J Clin Periodontol 2007; 34: 639–645 doi: 10.1111/j.1600-051X.2007.01105.x

Journal of Clinical Periodontology

Periodontal disease increases the risk of severe pre-eclampsia among pregnant women

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Canakci V, Canakci CF, Yildirim A, Ingec M, Eltas A, Erturk A. Periodontal disease increases the risk of severe pre-eclampsia among pregnant women. J Clin Periodontol 2007; 34: 639–645. doi: 10.1111/j.1600-051X.2007.01105.x.

Abstract

Aim: To evaluate the possible link between the severity of periodontal disease and pre-eclampsia and to correlate this link to clinical periodontal parameters and interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), and prostaglandins (PGE_2) levels in both gingival crevicular fluid (GCF) and serum.

Material and Methods: Fifty-nine pregnant women (20 mild pre-eclampsia, 18 severe pre-eclampsia, and 21 healthy pregnant women) were included in the study. Dental and periodontal recordings as well as GCF and blood samples were obtained within 48 h preceding delivery.

Results: The results of multivariate logistic regression showed a highly significant association between mild to severe pre-eclampsia and severe periodontal disease ($p<0.001$). After adjusting for potential confounders (smoking, body weight, socioeconomic status, education level, and age), severe pre-eclamptic women were 3.78 (1.77–12.74) times more likely to present severe periodontal disease than normotensive pregnant women. This odds ratio (OR) was 2.43 (1.13–8.19) for mild pre-eclamptic women. IL-1β, TNF-α, and PGE_2 levels in both serum and GCF were also significantly higher in the pre-eclamptic groups than the normotensive women.

Conclusions: These results indicate that the presence and severity of periodontal disease seems to increase the risk for not only the occurrence but also the severity of pre-eclampsia in pregnant women.

Key words: mild pre-eclampsia; periodontal diseases; pro-inflammatory cytokines; prostaglandins; severe pre-eclampsia

Accepted for publication 12 May 2007

Pre-eclampsia is a threatening condition for both the mother and the foetus (Carreiras et al. 2002). It is a common obstetric syndrome affecting about 7–10% of pregnant women, and remains as one of the two most common causes of maternal mortality in developed countries (Darmochwal-Kolarz et al. 2002, Von Dadelszen & Magee 2002). Despite intense efforts to find mechanisms and molecules that induce pre-eclampsia, no specific aetiological factor has been identified so far. The known risk factors for pre-eclampsia include primiparity, multigravidity, obesity, renal disease, uterine malformation, foetal hydrops, elevated serum lipid ratio, non-smoking, no pre-natal care, and diabetes (Erkkola 1997, Odegard et al. 2000, Riche et al. 2002).

Periodontal diseases are a group of infectious diseases caused by predominantly Gram-negative, anaerobic, and microaerophilic bacteria that induce local and systemic elevations of pro-inflammatory prostaglandins (PGE_2) and cytokines (Page 1991, Page & Kornman 1997). Numerous studies suggest that periodontal disease, as a source of subclinical and persistent infection, may induce systemic inflammatory responses that increase the risk of adverse pregnancy outcomes (Offenbacher et al. 1996, Bobetsis et al. 2006, Shub et al. 2006, Xiong et al. 2006). Adverse pregnancy outcomes that have been linked to periodontal disease include pre-term birth, low birth weight, miscarriage or early pregnancy loss, and pre-eclampsia (Offenbacher et al. 1996, Boggess et al. 2003, Canakci et al. 2004, Buduneli et al. 2005, Bobetsis et al. 2006, Xiong et al. 2006).

Recently, pre-eclampsia has been proposed to be a syndrome caused by an excessive systemic inflammatory response to pregnancy. Recent studies suggest that maternal periodontal

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
This study was supported by a grant (PN-2005/105) from Atatürk University, Erzurum, Turkey.

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disease is associated with an increased risk for development of pre-eclampsia (Boggess et al. 2003, Canakci et al. 2004, Oettinger-Barak et al. 2005, Contreras et al. 2006, Cota et al. 2006, Kunnen et al. 2007). Periodontal disease may burden pregnant women systemically with endotoxin, inflammatory cytokines, and oxidative stressors at the maternal–foetal interface (Contreras et al. 2006). Thus, it may be a vascular stressor that plays a role in the development of pre-eclampsia in pregnant women. Furthermore, there is ample evidence that periodontal bacteria frequently enter the circulation (Beck et al. 1996). Infected periodontium can also be regarded as a reservoir for both microbial products and inflammatory mediators like PGE_2, interleukins (ILs), and other cytokines. Local PGE_2 and both local and systemic tumour necrosis factor-α (TNF-α) levels were increased in periodontitis (Moss et al. 1995). Therefore, understanding the initiating aetiologic factor may help to design preventive and therapeutic strategies properly.

Alterations in major inflammatory mediator levels in gingival crevicular fluid (GCF) and serum may at least partly play a role in the interactions of these two diseases. To our knowledge, there is no published study on GCF and serum levels of pro-inflammatory mediators in pre-eclamptic women. Furthermore, Roberts (2001) emphasised the importance of assessing the level of severity of pre-eclampsia which has not been addressed in previous studies. Therefore, the aim of this study was to evaluate the possible link between severity of periodontal disease and pre-eclampsia, and to correlate this link to clinical periodontal parameters and IL-1β, TNF-α, and PGE_2 levels in both GCF and serum.

Material and Methods

Study population

Fifty-nine pregnant women (20 mild pre-eclampsia, 18 severe pre-eclampsia, and 21 healthy, normotensive pregnant women) were admitted to the Obstetrics Department of School of Medicine, Atatürk University. According to the American College of Obstetricians and Gynecologists recommendations (2000), pre-eclampsia was defined as a persisting elevated diastolic blood pressure ($\geqslant$90 mm Hg), a proteinuria ($>$300 mg in a 24-h urine sample), and the presence of oedema. Mild pre-eclampsia was diagnosed if a blood pressure of 140/90 mm Hg was observed at least on two occasions 6 h apart, with or without proteinuria. Severe pre-eclampsia was diagnosed when the following criteria were present: (1) a systolic blood pressure of $\geqslant$160 mm Hg or a diastolic blood pressure of $\geqslant$110 mm Hg on two occasions at least 6 h apart, with the patients resting in bed and (2) a proteinuria of $\geqslant$5 g in a 24-h urine collection or of $\geqslant$3+ on dipstick in at least two random clean-catch samples at least 4 h apart. Inclusion criteria were pre-eclampsia; normal response to glucose tolerance testing; no evidence of recent infections such as rubella, toxoplasma, hepatitis B or C, cytomegalovirus, or syphilis; absence of uterine contractions; non-smoker status; singleton pregnancy; gestational age corroborated by ultrasonography before the 20th week of gestation; and no foetal structural anomaly.

Age, marital status, prenatal care, and medical histories pertaining to the exclusion criteria were obtained from the hospital records. Education level of the mother, household income, and information about smoking (smoker, who smokes at least three cigarettes/day) and alcohol use were obtained through a personal interview conducted by a trained physician at the first prenatal visit. Furthermore, pregnant women requiring antibiotic prophylaxis for dental treatment, taking any medication that may influence sex steroid metabolism, and a history of periodontal treatment within the last 6 months were excluded from the study. The study protocol was reviewed and approved by the Ethics Committee of Atatürk University, and a written informed consent was obtained from each pregnant woman before their enrolment into the study.

Clinical examination

The number of restorations and carious lesions, as well as clinical periodontal measurements were recorded on all teeth present excluding the third molars within 48 h preceding delivery. All examinations were performed by two trained and pre-calibrated examiners (periodontists) who were not aware of the interview information. Intra-examiner variability in using the dental examination criteria was tested by performing duplicate examinations on 12 randomly selected mothers on consecutive days. The corresponding percentages of agreement were 87% for probing depth and bleeding and 92% for clinical attachment level (CAL). Pocket depth (PD) and CAL, bleeding on probing (BOP) (positive if bleeding occurs within 15 s after probing) were measured at six sites/tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) with a Williams' probe with Michigan markings (Hu-Friedy, Chicago, MI, USA). Dental plaque was scored as being present or absent at four points (mesial, buccal, lingual, and distal) on each tooth.

The periodontal condition was further stratified by severity according to the criteria used by Boggess et al. (2003). Periodontal health was defined as the absence of PD $\geqslant$4 mm. Mild periodontal disease was defined as one to 15 tooth sites with $\geqslant$4 mm PD and BOP. Severe periodontal disease was defined as $\geqslant$15 tooth sites with $\geqslant$4 mm PD and BOP.

Serum samples

Blood samples from 59 pregnant women were collected in vacutainer tubes without an additive (for cytokine assay) or in tubes containing indomethacin (10 μM final concentration, for PGE_2 assay). Indomethacin prevents ex vivo formation of eicosanoids. After clotting, they were centrifuged at 3500 g for 5 min. to separate the serum. Serum aliquots were stored at -80°C until laboratory analysis.

GCF samples

GCF samples were collected from a mesio-buccal and disto-palatal site on each of three teeth in each quadrant of women (molar, pre-molar, canine/incisor). The samples were obtained before clinical measurements and between 08:00 and 10:00 hours in the morning. The area was isolated with cotton rolls to eliminate saliva contamination and slightly air-dried. The samples were obtained within 30 s with Periopaper strips (Proflow Inc., Amityville, NY, USA) using the orifice method, and volume was measured on a pre-calibrated Periotron® 8000 (Oroflow Inc., Plainview, NY, USA). The sampling was performed before periodontal probing and clinical measurement. Care was taken to avoid mechanical injury. Strips contamined with blood were discarded. Twenty-four strips were used for each subject. Paper strips that absorbed GCF were placed in microcentrifuge tubes containing phosphate-buffered saline

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(PBS) (pH 7). Tubes were vortexed and centrifuged at $1500 \times g$ for 10 min. The supernatants were transferred into eppendorf tubes and stored at $-80°C$ until analysis. Samples were thawed and assayed immediately to ensure minimal deterioration, and samples of each patient were assayed at the same time as the matched control samples.

Laboratory analysis

IL-1β and TNF-α levels in serum and GCF samples were assayed by commercial ELISA kits (Biosource International, Camarillo, CA, USA) according to the manufacturer's instructions. Specimens were thawed and assayed immediately to ensure minimal deterioration, and each patient's samples were assayed at the same time as the matched control samples. The standards ranged from 0 to 250 pg/ml human IL-1β and from 0 to 1000 pg/ml human TNF-α. The lower limits of detection were 1 and 1.7 pg/ml for IL-1β and TNF-α, respectively. The intra- and inter-assay coefficients of variations were 4.4% and 6.7%, respectively, for IL-1β and 4.4% and 7.5%, respectively, for TNF-α.

PGE_2 levels were measured using an EIA kit from Cayman Chemical Co. (Ann Arbor, MI, USA) according to the manufacturer's directions. This method is based on the competition between PGE_2 and a PGE_2-acetylcholinesterase (AChE) conjugate (PGE_2 tracer) for a limited amount of PGE_2 monoclonal antibody. Briefly, all specimens were thawed just before the assay. Serum, saliva, and GCF samples were diluted with EIA buffer 1:50, 1:10, and 1:5, respectively. Samples were incubated 18 h at 4°C in polyclonal anti-mouse IgG-coated tubes with the PGE_2 AChE tracer and PGE_2 monoclonal antibody. After incubation, all wells were washed five times in washing buffer, following which Ellman's Reagent was added to each well. The standards ranged from 7.8 to 1000 pg/ml PGE_2 and the detection limit (80% B/B_0) was 15 pg/ml. The results were calculated using a four-parameter logistic curve fit and expressed as picogram per millilitre.

All absorbance values were read in an ELISA plate reader and the concentration of the samples was automatically calculated by software (Power Wave XS; BIO-TEK Instrument, Inc., KC Junior software). All samples were run in duplicate, and the mean values were used for statistical analysis.

Statistical analysis

SPSS for Windows (version 11.0) was used for statistical evaluation of the present data. The normality of data distribution was examined using the Shapiro–Wilk test. The differences between the three groups of normally distributed variables were assessed using one-way ANOVA and Tukey's multiple comparison tests. The Kruskall–Wallis one-way analysis of variance, followed by the Mann–Whitney U test with Bonferroni's correction was used to evaluate the differences between the groups in other variables (non-normal distribution). Correlations between clinical and biochemical parameters were determined by Spearman's correlation analysis. Multivariate logistic regression analysis was used to determine the association between periodontal disease and pre-eclampsia. From the logistic-regression analysis, odds ratios (ORs) were calculated with a 95% confidence interval (CI). A value of $p<0.05$ was considered to be significant.

Results

Demographic and pregnancy-related characteristics of the study groups are shown in Table 1. There were no significant differences in the mean age, gravidity, parity, pre-natal care, and education level between the groups ($p>0.05$). None of the pregnant women drank alcohol and all were married. There were significant differences in the gestational age, household income, and smoking between the pre-eclamptic and normotensive women ($p<0.05$ for others, $p<0.01$ for income). Birth weight showed significant differences between the three groups; the difference was significant between mild and severe pre-eclamptic groups ($p<0.01$) and between normotensive and mild pre-eclamptic groups ($p<0.01$), and was more significant between normotensive and severe pre-eclamptic groups ($p<0.001$). Systolic and diastolic blood pressures at delivery were significantly higher in the severe ($p<0.001$) and mild pre-eclamptic women ($p<0.01$) than in the normotensive women. The difference in systolic and diastolic blood pressures at delivery was also significantly different between the mild and severe pre-eclamptic women ($p<0.01$).

Table 2 displays the clinical dental and periodontal variables in the study groups. The number of teeth present and the number of restorations in the three groups were similar ($p>0.05$), but the number of decayed tooth were higher in the severe pre-eclamptic women ($p<0.05$). Mean PD, percentage of sites with PD $\geqslant$4 mm, and percentage of sites exhibiting BOP were significantly higher in the severe pre-eclamptic women than the mild pre-eclamptic and normotensive groups ($p<0.01$ and 0.001, respectively). The pre-eclamptic women exhibited higher CAL and percentage of sites with CAL $\geqslant$4 mm than the normotensive group ($p<0.01$). No significant difference in percentage of sites with plaque was found between the study groups ($p>0.05$).

Severe periodontal disease was found in 13 of the 18 (72.2%) severe pre-eclamptic

Table 1. Demographic and pregnancy-related characteristics of study groups (pre-eclamptic and normotensive)

Characteristic	Severe pre-eclampsia ($n=18$)	Mild pre-eclampsia ($n=20$)	Normotensive controls ($n=21$)
Age (year)	23.6 (4.2)	24.1 (3.9)	24.7 (4.5)
Gravidity	2.1 (1.3)	2.5 (1.2)	2.7 (1.2)
Parity	1.1 (0.9)	1.2 (0.8)	1.4 (0.9)
Education (years)	8.1 (3.1)	8.5 (2.9)	9.8 (3.3)
Household income ($)	307.1 (82.2)**	378.5 (73.1)**	489.4 (67.1)
Smoking (%) (yes)	2 (11.1%)*	2 (10.0%)*	6 (28.6%)
Prenatal care (%) (yes)	5 (27.8%)	6 (30.0%)	6 (28.6%)
Gestation age (week)	33.2 (1.8)*	34.5 (1.6)*	37.9 (2.1)
Body weight (kg)	78.0 (9.4)*	77.4 (8.1)*	69.3 (7.6)
SBP (mm Hg)	172.2 (11.3)***††	156.0 (7.7)**	105.0 (7.8)
DBP (mm Hg)	107.4 (12.6)***†	97.6 (7.5)***	63.3 (7.2)
Birth weight (g)	2021.4 (404.9)***††	2395.7 (382.4)**	3219.1 (477.5)

Values are expressed as means (SD) or number (%) unless otherwise stated.
$^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, significantly different than the normotensive women. $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$, significantly different than the mild pre-eclamptic women.
SBP, systolic blood pressure; DBP, diastolic blood pressure.

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Table 2. Clinical dental and periodontal variables in pre-eclamptic and normotensive pregnant women

Variable	Severe pre-eclampsia ($n = 18$)	Mild pre-eclampsia ($n = 20$)	Normotensive controls ($n = 21$)
Dental parameters			
Number of teeth	22.7 (5.9)	24.4 (6.8)	25.2 (6.7)
Decayed	5.6 (2.7)*	4.0 (3.2)	3.1 (2.1)
Restored	6.1 (4.9)	7.4 (5.3)	7.2 (7.1)
Periodontal parameters			
Mean PD (mm)	3.7 (0.5)***††	3.1 (0.4)**	2.6 (0.3)
Mean CAL (mm)	3.9 (0.5)**	3.6 (0.5)**	2.8 (0.5)
% of sites			
PD $\geqslant$4 mm	29.4 (9.2)***†	20.9 (8.1)**	14.7 (8.6)
CAL $\geqslant$4 mm	34.1 (12.4)**	31.9 (10.3)**	24.8 (11.3)
% sites with plaque	68.6 (37.2)	69.9 (30.8)	61.7 (22.6)
% sites exhibiting BOP	57.9 (13.6)***†	40.7 (11.5)**	22.4 (9.6)
SPD (yes) (n and %)	13 (72.2%)***†	10 (50.0%)**	7 (33.3%)

Values are expressed as means (SD) or number (%) unless otherwise stated.
*$p<0.05$, **$p<0.01$ and ***$p<0.001$, significantly different than the normotensive women. †$p<0.05$ and ††$p<0.01$, significantly different than the mild pre-eclamptic women.
SPD, severe periodontal disease, PD, periodontal pocket depth, CAL, clinical attachment level, BOP, bleeding on probing.

Table 3. Serum and GCF levels of IL-1β, TNF-α and PGE_2 in pre-eclamptic and normotensive (control) women

Variable	Severe pre-eclampsia ($n = 18$)	Mild pre-eclampsia ($n = 20$)	Normotensive control ($n = 21$)
GCF (pg/ml)			
IL-1β	7.2 (2.1)**	6.7 (1.5)**	4.1 (1.3)
TNF-α	9.6 (3.2)***††	6 .4 (2.2)**	3.7 (1.6)
PGE_2	76.1 (17.1)**†	57.4 (19.3)*	39.2 (16.8)
Serum (pg/ml)			
IL-1β	1.6 (0.5)**	1.4 (0.4)**	0.6 (0.3)
TNF-α	74 (9.1)***††	56.5 (8.5)**	32.8 (6.3)
PGE_2	90 (16.2)**	84.9 (14.6)**	57.9 (13.1)

Values are expressed as means (SD) or number (%) unless otherwise stated.
*$p<0.05$, **$p<0.01$ and ***$p<0.001$, significantly different than the normotensive women. †$p<0.05$ and ††$p<0.01$, significantly different than the mild pre-eclamptic women.
GCF, gingival crevicular fluid; PGE_2, prostaglandins; TNF-α, tumour necrosis factor; IL-1β, interleukin-1β.

women and in 10 of the 20 (50.0%) mild pre-eclamptic women and in seven of the 21 (33.3%) normotensive pregnant women. Mild periodontal disease was found in three of the 18 (16.7%) severe pre-eclamptic women and in five of the 20 (25.0%) mild pre-eclamptic women and in six of the 21 (28.6%) normotensive pregnant women. Two women (11.1%) in the severe pre-eclamptic group, five women (25.0%) in the mild pre-eclamptic group, and eight women (38.1%) in the normotensive group presented with a clinically healthy periodontium.

The results of multivariate logistic regression analysis showed a highly significant association between mild to severe pre-eclampsia and severe periodontal disease ($p<0.001$). After adjusting for potential confounders (smoking, body weight, socioeconomic status, education level, and age), severe pre-eclamptic women were 3.78 (1.77–12.74) times more likely to present a severe periodontal disease than normotensive pregnant women. This OR for mild pre-eclamptic women was 2.43 (1.13–8.19).

The IL-1β, TNF-α, and PGE_2 levels in serum and GCF samples of the study groups are presented in Table 3. Serum and GCF TNF-α, and GCF PGE_2 levels were significantly higher in the severe pre-eclamptic women than in the mild pre-eclamptic women ($p<0.05$ and 0.01, respectively). GCF and serum IL-1β and serum PGE_2 levels were similar in the two pre-eclamptic groups ($p>0.05$). All GCF and serum IL-1β, TNF-α, and PGE_2 values were significantly higher in pre-eclamptic groups than those in the normotensive group ($p<0.01$ and 0.001).

Correlations

Correlations between clinical periodontal parameters and biochemical data are shown in Table 4. The two pre-eclamptic groups exhibited several significant correlations: GCF IL-1β level was positively correlated with the number of sites with CAL $\geqslant$4 mm ($p<0.05$), PD ($p<0.05$), and BOP ($p<0.01$), whereas serum IL-1β and TNF-α levels were correlated with BOP ($p<0.05$ and 0.01, respectively), and GCF TNF-α level was correlated with BOP and PD ($p<0.05$ and 0.01, respectively). GCF PGE_2 level was correlated with the number of sites with CAL $\geqslant$4 mm, PD and BOP ($p<0.05$), serum PGE_2 level was positively correlated with PD ($p<0.05$), IL-1β, TNF-α, and PGE_2 levels in both serum and GCF (except serum PGE_2 level) were negatively correlated with birth weight ($p<0.05$ for other and $p<0.01$ for GCF TNF-α).

Significant correlations between GCF levels of the investigated pro-inflammatory mediators in the two pre-eclamptic groups are given in Table 5. GCF IL-1β was positively correlated with GCF TNF-α, GCF PGE_2 and serum IL-1β ($p<0.01$ for other and $p<0.05$ for GCF PGE_2). GCF TNF-α was positively correlated with GCF PGE_2, serum TNF-α, and serum IL-1β ($p<0.05$ for other and $p<0.01$ for serum TNF-α). A positive correlation was also seen between GCF PGE_2 and serum PGE_2 ($p<0.01$). Serum IL-1β was significantly correlated with serum TNF-α ($p<0.05$).

Discussion

The findings of the present study provide further support for the hypothesis that periodontitis measured by the presence of PD $\geqslant$4 mm, CAL $\geqslant$3 mm, and increased percentage of sites with BOP is associated with an increased risk for pre-eclampsia, which is in agreement with previous studies (Boggess et al. 2003, Canakci et al. 2004, Oettinger-Barak et al. 2005, Contreras et al. 2006, Cota et al. 2006). Severe pre-eclamptic women were 3.78 times more likely to present severe periodontal disease than normotensive pregnant women. This OR for mild pre-eclamptic women was

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Table 4. Spearman correlation coefficient (*r*) values between various periodontal parameters and birth weight and mean IL-1β, TNF-α and PGE_2 levels in GCF and serum of pre-eclamptic pregnant women

Variable	IL-1β		TNF-α		PGE_2	
	serum	GCF	serum	GCF	serum	GCF
CAL $\geqslant$4 mm	0.066	0.318*	0.151	0.107	− 0.072	0.273*
PD	0.117	0.311*	0.042	0.418**	0.320*	0.244*
BOP	0.256*	0.389**	0.454**	0.397*	0.132	0.255*
PI	0.016	0.031	− 0.023	0.121	− 0.014	− 0.077
Birth weight (g)	− 0.269*	− 0.301*	− 0.351*	− 0.376**	− 0.159	− 0.277*

*$p<0.05$ and **$p<0.01$, significant correlation.
PD, periodontal pocket depth; CAL, clinical attachment level; BOP, bleeding on probing; PI, plaque; GCF, gingival crevicular fluid; PGE_2, prostaglandins; TNF-α, tumour necrosis factor; IL-1β, interleukin-1β.

Table 5. Spearman correlation coefficient (*r*) values between GCF and serum IL-1β, TNF-α and PGE_2 levels in pre-eclamptic pregnant women

	GCF IL-1β	GCF TNF-α	GCF PGE_2	Serum IL-1β	Serum TNF-α	Serum PGE_2
GCF IL-1β	–					
GCF TNF-α	0.374**	–				
GCF PGE_2	0.318*	0.287*	–			
Serum IL-1β	0.411**	0.322*	0.112	–		
Serum TNF-α	0.077	0.407**	0.047	0.293*	–	
Serum PGE_2	0.104	− 0.054	0.368**	0.023	0.031	–

*$p<0.05$ and **$p<0.01$, significant correlation.
GCF, gingival crevicular fluid; PGE_2, prostaglandins; TNF-α, tumour necrosis factor; IL-1β, interleukin-1β.

2.43. The observation that PD and BOP were greater in the severe pre-eclamptic group as compared with the mild pre-eclamptic and normotensive group, confirms that severe periodontitis might be associated with severe pre-eclampsia. The presence and severity of periodontal disease seems to increase the risk for not only the occurrence but also the severity of pre-eclampsia in pregnant women.

One of the interesting findings in this study was the association between the number of decayed surfaces and severe pre-eclampsia. This finding is consistent with the study of Khader et al. (2006) whereas it was not the case in our previous study (Canakci et al. 2004). Stratifying the severity of pre-eclampsia in the present study and/or differences in sampling methods may have played a role in these conflicting findings.

In the present study, both serum and GCF levels of IL-1β, TNF-α, and PGE_2 were significantly higher in the pre-eclamptic groups than the normotensive women, with several statistically significant correlations between biochemical and clinical periodontal parameters. Numerous studies reported significantly higher serum levels of IL-1β, TNF-α, and PGE_2 in pre-eclamptic than normotensive women (Kupferminc et al. 1994, Vince et al. 1995, Ding et al. 1997, Ellis et al. 2001, Teran et al. 2001, Kocyigit et al. 2004). The present findings provide further support for the hypothesis that pre-eclampsia is associated with inflammation manifested with increased serum levels of pro-inflammatory mediators. Recently, Oettinger-Barak et al. (2005) reported significantly higher GCF levels of IL-1β, TNF-α, and PGE_2 in pre-eclamptic than normotensive women and our findings are in agreement with theirs. Furthermore, the present findings suggest that serum and GCF TNF-α, as well as GCF PGE_2 levels of pre-eclamptic women were influenced by the severity of pre-eclampsia. A plausible explanation of such an association might be hyperproduction of pro-inflammatory cytokines by severe pre-eclamptic women. The present observation of significant correlations between GCF and serum levels of pro-inflammatory mediators and clinical periodontal parameters is in agreement with the findings of Oettinger-Barak et al. (2005).

Gestational age at birth was less in pre-eclamptic women than in normotensive pregnant women. Birth weight was significantly lower in the pre-eclampsia groups (mean 2021.4 g for severe pre-eclamptic women and mean 2395.7 for mild pre-eclamptic women) than in the normotensive group (3219.1 g). Moreover, serum and GCF levels of pro-inflammatory cytokines were negatively correlated with birth weight, which is consistent with previous reports (Offenbacher et al. 2001, Bobetsis et al. 2006).

It was interesting to observe positive correlations between clinical periodontal parameters (especially BOP and PD) and GCF and serum pro-inflammatory cytokine levels in pre-eclamptic pregnant women.

Periodontal disease has been suggested to be associated with an increased risk for pre-eclampsia but, possible mechanisms have not been clarified. However, these data should be interpreted with caution, as the aetiology of both periodontal disease and pre-eclampsia is probably multifactorial and have common risk factors. A number of studies have reported that infection may be important in the pathogenesis of pre-eclampsia (Trogstad et al. 2001, Von Dadelszen & Magee 2002) and periodontal disease (Genco et al. 1988, Iacopino & Cutler 2000). It has been well demonstrated that cytokines are considered to play a key role in the inflammation process (Offenbacher et al. 1986, Genco et al. 2002, Kim & Amar 2006).

GCF levels of IL-1β, TNF-α, and PGE_2 are closely associated with the severity of gingival inflammation and/or periodontal tissue destruction (Page 1991, Page & Kornman 1997, Genco et al. 2002). Pro-inflammatory cytokines and PGE_2 may also be involved in the mechanism of periodontal-induced pregnancy complications. Cytokines such as IL-1β, IL-6, and TNF-α, together with PGE_2, are produced locally within the periodontal pocket and move into GCF. Owing to its high vascularity, the periodontium may act as a potential source of systemic inflammatory mediators (Shub et al. 2006). Moreover, Gorska et al. (2003) showed that the concentrations of IL-1β, IL-2, IFN-λ, and TNF-α were significantly higher in the serum samples of subjects with periodontitis than in healthy controls. It is possible that effects of inflammatory mediators from the periodontal reservoir on the fetoplacental unit

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(Boggess et al. 2005). It has been hypothesized that periodontal disease generates an inflammatory reaction leading to elevated systemic levels of cytokines, such as TNF-α, PGE_2, IL-1β, and IL-8 (Scannapieco 2004). This host response to a long-term exposure of periodontal pathogens may provoke systemic maternal and placental pro-inflammatory endothelial activation and dysfunction, which represents a significant risk factor for diseases of vascular origin, such as pre-eclampsia (Roberts 1998, Dekker & Sibai 1999, Redman et al. 1999, Blaauw et al. 2005).

The present study was undertaken to further investigate whether there is an association between periodontal disease and pre-eclampsia. In agreement with our previous study, the present study suggests that increased levels of IL-1β, TNF-α, and PGE_2 in GCF may be associated with pre-eclampsia. In conclusion, elevated pro-inflammatory cytokines present, in part, a plausible explanation for the increased severity of periodontal disease in patients with pre-eclampsia. Periodontal disease is not only associated with increased risk and severity of pre-eclampsia, but GCF markers of inflammation are associated with increased serum levels with worsening pre-eclamptic state. If ongoing studies show that treatment of periodontal infections substantially reduces the risk of pre-eclampsia as is the case with other adverse pregnancy outcomes, then periodontal therapy may be considered a vital part of prenatal care in pre-eclamptic women.

References

American College of Obstetricians and Gynecologists (2000) National high blood pressure education program working group on high blood pressure in pregnancy. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. *American Journal of Obstetrics and Gynecology* **183** (Suppl.), S1–22.

Beck, J., Garcia, R., Heiss, G., Vokonas, P. S. & Offenbacher, S. (1996) Periodontal disease and cardiovascular disease. *Journal of Periodontology* **67**, 1123–1137.

Blaauw, J., Graaff, R., van Pampus, M. G., van Doormaal, J. J., Smit, A. J., Rakhorst, G. & Aarnoudse, J. G. (2005) Abnormal endothelium- dependent microvascular reactivity in recently preeclamptic women. *Obstetrics and Gynecology* **105**, 626–632.

Bobetsis, Y. A., Barros, S. P. & Offenbacher, S. (2006) Exploring the relationship between periodontal disease and pregnancy complications. *Journal of the American Dental Association* **137** (Suppl. 10), 7S–13S.

Boggess, K. A., Lieff, S., Murtha, A. P., Moss, K., Beck, J. & Offenbacher, S. (2003) Maternal periodontal disease is associated with an increased risk for preeclampsia. *Obstetrics and Gynecology* **101**, 227–231.

Boggess, K. A., Moss, K., Madianos, P., Murtha, A. P., Beck, J. & Offenbacher, S. (2005) Fetal immune response to oral pathogens and risk of preterm birth. *American Journal of Obstetrics and Gynecology* **193**, 1121–1126.

Buduneli, N., Baylas, H., Buduneli, E., Türkoğlu, O., Köse, T. & Dahlen, G. (2005) Periodontal infections and pre-term low birth weight: a case-control study. *Journal of Clinical Periodontology* **32**, 174–181.

Canakci, V., Canakci, C. F., Canakci, H., Canakci, E., Cicek, Y., Ingec, M., Ozgoz, M., Demir, T., Dilsiz, A. & Yagiz, H. (2004) Periodontal disease as a risk factor for pre-eclampsia: a case–control study. *Australian and New Zealand Journal of Obstetrics and Gynaecology* **44**, 568–573.

Carreiras, M., Montagnani, S. & Layrisse, Z. (2002) Preeclampsia: a multifactorial disease resulting from the interaction of the feto-maternal HLA genotype and HCMV infection. *American Journal of Reproductive Immunology* **48**, 176–183.

Contreras, A., Herrera, J. A., Soto, J. E., Arce, R. M., Jaramillo, A. & Botero, J. E. (2006) Periodontitis is associated with preeclampsia in pregnant women. *Journal of Periodontology* **77**, 182–188.

Cota, L. O. M., Guimaraes, A. N., Costa, J. E., Lorentz, T. C. M. & Costa, F. O. (2006) Association between maternal periodontitis and an increased risk of preeclampsia. *Journal of Periodontology* **77**, 2063–2069.

Darmochwal-Kolarz, D., Rolinski, J., Leszczynska-Gorzelak, B. & Oleszczuk, J. (2002) The expressions of intracellular cytokines in the lymphocytes of preeclamptic patients. *American Journal of Reproductive Immunology* **48**, 381–386.

Dekker, G. A. & Sibai, B. M. (1999) The mmunology of preeclampsia. *Seminars in Perinatology* **23**, 24–33.

Ding, Z., Rowe, J., Sinosich, M. J., Saunders, DM., Hawkins, T. & Gallery, E. D. M. (1997) Serum from women with preeclampsia partially corrects the abnormal in vitro prostacyclin secretion of preeclamptic villous cytotrophoblasts but not that of prostaglandin E 2 or endothelin-1. *American Journal of Obstetrics and Gynecology* **177**, 1491–1495.

Ellis, J., Wennerholm, U.-B., Bengtsson, A., Lilja, H., Pettersson, A., Sultan, B., Wennergren, M. & Hagberg, H. (2001) Levels of dimethylarginines and cytokines in mild and severe preeclampsia. *Acta Obstetricia Et Gynecologica Scandinavica* **80**, 602–608.

Erkkola, R. (1997) Can pre-eclampsia be predicted and prevented? *Acta Obstetricia et Gynecologica Scandinavica* **76** (Suppl. 164), 98–100.

Genco, J. R., Offenbacher, S. & Beck, J. D. (2002) Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *Journal of the American Dental Association* **133** (Suppl.), 14S–22S.

Genco, R. J., Zambon, J. J. & Christersson, L. A. (1988) The origin of periodontal infections. *Advances in Dental Research* **2**, 245–259.

Gorska, R., Gregorek, H., Kowalski, J., Laskus-Perendyk, A., Syczewska, M. & Madalinski, K. (2003) Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. *Journal of Clinical Periodontology* **30**, 1046–1052.

Iacopino, A. M. & Cutler, C. W. (2000) Pathophysiological relationships between periodontitis and systemic disease: recent concepts involving serum lipits. *Journal of Periodontolology* **71**, 1375–1384.

Khader, Y. S., Jibreal, M., Al-Omiri, M. & Amarin, Z. (2006) Lack of association between periodontal parameters and preeclampsia. *Journal of Periodontolology* **77**, 1681–1687.

Kim, J. & Amar, S. (2006) Periodontal disease and systemic conditions: a bidirectional relationship. *Odontology* **94**, 10–21.

Kocyigit, Y., Atamer, Y., Atamer, A., Tuzcu, A. & Akkus, Z. (2004) Changes in serum levels of leptin, cytokines and lipoprotein in pre-eclamptic and normotensive pregnant women. *Gynecological Endocrinology* **19**, 267–273.

Kunnen, A., Blaauw, J., van Doormaal, J. J., van Pampus, M. G., van der Schans, C. P., Aarnoudse, J. G., van Winkelhoff, A. J. & Abbas, F. (2007) Women with a recent history of earlyonset pre-eclampsia have a worse periodontal condition. *Journal of Clinical Periodontology* **34**, 202–207.

Kupferminc, M. J., Peaceman, A. M., Wigton, T. R., Tamura, R. K., Rehnberg, K. A. & Socol, M. L. (1994) Immunoreactive tumor necrosis factor-alpha is elevated in maternal plasma but undetected in amniotic fluid in the second trimester. *American Journal of Obstetrics and Gynecology* **171**, 976–979.

Moss, M., Beck, J., Genco, R., Salvi, G. & Offenbacher, S. (1995) Progressing periodontitis is associated with increased serum tumor necrosis factor alpha (TNF-a). *Journal of Dental Research* **74** (Special Issue), 158 (Abstr. 1172).

Odegard, R. A., Nilsen, S. T. & Austgulen, R. (2000) Preeclampsia and fetal growth. *Obstetrics and Gynecology* **96**, 950–955.

Oettinger-Barak, O., Barak, S., Ohel, G., Oettinger, M., Kreutzer, H., Peled, M. & Machtei, E. E. (2005) Severe pregnancy complication (preeclampsia) is associated with greater periodontal destruction. *Journal of Periodontology* **76**, 134–137.

Offenbacher, S., Katz, V., Fertik, G., Collins, J., Boyd, D., Maynor, G., McKaig, R. & Beck, J. (1996) Periodontal infection as a possible risk factor for preterm low birth weight. *Journal of Periodontology* **67**, 1103–1113.

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Offenbacher, S., Lieff, S., Boggess, K. A., Murtha, A. P., Madianos, P. N., Champagne, C. M. E., McKaig, R. G., Jared, H. L., Mauriello, S. M., Auten, R. L. Jr., Herbert, W. N. P. & Beck, J. D. (2001) Maternal periodontitis and prematurity. Part I: obstetric outcome of prematurity and growth restriction. *Annals of Periodontology* **6**, 164–174.

Offenbacher, S., Odle, B. M. & Van Dyke, T.E (1986) The use of crevicular fluid PGE_2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Page, R. C. (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. *Journal of Periodontal Research* **26**, 230–242.

Page, R. C. & Kornman, K. S. (1997) The pathogenesis of human periodontitis. *Periodontology 2000* **14**, 9–11.

Redman, C. W., Sacks, G. P. & Sargent, I. L. (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. *American Journal of Obstetrics and Gynecology* **180**, 499–506.

Riche, E. L., Boggess, K. A., Lieff, S., Murtha, A. P., Auten, R. L., Beck, J. D. & Offenbacher, S. (2002) Periodontal disease increases the risk of preterm delivery among preeclamptic women. *Annals of Periodontology* **7**, 95–101.

Roberts, J. M. (1998) Endothelial dysfunction in preeclampsia. *Seminars in Reproductive Endocrinology* **16**, 5–15.

Roberts, J. M. (2001) Preeclampsia: is there value in assesing before clinically evident disease? *Obstetrics and Gynecology* **98**, 596–599.

Scannapieco, F. A. (2004) Periodontal inflammation: from gingivitis to systemic disease? *Compendium of Continuing Education in Dentistry* **25**, 16–25.

Shub, A., Swain, J. R. & Newnham, J. P. (2006) Periodontal disease and adverse pregnancy outcomes. *The Journal of Maternal–Fetal and Neonatal Medicine* **19**, 521–528.

Teran, E., Escudero, C., Moya, W., Flores, M., Vallance, P. & Lopes-Jaramillo, P. (2001) Elevated C- reactive protein and pro-inflammatory cytokines in Andean women with preeclampsia. *International Journal of Gynaecology and Obstetrics* **75**, 243–249.

Trogstad, L. I. S., Eskild, A., Bruu, A. L., Jeansson, S. & Jenum, P. A. (2001) Is preeclampsia an infectious disease? *Acta Obstetricia et Gynecologica Scandinavica* **80**, 1036–1038.

Vince, G. S., Starkey, P. M., Augustulen, R., Kwiatkowski, D. & Redman, C. W. (1995) Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. *British Journal of Obstetrics and Gynaecology* **102**, 20–25.

Von Dadelszen, P. & Magee, L. A. (2002) Could an infectious trigger explainthe differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? *Acta Obstetricia et Gynecologica Scandinavica* **81**, 642–648.

Xiong, X., Buekens, P., Fraser, W. D., Beck, J. & Offenbacher, S. (2006) Periodontal disease and adverse pregnancy outcomes: a systematic review. *British Journal of Obstetrics and Gynaecology* **113**, 135–143.

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Clinical Relevance

Scientific rationale for the study: Recent studies suggest that periodontal disease during pregnancy is associated with an increased risk for the pre-eclampsia. We investigated in a case–control study the possible link between the severity of periodontal disease and pre-eclampsia and to correlate this link to clinical periodontal parameters and IL-1β, TNF-α, and PGE_2 levels in both GCF and serum.

Principal findings: The presence and severity of periodontal disease seems to increase the risk for not only the prevalence but also the severity of pre-eclampsia in pregnant women.

Practical implications: If ongoing studies show that treatment of periodontal infections substantially reduces the risk of pre-eclampsia as is the case with other adverse pregnancy outcomes, then periodontal therapy may be considered a vital part of pre-natal care in pre-eclamptic women.

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