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# Evaluation of the relationship between smoking during pregnancy and subgingival microbiota

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***Abstract***

**Background:** Numerous studies have shown that smoking negatively affects periodontal health. Hormonal changes, which occur during pregnancy have also been reported to have adverse effects on the periodontal tissues or indirectly through alterations in the subgingival bacterial flora. At present, no knowledge exists concerning possible effects of smoking on the composition of subgingival plaque in pregnancy. The purpose of the present study was to evaluate the effects of smoking during pregnancy on the subgingival plaque bacteria most commonly associated with periodontal disease.

**Methods:** A total number of 181 women were examined within 72 h post-partum. Smoking status was recorded by means of a self-reported questionnaire and the study population was divided into three groups; non-smokers, light smokers, and heavy smokers. In each woman, two subgingival plaque samples were obtained from mesio- or disto-buccal aspect of randomly selected one molar and one incisor tooth by sterile paperpoints. Clinical periodontal recordings comprising presence of dental plaque, bleeding on probing (BOP), and probing pocket depth (PPD) were performed at six sites per each tooth at all teeth. Plaque samples 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 $\chi^2$ test, Kruskal–Wallis test and Mann–Whitney *U*-test.

**Results:** Mean ages were similar in the study groups. Plaque, BOP and PPD recordings were lower in the heavy-smoker group, but the differences were not statistically significant ($p > 0.05$). The detection rates and bacterial loads of the specific subgingival bacteria exhibited no significant differences between the groups. No correlation could be found between smoking status and detection rates and bacterial loads of various bacterial species.

**Conclusion:** The present findings suggest that smoking during pregnancy does not have a significant effect on the composition of subgingival plaque bacteria.

Key words: DNA–DNA hybridization; periodontal diseases/microbiology; pregnancy; risk factors; smoking/adverse effects; subgingival microbiota

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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 resulting in tissue destruction. Although, the basic aetiological factor in the pathogenesis of periodontal diseases is the infection with plaque bacteria, there are also risk factors that may modify the periodontal response to the microbial challenge. Tobacco smoking is accepted among these modifying risk factors (Bergström 2003, Bergström & Preber 1994). Smokers have been reported to be more susceptible to advanced and

aggressive forms of periodontal disease than are non-smokers (Calsina et al. 2002, Haber et al. 1993, Ketabi & Hirsch 1997, Rivera-Hidalgo 2003). A complex relationship between periodontopathogens and smoking has been suggested, and increased occurrence of specific bacteria has been reported in smokers (Grossi et al. 1997, Machtei et al. 1997, Zambon et al. 1996). In another study on aggressive periodontitis, smokers have been reported to harbour higher levels of some periodontopathogens compared with the non-smokers (Kamma et al. 1999). Accordingly, the likelihood of disease progression in smokers has been stated to be significantly higher than non-smokers (Machtei et al. 1997).

Pregnancy gingivitis is a non-specific, vascularizing, and proliferative inflammation with large amounts of infiltrated inflammatory cells. Löe and Silness (1963) have shown that pregnant women have more pronounced gingivitis than their non-pregnant peers. Because of the vascular changes, the gingiva is dark red and bleeds easily. Oedema-related smooth appearance of the gingiva, thickening of the gingival margin, and hyperplastic interdental papillae are other prominent clinical features. The gingival changes have been reported to be worst at the third and eighth month of pregnancy. Oestrogen and progesterone concentrations in the blood rise at least 100 times from the beginning of pregnancy (Fox 1993). Although, pregnancy-related hormonal changes seem to be the main reason of pronounced gingival inflammation, the exact mechanisms remain to be elucidated. Increased vascularity and vascular flow are among the main proposed factors relating directly or indirectly the hormonal changes to the gingival inflammation. Both cross-sectional and longitudinal studies on microbial dental plaque have shown that the numbers of some microorganisms increase during pregnancy. Kornman and Loesche (1980) reported that the ratio of bacterial anaerobes to aerobes and the proportion of *Bacteroides melaninogenicus, Prevotella intermedia* and *Porphyromonas gingivalis* increased. *Prevotella intermedia* and *P. melaninogenica* can metabolize the sex hormones and use them as their essential growth factors (Kornman & Loesche 1982). These ecological changes and possible additive effects of smoking may directly and indirectly lead to changes in the composition of subgingival microflora during pregnancy and may eventually act in the clinical periodontal manifestations. However, as yet there are no published studies on the relation between smoking during pregnancy and subgingival bacteria most commonly associated with periodontal disease. Smokers may have reduced capacity to maintain the inflammatory response to the microbial challenge and pregnancy may have a further effect on this impairment when combined in smokers.

The purpose of the present study was to compare the subgingival microbial composition in smoker and non-smoker women shortly after delivery and to determine whether smoking during pregnancy has an effect on the prevalence and proportions of main periodontopathogens that colonize in subgingival plaque in an attempt to relate the findings to the periodontal problems faced during pregnancy.

## Material and methods

### Study population

The study population was drawn from women with low socioeconomic status between the ages of 18 and 35 who gave birth in a special maternity hospital in İzmir, Turkey. A total number of 181 women who were not known to have any systemic disease or to have received treatment with antibiotics for the past 3 months participated in the present study. Smoking habits for each woman was recorded by means of a standard questionnaire and the study population was divided into three groups according to the individual's smoking status; non-smokers (women who have never smoked), light smokers ($<10$ cigarettes/day for less than 5 years), and heavy smokers ($\geqslant 10$ cigarettes/day for more than 5 years). Former smokers who had quit smoking were excluded. Women who smoke $\geqslant 10$ cigarettes/day for less than 5 years, and those who smoke $<10$ cigarettes/day for more than 5 years were also excluded in an attempt to make a clear discrimination between the smoking categories. All data were collected within 72 h of delivery. Informed consent was obtained from all the subjects before their enrolment in the study.

### Subgingival plaque sampling

Subgingival plaque samples were obtained prior to the clinical examination, from two different sites per subject. All plaque samples were collected from one molar and one incisor tooth of each subject selected according to the presence of clearer expression of visual signs of clinical inflammation. No attempt was made to standardize the location of sampling sites in upper or lower jaw. Sampling sites were accessed from buccal aspects of the mesial or distal surfaces at the interproximal sites. Prior to subgingival plaque sampling, dichotomous plaque recording was performed as present or absent and supragingival plaque was then removed 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 in each of the two subgingival plaque sampling sites in each woman. The paperpoints were then removed, placed in sterile dry Eppendorf tubes and transported to the laboratory of Oral Microbiology, Göteborg University, Göteborg, Sweden. The subgingival plaque samples obtained from two different sites in each subject were analysed separately.

### Clinical examination

Subsequent to dichotomous recording of supragingival microbial plaque and subgingival plaque sampling, clinical periodontal recordings were 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, and dental and periodontal probes. The periodontal examination 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. PPD measurements were recorded to the nearest millimetre using a William's probe. All measurements were carried out by two precalibrated 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: *P. 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), and *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, Massachusetts, USA) at 42°C. After a series of stringency wash, hybrids were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubated with an appropriate chemiluminescent substrate (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). For each of the investigated species, score 1 was selected as cutoff level to contrast colonized *versus* non-colonized women.

#### Statistical analyses

Clinical data were expressed as means and standard deviations (SD) and tested for significant differences between smokers and non-smokers using the Mann–Whitney *U*-test. Differences in prevalence of the various periodontal pathogens between smokers and non-smokers were determined using Pearson's $\chi^2$ test. Kruskal–Wallis test was used to compare the mean proportions of various bacterial species between the three study groups. Further differences between smokers and non-smokers were determined by using Mann–Whitney *U*-test. The relationship between smoking and the prevalence and proportions of different combinations (clusters) of bacterial species was determined by Pearson's $\chi^2$ analysis. Spearman' rank correlations were used to look at the relationships between the smoking status and the prevalence and proportions of various bacterial species. Multiple logistic regression analysis was also performed with the species, one at a time, as the response variable. Score 1 was used as cutoff level and the scores were transformed into a 0/1 variable, where 0 denoted score 0 and 1 denoted scores 1–5. The level of statistically significant difference was selected as $p<0.05$.

*Table 1.* Clinical characteristics of the study groups (mean ± standard deviation)

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| | non-smokers | light smokers | heavy smokers |
| *n* | 117 | 42 | 22 |
| Age | 25.08 ± 5.1 | 24.23 ± 4.3 | 25.72 ± 4.6 |
| PPD (mm)* | 3.39 ± 0.9 | 3.45 ± 0.8 | 3.13 ± 0.7 |
| BOP* | 0.68 ± 0.41 | 0.72 ± 0.38 | 0.56 ± 0.47 |
| PI* | 0.84 ± 0.34 | 0.85 ± 0.33 | 0.70 ± 0.45 |
| Number of teeth present | 26.84 ± 1.65 | 27.07 ± 1.77 | 26.45 ± 1.59 |
| Number of sites with PPD ($\geqslant$4 mm)† | 8.50 ± 10.74 | 9.92 ± 13.59 | 6.18 ± 7.66 |
| % of sites with BOP (+)† | 42.43 ± 29.12 | 44.88 ± 30.53 | 37.27 ± 32.35 |
| % of sites with plaque† | 82.39 ± 25.16 | 86.54 ± 19.80 | 71.59 ± 26.42 |

PPD, probing pocket depth; BOP, bleeding on probing; PI, plaque index as present or absent.
*Clinical periodontal recordings at subgingival plaque sampling sites.
†Mean values of clinical periodontal recordings covering all teeth present. No significant differences were found between the study groups with regard to the mean ages and clinical periodontal parameters ($p>0.05$).

## Results

#### Clinical characteristics

There were 117 women in the non-smoker group, while 42 women made up the light-smoker group and 22 the heavy-smoker group. Table 1 presents the clinical characteristics of the study population and the mean values of the clinical parameters of the sampling sites. The women included in the present study population were rather young and most of them had moderate-to-severe gingivitis, while only very few had mild periodontitis. There were no statistically significant differences between the study groups with regard to the mean age of the individuals ($p>0.05$) (Table 1). Although, the differences were not significant statistically, the heavy-smoker group showed lower values in plaque, BOP and PPD recordings at the sampling sites ($p>0.05$). The overall percentages of sites with BOP and plaque as well as the number of sites with a PPD of $\geqslant$4 mm were also lower in the heavy-smoker group, but the differences with the other groups were not statistically significant ($p>0.05$).

#### Microbiological analysis

Loads of specific subgingival bacteria did not exhibit any statistically significant differences between the study groups when the comparisons were made by Kruskal–Wallis test ($p>0.05$) (Table 2). When score 1 was used as the cutoff level, Pearson's $\chi^2$ test showed no significant difference in the detection rates of specific bacterial species between the groups (Table 3). The occurrence rates of *T. forsythensis, A. actinomycetemcomitans* and *F. nucleatum* were higher in the heavy-smoker group, compared with the other groups, but the differences did not reach the level of significance ($p>0.05$). *P. nigrescens*, *F. nucleatum*, *P. micros* and *S. intermedius* species exhibited 100% detection rates in the heavy smokers. *P. nigrescens* and *F. nucleatum* were detected in all of the plaque samples in the light smokers, while *S. intermedius* was the only bacteria detected in all of the samples

*Table 2*. The median and range (min–max) of scores of bacteria in sampling sites

| | Group 1 non-smokers ($n = 117$) | Group 2 light smokers ($n = 42$) | Group 3 heavy smokers ($n = 22$) |
|---|---|---|---|
| *Porphyromonas gingivalis* | 1 (0–5) | 1 (0–4) | 1 (0–3.5) |
| *Prevotella intermedia* | 2 (0–4.5) | 2 (0–5) | 2 (0–4) |
| *Prevotella nigrescens* | 2.5 (0–4.5) | 2.5 (0.5–5) | 2 (1–3.5) |
| *Tannerella forsythensis* | 1.5 (0–5) | 1.25 (0–4) | 1.5 (0–4) |
| *Actinobacillus actinomycetemcomitans* | 1 (0–3) | 0.75 (0–3) | 0.5 (0–2) |
| *Fusobaterium nucleatum* | 1 (0–3) | 1 (0.5–3) | 1.25 (0.5–2.5) |
| *Treponema denticola* | 1 (0–3.5) | 1 (0–3) | 1 (0–3) |
| *Peptostreptococcus micros* | 2 (0–4) | 2 (0–3.5) | 1.5 (0.5–4) |
| *Camphylobacter rectus* | 0.5 (0–2.5) | 0 (0–1.5) | 0.5 (0–2.5) |
| *Eikenella corrodens* | 1 (0–3) | 1 (0–3.5) | 1 (0–3) |
| *Selenomonas noxia* | 0 (0–2) | 0 (0–2) | 0 (0–2) |
| *Streptococcus intermedius* | 2 (1–4) | 2 (0–3.5) | 2.25 (1–3) |

No significant differences were found between the study groups with regard to the DNA–DNA hybridization scores of various bacteria ($p > 0.05$). The chemiluminescence units obtained were transformed into a scale of 0–5: 0, no signal; 1, a signal lower than that of the low standard (i.e. $< 10^5$); 2, 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); 5, a signal higher than the one of the high standard ( $> 10^6$ bacteria).

*Table 3*. Proportions (%) of smokers and non-smokers infected with the 12 species at cutoff score 1

| | Group 1 non-smokers ($n = 117$) | Group 2 light-smokers ($n = 42$) | Group 3 heavy-smokers ($n = 22$) |
|---|---|---|---|
| *Porphyromonas gingivalis* | 84.6 | 83.3 | 81.8 |
| *Prevotella intermedia* | 94.9 | 92.9 | 90.9 |
| *Prevotella nigrescens* | 97.4 | 100.0 | 100.0 |
| *Tannerella forsythensis* | 82.1 | 85.7 | 95.5 |
| *Actinobacillus actinomycetemcomitans* | 61.5 | 66.7 | 72.7 |
| *Fusobaterium nucleatum* | 88.9 | 100.0 | 100.0 |
| *Treponema denticola* | 91.5 | 90.5 | 86.4 |
| *Peptostreptococcus micros* | 95.7 | 97.6 | 100.0 |
| *Camphylobacter rectus* | 57.3 | 45.2 | 54.5 |
| *Eikenella corrodens* | 78.6 | 85.7 | 77.3 |
| *Selenomonas noxia* | 38.5 | 38.1 | 36.4 |
| *Streptococcus intermedius* | 100.0 | 97.6 | 100.0 |

No significant differences were found between the study groups with regard to proportions (%) of subjects infected with various bacteria ($p > 0.05$).

*Table 4*. Proportions (%) of smokers and non-smokers infected with the 12 species at cutoff score 4

| | Group 1 non-smokers ($n = 117$) | Group 2 light smokers ($n = 42$) | Group 3 heavy smokers ($n = 22$) |
|---|---|---|---|
| *Porphyromonas gingivalis* | 3.4 | 4.8 | 0 |
| *Prevotella intermedia* | 6.0 | 7.1 | 4.5 |
| *Prevotella nigrescens* | 2.6 | 7.1 | 0 |
| *Tannerella forsythensis* | 2.6 | 0 | 9.1 |
| *Actinobacillus actinomycetemcomitans* | 0 | 0 | 0 |
| *Fusobaterium nucleatum* | 0 | 0 | 0 |
| *Treponema denticola* | 0.9 | 0 | 4.5 |
| *Peptostreptococcus micros* | 0 | 0 | 4.5 |
| *Camphylobacter rectus* | 0 | 0 | 0 |
| *Eikenella corrodens* | 0 | 0 | 0 |
| *Selenomonas noxia* | 0 | 0 | 0 |
| *Streptococcus intermedius* | 0.9 | 0 | 0 |

No significant differences were found between the study groups with regard to proportions (%) of subjects heavily infected with various bacteria ($p > 0.05$).

in the non-smoker group. No significant differences were found between the detection rates of specific bacterial species between the study groups when score 4 was used as the cutoff level (Table 4). The occurrence rates of *T. forsythensis*, *T. denticola* and *P. micros* were higher in the heavy-smoker group, but the differences with the other groups were not significant ($p > 0.05$).

Spearman' rank correlation analysis revealed no significant correlation between the smoking statuses of the women who participated in the study and any of the bacterial species investigated (Table 5). There were significant positive correlations between clinical periodontal parameters and various bacterial species. Rates of various bacterial species exhibited significant correlations with other bacterial species.

## Discussion

The present cross-sectional study was undertaken to investigate whether or not smoking during pregnancy might favour a specific subgingival periodontopathogenic microflora. To our knowledge, this is the first study investigating the relationship between smoking during pregnancy and subgingival plaque composition. Subgingival plaque samples from two different interproximal sites in each woman have been evaluated for the presence as well 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 smokers as well as the non-smokers 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.

In the present study, clinical periodontal parameters were recorded to find out the effect of smoking on the periodontium. The statistical analyses

*Table 5*. Spearman's rank correlations

| | *Smoking* | *Age* | *BOP* | *PI* | *PPD* | *Porphyromonas gingivalis* | *Prevotella intermedia* | *Prevotella nigrescens* | *Tannerella forsythensis* | *Actinobacillus actinomycetemcomitans* | *Fusobacterium nucleatum* | *Treponema denticola* | *Peptostrptococcus micros* | *Campylobacter rectus* | *Eikenella corrodens* | *Selenomas noxia* | *Streptococcus intermedius* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Smoking | | 0.004 | −0.030 | −0.072 | −0.047 | 0.056 | 0.050 | 0.043 | 0.072 | −0.053 | 0.008 | 0.008 | 0.038 | −0.076 | −0.039 | 0.024 | 0.118 |
| Age | | | −0.043 | −0.122 | 0.005 | 0.147 | 0.044 | 0.011 | 0.132 | 0.054 | 0.050 | 0.061 | −0.049 | −0.041 | 0.045 | 0.047 | 0.007 |
| BOP | | | | 0.530† | 0.236† | 0.164* | 0.069 | 0.044 | 0.047 | 0.005 | 0.022 | 0.114 | 0.049 | 0.076 | 0.050 | −0.047 | −0.101 |
| PI | | | | | 0.117 | −0.025 | 0.077 | 0.014 | −0.044 | −0.102 | −0.112 | 0.056 | −0.128 | 0.047 | −0.110 | −0.139 | −0.125 |
| PPD | | | | | | 0.352† | 0.361† | 0.185* | 0.504† | −0.024 | 0.111 | 0.249† | 0.243† | 0.382† | 0.063 | −0.015 | 0.111 |
| *P. gingivalis* | | | | | | | 0.578† | 0.429† | 0.603† | 0.310† | 0.509† | 0.348† | 0.267† | 0.376† | 0.244† | 0.145 | 0.307† |
| *P. intermedia* | | | | | | | | 0.676† | 0.533† | 0.179* | 0.506† | 0.349† | 0.335† | 0.377† | 0.300† | 0.308† | 0.314† |
| *P. nigrescens* | | | | | | | | | 0.350† | 0.157* | 0.400† | 0.411† | 0.303† | 0.232† | 0.190* | 0.167* | 0.357† |
| *T. forsythensis* | | | | | | | | | | 0.103 | 0.281† | 0.446† | 0.390† | 0.564† | 0.134 | 0.032 | 0.177* |
| *A. actinomycetemcomitans* | | | | | | | | | | | 0.394† | 0.109 | 0.208† | 0.079 | 0.569† | 0.203† | 0.334† |
| *F. nucleatum* | | | | | | | | | | | | 0.198† | 0.385† | 0.299† | 0.406† | 0.350† | 0.375† |
| *T. denticola* | | | | | | | | | | | | | 0.381† | 0.292† | 0.069 | 0.180* | 0.215† |
| *P. micros* | | | | | | | | | | | | | | 0.278† | 0.280† | 0.354† | 0.422† |
| *C. rectus* | | | | | | | | | | | | | | | 0.129 | 0.159* | 0.029 |
| *E. corrodens* | | | | | | | | | | | | | | | | 0.306† | 0.325† |
| *S. noxia* | | | | | | | | | | | | | | | | | 0.219† |
| *S. intermedius* | | | | | | | | | | | | | | | | | |

BOP, bleeding on probing; PI, plaque index; PPD, probing pocket depth.
Correlation is significant at $^{*}p<0.05$ level (2-tailed) and at $^{\dagger}p<0.01$ level (2-tailed).

comparing the clinical periodontal parameters revealed no significant differences between the study groups neither in the subgingival plaque sampling sites nor in the overall periodontal health status. However, plaque index, BOP and also PPD values were all lower in the heavy-smoker group compared with the light- and non-smokers. Although the differences between the groups were not statistically significant, the lower BOP scores observed in the heavy-smoker group might be because of the vascular changes that occur as a response to smoking. Gingival blood flow in smokers has been suggested to be less in comparison with non-smokers, which will also induce a decreased local host defence (Preber & Bergström 1985). Recently, Nair et al. (2003) have showed that BOP with a constant force probe increased from 16% of sites to 32% of sites after quitting smoking, despite improvements in the subjects' oral hygiene. Smoking is well known to make periodontal tissues appear normal by suppressing the clinical signs of inflammation (Bergström et al. 1988). The slightly lower plaque index values observed in the heavy-smoker group might have also had a role in less BOP found in this study group.

The women included in the present study population were rather young and most of them had moderate-to-severe gingivitis, while only very few had mild periodontitis. In older ages, periodontal disease is more likely to be more pronounced. Moreover, in case of periodontitis, smoking is more likely to have a detectable effect on the severity and distribution of clinical periodontal findings. Therefore, the young age range of the study population together with the presence of gingivitis rather than periodontitis might have resulted in the lack of statistically significant differences between the study groups with regard to the clinical periodontal parameters and the composition of subgingival microbiota.

The results of the present study demonstrated that, the women who smoke during their pregnancy periods exhibit more or less the same frequencies as well as the loads of the 12 bacterial species with the women who have never smoked. This finding suggests that smoking has a limited, if any, influence on the subgingival periodontopathogenic microflora. Moreover, the socioeconomic homogeneity of the study population and the lack of regular

home care as well as professional care may explain the positive detection of all the 12 bacterial species in all the plaque samples evaluated. Accordingly, many studies have failed to find significant effects of smoking on the occurrence and the loads of main periodontopathogens (Haffajee et al. 1997, Lie et al. 1998, Preber et al. 1992, 1995, Renvert et al. 1998, Stoltenberg et al. 1993, van der Velden et al. 2003). Haffajee and Socransky (2001) have evaluated up to 28 plaque samples per subject for 29 different bacterial species by DNA–DNA hybridization technique. In that study of never, past, and current smokers, the prevalence (percentage of sites colonized) but not the counts or proportions of *Eubacterium nodatum*, *F. nucleatum* ss *vincentii*, *P. intermedia*, *P. micros*, *P. nigrescens*, *T. forsythensis*, *P. gingivalis*, and *T. denticola* were found to be significantly higher in current smokers than in the other two groups. The reported differences in prevalence between smokers and non-smokers were because of greater colonization at sites with pocket depth $<4$ mm and the authors concluded that smoking does not seem to significantly affect counts of subgingival species. In a study of 145 patients of whom 83 were smokers, no difference in the counts of *A. actinomycetemcomitans, P. gingivalis, P. intermedia* were found between smokers and non-smokers (Preber et al. 1992). Furthermore, Boström et al. (2001) have found similar frequencies of 12 species investigated with DNA–DNA hybridization technique in microbial samples of smoker *versus* non-smoker moderate-to-severe periodontitis patients. In a recent retrospective study, van der Velden et al. (2003) have analysed microbial samples before and after periodontal treatment in 59 periodontitis patients. They found no difference in the prevalence of various bacteria between smokers and non-smokers before treatment, while after treatment values exhibited significant differences between the study groups.

On the other hand, van Winkelhoff et al. (2001) have found a higher prevalence of *P. intermedia/nigrescens* and higher mean counts of *P. micros* and *F. nucleatum* in untreated smoker patients with chronic periodontitis. They also had a group of treated smoker patients with chronic periodontitis and those patients were characterized by higher prevalence of *T. forsythensis*, *P. micros* and *C. rectus* and again higher mean levels of *P. micros* and *F. nucleatum*. It has been speculated from these results that smoking is a determining factor for the composition of the subgingival microflora in adult periodontitis patients. Moreover, Eggert et al. (2001) have demonstrated that smoking extends a favourable habitat for bacteria like *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* to shallow sites with a pocket depth $\leqslant 5$ mm and they have hypothesized that molecular byproducts of smoking may interfere with mechanisms that normally contain growth of damaging bacteria at the surface of the oral mucosa in gingival crevices. However, our present findings do not indicate any significant effects of smoking during pregnancy on the composition of subgingival microbiota.

Our present findings taken together with the previous reports may suggest that smoking damages host mechanisms rather than directly affecting the composition of subgingival plaque bacteria and eventually it may exaggerate gingival inflammation during pregnancy. Accordingly, Eggert et al. (2001) proposed a mechanism through which the molecular byproducts of smoking might influence progression of periodontitis via damage to cells that normally protect the periodontal environment. Therefore, smoking during pregnancy may have an indirect additive effect on the occurrence of clinical periodontal findings. It appears to be more likely that smoking during pregnancy affects host response component of the gingival inflammation rather than the microbial insult.

Changes in hormone levels during pregnancy have long been associated with the development of gingivitis and are well-documented in the literature (Laine 2002, Raber-Durlacher et al. 1994, Sooriyamoorthy & Gower 1989). Shoji et al. (2000) have speculated that pregnancy could be considered as a possible risk factor for alveolar bone loss. On the other hand, Jensen et al. (1981) have reported that the pregnant women had a much higher level of black pigmented *Bacteroides* species (now classified into *Porphyromonas* and *Prevotella* spp. including *P. intermedia*) than the non-pregnant controls. Although, the increased susceptibility of gingival tissues to inflammation in pregnancy seems to be connected to pregnancy-related hormonal changes, it is not clear by which mechanisms these hormones affect the periodontium. Besides the increased vascularity and vascular flow, changes in the immune system or changes in connective tissue metabolism are among the proposed explanations. Another possible mechanism is modification of subgingival flora by pregnancy. The proportion of *P. intermedia* has been found to increase during pregnancy, but the relation of *P. intermedia* to the clinical signs of gingival inflammation has been controversial (Jensen et al. 1981, Kornman & Loesche 1980, Muramatsu & Takaesu 1994, Raber-Durlacher et al. 1994).

The present cross-sectional study was undertaken to evaluate whether or not smoking during pregnancy might favour a specific subgingival periodontopathogenic microflora. However, the findings of the present study do not support the hypothesis that smoking during pregnancy affects the composition of subgingival plaque bacteria. It may be concluded from the findings of the present study that smoking exerts little, if any, influence on the subgingival occurrence of the 12 different species of bacteria most commonly associated with periodontal disease.

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