Porphyromonas gingivalis in dental plaque and serum C-reactive protein levels in pregnancy

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Abstract
Background: The periodontopathogen Porphyromonas gingivalis (Pg) has been reported as a risk factor for preterm labour. Its pathogenesis and role in pregnancy have not been investigated in Lebanon. Elevated C-reactive protein (CRP) levels in pregnant women with periodontitis also appear to mediate preterm labour.

Methodology: The study included 20 pregnant women with periodontitis and 20 with normal periodontium. PCR was done for Pg detection in oral plaque and vaginal samples. Serum CRP levels were determined by ELISA.

Results: Pg was detected in the oral plaque of 13 of 20 pregnant subjects with clinical periodontitis (patients) and 2 of 20 controls with a healthy periodontium. Vaginal swabs were all Pg-negative, ruling out systemic infection. Serum CRP levels were elevated in 12 of 20 patients and 8 of 20 controls. None of the participants experienced preterm labour.

Conclusions: This is the first report that implicates Pg in Lebanese periodontitis patients. Preliminary results do not indicate a relationship among Pg, periodontitis, CRP levels and preterm labour.

Key words: C-reactive protein, periodontitis, Porphyromonas gingivalis, preterm labour


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Introduction
Porphyromonas gingivalis (Pg), a Gram-negative non motile, asaccharolytic obligate anaerobic coccobacillus, is one of the most studied periodontal pathogens. Pg possesses a number of virulence factors including the lipopolysaccharide (LPS) component of its cell wall and the tissue-damaging enzymes it produces [1].

The detection of Pg from subgingival samples is related to periodontal inflammation, increased probing depth, poor oral hygiene and attachment loss [2]. Although the pathogen can be horizontally transmitted, the patient’s own oral flora seems to be the main source of re-emerging periodontal disease after treatment [3,4]. Pg is generally detected by culture, especially when antibiotic sensitivity is needed, or by molecular methods, mainly polymerase chain reaction (PCR), which provides an excellent detection threshold and is highly specific [5].

Maternal periodontal disease has been reported as a risk factor for preterm labour in the United States [6]; however, this association was not observed in studies of Asian emigrants in European and Sri Lankan women [7,8].

Elevated levels of serum C-reactive protein (CRP) are detected in low-grade inflammation such as periodontitis [9]. As mentioned by van Winkelhoff and Slots [10], van Winkelhoff et al. failed to isolate Pg from the vagina of pregnant women with periodontitis; however, its presence in a higher vaginal location could not be ruled out. If Pg is absent in the vagina, products of causative agents of periodontitis such as Pg are thought to trigger the release of cytokines which in turn signal increased production of acute phase reactants such as CRP by the liver [11,12]. Reports on the role of elevated CRP levels in pregnant women with periodontitis and preterm labour are not conclusive. Several reports indicated that elevated CRP levels in pregnant women with periodontitis appear to mediate preterm labour [9,13-15]. While Ghezzi et al. [16] did not find a relationship between elevated circulating CRP levels and preterm labour, they reported an
Table 1. Inclusion and exclusion criteria used for patient enrollment in the study

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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</tr>
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<tbody>
<tr>
<td>- Between 18 and 39 years of age (inclusive)</td>
<td>- Unable to provide informed consent or comply with study protocol</td>
</tr>
<tr>
<td>- At least 20 teeth</td>
<td>- At medical risk as a result of participation</td>
</tr>
<tr>
<td>- No vaginosis</td>
<td>- Have multiple fetuses as diagnosed by ultrasound</td>
</tr>
<tr>
<td>- No urinary tract infection</td>
<td>- Require antibiotic treatment for any medical/dental reason</td>
</tr>
<tr>
<td>- Periodontal disease</td>
<td></td>
</tr>
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</table>
ImmunoAssay kit (BioCheck Inc, Vintage Park, California, USA) according to the manufacturer’s instructions. Specimens were run in duplicates. Absorbance values were read at 450nm. CRP concentrations in mg/l were then calculated using a quadratic regression curve. Based on the manufacturer’s instructions, all values greater than 8.2 mg/l (norm 0.068-8.2 mg/l) were considered elevated.

Statistical analysis
Differences between groups were tested with the two-tailed Student’s t-test (p ≤ 0.05).

Results
Population and dental analysis
There were no significant differences between the two groups concerning the number of pregnancies, smoking, and level of education. None of the patients or controls experienced preterm labour.

All dental parameters had a statistically significant difference between patient and control groups (P ≤ 0.05). The average values of tested indices for the patient group indicated a moderate periodontitis. Some women in the control group had gingivitis and thus presented false pockets and bleeding on probing (Table 2).

Detection of Pg
Pg was detected in the dental plaque of 13 (65%) patients and two (10%) controls (Table 3). The agarose electrophoretic pattern obtained using specimens obtained from five patients are shown in Figure 1. The vaginal specimens were all negative for IS1126.

CRP levels
Nine of the 13 IS1126-positive patients and one of the two IS1126-positive controls had elevated CRP levels. Three of the IS1126-negative patients and seven of the 18 IS1126-negative controls had elevated CRP levels (Table 3).

Discussion
The pregnant women enrolled in this study were all healthy except for periodontal disease. This selection avoided any bias related to previous oral infections that would have necessitated antibiotic intake; thus the differences between the study and control groups emerge with greater significance regarding periodontal condition.

Bleeding on probing is a sign of gingival inflammation. Both groups presented elevated bleeding indices, compatible with their hormonal status and poor hygiene. Since both groups were of low educational level, these observations further emphasize low socioeconomic status as a risk factor for periodontal disease [20].

The mesial surfaces of the teeth present periodontal pockets more frequently than the buccal, distal or lingual surfaces. This prevalence is, in part, related to tooth anatomy and the presence of grooves that facilitate plaque accumulation [21,22].

Table 2. Baseline socio-economic and dental characteristics of pregnant women

<table>
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<th>Periodontal disease</th>
<th>Periodontal health</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>34.3 ± 5.36</td>
<td>26.10 ± 4.57</td>
<td>0.017</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>2.3 ± 1.62</td>
<td>1.53 ± 0.79</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>26.5%</td>
<td>23%</td>
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</tr>
<tr>
<td>Educational level</td>
<td>Low</td>
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</tr>
<tr>
<td>Probing depth (mm)</td>
<td>5.3 ± 3.2</td>
<td>3.2 ± 1.5</td>
<td>0.027</td>
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<td>Clinical attachment loss</td>
<td>4.2 ± 2.3</td>
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<td>Bleeding on probing</td>
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NS = Non Significant
-IS1126; insertion sequence specific for Porphyromonas gingivalis. CRP; C-Reactive Protein
-CRP level greater than 8.2 mg/l is considered elevated

Table 3. IS1126-status and CRP levels in IS1126-positive and -negative patients and controls

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study, the plaque samples were taken from the deepest pockets, which were most often located on the mesial surface of maxillary and mandibular molars. While this sampling method proved to be effective, not all the periodontitis patients tested positive for Pg. Thirteen of 20 (65%) patients and two of 20 (10%) healthy controls were Pg-positive. These results concur with those of Griffen et al. [23] who reported that 103 of 130 (79%) patients and 46 of 181 (25%) controls were Pg-positive. They concluded that their data implicate Pg in periodontitis.

The fact that some periodontally healthy controls were Pg-positive is in agreement with studies that demonstrated the presence of periodontopathogens in even healthy adolescents and young adults [5].

The reports that periodontitis in pregnancy leads to preterm labour suggest that the causative agent is not confined to the oral cavity. Rather, it becomes systemic and exerts its effect in the uterus. Hu et al. [24] reported that in a mouse model infected with Pg, remote lesions from the site of infection were observed. They suggested that activation of the kinin system is involved in allowing Pg to disseminate. The inability to detect Pg in the vagina argues against a systemic infection. This concurs with the report of van Winkelhoff and Slots [10] who failed to isolate Pg from the vagina of pregnant women with periodontitis, but its presence in a higher vaginal location is possible. If Pg is absent in the vagina, products of causative agents of periodontitis such as Pg are thought to trigger the release of cytokines such as IL-6, which in turn signal increased production of acute phase reactants such as CRP by the liver [11,12].

CRP is an important biological marker of inflammation. It has been reported that periodontal disease accompanied by elevated CRP levels is associated with adverse pregnancy outcome [16]. Twelve of 20 (60%) patients had elevated CRP levels. In a study of 1,351 women, Miller [25] reported that CRP levels were higher in healthy pregnant women than non-pregnant women. Concurring with this report, eight of 20 (40%) healthy pregnant controls had elevated levels of CRP. This finding suggests that an elevated CRP level in pregnancy is a normal finding and not related to periodontitis.

Although the number of participants is small, the fact that none of the patients or controls experienced preterm delivery tends to indicate the absence of a relationship between periodontitis and preterm labour. Lebanese women fit the known profile of other Caucasian women in terms of the detection of Pg as a causative agent of periodontitis.

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**References**


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Conflict of interests: No conflict of interests is declared.