections/treatment during pregnancy	15.6	24.5	0.158
Prenatal controls			0.706
Regular	63.2	58.8	
Not regular	36.8	41.2	
Weight gain	12.53 ± 4.7	10.51 ± 3.4	0.010*
Type of delivery			0.206
Normal delivery	86.7	79.2	
Caesarean	13.3	20.8	

PLBW, pre-term low birth weight. All data are given as percentages.
*$p < 0.05$.
[†]Mean ± SD.

Table 2. Dental and clinical periodontal characteristics of the study groups

	Group 1 controls (normal birth) ($n = 128$)	Group 2 cases (PLBW) ($n = 53$)	p value
Dental complaints/treatment during pregnancy	3.9*	0.00	0.323
Number of teeth present	26.96 ± 1.5	26.58 ± 1.93	0.170
Number of decayed teeth	1.43 ± 1.8	1.45 ± 2.03	0.940
Number of teeth with restoration	0.32 ± 1.06	0.26 ± 1.15	0.753
Number of sites with PPD ⩾ 4 mm	8.96 ± 11.58	7.57 ± 10.08	0.445
Number of sites with PPD = 6–7 mm	0.57 ± 1.83	0.26 ± 1.06	0.460
% of sites with BOP (+)	42.27 ± 30.33	42.64 ± 28.61	0.939
% of sites with plaque	80.54 ± 26.02	85.66 ± 19.86	0.154
PPD (mm) (of sampling sites)	3.45 ± 0.93	3.20 ± 0.80	0.850
BOP (of sampling sites)	0.68 ± 0.42	0.69 ± 0.40	0.695
PI (of sampling sites)	0.82 ± 0.37	0.85 ± 0.33	0.471

PLBW, pre-term low birth weight; PPD, probing pocket depth; BOP, bleeding on probing; PI, plaque index. All data are given as mean ± SD.
*Percentage.
No significant differences were found between the study groups with regard to the dental and periodontal parameters recorded for the whole dentition or for the subgingival plaque sampling sites ($p > 0.05$).

cant difference between the cases and the controls was found with regard to the weight gain during pregnancy, and cases have gained significantly less weight than the controls ($p < 0.05$). Clinical dental characteristics of the study population and the mean values of the clinical periodontal parameters of the sampling sites are presented in Table 2. There were no statistically significant differences between the cases and controls with regard to the dental and periodontal parameters and the values of clinical periodontal recordings were found to be very similar ($p > 0.05$) (Table 2).

Microbiological analysis

Loads of *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *A. actinomycetemcomitans*, and *S. intermedius* exhibited statistically significant differences ($p < 0.05$) between the cases and the controls when comparisons were made by Mann–Whitney *U*-test (Table 3). Mean and median scores (bacterial loads) of these bacteria in the subgingival plaque sampling sites were significantly higher in the controls than in those of the cases. The occurrence rates of *P. intermedia, F. nucleatum, P. micros, C. rectus, E. corrodens, S. noxia,* and *S. intermedius* were higher in the cases compared with the controls, but the differences were not statistically significant ($p > 0.05$) (Table 4). The rest of the periodontopathogenic bacteria showed very similar occurrence rates in the cases and the controls. The occurrence rate of *T. forsythensis* was higher in the controls compared with the cases, but the difference did not reach the level of significance ($p > 0.05$). When score 1 was used as the cutoff level, Pearson's χ^2 test showed significant differences between the two study groups, that is, the detection rates of *P. gingivalis* and *A. actinomycetemcomitans* in the controls were significantly higher than those in the cases ($p < 0.05$) (Table 4). When score 4 was used as the cutoff level, Pearson's χ^2 test showed significant differences between the study groups in that the detection rates of *P. gingivalis, P. intermedia, P. nigrescens, A. actinomycetemcomitans* and *F. nucleatum* were significantly higher in the controls than in the cases (Table 5). At the end of the logistic regression analysis performed with the forward selection method, a model was created (Table 6). According to this model, *P. micros* and *C. rectus* were found to significantly increase the risk of PLBW ($p < 0.01$ and $p < 0.05$ respectively), while *P. nigrescens* and *A. actinomycetemcomitans* decreased this risk ($p < 0.01$). The accurate predict values were 78.9% for the controls and 79.4% for the cases. The rest of the evaluated demographic and clinical parameters did not show any significant effect on predicting birth weight.

Discussion

The present case–control study was undertaken to investigate whether maternal periodontal infection might create a higher risk for PLBW. For this purpose, subgingival plaque samples from two different interproximal sites in each woman have been collected within 72 h of delivery. These samples were evaluated for the presence as well

Table 3. Mean and median values of the scores of bacteria in the sampling sites

	Group 1 controls (normal birth) ($n = 128$)		Group 2 cases (PLBW) ($n = 53$)		p value
	mean ± SD	median (min–max)	mean ± S.D.	median (min–max)	
Porphyromonas gingivalis	1.39 ± 1.11	1 (0–5)	0.87 ± 0.91	0.5 (0–4)	0.000†
Prevotella intermedia	2.10 ± 1.22	2 (0–5)	1.61 ± 1.05	1.5 (0–4.5)	0.028*
Prevotella nigrescens	2.58 ± 0.95	2.5 (0–5)	1.92 ± 0.99	2 (0–4)	0.000†
Tannerella forsythensis	1.57 ± 1.19	1.5 (0–5)	1.42 ± 1.20	1.5 (0–3.5)	0.523
Actinobacillus actinomycetemcomitans	1.03 ± 0.91	1 (0–3)	0.46 ± 0.59	0 (0–2)	0.000†
Fusobacterium nucleatum	1.40 ± 0.86	1.5 (0–3)	1.18 ± 0.59	1 (0–3)	0.074
Treponema denticola	1.21 ± 0.74	1 (0–3.5)	1.19 ± 0.76	1 (0–3.5)	1.000
Peptostreptococcus micros	1.75 ± 0.92	2 (0–4)	1.83 ± 0.86	2 (0–3.5)	0.601
Campylobacter rectus	0.39 ± 0.46	0.5 (0–2.5)	0.52 ± 0.63	0.5 (0–2.5)	0.327
Eikenella corrodens	1.19 ± 0.97	1 (0–3.5)	0.94 ± 0.80	1 (0–3)	0.079
Selenomonas noxia	0.29 ± 0.48	0 (0–2)	0.31 ± 0.36	0 (0–1)	0.297
Streptococcus intermedius	2.20 ± 0.70	2 (0–4)	1.77 ± 0.71	1.5 (1–3)	0.000†

PLBW: Pre-term low birth weight.
Mann–Whitney *U*-test was performed to compare the two study groups with regard to the bacterial loads.
*$p < 0.05$, †$p < 0.01$.

Table 4. Occurrence rates (%) of 12 species in cases and controls at cutoff score 1

	Group 1 controls (normal birth) ($n = 128$)	Group 2 cases (PLBW) ($n = 53$)	p value
Porphyromonas gingivalis	87.5	75.5	0.045*
Prevotella intermedia	93.8	94.3	1.000
Prevotella nigrescens	98.4	98.1	1.000
Tannerella forsythensis	87.5	77.4	0.086
Actinobacillus actinomycetemcomitans	70.3	49.1	0.007†
Fusobacterium nucleatum	90.6	98.1	0.112
Treponema denticola	90.6	90.6	1.000
Peptostreptococcus micros	96.1	98.1	0.673
Campylobacter rectus	53.1	56.6	0.669
Eikenella corrodens	79.7	81.1	0.825
Selenomonas noxia	34.4	47.2	0.107
Streptococcus intermedius	99.2	100.0	1.000

PLBW, Pre-term low birth weight.
Fisher's exact test or Pearson χ^2 test was used to compare the two study groups according to the relation between the groups.
*$p < 0.05$, †$p < 0.01$

Table 5. Proportions (%) of women heavily infected with the 12 species at cutoff score 4

	Group 1 controls (normal birth) ($n = 128$)	Group 2 cases (PLBW) ($n = 53$)	p value
Porphyromonas gingivalis	25.2	7.7	0.008†
Prevotella intermedia	38.7	21.2	0.026*
Prevotella nigrescens	61.3	36.5	0.003†
Tannerella forsythensis	29.4	25.0	0.555
Actinobacillus actinomycetemcomitans	11.8	0	0.006†
Fusobacterium nucleatum	16.8	3.8	0.020*
Treponema denticola	7.6	9.6	0.763
Peptostreptococcus micros	29.4	26.9	0.741
Campylobacter rectus	0.8	1.9	0.517
Eikenella corrodens	16.8	7.7	0.114
Selenomonas noxia	0	0	–
Streptococcus intermedius	46.2	30.8	0.059

PLBW, Preterm low birth weight.
Fisher's exact test or Pearson χ^2 test was used to compare the two study groups according to the relation between the groups.
*$p < 0.05$, †$p < 0.01$

as the loads of 12 different bacterial species most commonly associated with periodontal disease. We collected plaque samples from one posterior and one anterior site selected on a random basis to better discriminate the likelihood of accumulation of any specific bacterial species. The women enrolled in the present study had a low socioeconomic level and most of them have not visited a dentist in their lives, nor used antibiotics frequently, if ever. This situation suggests that the periodontal disease status in this population may be considered as naïve. The study comprized a well-defined population, namely mothers giving birth at a particular hospital and the cases as well as the controls had oral hygiene habits far from the satisfactory level. Therefore, we can claim that the study population had a socioeconomic homogeneity, which we think is important in particularly cross-sectional studies. The control mothers were selected randomly at the time that cases were recruited. We excluded women with maternal age under 18 and over 35 years, since age outside this range is known as a risk factor for PLBW. Both the cases and the controls were similar with respect to the major risk factors for PLBW such as socioeconomic status, and maternal age. Thus, the present results may be considered as free of selection biases.

The oral health status of the study population was determined by a thorough structured clinical examination. However, we had no opportunity to study radiological evidence of alveolar bone loss as a possible indicator of severe periodontal infections, because no radiographs were available. Despite this limitation, accurate diagnosis of periodontal pockets was made.

Periodontal examinations were performed soon after the birth, allowing for a clear comparison of the influence of periodontal infections on the pregnancy outcome. Mitchell-Lewis et al. (2001) have suggested that a study of the impact of infection by specific periodontal microbiota on pregnancy outcomes should involve only women with undisturbed subgingival plaques during pregnancy. Accordingly, our study population is consisted of women enrolled immediately after delivery and almost none of these women had visited a dentist during their pregnancy periods. Moreover, the level of periodontal disease in the study population deserves attention. In the present study,

Table 6. Predictors of pre-term low birth weight (full model)

Variable	Coefficient	Standard error	*p*	Odds ratio
Prevotella nigrescens	− 1.063	0.379	0.005	0.35
Actinobacillus actinomycetemcomitans	− 1.692	0.510	0.001	0.18
Peptostreptococcus micros	1.339	0.508	0.008	3.82
Campylobacter rectus	1.967	0.802	0.014	7.15

both the cases and the controls can be viewed as a sample of women with poor oral hygiene habits, abundant plaque, overt gingivitis, but low prevalence of destructive periodontal disease. The proportion of smokers was similar in both groups and smoking did not show an association with PLBW. On the contrary, a dose–response relationship between smoking and low birth weight has been reported, with birth weight impairments increasing with the number of cigarettes smoked (Berkowitz & Papiernik 1993). In the present study ex-smokers have not been determined and it is likely that ex-smokers may exist among the non-smoker group and this may have affected to some extend the results of comparisons.

In the present study, the accepted risk factors for PLBW; like smoking, PLBW history in the close relatives, history of previous PLBW, infections during pregnancy and lack of regular pre-natal controls were found to be more prominent or common among the cases when compared with the controls. However, the differences between the study groups did not reach the level of significance.

Effects of systemic diseases or conditions on periodontal health and disease status have long been recognized. However, it is quite a new era dealing with the possible effects of periodontal diseases on the systemic health. Recent studies suggest a mild to moderate association between periodontal disease and certain systemic disorders such as diabetes mellitus, cardiovascular diseases, pneumonia and PLBW.

The first human investigation of the theory that remote sites of infection might contribute to PLBW was conducted by Offenbacher et al. (1996) in a case-control study of 93 mothers of PLBW infants. After adjusting for all other risk factors, the authors concluded that the mothers with periodontal disease, which was defined by clinical attachment level measurements, had more than seven times the risk of delivering a PLBW infant. In another study by the same researchers, gingival crevicular fluid (GCF) levels of PGE_2 and IL-1 as well as the levels of *P. gingivalis, A. actinomycetemcomitans, T. denticola,* and *T. forsythensis* were investigated (Offenbacher et al. 1998). They reported that the loads of four periodontal pathogens were significantly higher in the mothers with PLBW and also suggested a dose–response relationship for increased GCF PGE_2 level as a marker of current periodontal disease activity and decreasing birth weight. More recently, Offenbacher's group analysed blood samples from fetal cords for the presence of immunoglobulin M (IgM) antibody against various periodontal pathogens (Offenbacher et al. 1999). It was reported that 33.3% of the PLBW samples were positive for IgM against test bacteria, whereas only 17.9% of the normal birth weight samples tested positive. Romero et al. (2002) have reported highly significant clinical relationship between severity of maternal periodontal disease which was determined by Russell's periodontal index and low birth weight. Periodontal disease has been suggested as a significant risk factor for PLBW also in some other clinical studies (Dasanayake 1998, Jeffcoat et al. 2001, Lopez et al. 2002a, b). However, these data should be interpreted with caution, as the aetiology of both periodontal disease and PLBW is probably multifactorial and have common risk factors.

On the contrary, Davenport et al. (1998) have conducted a case-control study over 700 mothers and found no difference between the study cases and controls for any of the periodontal indices [pocket depth, bleeding index or community periodontal index of treatment needs (CPITN)]. Furthermore, after controlling for confounding factors, they concluded that periodontal disease does not create any greater risk for PLBW. In another study, the same researchers evaluated pocket depth, bleeding index, CPITN and attachment loss in 236 cases and 507 controls, and again failed to find any evidence for an association between PLBW and periodontal disease (Davenport et al. 2002). Madianos et al. (2001) have evaluated by the checkerboard DNA–DNA hybridization method the presence of 15 bacterial species in subgingival plaque samples from all four first molars obtained postpartum (within 48 h of delivery). They reported no significant differences in the prevalence of pathogens between pre-term mothers and full-term mothers at delivery. Accordingly, our present study has found no significant differences in the loads and occurrence rates of individual periodontopathogens in the subgingival plaque between the cases and the controls. Just the opposite, the occurrence rates of *P. nigrescens* and *A. actinomycetemcomitans* were found to be significantly higher in the control mothers than in the case mothers. However, when multiple bacteria were evaluated together, the regression analysis model indicated that *C. rectus* and *P. micros* might increase the risk of PLBW. Therefore, we suggest that the possible relationship between PLBW and periodontal disease reported previously may probably be explained by complex actions of different bacteria rather than the presence or loads of individual species in the subgingival plaque. It is also likely that other mechanisms like increases in the circulating levels of cytokines or prostaglandins rather than the clinical periodontal indices and the prevalence of subgingival pathogens may increase the risk for PLBW.

However, another study from a cohort of young minority women in New York, have found that PLBW mothers had significantly higher levels of *T. forsythensis* and *C. rectus*, although clinical periodontal status revealed no significant differences between the two groups (Mitchell-Lewis et al. 2001). The differences between various studies including our present study may be explained in different ways; there may in fact be no association, the differences in study populations may have led the differences in the data, and finally periodontal diseases and PLBW may be associated only in the presence of other specific environmental or genetic risk factors. Failure to control adequately for potential confounding factors may have a role in the inconsistencies between the results of different studies (Hujoel et al. 2000).

Intra-uterine infections are recognized as one of the most important

etiological factors in pre-term birth (Romero et al. 1989). However, it is not yet been clarified how these organisms initiate pre-term delivery since studies of term labour are still in progress (Lamont et al. 1990). It may be speculated that organisms associated with periodontal disease progression may enable other organisms' access to systemic dissemination. The proposed association between periodontal disease and PLBW may be a reflection of some alterations in the patient's inflammatory phenotype, which creates considerable risk for both conditions. It is also likely that a genetically determined trait predisposes an individual to periodontal disease and also to other multifactorial conditions of which inflammation is an important component.

It has been suggested that many PLBW cases occur as a result of infections of unknown origin (Gibbs et al. 1992). The present case-control study was undertaken to evaluate whether or not maternal periodontal infection might create higher risk for PLBW infants. Our findings suggest that maternal periodontal infection may have a contributory role in the risk of PLBW. We conclude that the association between periodontal diseases and pregnancy outcome may be explained mainly by confounding factors; particularly those relating to health behaviour, but periodontopathogens may also have either a direct or indirect role. Further research in this area is required, particularly with respect to the effect of population differences on this potential association between periodontal diseases and PLBW. Moreover, whether the relationship between periodontal diseases and PLBW is causal or simply associative needs to be better clarified.

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