Periodontal conditions during the pregnancy associated with periodontal pathogens

Maria Matilde Usin¹, Sandra M. Tabares², Ricardo J. Parodi¹ & Adela Sembaj²

¹ Department Periodontics, Faculty of Dentistry, National University of Cordoba, Córdoba, Argentina
² Department of Biochemistry and Molecular Biology, Faculty of Medical Science, National University of Cordoba, Córdoba, Argentina

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Correspondence
Dr Adela Sembaj, Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas Segundo Piso Pabellón Argentina, Universidad Nacional de Córdoba, Ciudad Universitaria, Haya de la Torre s/n 5016, Córdoba, Argentina.
Tel: +54-351-4333024
Email: asembaj@biomed.uncor.edu

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Abstract
Aim: To describe the bacterial associations in the periodontal pockets of pregnant women and to correlate the presence of Prevotella intermedia, Tannerella forsythia (T. forsythia), Treponema denticola (T. denticola), Aggregatibacter actinomycetemcomitans, and Porphyromona gingivalis (P. gingivalis) with periodontal parameters of severity.

Methods: The analysis was performed with 150 pregnant women. The examination consisted of an evaluation of bleeding, suppuration, probing depths, clinical attachment levels, hypermobility scores, the Silness and Löe Plaque Index, and the Löe and Silness Gingival Index. Each periodontal pathogen was identified by polymerase chain reaction.

Results: A statistically-significant association was observed ($P < 0.01$) between $P. gingivalis$ and $T. forsythia$, between $P. gingivalis$ and $T. denticola$, and between $T. forsythia$ and $T. denticola$. Age was observed to be a risk factor in the development of moderate periodontitis (odds ratio [OR] = 4.92, 95% confidence interval [CI] = 1.1–21.3, $P = 0.0328$). Age was significantly associated with increased pocket depth and plaque index (OR = 6.36, 95% CI = 1.8–22.2, $P = 0.0037$). In pregnant women, the presence of $P. gingivalis$ was found to increase the risk of developing a clinical attachment level $\geq 5$ mm.

Conclusion: A high prevalence of $P. gingivalis$ in pregnant women, especially in combination with $T. forsythia$ and $T. denticola$, was associated with an increased risk of developing moderate periodontitis, and that association was more marked in pregnant women aged 30 years or older.

Introduction
During pregnancy, some women experience gingivitis, which is defined by gingival inflammation initiated by dental plaque and exacerbated by endogenous steroid hormones. It is associated with edema, bleeding, and halitosis.¹ The etiology of gingivitis during pregnancy involves changes in subgingival or supragingival biofilms and the influence of endogenous sex steroid hormones on the periodontium.²⁻⁴

Kornman and Loesche² demonstrated that Prevotella intermedia (P. intermedia) can utilize female sex hormones such as progesterone or estrogen as a source of nutrients. Clark and Soory⁵ showed that Treponema denticola (T. denticola) in cultures can also use steroid hormones. T. denticola can exert its influence as a component of the total plaque and the local host cells’ steroid metabolism process. Similarly, the modulation of inflammation brought about by changes in the local steroid metabolites will influence the inflammatory mediators produced by T. denticola, other plaque organisms, and the host cells.⁵ In addition, there is a decreased response by the maternal T cells during pregnancy, which could alter the tissue response to bacterial plaque.⁶ For this reason, pregnant women are more susceptible to gingival sulci or periodontal pockets.⁷
It is widely accepted that the initiation and progression of periodontal diseases depend on the presence of certain microorganisms capable of causing them. In addition to those species with recognized pathological actions, it is possible to include the following: Porphyromonas gingivalis (P. gingivalis), P. intermedia, T. denticola, Tannerella forsythia (T. forsythia), and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans). Pathological mechanisms have enabled these bacterial strains to colonize the host, multiply, and negatively alter their physiology. Previous studies have shown that many of these species are generally present together in subgingival biofilms. Quantitative changes in the biofilm, especially due to the overgrowth of P. intermedia, have been implicated in the increased severity of gingivitis during pregnancy.

It is known that these periodontopathogens and their subspecies are capable of spreading throughout the circulatory system and affecting other organs. Several researchers have suggested that gingivitis during pregnancy and maternal periodontitis are important risk factors for preterm births and low birth weight.

Given the high prevalence of gingivo-periodontal diseases and their risk factors for several diseases and pregnancy complications, we conducted the present study to identify P. gingivalis, P. intermedia, T. forsythia, T. denticola, and A. actinomycetemcomitans in the periodontal pockets or gingival sulci of pregnant women. In the present study, we also assessed the association of these periodontopathogens with the loss of healthy periodontia to correlate microbiological patterns with periodontal status in pregnancy.

Materials and methods

Study population

The present study was an analytical, cross-sectional study, and the present data represent cases from regular gynecological examinations of 150 healthy pregnant women from the Provincial Maternity Hospital in Córdoba, Argentina. This work was approved by the Ethics Committee of the Provincial Maternity Hospital. All participating participants signed an informed consent according to the Declaration of Helsinki. The women were aged between 18 and 40 years, and were in different stages of their normal pregnancies. Patients with the following selection criteria were included in the study: (a) signs of gingival inflammation; (b) no periodontal treatment of any kind in the past year; (c) no antibiotic therapy in the past 6 months; (d) no contraceptive drug therapy or treatment; and (e) good systemic health (i.e. no diabetes, hormonal disease, or hypertension).

Clinical dental examination and microbiologic sampling

At the first visit, the examiner (MMU) performed a full-mouth examination on all existing teeth. The periodontal condition was evaluated with a periodontal probe (Periopaper Dental Products, Plymouth, PA, USA) at six sites using the following parameters: bleeding on probing, suppurination, probing depth (PD) scores, clinical attachment level (CAL), hypermobility score, Silness and Löe Plaque Index (PI), and Löe and Silness Gingival Index (GI). The same dentist selected the deepest periodontal pocket for microbial sampling from each patient. These periodontal pockets were selected from the proximal, mesial, and distal sites of the anterior upper and lower teeth.

The supragingival plaque was gently removed with a cotton swab; the site of collection was isolated with cotton rolls and dried with air. Next, six paper points made of sterile absorbent paper size 40 were introduced into the bottom of the depth pocket. The paper points remained in situ for 30 s with a catheterization movement, and were then placed into a sterile Eppendorf tube containing 0.5 mL sterile, double-distilled water. The samples were stored at −20°C until they were processed for bacterial identification.

Bacterial identification by polymerase chain reaction amplification

The sterile, double-distilled water-suspended specimen was warmed to 37°C for 10 min, and thoroughly vortex mixed before centrifugation at 14 000 × g for 5 min. The pellet was suspended in 200 μL water, boiled for 10 min, chilled on ice, and centrifuged. The DNA was purified from the supernatant by conventional techniques, and then visualized by electrophoresis on ethidium bromide-stained agarose gels.

The polymerase chain reaction (PCR) method has been described in detail by Ashimoto et al. First, we tested each sample with a specific and highly-conserved sequence of the 16S rRNA gene to detect the presence of Gram-negative bacteria. If we observed a 960-bp fragment, PCR for each species of the periodontal pathogens was performed using a specific oligonucleotide, as previously described. The amplification was performed in a Perkin–Elmer Cetus thermocycler (Waltham, MA, USA). Each sample was performed in duplicate, and each reaction was conducted using a negative control without any DNA. A positive control, with DNA isolated from each bacterial species, was obtained from the culture (donated by the bacteriology laboratory of Reina Fabiola Hospital, Catholic University of Córdoba, Córdoba, Argentina). The PCR products were analyzed by 1.6% agarose gel.
electrophoresis in a tris-borate ethylenediaminetetraacetic acid buffer. The gel was stained with 0.5 μg/mL ethidium bromide and visualized under UV light.21

Statistical analysis

A grouping analysis was performed using pairs of bacteria (10 pairs), including the numbers of concordant positive cases (both bacteria were detected) and the numbers of concordant negative cases (both bacteria were not detected). All of the observed concordant cases (positive + negative) were recorded. We then calculated the κ concordance coefficient and the φ correlation coefficient using the Sokal-Sneath method to identify significant bacterial associations.

A univariate association was established between each germ and the more severe grade of each clinical parameter (CAL ≥ 3 mm and < 5 mm, PD ≥ 5 mm, PI > 2, GI ≥ 2, and spontaneous bleeding). Increased prevalence of the disease was assessed through the addition of independent risk factors (e.g. age, trimester, and the presence of P. gingivalis, T. forsythia, T. denticola, A. actinomycescomitans, and P. intermedia). We analyzed the association of each microbe with each clinical variable using Pearson’s Chi-square test. Statistical significance was established at 5% (P < 0.05). We calculated the crude odds ratio (OR) and 95% confidence interval (CI); the relative risk of each factor was calculated via multiple logistic regression.

Results

The demographic and clinical characteristics of our study population are presented in Table 1. Notably, 22% of the pregnant women were ≥ 30 years of age at the time of selection and sampling. All patients showed bleeding on probing. We identified bacteria in 85.1% of the 68 pockets with PD ≥ 5 mm + CAL = 1–2 mm, which was defined as mild periodontitis. In 15 pockets with PD ≥ 5 mm + CAL = 3–4 mm, which was categorized as moderate periodontitis, 92% contained bacteria. However, in 67 samples with PD = 4–5 mm and CAL = 0, which was diagnosed as only gingivitis, bacteria were identified in only 1.4% of cases (Table 1).

Overall, the bacterial frequencies identified by PCR were as follows: 39.3% P. gingivalis, 34% T. denticola, 3.3% A. actinomycescomitans, 21.3% T. forsythia, and 3.3% P. intermedia. Only P. intermedia was found in pockets from women in their first trimester of pregnancy.

The presence of more than one bacterium was a common feature in our patients. Therefore, to detect whether the bacterial associations occur randomly, and if certain species are preferentially linked among one another, we performed a pair-association analysis of the periodontopathogens (Table 2). We found statistical significance (P < 0.0001), with a coefficient φ of proximity between 0.36 and 0.44 and a κ index > 30 between P. gingivalis and T. forsythia, between P. gingivalis, and T. denticola and between T. forsythia and T. denticola. In these cases, the observed percentage matches were higher than random expected percentages. All of the other associations among the bacteria were not statistically significant.

We analyzed the association between the presence of pathogens and clinical parameters of the severity of periodontitis. The univariate analysis, with respect to the clinical diagnosis of moderate periodontitis, showed that the presence of P. gingivalis, T. denticola, and T. forsythia was statistically associated with a CAL between 3 and 4 mm (P < 0.0001). The percentage of pregnant women with a CAL between 3 and 4 mm and with P. gingivalis was 57.6%, while 17.1% did not have P. gingivalis. The percentage of pregnant women with a CAL between 3 and 4 mm and with P. gingivalis was 57.6%, while 17.1% did not have P. gingivalis. The percentage of pregnant women with a CAL between 3 and 4 mm and with P. gingivalis was 57.6%, while 17.1% did not have P. gingivalis. Therefore, the presence of these bacterial associations might be considered risk factors for an increased CAL and for the development of moderate-to-severe periodontitis. In the multivariate analysis, P. gingivalis and T. denticola were found to be risk factors for moderate

| Table 1. Demographic and clinical characteristics of the pregnant women |
| --- | --- |
| Characteristics | Percentage with bacteria detected |
| Age (range) | 26 ± 5.7 (18–40) |
| Women ≥ 30 years (%) | 22 |
| Pregnant women smokers (%) | 24 |
| Women in the first trimester (%) | 35 (23) |
| Women in the second trimester (%) | 72 (48) |
| Women in the third trimester (%) | 43 (29) |

<table>
<thead>
<tr>
<th>Patients with</th>
<th>Diagnosis (n)</th>
<th>Percentage with bacteria detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD = 4–5 mm + CAL = 0 (%)</td>
<td>Gingivitis (67)</td>
<td>1.4</td>
</tr>
<tr>
<td>PD ≥ 5 mm + CAL = 1–2 (%)</td>
<td>Mild periodontitis (68)</td>
<td>85.1</td>
</tr>
<tr>
<td>PD ≥ 5 mm + CAL = 3–4 (%)</td>
<td>Moderate periodontitis (15)</td>
<td>92</td>
</tr>
</tbody>
</table>

Age in years, mean ± SD.
Percentages represent the number of samples identified with pathogens in each group.
CAL, clinical attachment loss; PD, probing depth.
periodontitis. The risk of an increased CAL was 14 times higher with the presence of *P. gingivalis* (OR = 14.6, 95% CI = 4.2–50.6), and approximately five times higher with the presence of *T. denticola* (OR = 4.98, 95% CI = 1.6–15; Table 3).

The multivariate analysis for PD ≥ 5 mm showed that a fourfold risk of increased depth of the periodontal pocket when the patients were older than 30 years (OR = 4.55, 95% CI = 1.7–12.0, *P* = 0.0022), and a threefold risk with the presence of *P. gingivalis* (OR = 3.19, 95% CI = 1.3–8.0, *P* = 0.0136; Table 3). In addition, maternal age ≥ 30 years was found to cause a sixfold increase in the development of PI > 2 (OR = 6.36, 95% CI = 1.8–22.2, *P* = 0.0037). With the presence of *P. gingivalis*, the PI could increase as much as fivefold (OR = 4.92, 95% CI = 1.8–22.2, *P* = 0.0328; Table 3).

When we analyzed the risk increases to GI > 2, the data showed that *P. gingivalis*, *T. forsythia*, or *P. intermedia* in pregnant women were independent risk factors. The presence of *P. gingivalis* might increase the risk of GI > 2 by as much as sevenfold (OR = 7.34, 95% CI = 1.3–43.0, *P* = 0.0270). *T. forsythia* might be associated with a sixfold increase (OR = 6.39, 95% CI = 1.3–31.7, *P* = 0.0232), and when *P. intermedia* was detected, the risk for GI > 2 could increase as much as 51-fold. (OR = 51, 95% CI = 6.51–51.86, *P* = 0.0008).

### Discussion

The results of the present study, which were based on a small sample of consecutive pregnant women with normal pregnancy outcomes, provide information regarding regional microbiological compositions in normal pregnancies and their relationships with clinical periodontal status. We observed that *P. gingivalis* and *T. denticola* are frequently detected in the sulci or pockets, and that their presence is associated with a severe PD and CAL, as well as an increased risk of developing moderate periodontitis. Several observational and interventional studies have shown an association between periodontal disease and adverse pregnancy outcomes, such as preterm labor and low birth weight, but other studies have shown no relationships between periodontal disease and pregnancy outcomes. In a study in Argentina that followed 1562 pregnant women, there was no significant association between periodontal disease and premature labor, low birth weight, and preclampsia. In our study, we observed that all pregnant women selected according to the inclusion criteria had their babies to term and with normal birth weights. Recently, Albert et al. showed that women who received preventive dental care had better birth outcomes than those who did not receive any treatment. They observed no evidence of increased risk of adverse birth outcomes from dental or periodontal treatment. According to these observations and our results, developing preventive oral hygiene practices during pregnancy to improve the oral health is needed.

In the present study, we also described bacterial associations in pregnant women. The analysis of pathogen pairs resulted in a statistically-significant association (P < 0.0001) between *P. gingivalis* and *T. denticola*, between *P. gingivalis* and *T. forsythia*, and between *T. forsythia* and *T. denticola*. Based on these bacterial combinations, a lesion might progress if patients are not

### Table 2. Analysis of the associations of bacteria identified by polymerase chain reaction in the periodontal pockets of pregnant women

<table>
<thead>
<tr>
<th>Bacterial pairs</th>
<th>n (+)</th>
<th>n (-)</th>
<th>Total observed, n (%)</th>
<th>( \kappa ) (%)</th>
<th>( \phi )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. gingivalis/T. forsythia</em></td>
<td>23</td>
<td>82</td>
<td>106 (71)</td>
<td>33</td>
<td>0.36</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>P. gingivalis/T. denticola</em></td>
<td>39</td>
<td>80</td>
<td>119 (79)</td>
<td>55</td>
<td>0.56</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>T. forsythia/T. denticola</em></td>
<td>23</td>
<td>92</td>
<td>115 (77)</td>
<td>43</td>
<td>0.44</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

\( n (+) \), concordant positive cases; \( n (-) \), concordant negative cases. *P. gingivalis*, *Porphyromonas gingivalis*; *T. denticola*, *Treponema denticola*; *T. forsythia*, *Tannerella forsythia*.

### Table 3. Odds ratios (OR) and 95% confidence intervals (CI) obtained through logistic regression analyses for the association between periodontal parameters of severity and pregnant women

<table>
<thead>
<tr>
<th>Moderate periodontal parameters</th>
<th>Model covariability</th>
<th>n</th>
<th>OR†</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL 3–4 mm</td>
<td><em>P. gingivalis</em></td>
<td>59</td>
<td>14.6</td>
<td>4.2–51</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td><em>T. denticola</em></td>
<td>31</td>
<td>4.98</td>
<td>1.6–15</td>
<td>0.0044</td>
</tr>
<tr>
<td>PD ≥ 5 mm</td>
<td>Age ≥ 30 years</td>
<td>33</td>
<td>4.55</td>
<td>1.7–12</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td><em>P. gingivalis</em></td>
<td>40</td>
<td>3.19</td>
<td>1.3–8</td>
<td>0.0136</td>
</tr>
<tr>
<td></td>
<td><em>T. denticola</em></td>
<td>31</td>
<td>2.43</td>
<td>0.9–6.5</td>
<td>0.0766</td>
</tr>
<tr>
<td>PI ≥2</td>
<td>Age ≥ 30 years</td>
<td>33</td>
<td>6.36</td>
<td>1.8–22</td>
<td>0.0037</td>
</tr>
<tr>
<td></td>
<td><em>P. gingivalis</em></td>
<td>59</td>
<td>4.92</td>
<td>1.1–21</td>
<td>0.0328</td>
</tr>
<tr>
<td>Gl ≥2</td>
<td><em>P. gingivalis</em></td>
<td>59</td>
<td>7.3</td>
<td>1.3–43</td>
<td>0.0270</td>
</tr>
<tr>
<td></td>
<td><em>T. forsythia</em></td>
<td>50</td>
<td>6.39</td>
<td>1.3–31</td>
<td>0.0232</td>
</tr>
<tr>
<td></td>
<td><em>P. intermedia</em></td>
<td>6</td>
<td>5.1</td>
<td>5.1–518</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

CAL, clinical attachment level; Gl, Löe and Silness Gingival Index; *P. gingivalis*, *Porphyromonas gingivalis*; PD, probing depth; PI, Silness and Löe Plaque Index; *T. denticola*, *Treponema denticola*; *T. forsythia*, *Tannerella forsythia*.

†Adjusted for age, each periodontal clinical parameter and periodontal pathogens.
treated. A strong association between P. gingivalis and T. denticola in pregnant women could be considered a risk factor for developing severe periodontitis, because these strains can increase the CAL by more than 5 mm. Africa et al. demonstrated that in maternal gingival crevicular fluid, certain bacterial combinations yield significant correlations with gingival bleeding, especially when A. actinomycetemcomitans, T. forsythia, and P. intermedia are present. We found that the CAL was less than 4% in samples in which these pathogens were absent; however, when the association of P. gingivalis and T. denticola was present, the CAL increased by 60% in pregnant patients. Kesavalu et al. noted that a polymicrobial infection with T. denticola and P. gingivalis in mice was significantly more virulent than a separate infection with each microorganism.

Our results are in agreement with published data that state that the presence of gingival inflammation affects the composition of microflora. Members of the red complex (P. gingivalis, T. forsythia, and T. denticola) and the orange complex (P. intermedia) are found in large amounts in areas that bleed during periodontal probing, which is considered a clinical indicator of periodontal inflammation. These species might benefit because they are closer to the source of the nutrients. They also benefit because gingival crevicular fluid in this case can be enriched by the products of tissue degradation, which favors the growth of many anaerobic species, such as P. gingivalis and T. denticola. The findings in pregnant women, in whom the presence of P. gingivalis, T. denticola and P. intermedia was identified, could be related to the risk of GI > 2, which, when translated clinically, is an increase in the degree of gingivo-periodontal tissue inflammation.

Renvert et al. attempted to correlate microbiological composition with clinical parameters. They noted a significant relationship between the CAL and the presence of bacterial associations in the subgingival biofilm, establishing that P. gingivalis and T. forsythia increase the risk of developing severe periodontitis and reduce the likelihood of successful treatment. Similar results we observed in pregnant women, which demonstrated that the presence of P. gingivalis and T. denticola increases the risk of developing a more severe CAL. However, T. forsythia does not appear to be a statistically-independent risk factor and can aggravate CAL to levels ≥ 5 mm.

Another variable that appears to be a risk factor is age. We observed that pregnant women ≥ 30 years of age could have a greater risk of developing PD ≥ 5 mm in depth, which, along with the presence of P. gingivalis, increases the risk of developing severe periodontitis. However, we were unable to determine the number of previous pregnancies experienced by each woman. The literature shows that in patients with periodontitis, this pathogen increases depending on age. We found a 39.33% prevalence of P. gingivalis in pregnant women compared to 22% in non-pregnant women of the same age in our community. Additionally, we detected a prevalence of 22% in pregnant women aged ≥ 30 years. For this reason, pregnant women aged ≥ 30 years could be considered a particular risk group, with an increased risk of having an aggravated periodontal status and dental element loss.

The results obtained from this study showed that the presence of P. gingivalis, P. intermedia, and T. denticola detected in the periodontal pockets of pregnant women aged ≥ 30 years is associated with an increased risk of aggravated clinical parameters of periodontitis, such as PD, CAL, PI, and PG. Given the possibility that periodontal disease might affect pregnancy outcomes, dentists need to play a proactive role in the oral health maintenance of pregnant women. Odontogenic infections should be treated promptly during pregnancy. Further research is needed to establish the pathogenic mechanisms of active periodontal disease and subgingival periodontal pathogens common in pregnant women to guarantee better pregnancy outcomes.

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