Role of cytokines in development of pre-eclampsia associated with periodontal disease – Cohort Study


Abstract

Aim: The present study was designed to find any association of cytokines in women with periodontal disease and development of pre-eclampsia in North Indian population.

Materials and Methods: A total of 504 consecutively registered primigravida with a single live pregnancy were recruited at 14–18 weeks of gestation from antenatal clinic of Maulana Azad Medical College & associated Lok Nayak Hospital and Maulana Azad Institute of Dental Sciences, New Delhi. One periodontist performed oral health examination of all patients at inclusion into study. Blood samples were collected to measure the level of cytokines IL-4, IL-10, TNF-α and IFN-γ.

Results: The profile of blood levels of cytokines from women with periodontal disease was observed. The log serum levels of TNF-α & IL-4 at 16–18 weeks of gestation were significantly higher in women with periodontal disease (4.13 ± 2.06; 0.47 ± 1.56 pg/ml respectively) than in women with healthy gums (2.16 ± 1.51; 0.02 ± 1.84 pg/ml respectively, p < 0.001). Periodontal disease is associated with log serum TNF-α levels at cut-off ≥14.43 pg/ml at sensitivity 71.2% and specificity 62% (OR = 4.04; 95%CI = 2.77–5.87). Woman with periodontal disease who later developed pre-eclampsia had lower levels of TNF-α (3.72 ± 1.33 pg/ml) than those with periodontal disease who did not develop pre-eclampsia (4.20 ± 2.15 pg/ml, p ≥ 0.05).

Conclusion: Reduced TNF-α level secretion in the early second trimester in women with periodontal disease appears to be associated with the development of pre-eclampsia.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests. This study was financially supported by University Grants Commission, New Delhi, India.

Periodontal disease is one of the most common chronic infectious disorders in humans with a prevalence between 10% and 60% depending on the definition and the population being studied (Albandar & Rams 2002). It affects 20–50% of pregnant women, especially economically disadvantaged women (Offenbacher et al. 2001, Xiong et al. 2006). Periodontal disease, a chronic infection of gingiva and the dental supportive structures, is caused by periopathogen microorganisms. The mechanism of damage to the dental supportive structures includes direct
tissue damage as a result of bacterial plaque, and indirect damage due to bacterial effects on the immune system and the host inflammatory response. Periodontal disease has been identified as a vascular stressor that causes increases in inflammatory mediators which lead to endothelial damage (Oettinger-Barak et al. 2005). The evidence suggests that the localized periodontitis can have significant effects on systemic health. Periodontal disease is associated with many adverse pregnancy outcomes such as pre-term delivery (Offenbacher et al. 2001, Davenport et al. 2002, Lopez et al. 2002, Jeffcoat et al. 2003), pre-eclampsia (Boggess et al. 2003, Canacik et al. 2004), abortion and stillbirth (Moore et al. 2004), low birth weight infants (Offenbacher et al. 2001, Lopez et al. 2002, Jarjoura et al. 2005) and preterm low birth weight infants (Jeffcoat et al. 2003, Mokeem et al. 2004). However, studies from Argentina (Castaldi et al. 2006), Turkey (Buduneli et al. 2005), Denmark (Skudbol et al. 2006), Iceland (Holbrook et al. 2004), Sri Lanka (Rajapakse et al. 2005), and the United Kingdom (Davenport et al. 2002, Moore et al. 2004, 2005) failed to demonstrate any association. It is possible that this varying relationship of periodontal disease and adverse pregnancy outcomes may be due to varying aetiological factors in such different populations.

Pre-eclampsia is a complication that is detected in the second half of pregnancy (after the 20th week of gestation), but most probably has its onset during the early stages of gestation. The incidence of pre-eclampsia is 5–10% of all pregnancies. It is a placenta-dependent disorder with both local intra-uterine and systemic signs and symptoms. Trophoblastic invasion is under the influence of several cytokines produced at the maternal–foetal interface by cells of immune and non-immune origin, such as leucocytes including NK cells, trophoblasts, stromal cells and glandular endothelium (Saito & Sakai 2003).

TNF-α promotes apoptosis and leakage of the endothelial vessels, leading to systemic endothelial activation and signs associated with pre-eclampsia (Rinehart et al. 1999). IL-1 and TNF-α both promote structural and functional changes in endothelial cells including oxidative stress, activation of the complement cascade, secretion of vasoconstrictors, microthrombosis and infarction, and elevated thromboxane levels. All these changes are seen in pre-eclampsia, and the effects of increased expression of TNF-α seem to be involved in the pathophysiological mechanisms leading to the clinical signs (Roberts et al. 1989, Anim-Nyame et al. 2003). Excessive amounts of IFN-γ in conjunction with TNF-α and IL-1 can lead to apoptosis of trophoblasts (Ashkar et al. 2000, Moffett-King 2002). Normotensive pregnancy is associated with a high increase in IL-4 in the first half of the pregnancy. In contrast, high levels of IL-4 are associated with pre-eclampsia in the second half of pregnancy and puerperium (Omu et al. 1999).

As is evident from the literature, periodontal disease is associated with pre-eclampsia. It is hypothesized that the association between these two diseases is probably based on hypertension-related cytokine alterations. Therefore, the present study is designed to find any association of cytokines in women with periodontal disease and development of pre-eclampsia in North Indian population.

**Materials and Methods**

A cohort study was conducted in the department of Obstetrics and Gynaecology at Maulana Azad Medical College and associated Lok Nayak Hospital and Maulana Azad Institute of Dental Sciences, New Delhi from November 2009 to March 2012. It is a main public hospital of the city Government and caters to the population of Delhi and surrounding areas.

Taking the prevalence of pre-eclampsia as 10% and odd ratio for periodontal disease as 1.6, the sample size was calculated with 90% power and 5% level of significance for comparing the cytokine levels in women having pre-eclampsia with periodontal disease. The sample size was estimated to be 225. However, to take care of confounding factors and “loss to follow-up” during pregnancy, and the feasibility, it was proposed to include 530 cases. All consecutively registered primigravidae, in the age group of 20–35 years with a single live pregnancy were recruited at 14–18 weeks of gestation from the antenatal clinic. Women were sure of dates, and gestation age was determined with best obstetric estimates using definite menstrual history and ultrasonography done in the first trimester.

Women with chronic hypertension, diabetes mellitus, renal disease, parathyroid disease, urolithiasis, polyhydramnios, congenital malformation of foetus, and those having systolic blood pressure measurements at the start of pregnancy ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg, and women with any evidence of infection were excluded from the study.

After obtaining written informed consent, eligible women were evaluated on the basis of pre-designed and pre-tested proforma with respect to history, clinical and obstetric examination and ultrasonography. Socioeconomic status of the subjects was evaluated according to Kuppuswamy Scale, which utilizes three variables namely education, occupation and income (Mishra & Singh 2003). The study followed the principles outlined in the Declaration of Helsinki. The study was approved by the Institutional Ethics Committee, Maulana Azad Medical College, New Delhi.

One periodontist performed the oral health examination of all patients at the time of inclusion into the study. The oral examination involved examining the patient’s dentition, periodontium, soft tissues and fillings, if present. Periodontal pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) were also recorded for each site.

Blood samples were collected at 14–18 weeks of gestation to study the levels of cytokines. Serum cytokine levels of IL-4, IL-10, TNF-α and IFN-γ titres were measured using commercially available ELISA kits (Gen-Probe Diaclone SAS, Besançon Cedex, France). Sensitivity, minimum detectable dose for IL-4 was 0.7 pg/ml; IL-10 < 5 pg/ml, TNF-α < 80 pg/ml and IFN-γ < 5 pg/ml. The kit had no cross reactivity for other proteins. The overall intra-assay coefficient of variation for IL-4 was 3.5%, IL-10
Criteria for pre-eclampsia

Pre-eclampsia is defined as a reading of systolic blood pressure >140 mm of mercury and diastolic blood pressure >90 mm of mercury on two occasions at least 4 h apart after 20 weeks of gestation in a woman with previously normal blood pressure along with development of proteinuria (defined as >300 mg/24 h or ≥1+ on a clean–catch dipstick test in the absence of urinary infection) [ACOG (2002), Practice Bulletin No. 33].

Criteria for diagnosing periodontal disease

Periodontal examination was done by using mouth mirror, tweezers, and probe, and vestibular, lingual, mesial and distal sites of each tooth were evaluated. PPD, BOP, gingival recession and CAL were noted. Gingival index was determined as 0: normal; 1: mild inflammation, slight colour change and oedema and no BOP; 2: moderate inflammation, redness, oedema with BOP; 3: severe inflammation, marked redness and oedema, ulceration and spontaneous bleeding (Loe & Silness 1963). Gingival index scoring was calculated by adding gingival index around all four sites of each tooth divided by 4, and then total scores of all teeth divided by number of teeth. PPD was measured from the gingival margin to the most apical penetration of probe (base of the gingival sulcus or periodontal pocket). CAL was measured from the cementoenamel junction to the most apical penetration of the probe. CAL was measured at four sites of all the teeth.

Women with healthy gums had gingival index scores of 0 to 1, probing depths <4 mm with no CAL. Women with gingivitis had gingival index scores of 1, probing depths ≥4 mm and no CAL. Women having CAL and probing depths ≥4 mm in one or more sites were diagnosed as those with periodontitis.

Statistical analysis

Periodontal disease includes women with gingivitis and periodontitis. Quantitative data, age and haemoglobin were compared between different groups by using the student t-test. The serum cytokine levels had scattered values so log values were taken to normalize the data and were compared using non-parametric Wilcoxon Mann–Whitney test. Qualitative data such as years of education and socioeconomic status were compared between the groups using Chi-square test. Cut-off value of cytokines was calculated using receiver operating characteristic (ROC) curve. A p-value <0.05 was considered as the cut-off point for the level of significance.

Results

Among the 528 primigravida women recruited for the study, 24 were lost to follow-up, so analysis was performed for 504 cases (Fig. 1). The periodontal disease levels were comparable between the 528 and 504 women. The prevalence of periodontal disease in the study population was found to be 57.14% (288/504). The comparative demographic profile and pregnancy outcome of both the periodontal disease group and non-periodontal disease group are shown in Tables 1, 2 respectively. The incidence of pre-eclampsia in the study population was found to be 10.12% (51/504). The variables for occurrence of periodontal disease as well as pre-eclampsia such as age, parity, education, socioeconomic status and pre-pregnancy body mass index were comparable in subjects with periodontal disease and without periodontal disease (p > 0.05).

It was further observed that 39/288 (13.54%) women with periodontal disease later developed pre-eclampsia as compared to 12/216 (5.55%) women with healthy gums (p < 0.001). Development of pre-eclampsia in women with periodontal disease was almost three times more frequent (OR = 2.66; 95% CI = 1.32–5.73).

The log serum levels of IL-4, IL-10, TNF-α & IFN-γ were comparable for the gestational age between 14 and 18 weeks (p > 0.05). The log serum level of IFN-γ was significantly lower in women with periodontal disease (0.26 ± 2.12 pg/ml) than in women with healthy gums (0.71 ± 2.33 pg/ml, p < 0.001). However, the log serum levels of IL-4 and TNF-α were significantly higher in women with periodontal disease than in women with healthy gums (p < 0.001, Fig. 2). Univariate analysis was performed to relate the periodontal disease with log serum cytokine levels. It was found that periodontal disease is associated with log serum TNF-α levels at a cut-off of ≥14.43 pg/ml at sensitivity 71.2% and specificity 62% (OR = 4.04; 95% CI = 2.77–5.87, Table 3).

On further analysis, the log serum TNF-α level was significantly higher in women with periodontal disease who later developed pre-eclampsia (n = 39, 3.72 ± 1.33 pg/ml) as compared to women with healthy gums who later developed pre-eclampsia (n = 12, 1.96 ± 1.39 pg/ml, p < 0.001, Fig. 3a). The log serum TNF-α level was significantly higher in women with periodontal disease who later developed pre-eclampsia (n = 39, 3.72 ± 1.33 pg/ml) as compared to women with healthy gums who did not develop pre-eclampsia (n = 204, 2.18 ± 1.52 pg/ml, p < 0.001, Fig. 3b). The log serum level of TNF-α was lower in women with periodontal disease who later developed pre-eclampsia (n = 39, 3.72 ± 1.33 pg/ml) as compared to women with periodontal disease who did not develop pre-eclampsia (n = 249, 4.20 ± 2.153 pg/ml, p > 0.05, Fig. 3c). No significant difference was observed in the log serum levels of IL-4, IL-10, TNF-α and IFN-γ in women with healthy gums who later developed pre-eclampsia (n = 12) compared to those who did not develop pre-eclampsia (n = 204, p > 0.05, Fig. 3d). Further, it was found that the log serum levels of IL-4, IL-10 and TNF-α were significantly higher, and the level of IFN-γ was significantly lower in women who had periodontal disease and did not develop pre-eclampsia (n = 249) as compared to women with healthy gums who did not develop pre-eclampsia (n = 204, p < 0.001.
Fig. 3e). The univariate analysis relating periodontal disease and pre-eclampsia with log serum cytokine levels showed that periodontal disease with pre-eclampsia is associated with the log serum TNF-α level at a cut-off of ≥24.88 pg/ml at sensitivity 66.7% and specificity 41.4% (OR = 3.03; 95% CI = 1.52–6.04, Table 3).

The women with periodontal disease were divided into gingivitis (no BOP and mild inflammation) and periodontitis (patients with attachment loss and severe inflammation) and data were analysed (Table 4). It was observed that the log serum level of TNF-α was significantly higher in women with gingivitis (n = 191) and periodontitis (n = 58) who did not develop pre-eclampsia (3.65 ± 2.11 and 5.75 ± 1.62 pg/ml respectively) as compared to women with healthy gums who did not develop pre-eclampsia (n = 204, 2.18 ± 1.52 pg/ml, p = 0.04 and 0.01). The log serum level of TNF-α was significantly higher in women with gingivitis (n = 18) and periodontitis who developed pre-eclampsia (n = 21, 3.01 ± 1.05 and 4.32 ± 1.27 pg/ml respectively) as compared to women with healthy gums who did not develop pre-eclampsia (n = 204, 2.18 ± 1.52 pg/ml, p = 0.02 and 0.001 respectively). No significant difference was observed in log serum levels of IL-4, IL-10, TNF-α and IFN-γ in women with gingivitis who did not develop pre-eclampsia (n = 18) compared to women with gingivitis who did not develop pre-eclampsia (n = 204, 2.18 ± 1.52 pg/ml, p > 0.05). However, log serum levels of TNF-α was significantly lower in women with periodontitis who developed pre-eclampsia (n = 21, 4.32 ± 1.27 pg/ml) compared to women with periodontitis who did not develop pre-eclampsia (n = 58, 5.75 ± 1.62 pg/ml, p = 0.01, Table 4).

Discussion

In the present study, the prevalence of pre-eclampsia in women with healthy gums was 5.5%. In our previous studies from New Delhi, primigravidae were recruited before 20 weeks of gestational age and 7.8% of these developed pre-eclampsia in the second half of pregnancy (Kumar et al. 2009). The prevalence of periodontal disease in the present study population was found to be 57.14% which is similar with other studies on Indian populations. In a study by Agarwal et al. (2010), periodontal diseases were found to be one of the more prevalent oral diseases affecting more than 50% of

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Table 1. Comparative demographic details of periodontal disease and non-periodontal disease groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Periodontal disease</th>
<th>Non-periodontal disease</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.14 ± 2.59</td>
<td>22.26 ± 2.66</td>
<td>0.61</td>
</tr>
<tr>
<td>Years of education (%)</td>
<td>212 (73.61%)</td>
<td>142 (65.74%)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>47 (16.32%)</td>
<td>37 (17.13%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 (10.07%)</td>
<td>37 (17.13%)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.26 ± 3.40</td>
<td>21.56 ± 3.88</td>
<td>0.36</td>
</tr>
<tr>
<td>Socioeconomic status (%)</td>
<td>6 (2.08%)</td>
<td>0 (0%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Lower (&lt;5)</td>
<td>203 (70.49%)</td>
<td>151 (69.91%)</td>
<td></td>
</tr>
<tr>
<td>Upper lower (5-10)</td>
<td>73 (25.35%)</td>
<td>61 (28.24%)</td>
<td></td>
</tr>
<tr>
<td>Upper middle (16-25)</td>
<td>5 (1.74%)</td>
<td>4 (1.85%)</td>
<td></td>
</tr>
<tr>
<td>Upper (26-29)</td>
<td>1 (0.34%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

*Based on Kuppuswamy Scale (Mishra & Singh 2003).

Fig. 1. Descriptive representation of the study subjects with log serum cytokine levels in pg/ml.

Table 3. Comparative demographic details of periodontal disease and non-periodontal disease groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Periodontal disease</th>
<th>Non-periodontal disease</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>0.47 ± 1.58</td>
<td>0.02 ± 1.84</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>2.72 ± 1.86</td>
<td>2.46 ± 1.67</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>4.13 ± 2.06</td>
<td>2.16 ± 1.51</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.26 ± 2.12</td>
<td>0.71 ± 2.33</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-eclampsia (n = 39)</th>
<th>No-pre-eclampsia (n = 249)</th>
<th>Pre-eclampsia (n = 12)</th>
<th>No-pre-eclampsia (n = 204)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>0.60 ± 1.40</td>
<td>0.45 ± 1.59</td>
<td>0.55 ± 1.69</td>
<td>0.01 ± 1.85</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>2.08 ± 1.68</td>
<td>2.82 ± 1.87</td>
<td>2.17 ± 1.67</td>
<td>2.47 ± 1.67</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.72 ± 1.33</td>
<td>4.20 ± 2.15</td>
<td>1.96 ± 1.39</td>
<td>2.18 ± 1.52</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.56 ± 2.01</td>
<td>0.21 ± 2.13</td>
<td>0.28 ± 2.27</td>
<td>0.73 ± 2.34</td>
<td></td>
</tr>
</tbody>
</table>

*Based on Kuppuswamy Scale (Mishra & Singh 2003).
Indian community. Another study by Pralhad et al. (2013) observed a prevalence of periodontal disease of 65.5%, which was found to be significantly higher in females with hypertension. Shah (2005) in her report for the National Commission on Macroeconomics observed periodontal disease in 40% to 45% of the population of India.

The present prospective study was conducted to (1) look for the cytokine levels in the primigravidae at 14–18 weeks of gestation and correlate it with periodontal disease; and (2) determine if the change in the serum cytokine levels due to periodontal disease is responsible for pre-eclampsia. The main finding was that the serum levels of IL-4 and TNF-α were significantly higher in the women with periodontal disease than women with healthy gums. The cytokine levels were higher in pregnant women with gingivitis as well as with periodontitis than in healthy controls. These increased levels of cytokines may be due to the defence mechanism, which comes into action to stop gum infection. A study by Gokul (2012) showed elevated levels of TNF-α in patients with gingivitis and periodontitis as compared to healthy controls in both gingival crevicular fluid and serum, suggesting an association between periodontal disease and levels of TNF-α.

It has been speculated for a long time that in the latent phase of pre-eclampsia, cytokine production at the foeto-maternal interface may be deranged possibly due to abnormal foeto-maternal immune inter-play which results in impaired placental development (Conrad & Benyo 1990). In the present study, serum TNF-α level in early second trimester was significantly lower in women who developed pre-eclampsia than in those who did not develop pre-eclampsia. There are three studies which have estimated the levels of TNF-α in second trimester in which two are on plasma levels and one on amniotic fluid. A study by Serin et al. (2002) showed lower levels of TNF-α in second trimester in women who later developed pre-eclampsia than those who did not develop pre-eclampsia, though the difference was not significant. Similarly, another study showed no significant difference in the levels of TNF-α at 11–14 weeks of gestation in women who later developed pre-eclampsia and those who did not develop pre-eclampsia (Chaparro et al. 2012). These might be because of a smaller number of women with pre-eclampsia in their study. However, the study on amniotic fluid showed higher levels of TNF-α in women who later developed severe pre-eclampsia. The difference was again statistically not significant (Heikkinen et al. 2001). In this study also, the number of women with severe pre-eclampsia was small.

In the present study, the level of TNF-α was lower in women with periodontal disease who later developed pre-eclampsia than in women with periodontal disease who did not develop pre-eclampsia. This suggests that the level of TNF-α, which should increase due to periodontal disease, was not functioning normally in women who were later going to develop pre-eclampsia. There is a lack of studies on the levels of cytokines in women with periodontal disease and development of pre-eclampsia. However, a recent case–control study suggested that periodontitis was clinically related to pre-eclampsia, but failed to show a correlation of periodontal disease and pre-eclampsia with cytokine expression (Chaparro et al. 2013).

The level of TNF-α was found to be higher in women with periodontal disease who did not develop pre-eclampsia than women with healthy gums who did not develop pre-eclampsia. This increased level of TNF-α might be due to the inflammation of the gums, that is, periodontal disease (Gokul 2012). The response of TNF-α level in the women with periodontal disease who later developed pre-eclampsia was reduced as...
compared to women with periodontal disease who did not develop pre-eclampsia. In cross-sectional studies, TNF-α levels increase in women with pre-eclampsia and it has been found to be associated with adverse pregnancy outcomes (Saito et al. 1999, Koçyigit et al. 2004, Azizieh et al. 2005, Bakheit et al. 2009). In the present study, the titre of TNF-α was measured in mid-second trimester (mean ± SD: 16.22 ± 1.93 weeks) and it was found to be reduced in women who later developed pre-eclampsia (51 cases) compared to women who did not develop pre-eclampsia (453 cases) (61.27 ± 59.12; 95.64 ± 100.35 pg/ml respectively). It would have been better if the cytokine levels are evaluated at the development of pre-eclampsia. This probably would have provided further insight into the aetiopathogenesis of pre-eclampsia.

The level of TNF-α in women with periodontal disease who later developed pre-eclampsia (39 cases) (3.72 ± 1.33 pg/ml) was higher than women with healthy gums who later developed pre-eclampsia (12 cases) (1.96 ± 1.39 pg/ml), but lower than women with periodontal disease who did not develop pre-eclampsia (249 cases) (4.20 ± 2.15 pg/ml). A hypothetical model of the biological association between periodontal disease and adverse outcomes have been proposed by Bobetsis et al. (2006). This includes (1) Direct pathway: Periodontal bacteria and/or their pathogenic products disseminate to the foeto-placental unit where they initiate an ectopic infection and/or trigger a local inflammatory response that results in the elevation of inflammatory cytokines and mediators that contribute to pregnancy complications, (2) Indirect pathway: Inflammatory cytokines and mediators produced at the gingival level in response to periodontal pathogens enter the blood circulation and reach the foeto-placental unit and enhance the accumulation of larger amounts of these mediators in this compartment and (ii) the liver where they stimulate a systemic inflammatory response by the production of acute phase reactants. These products gain access to the blood circulation and may enter the foeto-placental unit exacerbating intra-uterine inflammation (Madianos et al. 2013, Sanz & Kornman 2013). Moreover, infection/inflammation in the placenta will downregulate the expression of genes related to the growth and development of the placenta and the foetus. This will contribute to a significant alteration in the structure of the placenta, especially in areas that are critical for the exchange of nutrients between the mother and the foetus. Impaired nutrient transportation via the placenta may lead to low birth weight baby, while structural damage of the blood vessel-rich placenta may disrupt normal blood flow and increase maternal blood pressure, initiating preeclampsia (Madianos et al. 2013). There is a complex interplay of various factors resulting in alteration of host immunological response to periodontitis in pregnant women. It is possible that the response of cytokines is blunted in women with periodontitis and pre-eclampsia. This suggests that reduced levels of TNF-α in women with periodontal disease who later developed pre-eclampsia may be due to an underlying immunological response to the pre-eclampsia. However, further targeted studies are required to determine a “cause and effect” relationship.

Although, TNF-α level was higher in women with gingivitis (no BOP with mild inflammation) and

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**Table 3. Univariate analysis relating periodontal disease with log serum cytokine levels**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cut-off</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontal disease (N = 288) versus healthy gums (N = 216)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>( \geq 1.75 )</td>
<td>1.42</td>
<td>0.99–2.02</td>
<td>0.05</td>
<td>0.007</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>( \geq 12.275 )</td>
<td>1.29</td>
<td>0.97–1.84</td>
<td>0.16</td>
<td>0.004</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>( \geq 14.43 )</td>
<td>4.04</td>
<td>2.77–5.87</td>
<td>(&lt; 0.04^* )</td>
<td>0.106</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>( \leq 0.945 )</td>
<td>0.70</td>
<td>0.49–0.99</td>
<td>0.05</td>
<td>0.008</td>
</tr>
<tr>
<td>Periodontal disease with pre-eclampsia (39) versus periodontal disease without pre-eclampsia (249)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>( \geq 2.25 )</td>
<td>1.21</td>
<td>0.66–2.33</td>
<td>0.56</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>( \leq 11.91 )</td>
<td>0.48</td>
<td>0.25–0.94</td>
<td>0.03*</td>
<td>0.009</td>
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<tr>
<td>TNF-α (pg/ml)</td>
<td>( \leq 24.88 )</td>
<td>3.03</td>
<td>1.52–6.04</td>
<td>0.002*</td>
<td>0.021</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>( \leq 0.63 )</td>
<td>0.33</td>
<td>0.13–0.86</td>
<td>0.023*</td>
<td>0.013</td>
</tr>
</tbody>
</table>

*Significant p-value; CI, confidence interval.

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**Fig. 3.** Comparison of log serum cytokines levels in pg/ml (mean ± SE) among women who had healthy gums with pre-eclampsia; periodontal disease with pre-eclampsia; healthy gums without pre-eclampsia; periodontal disease without pre-eclampsia.
Table 4. Comparison of log serum cytokine levels in various groups of study population

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 58)</td>
<td>(n = 29)</td>
<td>(n = 9)</td>
<td>(n = 21)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Log serum IL-4 (pg/ml)</td>
<td>1.41</td>
<td>0.22</td>
<td>0.98</td>
<td>0.14</td>
</tr>
<tr>
<td>Log serum IL-10 (pg/ml)</td>
<td>1.58</td>
<td>0.34</td>
<td>0.64</td>
<td>0.22</td>
</tr>
<tr>
<td>Log serum TNF-a (pg/ml)</td>
<td>1.52</td>
<td>1.05</td>
<td>1.48</td>
<td>1.62</td>
</tr>
<tr>
<td>Log serum IFN-c (pg/ml)</td>
<td>1.92</td>
<td>0.08</td>
<td>2.11</td>
<td>1.61</td>
</tr>
</tbody>
</table>

**Notes:**
- *p*-value
- IL-4: 0.003, IL-10: 0.02, TNF-a: 0.001, IFN-c: 0.007
- Significant at *p*-value

Various groups of study population consist of women with: (i) healthy gums without pre-eclampsia and periodontal disease without pre-eclampsia, (ii) gingivitis who developed pre-eclampsia, (iii) gingivitis who did not develop pre-eclampsia.

The levels of cytokine interleukin-1β, TNF-α and prostaglandin E2 were increased in the gingival crevicular fluid and serum of pregnant women with pre-eclampsia when compared with those of normotensive pregnant women (Canakci et al. 2007). The extensive vascularization of the periodontal ligament leads one to suppose that chronic local infections could be the source of cytokines that disseminate and act systemically in the vascular endothelium, promoting endothelial lesions. TNF-α promotes apoptosis and leakage of the endothelial vessels, leading to systemic endothelial activation (Rinehart et al. 1999). IL-1 and TNF-α both promote structural and functional changes in endothelial cells including oxidative stress, activation of the complement cascade, secretion of vasoconstrictors, microthrombosis and infarction and elevated thrombomodulin levels. All these changes are seen in pre-eclampsia.

The effects of increased expression of TNF-α seem to be involved in the pathophysiological mechanisms leading to the clinical signs (Rinehart et al. 1999, Anim-Nyame et al. 2003). Therefore, it is possible that the low level of TNF-α in mid-second trimester of pregnancy in women who are going to develop pre-eclampsia might be modulating the aetio-pathogenesis response, that is, trophoblastic invasion and endothelial dysfunction. This is especially occurring in pregnant patients having CAL with severe inflammation.

It was found that the maternal age, education and socioeconomic status were similar in both periodontal and non-periodontal groups. In contrast, a study by Levine et al. (2013) reported that the risk of periodontal disease is higher among black and/or low-income individuals (Levine et al. 2013). A study from France reported that tooth decay was statistically associated with lower age (OR = 1.58, 95% CI [1.03, 2.45]) and lower educational level (OR = 1.53, 95% CI [1.06, 2.23]) (Vergnes et al. 2012).

However, in the present study, the cytokine levels are measured at 14–18 weeks of gestation only. It would have been better if the cytokine levels had been performed twice: once at 14–18 weeks of gestation and another at the time of clinical development of pre-eclampsia. This would have given a better insight on the role of cytokines in the development of pre-eclampsia associated with periodontal disease. Moreover, a large number of studies have investigated potential associations between maternal periodontitis and adverse pregnancy outcomes. There is a high degree of variability in study populations, as well as in recruitment and assessment methods (full-mouth or partial-mouth) including the definitions adopted, resulting in diversified outcomes (Ide & Papapanou 2013).

In conclusion, reduced TNF-α level secretion in the early second trimester in women with periodontal disease appears to be associated with the development of pre-eclampsia. Therefore, if health professionals provide early and regular oral health care to pregnant women as a part of their antenatal care, it might prove to be beneficial for reducing adverse pregnancy outcomes.

References


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**Clinical Relevance**

*Scientific rationale for the study:* Periodontal disease is common in pregnant women and is associated with pre-eclampsia. It is hypothesized that the association between these two diseases is probably based on hypertension-related cytokine alteration.

*Principal findings:* Low levels of TNF-\(\alpha\) secretion was found in the early second trimester in women with periodontal disease who later developed pre-eclampsia.

*Practical implications:* This finding may act as an early biomarker and help to identify which pregnant women with periodontal disease will later develop pre-eclampsia. Once the risk population is identified, it will be easier for the clinician to take special care and provide appropriate management.