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Is pre-term labour associated with periodontitis in a Danish maternity ward?

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Abstract

Objectives: To reveal differences in periodontal status and presence of subgingival bacteria in a Scandinavian population of women with pre-term birth compared with women who delivered at term.

Materials and Methods: Twenty-one women with pre-term labour (before week 35) and 33 women with term labour (between weeks 38 and 41) were included in this case–control study. Periodontal measurements included plaque index (PlI), probing pocket depth (PPD) and bleeding on probing (BOP). Inter-proximal distances from the cemento-enamel junction (CEJ) to the marginal bone crest (MBC) were measured on bitewing radiographs. In 31 patients (16 cases and 15 controls) the subgingival plaque was analysed using ''checkerboard'' DNA–DNA hybridization.

Results: Differences between the two examined groups were found related to ''Twin births'' ($p = 0.0064$) and ''Smokers'' ($p = 0.03$). None of the periodontal measurements showed any association. Significant differences were found concerning presence of *Tannerella forsythensis*, *Treponema denticola*, *Peptostreptococcus micros*, *Streptococcus intermedius*, *Streptococcus oralis*, *Streptococcus sanguis* and *Capnocytophaga ochracea* but when defining sites with $>10^5$ bacteria as heavily colonized, no statistical difference was found between the two groups.

Conclusion: A relation between pre-term birth and periodontitis was not revealed in the present study.

Key words: periodontitis; pre-term delivery; subgingival bacteria

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Spontaneous pre-term birth is associated with a series of risk factors such as high/low maternal age, over weight and underweight, parity, primiparous mothers, low socio-economic status, little or no education, alcohol and drug abuse, genital and urinary tract infections, hypertension and Afro-American race. However, these risk factors are not present in about 25% of the cases (Mealey 1999), and other causes have gained increasing attention. As it is well known that there is an association between maternal genitourinary tract infections and pre-term birth (Niswander & Gordon 1972), it has been hypothesized that some of the cases of pre-term labour could be explained by infections outside the urogenital area, and periodontitis has gained attention as a possible cause.

The possible association between pre-term birth and periodontitis is explained as follows: bacteraemia caused by periodontitis consists of various oral bacteria including Gram-negative bacteria releasing lipopolysaccharides (Silver et al. 1977). The bacteria and their products initiate the production of cytokines (IL-1, IL-6 and tumour necrosis factor (TNF)) by inflammatory cells (Mealey 1999). These cytokines stimulate the production of prostaglandins in chorion. As prostaglandins mediate cervical ripening and stimulate uterine contractions, the increased prostaglandin output is decisive for the onset of labour (Haram et al. 2003). This pathway has been supported by results of experimental studies.

In a hamster model, subcutaneous implantation of vital or heat-killed *Porphyromonas gingivalis* caused both significantly higher levels of TNF and prostaglandin E_2 (PGE_2), significantly increased mortality of foetuses and lower weight of viable foetuses as compared with the control group (Collins et al. 1994).

A study including 18 women, examined in the second trimester, showed a relation between oral infection and the amount of cytokines in the amniotic fluid. Further, the concentrations of PGE_2 and IL-1β in the crevicular fluid

were positively correlated with the corresponding concentrations in the amniotic fluid (Damare et al. 1998). The first study connecting pre-term birth and periodontitis estimated that spontaneous pre-term birth might be caused by periodontal disease in 18% of the cases (Offenbacher et al. 1996). Later studies, however, did not allow a firm conclusion regarding differences in periodontal health status between women with normal birth outcome and women with pre-term labour (Dasanayake 1998, Offenbacher et al. 1998, 2001, Dasanayake et al. 2001, Jeffcoat et al. 2001, 2003, Madianos et al. 2001, Mitchell-Lewis et al. 2001, Davenport et al. 2002, López et al. 2002, Romero et al. 2002, Konopka et al. 2003, Carta et al. 2004, Goepfert et al. 2004, Holbrook et al. 2004, Moore et al. 2004, 2005, Radnai et al. 2004, Buduneli et al. 2005, Moliterno et al 2005, Moreu et al. 2005, Noack et al. 2005). Moreover, the majority of studies published include women who may differ greatly from average Scandinavian women with respect to race, socio-economic status and easy access to health services in general. To reveal whether the suggested association between periodontal disease and pre-term birth could be found in a Danish female population, a study among women in a Danish maternity ward was designed.

The aim of this study was to reveal possible differences in periodontal status and in the presence of subgingival bacteria in a population of women with pre-term birth compared with a control group of women delivering at term.

Materials and Methods

Patient selection

The study was designed as a case–control study. All women (72 individuals) who in the year 2002 had idiopathic spontaneous labour before 35 completed weeks of gestation at a Danish hospital (Hillerød Sygehus) were invited to participate. Fifty-five women responded and 22 women fulfilled the inclusion criteria. The group of cases was examined during 2003 within 11 months post-partum.

The control group was recruited from the maternity ward at the same hospital, during the spring, year 2002. During hospitalization, 103 women who matched the inclusion criteria were invited to participate. Thirty-eight accepted the invitation. The control group was examined within 8 days post-partum.

The regional ethical committee approved the study. All participants were informed about procedures and informed consent was obtained in writing.

Inclusion and exclusion criteria

Pre-term birth was defined as spontaneous labour or rupture of membranes at less than 35 completed weeks of gestation. Term birth was defined as spontaneous delivery at gestational age between 38 and 41 completed weeks.

Criteria for exclusion were present and untreated chronic infections besides periodontitis (including untreated genital infections and urinary tract infections), treatment with antibiotics 6 weeks before the examination and systemic diseases that would require prophylactic antibiotics for the periodontal examination.

Medical data collection

In order to control for known risk factors, all participants were asked to fill out a questionnaire covering obstetrical data, health and medication during pregnancy, general health and medication, oral health history, smoking habits, alcohol consumption during pregnancy, living conditions, level of education, height and weight and dental care habits.

Oral examination

All patients were examined by the same clinician (T. S.) at the Department of Oral Surgery, Hillerød Sygehus, Denmark. The registrations included plaque index (PlI), probing pocket depth (PPD) and bleeding on probing (BOP).

PlI was registered (Silness & Loe 1966) on three index teeth in each quadrant (one molar, one bicuspid and the central incisor), six sites each tooth. If a tooth was missing, the nearest posterior tooth was used. PPD was registered at six sites of all teeth present except third molars. BOP was registered at six sites of all teeth 15 s after probing.

Radiographical examination

In each individual two bitewing radiographs were taken. The radiographs were scanned and digitized (Epson expression 1680 pro, Seiko Epson Corporation, Nagano, Japan) with a resolution of 600 dpi, whereafter the interproximal distances in mm from the cemento-enamel junction (CEJ) to the marginal bone crest (MBC) were measured by the program *DP-soft ver. 3.2* (Olympus Europa GMBH, Hamburg, Germany). The following sites were measured: mesial surface of the second molar, mesial and distal surfaces on the first molar and second bicuspid and the distal surface of the first bicuspid.

Subgingival plaque samples

Fifteen women with normal birth outcome and 16 with pre-term labour were randomly selected to participate in a microbiological examination.

Four subgingival plaque samples were obtained before the clinical examination from each individual, one from each quadrant at the mesio-facial surface of the first molar with a sterile paper point. The first molars were present in all patients. The area was thoroughly cleaned for supragingival plaque with a curette and a cotton pellet and then air dried. The paper point was inserted into the pocket and left for 10 s. The paper point was then immediately transferred to a sterile Eppendorf tube.

Processing of the bacterial plaque samples

The samples were transferred to 100 µl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6) and 100 µl 10.5 M NaOH were added and the suspensions were boiled for 5 min. After boiling 800 µl 5 M ammonium acetate were added to each tube and the samples were processed with the checkerboard methodology according to standardized procedures (Socransky et al. 1994, Papapanou et al. 1997). Immobilization of bacterial samples onto nylon membranes was completed within 4 weeks from sample collection.

Digoxigenin labeled, whole genomic probes were prepared by random priming using the High-Prime labelling kit (Boehringer-Mannheim, Mannheim, Germany) from the following 18 microbial strains: *Porphyromonas gingivalis* (FDC381), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Tannerella forsythensis* (ATCC43037), *Actinobacillus actinomycetemcomitans* (FDC Y4), *Fusobacterium nucleatum* (ATCC 10953), *Treponema denticola* (OMGS 3271), *Peptostreptococcus micros* (OMGS 2852), *Campylobacter rectus* (ATCC

33238), *Eikenella corrodens* (ATCC 23834), *Selemonas noxia* (OMGS 3119), *Streptococcus intermedius* (ATCC 27335), *Streptococcus oralis* (ATCC 33624), *Streptococcus sanguis* (OMGS2482), *Streptococcus mutans* (OMGS 2478), *Veillonella parvula* (ATCC 10790), *Capnocytophaga ochracea* (ATCC 33624) and *Actinomyces naeslundii* genospecies 2 (ATCC 15987).

The hybrids formed between the bacterial DNA and the probes were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubation with a chemiluminescent substitute (CSPD, Boehringer-Mannheim). Evaluation of the signal was performed at a LumiImager™ workstation (Boehringer-Mannheim) by comparing the obtained signals with those of pooled standard samples containing 10^6 (high standard) or 10^5 (low standard) of each of the 18 bacterial species. The sensitivity and specificity of whole genomic probes constructed as above have been described previously (Socransky et al. 1994), and a comparison between checkerboard hybridization and culture in the identification of subgingival microbiota has also been published (Papapanou et al. 1997). In addition, the probes were cross-tested against the 18 species of the panel in order to distinguish cross-hybridizations. The obtained chemiluminescent signals were transformed into a scale of scores from 0 to 5 according to Papapanou et al (1997). The occurrence of individuals positive for each of the investigated bacterial species was described at two different cut-off levels, Score 1 and Score 3. Score 1 cut-off level ($<10^5$) was selected to contrast colonized *versus* non-colonized sites and score 3 cut-off level ($>10^5$) to contrast heavily colonized (score 3 or more) *versus* non-colonized and less heavily colonized individuals.

Statistical Analysis

All calculations and statistical analyses were performed using the Statistical Analysis System (SAS) version 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Wilcoxon's test (non-parametric) was used to demonstrate differences between the two groups. Further, χ^2 tests were used to analyse the association between the distribution in the groups (dependent variable) and independent variables (e.g. variables expressing amount of periodontal disease and covariates like smoking and other known risk factors). Variables that achieved statistically significant association at $p \leqslant 0.20$ were then entered in a logistic regression analysis (PROC LOGISTIC) to estimate the increased risk of the variables (odds ratio). Level of significance was $p = 0.05$.

Results

One woman with pre-term birth and five women with normal birth outcome were lost because of drop-out. A total of 21 women with pre-term birth (case group) and 33 women with normal birth outcome (control group) participated in the complete study.

Mean age for women with pre-term birth was 31.5 years (SD 4.2) and for the control group was 30.8 years (SD 3.60). There were no significant differences in age between the two groups.

Questionnaire

Results obtained from the questionnaire are shown in Table 1. Only the variables birth of twins (''Twins'') and the number of smokers (''Smoker'') differed significantly between the two groups. No twin births were observed in the control group, and around five times more smokers were found in the pre-term group as compared with the control group.

Clinical and radiographical data

The mean values of clinical and radiographical data concerning PlI, PPD and BOP revealed no difference between the groups. The mean differences (term minus pre-term) between the two groups were as follows: PlI: -0.0054 ($p = 0.96$), PPD: -0.096 mm ($p = 0.11$) and BOP: 2.96% ($p = 0.40$). To further analyse the clinical and radiographical data the percentage of sites with PlI $\geqslant 2$, percentage of sites with PPD $\geqslant 4$ mm, percentage of sites with BOP and percentage of sites with distance between CEJ and MBC $\geqslant 2$ mm were calculated. Data are presented in Table 2, and again there were no significant differences between the groups.

Microbiological data

There were no differences between the two groups concerning PPD at sites included in the microbiological examination. None of the sites used for microbiological examination had PPD

Table 1. Data obtained from a questionnaire covering height and weight, health and medication, smoking habits, alcohol consumption, living conditions, level of education and dental care habits

	Term birth			Pre-term birth			*p*-value
	N	%	no.	*n*	%	no.	
Maternal age: >35 years	33	12	4	21	14	3	0.31
Maternal age: <20 years	33	0	0	21	0	0	n.r.
Underweight (BMI <18,5)	33	3	1	21	5	1	0.48
Overweight (BMI >25)	33	24	8	21	33	7	0.19*
Twins	33	0	0	21	24	5	0.0064†
Parity ⩾3 children	33	6	2	21	24	5	0.06*
Primiparous	33	39	13	21	57	12	0.10*
Marital status: single	33	0	0	21	5	1	0.40
Living conditions: <20 m^2/person	33	12	4	21	14	3	0.31
Unemployed	33	6	2	21	5	1	0.45
⩽10 years education	33	24	8	19	21	4	0.26
Irregular dental care	33	15	5	21	14	3	0.30
⩾4 servings of alcohol weekly	33	6	2	21	5	1	0.45
Use of drugs d.p.	33	0	0	21	0	0	n.r.
Smoker d.p.	33	3	1	21	24	5	0.03*†
Urinary infection d.p.	33	21	7	21	10	2	0.17*
Genital infections d.p.	33	3	1	21	0	0	0.61
Other infections d.p.	33	9	3	21	19	4	0.18*
Antibiotic therapy d.p.	33	21	7	21	19	4	0.27
Low blood pressure d.p.	33	0	0	21	5	1	0.38
High blood pressure d.p.	33	6	2	21	5	1	0.45
Race: not Caucasian	33	3	1	21	10	2	0.28

*Variables to be included in the logistic regression.

†Statistically significant differences between the two groups.

BMI, body mass index; n.r., not relevant; d.p., during pregnancy.

Table 2. Distribution of mean number of teeth and distribution of mean values of clinical and radiographical data used in the definitions of a periodontally diseased site (see text)

	Term birth			Pre-term birth		
	n	Mean	SD	*n*	Mean	SD
Number of teeth	33	27.47	1.21	21	27.90	0.45
Plaque score $\geqslant 2$ (% sites)	33	12.42	14.97	21	15.14	10.90
Probing pocket depth $\geqslant 4$ mm (% sites)	33	3.05	2.83	21	3.83	3.07
Bleeding on probing (% sites)	33	31.22	13.46	21	28.26	10.49
Distance from CEJ to MBC $\geqslant 2$ mm (%sites)	33	12.01	16.58	20	12.58	17.20

There are no statistically significant differences between the two groups.
CEJ, cemento-enamel junction; MBC, marginal bone crest.

Table 3. Microbiological data

	Term birth ($n = 15$)		Pre-term birth ($n = 16$)		*p*-value
	%	no.	%	no.	
Porphyromonas gingivalis	7	1	31	5	0.09
Prevotella intermedia	80	12	56	9	0.12
Prevotella nigrescens	67	10	69	11	0.30
Tannerella forsythensis	13	2	50	8	0.03†
Actinobacillus actinomycetemcomitans	0	0	25	4	0.06
Fusobacterium nucleatum	40	6	63	10	0.13
Treponema denticola	67	10	100	16	0.02†
Peptostreptococcus micros	20	3	94	15	<0.001†
Campylobacter rectus	20	3	50	8	0.07
Eikenella corrodens	20	3	44	7	0.12
Selemonas noxia	60	9	38	6	0.13
Streptococcus intermedius	74	11	100	16	0.04†
Streptococcus oralis	33	5	100	16	<0.001†
Streptococcus sanguis	40	6	100	16	<0.001†
Streptococcus mutans	80	12	100	16	0.10
Veillonella parvula	7	1	25	4	0.16
Capnocytophaga ochracea	13	2	50	8	0.03†
Actinomyces naeslundii	80	12	100	16	0.10

The number of participants in each group with the bacteria present on at least one of four sites tested.
†Statistically significant differences between the two groups.

>4 mm. The mean differences in PPD (term minus pre-term) at each site were (FDI nomenclature) 16: 0.0417 mm ($p = 0.07$), 26: 0.1833 mm ($p = 0.91$), 36: -0.096 mm ($p = 0.97$) and 46: -0.1 mm ($p = 1.0$). Table 3 shows the number of women in each group with a positive signal (checkerboard score 1–5) on at least one of four sites tested by the ''checkerboard'' DNA–DNA hybridization method. There were significant differences between the two groups concerning presence of six bacteria in the subgingival plaque (out of the 18 tested). These bacteria were *Tannerella forsythensis* (formerly known as *Bacteroides forsythus*), *Treponema denticola*, *P. micros*, *Streptococcus intermedius*, *Streptococcus oralis*, *Streptococcus sanguis* and *C. ochracea*.

Table 4 shows the number of participants in each group with an amount of bacteria $>10^5$ on at least one of four sites tested (a checkerboard score $\geqslant 3$). No statistical difference was found between the two groups with respect to the number of women with an amount of bacteria $>10^5$.

Variables indicating increased risk for pre-term birth

The overall description of clinical and radiographical variables as presented in Table 2 offers no information about an association between pre-term birth and periodontal disease. In an attempt to identify sites with active periodontal disease, a periodontally diseased site was defined either as a site with PPD $\geqslant 4$ mm and BOP (definition 1) or a site with a distance $\geqslant 2$ mm between CEJ and MBC and BOP (definition 2). The number of sites following definition 1 or 2 is calculated for each patient, and in Fig. 1 the distribution of patients in the two groups with 0, 1–5, 6–10 or >10 periodontally inflamed sites is shown. The number of sites following definition 1 or 2 is then considered the extent of active periodontal disease. Both variables showed the same distribution within the two groups and the lack of association is independent of how the threshold is set. It can therefore be concluded that the definitions of a periodontally diseased site used in the present study revealed no difference between the two groups and therefore no association between pre-term birth and periodontal disease.

Whether the known association between smoking and periodontal disease (Grossi et al. 1995) could be found in these groups of patients was further examined. Participants who recently ceased smoking or smoked during pregnancy were included in a ''group of smokers''. No effect of smoking on the amount of periodontal disease (definition 1 or definition 2) could be demonstrated.

To identify variables increasing the risk of pre-term birth a logistic regression analysis with group (term/pre-term) as dependent variable was executed. In Table 1 it can be seen that only the variables marked with ∗ should be included in the logistic regression. ''Twins'' fulfill the inclusion criteria, but twin births were only represented in the case group, and therefore cannot be included in the logistic regression analysis. The lack of association between microbiological data and pre-term birth and the used estimates of periodontal disease and pre-term birth ($p > 0.20$) hinders these variables from inclusion.

Table 5 shows the odds ratio estimates for pre-term birth. As the lower border of the 95% confidence intervals has to be greater than 1, significantly increased risk is seen with (a) parity $\geqslant 3$ children, (b) smoking and finally (c) primiparous mothers.

Discussion

In this study, pre-term birth was defined as labour before 35 completed weeks of gestation. Most other studies designed to evaluate the association between pre-term labour and periodontal disease chose labour before 37 completed weeks of gestation as the definition of pre-term birth and labour at term as labour at gestational age between 38 and 41 completed weeks of gestation. Hence, it

Table 4. Microbiological data

	Term birth ($n = 15$)		Pre-term birth ($n = 16$)		p value
	%	no.	%	no.	
Porphyromonas gingivalis	0	0	6	1	0.52
Prevotella intermedia	27	4	13	2	0.22
Prevotella nigrescens	7	1	13	2	0.40
Tannerella forsythensis	0	0	0	0	n.r.
Actinobacillus actinomycetemcomitans	0	0	13	2	0.26
Fusobacterium nucleatum	0	0	6	1	0.52
Treponema denticola	7	1	13	2	0.40
Peptostreptococcus micros	0	0	6	1	0.52
Campylobacter rectus	0	0	0	0	n.r.
Eikenella corrodens	0	0	6	1	0.52
Selemonas noxia	0	0	0	0	n.r.
Streptococcus intermedius	13	2	13	2	0.40
Streptococcus oralis	13	2	38	6	0.11
Streptococcus sanguis	0	0	6	1	0.52
Streptococcus mutans	20	3	31	5	0.25
Veillonella parvula	7	1	0	0	0.48
Capnocytophaga ochracea	0	0	13	2	0.26
Actinomyces naeslundii	33	5	31	5	0.30

The number of participants in each group with an amount of bacteria $>10^5$ on at least one of four sites tested (a checkerboard score >3). There are no statistically significant differences between the two groups.
n.r., not relevant.

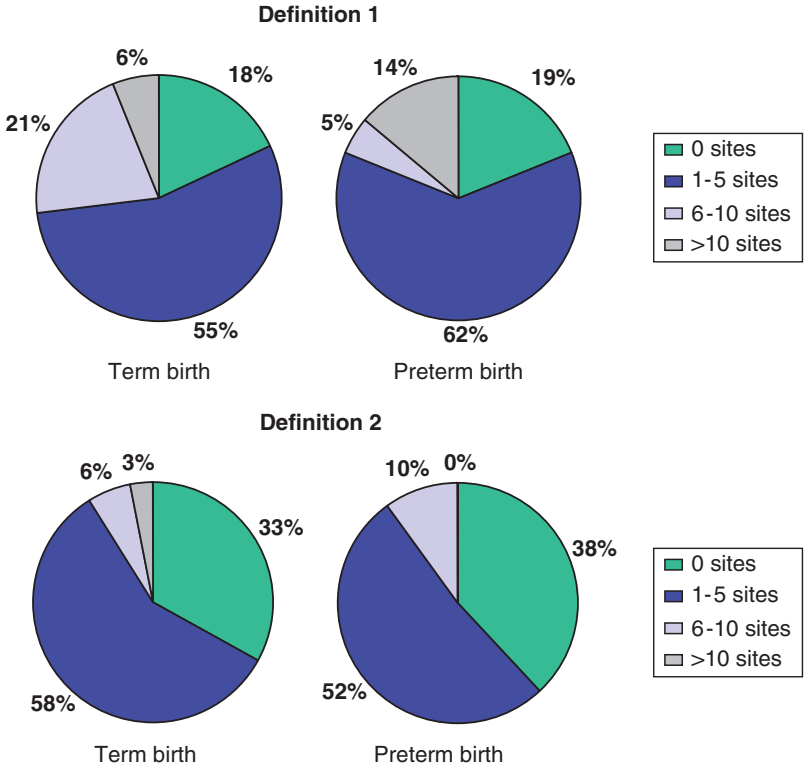

Fig. 1. The distribution of patients in the term/pre-term groups with 0, 1–5, 6–10 or >10 periodontally inflamed sites according to definition 1: a site with probing pocket depth greater than or equal to 4 mm and bleeding on probing (BOP), or definition 2: a site with a distance greater than or equal to 2 mm from the cemento-enamel junction to the marginal bone crest and BOP. There are no statistically significant differences between the two groups.

follows that the difference in gestational age between the two groups is limited for some of the individuals. By defining pre-term birth as labour before 35 completed weeks of gestation, well-defined groups were ensured, because all participants in the control group were women who delivered between 38 and 41 completed weeks of gestation.

This study failed to support a proposed link between pre-term birth and periodontitis. The women studied were a random selection of a Scandinavian population of general good health and high socio-economic status. The prevalence of periodontitis is considered low in a population of relatively young age as the population studied. In Denmark, there is free access to comprehensive health care for all citizens and public dental treatment for all until the age of 18, which may not be the case for many of the participants in the reported studies carried out in North-American populations. It could therefore be suggested that the lack of consistency between the present results and those of a positive association between periodontitis and pre-term birth (Offenbacher et al. 1996, 1998, 2001, Dasanayake 1998, Dasanayake et al. 2001, Jeffcoat et al. 2001, 2003, Madianos et al. 2001, López et al. 2002, Konopka et al. 2003, Carta et al. 2004, Goepfert et al. 2004, Radnai et al. 2004, Buduneli et al. 2005, Moreu et al. 2005, Moliterno et al. 2005) is due to population differences related to race, different lifestyle and poorer living conditions, including poorer access to comprehensive health care. Moreover, it is important to note that the prevalence of pre-term birth in Denmark is around 7% (Danish National Board of Health 2003) whereas the reported prevalence in North America is around 13% (Offenbacher et al. 1996). The results of our study are consistent with results from Iceland (Holbrook et al. 2004), Germany (Noack et al. 2005) and England (Moore et al 2005).

It is known that pregnancy, as a consequence of hormonal influences, can modify the periodontal situation (Loe & Silness 1963). In this study, the women in the case group were examined at a later stage in their postpartum period compared with the control group. This fact can be suspected to blur the difference between the two groups. However, this is not considered to be a problem in the present study, since it has to be realized that if the women with pre-term birth suffered

Table 5. Adjusted odds ratio estimates for pre-term birth

	Odds ratio	95% confidence interval
Parity $\geqslant 3$ children	16.70	1.77–157.72
Smoker d.p.	15.27	1.18–196.87
Primiparous	5.98	1.25–28.67
Other infections d.p.	3.83	0.59–24.81
Overweight (BMI >25)	1.66	0.38–7.40

d.p., during pregnancy; BMI, body mass index.

from more than a simple pregnancy gingivitis at the time of birth there could be a difference between the two groups with respect to radiographical boneloss. That was not the case.

It can be argued that the sample size is limited and therefore the power of the statistical analyses is weak. From the present study, estimates of standard deviations (SD) for the variables can be calculated. If as an example the maximum radiological measurements are used as effect variable, an SD of 0.56 mm in the term group and 0.58 mm in the pre-term group is found. A common SD of 0.57 mm is reasonable. This estimate indicates that with a least significant difference of 1 mm between the groups, a power of 99% (type I error: 0.05, type II error: 0.01) is achieved with 11 participants in each group. Further it should be noticed that the hypotheses is one sided, which will increase the power or lower the level of significance further. There is therefore no reason to believe that it was due to lack of power that the study failed to demonstrate differences between the two groups.

Smoking is a well-known risk factor for both periodontitis (Grossi et al. 1995) and pre-term birth (Castles et al. 1999). In this population the greater part of smokers were found in the case group, but even though former smokers were also included in the analysis and related to estimates of periodontitis, no relation between smoking and periodontal disease could be found.

Interestingly it appears from Table 3 that a difference in periodontal microbiota between the two groups was revealed even though there was no differences in PPD. When regarding the number of women harbouring each bacteria, it is apparent that the women with pre-term birth are over-represented (except for *P. intermedia* and *Selemonas noxia*). One explanation could be that the women with pre-term birth might harbour a higher subgingival load of periodontal pathogens, which is consistent with the results of another study that also failed to reveal differences in clinical periodontal status between the two groups (Mitchell-Lewis et al. 2001). The difference in periodontal microbiota may partly be explained by the difference in post-partum period before examination.

Several studies have shown that it is possible to isolate bacteria considered as periodontal pathogens from periodontal healthy persons (Dahlén et al. 1992, Ximénez-Fyvie et al. 2000). On this basis it is difficult to define a threshold for a relevant subgingival load of the selected bacteria. In this study $>10^5$ bacteria (checkerboard score 3) was chosen as the threshold for a well-colonized site, which is consistent with levels found in another study (Mitchell et al. 2001). As described, the use of this threshold in the statistical analysis eliminates the difference between the two groups, which is consistent with the fact that there was no difference in periodontal status between the two groups.

In conclusion, it is important to notice that the following four variables in this study turned out to differ significantly between the case group and the controls: giving birth to twins, parity $\geqslant 3$ children, smoking and primiparous mothers (Table 5). All of them are known risk factors for pre-term birth (Mealey 1999, Haram et al. 2003). A relation between pre-term birth and presence of periodontitis could not be demonstrated.

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Clinical Relevance

Scientific rationale for the study: No firm conclusion is yet revealed regarding the possible link between periodontal disease and pre-term labour. This study was carried out in a Danish maternity ward.

Principal findings: The prevalence of periodontitis is low in the population studied and none of the periodontal measurements differed between the two groups (term labour *versus* pre-term labour).

Practical implications: A relation between pre-term birth and periodontitis was not revealed in the present study.