Gingival changes during pregnancy: III. Impact of clinical, microbiological, immunological and socio-demographic factors on gingival inflammation

Carrillo-de-Albornoz A, Figuero E, Herrera D, Cuesta P, Bascones-Martínez A.


Abstract
Aims: To identify predictor variables involved in exacerbated gingival inflammation associated with pregnancy.

Material and Methods: In this cohort study, 48 pregnant and 28 non-pregnant women without periodontitis were included. The pregnant women were evaluated in the first, second and third trimester and at 3 months postpartum, whilst the non-pregnant women were evaluated twice, with a 6-month interval. At each visit, clinical (plaque index (PlI) and gingival index (GI)), hormonal (salivary progesterone and estradiol), immunological (gingival crevicular fluid interleukin-1β, interleukin-6, tumour necrosis factor-α (TNF-α) and prostaglandin-E2] and microbiological (periodontal pathogens culture) evaluations were performed. Statistical analysis was undertaken using exhaustive chi-square automatic interaction detection (exhaustive CHAID) to analyse the predictive value of the independent outcomes to develop pregnancy GI.

Results: PlI was the strongest predictor implicated in the GI throughout pregnancy and after delivery. During the second and third trimesters the presence of Porphyromonas gingivalis significantly contributed to the worsening of gingival inflammation. When compared with the non-pregnant group, significant differences were found in TNF-α amounts and concentrations and in the third trimester site-specific GI.

Conclusions: Bacterial challenge to the gingival tissues, both quantitatively (PlI) and qualitatively (harbouring P. gingivalis) appears to affect the level of gingival inflammation observed during pregnancy.

Key words: dental biofilm; IL-6; immunology; microbiology; Porphyromonas gingivalis; pregnancy gingivitis; Prevotella intermedia; TNF-α

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Conflict of interest and source of funding statement
This research was partially supported by grants from the Autonomous Community of Madrid (Spain) and the Education Ministry of Spain (AP2002-3116). Dentaid freely supplied toothbrushes (Vitis access®, Dentaid, Cerdanyola, Spain). Colgate freely supplied dentifrices (Colgate total®. Colgate, Piscataway, NJ, USA). The authors declare that they have no conflict of interests.

Homoeostasis in the periodontium involves multiple factors that can modify the clinical expression of plaque-induced gingivitis, including variations in sex hormone production (Armitage 1999). Clinical studies have reported a transient increase in the incidence and severity of
gingival inflammation during pregnancy, unrelated to variations in the amount of plaque present (Silness & Löe 1964, Hugoson 1971, Tila-karntne et al. 2000, Figuero et al. 2010).

During pregnancy, periodontal tissue responses to the biofilm challenge are strengthened, as female sex hormones are necessary but not sufficient to produce gingival changes by themselves and a minimum amount of plaque is required (Arafat 1974, Chaikin 1977). Despite extensive research linking periodontal conditions with sex hormones kinetics, more definitive molecular mechanisms still remain to be determined (Mascarenhas et al. 2003). Models for the hormonal role in the periodontium depend on the understanding of the actions and interactions of hormones with the resident population of cells (Mariotti 1994).

Different aetiological pathways have been proposed in an attempt to explain the increased gingival inflammation observed; however, the results so far have been inconclusive. The most prominent theories to describe pregnancy repercussion include hormone effects on the subgingival biofilm, the immune system, the vasculature and the specific cells of the periodontium. Different studies have dealt with these potential aetiologies, but the response of the periodontium is probably not related to a single mechanism but rather multifactorial in nature (Mariotti 1994). The simultaneous evaluation of the different potential mechanisms may therefore provide a broader understanding of this gingival endocrinopathy.

According to the immune-system theory, immuno-modulative changes developed for foetal tolerance would render periodontal tissues more prone to develop gingival inflammation during pregnancy (O’Neil 1979a, Lopatin et al. 1980, Raber-Durlacher et al. 1991). Interleukin-6 (IL-6), a pleiotropic proinflammatory cytokine, has been suggested to be modulated by hormones, but this proposal is still controversial. Some studies have shown a down-regulation of IL-6 production after sex hormones stimulation, hypothesizing that this would render the gingiva less efficient to bacterial challenge (Cohen-Solal et al. 1993, Lapp et al. 1995, Gornstein et al. 1999, Lapp & Lapp 2005), while other researches reported that the production of IL-6 was significantly enhanced by the stimulation of estradiol and progesterone (Yokoyama et al. 2005). TNF-α is another proinflammatory biomarker, possibly affected by hormonal variations. Oestrogen deficiency has been demonstrated to increase T-cell production of TNF-α (Weitzmann & Pacifi c 2006), supporting the concept of type 1-cytokine down-regulation by sex steroids (Szekeres-Bartho 2002, Piccinni 2010, Shiau & Reynolds 2010). On the other hand, animal models have shown that a bacterial challenge with Porphyromonas gingivalis, as a localized infection, in pregnant mice triggers an inflammatory response with an increase of TNF-α serum levels (Collins et al. 1994, Lin et al. 2003).

The explanation of an exacerbation of the gingival inflammation, as a consequence of changes in the supra- or subgingival biofilm, is one of the most solidly based hypotheses. A qualitative shift of the subgingival microbiota in a group of non-peri- odontitis pregnant women that showed an increase in gingival inflammation during pregnancy was recently reported. Significant differences in proportions were found for Aggregatibacter actinomycescomi- tans, P. gingivalis, Prevotella interme- dia/nigrescens, Tannerella forsythia, Parvimonas micra, Campylobacter rectus and Fusobacterium nucle- atum when comparing pregnancy to 3 months postpartum (Carrillo-de-Albornoz et al. 2010). This worsening of the periodontal condition during pregnancy, concomitant with a more pathogenic subgingival micro- biota, was in agreement with previous reports (Kornman & Loe- sche 1980, Raber-Durlacher et al. 1994, Adriaens et al. 2009). This report is the third of a series of articles designed to simultaneously evaluate the role of different potential aetiological pathways on gingival inflammation during pregnancy. In the previous reports, clinical findings, IL-1β and prostaglandin E2 (PGE2) (Figuero et al. 2010) and the composition of the subgingival micro- biota (Carrillo-de-Albornoz et al. 2010) were assessed. The present report aims to evaluate, by means of a multivariate analysis, the effect of all the analysed factors in the whole series of reports (clinical, socio-demo- graphic, immunological and microbiologi- cal) over the gingival inflammation developed in a cohort of pregnant women without periodontitis. Furthermore, the role of additional inflammatory mediators was assessed, specifically whether the higher gingival inflammatory reaction in pregnant women was associated with changes in IL-6 and TNF-α levels in GCF.

Patients and Methods

Study design and patient sample

This was an open cohort prospective study with parallel design, and the methodology, as well as the inclusion and exclusion criteria for subject enrolment have already been reported (Carrillo-de-Albornoz et al. 2010, Figuero et al. 2010). Briefly, two groups (42 pregnant and 20 non-pregnant) of non-smoking, systemically and periodontally healthy women were followed for a 9-month period. Data were gathered on the pregnant women at four visits: at the end of the first trimester (12–14 weeks of preg- nancy), second trimester (23–25 weeks of pregnancy) and third tri- mester (33–36 weeks of pregnancy) and at 3 months postpartum. Data on non-pregnant women were collected at two visits, 6 months apart, matching the first and the third visits of the pregnant group. Hormonal menstrual cycle status was controlled, scheduling visits during the luteal phase (days 17–21). Socio-demo- graphic parameters were registered at the first visit and at each visit clinical, hormonal, immunological and micro- biological evaluations were under- taken (Fig. 1).

After all evaluations and sam- pling procedures at the baseline visit, patients received oral hygiene instructions. To standardize supra- gingival plaque control home care devices, a manual toothbrush (Vitis access®, Dentaid, Cerdanyola, Spain) and a well-tolerated dentifrice among pregnant women (Colgate Total®, Palmolive, Piscataway, NJ, USA)(Kraiivaphan et al. 2006) were given. No other interventions were performed. At the end of the study, all subjects received a professional prophylaxis.
The study design was approved by the Research and Ethics Committee of San Carlos University Hospital (Madrid). Written informed consent was obtained from all participants.

**Study variables**

At each visit, the following evaluations were carried out, in the order listed below.

- **Socio-demographic features.** At the first visit, all women were asked to complete a self-reported questionnaire to assess their socioeconomic status (age, education level and profession) and dental care awareness (frequency of tooth brushing, last visit to the dentist and self-evaluation of their oral status).

- **Progestosterone and estradiol levels in saliva.** Unstimulated saliva was collected during 2 min. into a sterile glass tube and stored frozen at −20°C for a maximum time period of 1 year (Morishita et al. 1988, Meulenberg & Hofman 1989). Subsequently, progesterone and estradiol concentrations were calculated by means of a competitive immunoenzymatic colorimetric method (DIA. METRA S.r.l, Foligno, Italy).

- **IL-6, TNF-α, IL-1β and PGE2 analysis.** GCF was collected from the mesiobuccal sulcus of both upper canines (1.3 and 2.3), using Harco Periopaper (Harco, Irvine, CA, USA) (two samples per patient and per visit). Each strip was measured for fluid volume with a calibrated Periotron 8000® (Harco) (Chapple et al. 1999) and placed in a sterile Eppendorf tube. GCF sample from tooth 1.3 was used to measure TNF-α and IL-1β and that from tooth 2.3 to measure IL-6 and PGE2, using enzyme-linked immunosorbent assays (ELISA) (IL-6, TNF-α, IL-1β; BLK Diagnostics International, Badalona, Barcelona, Spain; PGE2: DRG Diagnostic, DRG Instruments GmbH, Marburg; Germany). Analyses were performed according to the manufacturer’s protocol. Results were calculated using the standard curves created for each assay. Concentrations were corrected for GCF volume and defined as nanograms per millilitre. The total amount of IL-6, TNF-α, IL-1β and PGE2 was expressed in picograms.

- **Plaque index (PlI), according to Silness & Löe (1964).** This parameter was recorded in all teeth at four sites per tooth (mesial, distal, buccal and lingual) with a CPC-12 periodontal probe (Hu-Friedy, Leimen, Germany). A site-specific evaluation was obtained from the immunological sampled sites (sPlI), in addition to full-mouth PlI recording.

- **Microbiological assessment.** A pooled subgingival sample was obtained from four sampled sites. At each visit, the sample was obtained from the four inter-proximal sites showing the most marked inflammation per quadrant, excluding those sites in which GCF samples were taken. Samples were obtained with two sterile consecutive #30 paper points per site (Zipperer s, United Dental MFRS Inc., West Palm Beach, FL, USA) and transferred to a vial containing 1.5 ml of reduced transport fluid (RTF) (Syed & Loesche 1972). At the laboratory, aliquots of 0.1 ml were plated manually for the detection of *A. actinomyctecomitans* on the specific medium Dental-1 (Alsina et al. 2001). Suspected isolates were identified on the basis of colony morphology and positive catalase reaction. Sample dilutions were also plated onto a non-selective blood agar plate (Blood Agar Base II®, Oxoid, Basingstoke, UK), supplemented with haemine (5 mg/l), menadione (1 mg/l) and 5% sterile horseblood. Suspected colonies were identified by microscopy, confirming their identification by means of Gram staining and cell morphology, aero tolerance, production of catalase and other biochemical reactions (Rapid ID 32A, BioMerieux SA, Le-Balmeles-Grottes, France). Total anaerobic counts were calculated, as well as counts of the detected periodontal pathogens (*P. intermedia* nigrescens, *P. gingivalis*, *A. actinomyctecomitans*, T. forsythia, F. nucleatum, P. microa, Eikenella corrodens, C. rectus and Capnocytophaga sp.). In addition to the quantitative microbiological data, the frequency of detection and proportions for each bacterial species were also calculated.

**Gingival index (GI), according to Löe & Silness (1963).** GI was recorded at all sites at four sites per tooth (mesial, distal, buccal and lingual) with a CPC-12 periodontal probe (Hu-Friedy, Leimen, Germany). A site-specific evaluation was obtained from the
immunological sampled sites (sGI), in addition to full-mouth GI recording.

All laboratory analyses (microbiological and immunological) were performed at the Research Laboratory, School of Dentistry, at the University Complutense, Madrid (Spain).

Data management and statistical analysis
Interleukin-6 and TNF-α analysis
A subject level analysis was performed for each study outcome. Biomarker data (IL-6 and TNF-α) were expressed as amounts (pg) and concentrations (ng/ml). Clinical measurements were averaged across subjects from the sites selected for GCF sampling for immunological analysis (sPlI and sGI).

Kolmogorov-Smirnov test was applied for each variable to assess the “goodness of fit” to normal distribution. As normality was not achieved rigorously for all the variables at the different time point intervals, non-parametric tests were used. Data were expressed by median and interquartile range. Intragroup differences to evaluate longitudinal variations over time were determined by Friedman’s test. Post hoc comparisons were performed using Bonferroni’s corrections. To determine differences between pregnant and non-pregnant women (inter-group comparison), the Mann–Whitney U-test was used.

Exhaustive CHAID algorithm
In order to identify predictors that could explain the increased gingival inflammation associated to pregnancy, the influence of the different aetiological potential factors evaluated in the present study was analysed in a multivariate analysis. A decision tree through the Chi-square automatic interaction detector (SPSS Exhaustive CHAID) was used, as it offered the capacity to combine categorical and continuous variables. Each predictor was added to the independent outcome, while 30 independent outcomes were classified in each predictor values of a dependent outcome. GI was set as the dependent outcome, while 30 independent outcomes were tested: (i) clinical (full mouth PlI), (ii) socio-demographic (age, education level, profession, frequency of tooth brushing, frequency of dentist visiting and self-perception of oral health); (iii) immunological (IL-1β, IL-6, TNF-α, PGE2) and (iv) microbiological variables (total anaerobic bacterial counts and specific counts and percentage of the following periodontal pathogens: P. intermedia/nigrescens, P. gingivalis, A. actinomycetemcomitans, T. forsythia, F. nucleatum, P. micra, E. corrodens, C. rectus and Capnocytophaga sp). Significance values for merging and splitting criteria were adjusted using the Bonferroni method. As inflammatory mediators were site-specific measured, to further explore these outcomes another exhaustive CHAID algorithm analysis was performed, where sGI was set as the dependent outcome and sPlI was added to the independent outcomes.

Exhaustive CHAID analysis results are presented in decision trees, which provide a hierarchical visual depiction of predictor variables interactions. The independent variables that were significantly associated with the dependent outcome are classified in nodes. The variable at the highest level of the tree is determined to have the closest statistical association with the dependent outcome, and is represented as the first node. Subsequent associations are classified in consecutive nodes. Groups were split under the following criteria: tree depth was limited to three levels and the minimum number of cases per node for parent nodes was ten. For child nodes, the minimum number of cases was set at 10% of the sample size.

Statistical significance was established at the 95% confidence level. SPSS for Windows (SPSS Inc. version 18) was used for the data analysis.

Results
The results for all the parameters, except IL-6 and TNF-α, have been previously analysed separately (Carrillo-de-Albornoz et al. 2010, Figuero et al. 2010).

Patient sample
Out of a sample of 60 pregnant women, 48 agreed to participate in the study. Forty-two women (30.15 years, range 20–35) completed the three visits during pregnancy and the postpartum visit was completed by 26 women. In the non-pregnant group, 20 non-pregnant women (24.38 years, range 22–26) completed the study from the 28 women initially enrolled in the study (Fig. 1).

Table 1. Site-specific plaque index (sPlI) and gingival index (sGI) scores [median (inter-quartile range)] in GCF sample sites for pregnant and non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Pregnant group</th>
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<tbody>
<tr>
<td></td>
<td>First trimester (n = 42)</td>
<td>Second trimester (n = 42)</td>
<td>Third trimester (n = 42)</td>
<td>Postpartum (n = 26)</td>
</tr>
<tr>
<td>sPlI</td>
<td>0.50 (0.0–1.0)</td>
<td>0.00 (0.0–0.50)</td>
<td>0.00 (0.0–0.50)</td>
<td>0.50 (0.0–0.50)</td>
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<tr>
<td>sGI</td>
<td>0.75 (0.00–1.50)</td>
<td>1.00† (0.50–2.00)</td>
<td>1.00† (0.50–2.00)</td>
<td>0.25‡ (0.00–1.00)</td>
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<tr>
<th></th>
<th>Non-pregnant group</th>
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<tbody>
<tr>
<td></td>
<td>Baseline (n = 20)</td>
<td>6 months (n = 20)</td>
<td></td>
</tr>
<tr>
<td>sPlI</td>
<td>0.00 (0.00–0.50)</td>
<td>0.00 (0.00–0.50)</td>
<td></td>
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<tr>
<td>sGI</td>
<td>0.25 (0.00–1.00)</td>
<td>0.50 (0.00–1.00)</td>
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</table>

Intragroup comparison: Friedman’s test with Bonferroni correction (†p < 0.05); intergroup comparison: Mann–Whitney U-test (‡p < 0.05).
### Table 2. IL-6 levels (amount and concentration) in gingival crevicular fluid of pregnant and non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Pregant group</th>
<th></th>
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<th>Postpartum (n = 26)</th>
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<tbody>
<tr>
<td></td>
<td>First trimester (n = 42)</td>
<td>Second trimester (n = 42)</td>
<td>Third trimester (n = 42)</td>
<td></td>
</tr>
<tr>
<td>Amount (pg)</td>
<td>0.08 (0.01–0.14)</td>
<td>0.12 (0.02–0.20)</td>
<td>0.16 (0.03–0.27)</td>
<td>0.04* (0.0–0.22)</td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>0.24 (0.02–0.62)</td>
<td>0.27 (0.06–0.81)</td>
<td>0.66 (0.16–1.02)</td>
<td>0.11* (0.0–0.56)</td>
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<tr>
<td></td>
<td>Non-pregnant group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount (pg)</td>
<td>0.01 (0.0–0.12)</td>
<td></td>
<td>0.06 (0.0–0.22)</td>
<td></td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>0.05 (0.0–0.3)</td>
<td></td>
<td>0.26 (0.0–1.39)</td>
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</tbody>
</table>

IL-6 levels are expressed as median (inter-quartile range). Intragroup comparison: Friedman’s test with Bonferroni correction (*p < 0.05); intergroup comparison: Mann–Whitney U-test.

### Clinical parameters

Site-specific plaque and gingivitis levels were analysed from the GCF isolated sampled teeth. sPlI showed a slight decrease during pregnancy and a tendency to increase after delivery, but changes were not significant. sGI increased significantly from the first trimester to the second trimester (p = 0.02), then maintained high levels at the third trimester, and decreased postpartum (p < 0.001) (Table 1).

### Interleukin-6 and TNF-α levels in GCF

Table 2 shows the longitudinal changes of IL-6 during pregnancy. Both IL-6 amounts and concentrations showed a gradual increase throughout pregnancy and decreased after delivery, concomitant with the decrease in the sGI. Changes were statistically significant for the reduction from the third trimester to postpartum visit (p < 0.001).

TNF-α behaviour in CGF throughout pregnancy is depicted in Table 3. Amounts and concentrations of this inflammatory mediator decreased progressively from the first to the third trimester. At the postpartum visit, the concentration of the biomarker increased. Changes were significant for the reduction in the absolute quantity from the second to the third trimester (p = 0.03).

### Comparison between pregnant and non-pregnant women

The non-pregnant group showed minor changes in the studied outcomes between the two visits (Tables 1–3). When compared with the pregnant group, significant differences were found in the sGI between the third trimester (pregnant group) and the 6-month visit for the non-pregnant group (Table 1). TNF-α amounts and concentrations were significantly higher in the pregnant group, both at the first and third trimester comparison.

### Gingival index CHAID decision tree analysis

Decision trees were built up for each trimester, analysing the impact of the 30 independent outcomes evaluated: (i) clinical (full mouth PII), (ii) socio-demographic (age, education level, profession, frequency of tooth brushing, frequency of dentist visiting and self-perception of oral health); (iii) immunological (IL-1β, IL-6, TNF-α, PGE2) and (iv) microbiological variables (total anaerobic bacterial counts and specific counts and percentage of the following periodontal pathogens: P. intermedia/migens, P. gingivalis, A. actinomycetemcomitans, T. forsythia, F. nucleatum, P. micra, E. corrodens, C. rectus and Capnocytophaga sp.), over the gingival inflammation (GI).

**First trimester**

PII was the only factor significantly involved with first term GI (Fig. 2). None of the other outcomes influenced GI at this stage.

Four nodes were obtained depending on PII values. At a given PII minor or equal to 0.35, the expected GI at the first term was 0.49 (node 1). In the second node, where the PII raised (0.35–0.51), the

### Table 3. TNF-α levels (amount and concentration) in gingival crevicular fluid of pregnant and non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Pregant group</th>
<th></th>
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<th>Postpartum (n = 26)</th>
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<tbody>
<tr>
<td></td>
<td>First trimester (n = 42)</td>
<td>Second trimester (n = 42)</td>
<td>Third trimester (n = 42)</td>
<td></td>
</tr>
<tr>
<td>Amount (pg)</td>
<td>3.99 (1.70–9.33)</td>
<td>2.37 (1.07–11.44)</td>
<td>1.30* (0.35–9.69)</td>
<td>1.31 (0.84–7.31)</td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>9.07 (5.77–30.68)</td>
<td>8.84 (4.01–48.10)</td>
<td>4.84* (0.99–24.25)</td>
<td>9.56 (1.75–26.98)</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant group</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Amount (pg)</td>
<td>0.45 (0.09–0.59)</td>
<td></td>
<td>0.40 (0.09–0.82)</td>
<td></td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>1.55 (0.21–2.94)</td>
<td></td>
<td>1.86 (0.74–2.92)</td>
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</table>

TNF-α levels are expressed as median (inter-quartile range). Intragroup comparison: Friedman’s test with Bonferroni correction (*p < 0.05); intergroup comparison: Mann–Whitney U-test (′p < 0.05).

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GI increased to 0.79. The same pattern was observed at the third and fourth nodes, where PlI progressively increased and so the GI did (1.14 and 1.56, respectively).

**Second trimester**

The predictors significantly associated with GI were PlI and counts of *P. gingivalis*. PlI was the main outcome implicated in the gingival inflammation during second trimester. Three groups were obtained depending on different second trimester PlI range scores (*p* < 0.001), with GI of 0.72, 1.23 and 1.85 for nodes 1, 2 and 3, respectively.

Node 2 was the most prevalent, including 61.9% of the cases and it was significantly associated with counts of *P. gingivalis*. Counts of *P. gingivalis* of less than 6600 colonies were associated with a GI of 1.09, while for counts exceeding 6600 colonies, the GI increased to 1.48 (Fig. 3).

**Third trimester**

Outcomes followed the same pattern as described for the second trimester. PlI presented on the second trimester was the main involved variable (*p* < 0.001). Three filial nodes were derived, with different GI scores depending on the PlI values. Node 2, again the most prevalent, presented a GI of 1.22 and was also associated with the counts of *P. gingivalis*. Absence of *P. gingivalis* was concomitant with less inflammation, while the presence of the pathogen was associated with an increased GI (Fig. 4).

**Postpartum**

PlI at first trimester during pregnancy was the main outcome responsible for GI observed after delivery. If pregnant women presented a PlI of less than 0.43 in the first trimester, the GI expected after delivery was 0.61 (node 1). On the other hand, for those women with a first trimester PlI value higher than 0.43, GI increased to 1.14 after delivery (node 2). The gingival inflammation also depended on the counts of *P. micra* and PlI during the second term (Fig. 5).

**Site-specific GI CHAID decision tree analysis**

Inflammatory mediators (IL-1β, IL-6, TNF-α, PGE2) were not significantly involved in the corresponding site-specific GI developed during pregnancy (data not shown).

**Discussion**

The present study aimed to evaluate the overall effect of different potential factors over gingival inflammation during pregnancy. A multivariate analysis was used to determine the impact of the clinical, socio-demographic, immunological and microbiological factors over the associated GI. This analysis revealed that PlI presented during pregnancy was the main implicated outcome in the GI developed throughout pregnancy and after delivery. During the second and third trimesters, *P. gingivalis* was implicated in the worsening of the clinical condition. This implies that both PlI and harbouring *P. gingivalis* could modify gingival inflammation during pregnancy.


To evaluate the exposure of gingival tissues to pregnancy, the present cohort study was designed in an attempt to standardize methodology and overcoming previous reported limitations, thus results were reported following the STROBE statement (von Elm et al. 2008). As detailed in the previous reports (Carrillo-de-Albornoz et al. 2010, Figuero et al. 2010), it is important to highlight the weaknesses of the study, including the high incidence of dropouts (specially after delivery) and the lack of homogeneity between the groups in terms of demographic characteristics and initial clinical status, which may limit...
Factors involved in pregnancy gingivitis

The gingival inflammation present during the second and third trimesters of pregnancy could be explained in 61.9% of the cases by qualitative changes in the microbial composition of the subgingival biofilm. This group of pregnant women cover the filial nodes generated from the parent node with intermediate plaque level (Node 2; PII 0.41–0.98). P. gingivalis counts were the principal factor responsible for the splitting into different GI filial nodes. Absence of P. gingivalis was associated with low GI scores, while the presence of the pathogen was associated with a worsening of the periodontal condition. This means, at least in part, that harbouring P. gingivalis at different counts and proportions during pregnancy favours the development of more severe forms of gingivitis and explains differences in the clinical outcomes. The gingival inflammation present during the second and third trimesters of pregnancy could be explained in 61.9% of the cases by qualitative changes in the microbial composition of the subgingival biofilm. This group of pregnant women cover the filial nodes generated from the parent node with intermediate plaque level (Node 2; PII 0.41–0.98). P. gingivalis counts were the principal factor responsible for the splitting into different GI filial nodes. Absence of P. gingivalis was associated with low GI scores, while the presence of the pathogen was associated with a worsening of the periodontal condition. This means, at least in part, that harbouring P. gingivalis at different counts and proportions during pregnancy favours the development of more severe forms of gingivitis and explains differences in the clinical outcomes.
pregnancy (35.7%) was relatively constant throughout the pregnancy of women. The frequency of detection of subgingival bacteria in comparison to other European countries, the Netherlands demonstrated a strong association with periodontitis, including A. actinomycescomitans, P. gingivalis, T. forsythia and T. denticola (aetiological factor in pregnancy gingivitis (Kornman & Loesche 1980, Jensen et al. 1981, Raber-Durlacher et al. 1994). Gürsoy et al. (2009) reported that the vast majority of isolates of P. intermedia sensu lato during pregnancy proved to be P. nigrescens (95.3%). Our research group observed a significant increase in the gingival inflammation in pregnant P. intermedia/nigrescens-positive women, but considering that this was concomitant with an increase in plaque levels (Carrillo-de-Albornoz et al. 2010). In the present study, P. intermedia/nigrescens was not a predictor outcome involved in the GI developed during pregnancy, what is in agreement with other reports that found no microbiological differences in the mentioned pathogens (Jonsson et al. 1988, Adriaens et al. 2009).

From an aetipathogenic point of view, the influence of bacterial challenge over gingival inflammation during pregnancy can be direct or

**Fig. 4.** Decision tree for gingival index (GI) in the third trimester (Exhaustive CHAID algorithm). PII, Plaque index; SD, Standard deviation; Cfu Pg, Colony forming units of P. gingivalis. *p < 0.05.

biofilm have been observed, although to date there are insufficient data to explain the basis of these differences (Marsh & Devine 2011, Sanz & van Winkelhoff 2011). In periodontally healthy patients the prevalence of P. gingivalis has been reported worldwide, being found in 25% in a US population (Griffen et al. 1998), 23.1% in Taiwanese subjects (Yang et al. 2004) and 22.1% in a Chinese adult population (Zhao et al. 2007).

P. gingivalis has repeatedly demonstrated a strong association with disease (Kebschull & Papapanou 2011). Bacterial species aetiologically related to periodontitis, including A. actinomycescomitans, P. gingivalis, T. forsythia and T. denticola (aetiological burden group), have been associated with increased bleeding on probing prevalence in sites with PD ≤ 3 mm (Demmer et al. 2008). In patients with periodontitis, P. gingivalis has been reported to be more frequently detected in destructive forms (Haffajee & Socransky 1994, Takeuchi et al. 2001, van Winkelhoff et al. 2002) and in subjects exhibiting periodontal disease progression (Grossi et al. 1995). In successfully treated patients it appears to be significantly reduced and it is commonly encountered in sites that exhibit recurrence and progression (Mombelli et al. 2000, Fujise et al. 2002). Two postpartum parenteral nodes were formed depending on the first trimester PII. The higher PII was related to a worsening of the clinical condition. The Node 2 group presented with an increased gingival inflammation (GI 1.14) that was associated with the presence and counts of P. micra and additionally with the second trimester PII. Counts of P. micra exceeding 7260 colonies were concomitant with a reduction of the GI, but it is important to highlight that no definitive conclusions can be drawn, as only three cases were included in this group.

Several authors have proposed the increase in P. intermedia/nigrescens counts during pregnancy as an aetiological factor in pregnancy gingivitis (Kornman & Loesche 1980, Jensen et al. 1981, Raber-Durlacher et al. 1994). Gürsoy et al. (2009) reported that the vast majority of isolates of P. intermedia sensu lato during pregnancy proved to be P. nigrescens (95.3%). Our research group observed a significant increase in the gingival inflammation in pregnant P. intermedia/nigrescens-positive women, but considering that this was concomitant with an increase in plaque levels (Carrillo-de-Albornoz et al. 2010). In the present study, P. intermedia/nigrescens was not a predictor outcome involved in the GI developed during pregnancy, what is in agreement with other reports that found no microbiological differences in the mentioned pathogens (Jonsson et al. 1988, Adriaens et al. 2009).

From an aetipathogenic point of view, the influence of bacterial challenge over gingival inflammation during pregnancy can be direct or
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Indirectly explained. According to the direct pathway, the increase in hormone levels would promote the overgrowth of specific bacteria that are responsible for the increased gingival inflammation (Kornman & Loesche 1982). However, an indirect pathway cannot be discarded, assuming that the presence of the pathogen would be the consequence and not the cause of the condition, as the greater exposure to sex steroid hormones would transform the gingiva into a more susceptible environment due to greater gingival probing depths (Miyazaki et al. 1991), a higher gingival crevicular flow rate (Lindhe & Branemark 1968), a lower degree of keratinization (Mariotti 1994) and reduced immunoresponsiveness.

None of the other microbiological or immunological factors evaluated in the present model were significantly implicated in the gingival inflammation developed during pregnancy. Further research is therefore warranted, analysing other aetiological variables and increasing the sample size to corroborate the present results.

Interleukin-6 and TNF-α levels

Sex steroids have been suggested to contribute to observed maternal immune-modulation during pregnancy (Mellor & Munn 2000, Szekeres-Bartho 2002, Shiau & Reynolds 2010). Immunologic tolerance during successful pregnancy seems to be associated with an increase in humoral immunity (type-2 cytokine production) and a decrease in cell-mediated immune response (type-1 cytokine production) (Wegmann et al. 1993). Repercussion of sex steroids over periodontal conditions is furthermore recognized in situations of oestrogen deficiency, as seen in post-menopausal women (Haas et al. 2009).

Different in vitro studies have shown a potential inhibitory effect of sex hormones on the IL-6 secretion (Cohen-Solal et al. 1993, Lapp et al. 1995, Gornstein et al. 1999, Lapp & Lapp 2005). Telleria et al. (1998) corroborated in an animal study the down-regulation of IL-6 production, although the expression of IL-6 mRNA increased after an in vivo injection of bacterial lipopolysaccharide. This contrasts with other studies in which IL-6 production increased following estradiol or progesterone stimulation, at concentrations comparable to those presented in the plasma of pregnant women. This finding suggests that large amounts of female sex hormones in pregnant women may directly stimulate the production of this cytokine by gingival fibroblasts (Yokoyama et al. 2005).

In the present study, IL-6 (amounts and concentrations) progressively increased during pregnancy and was significantly reduced after delivery, concomitant with the fall in hormone production. This is in agreement with previous human studies that correlate local levels of IL-6 with the periodontal status at different life stages. Furthermore, chronic gingivitis has been associated with an increase in IL-6 levels in women.
GCF when compared to periodontally healthy subjects (Offenbacher et al. 2007). Becerik et al. (2010) reported that GCF levels of IL-6 were significantly higher in a group of women with gingivitis (bleeding on probing – BoP – in more than 50% of the probing sites) compared to a group of periodontally healthy women (BoP < 10%), although no differences were found at the different menstrual cycle stages in both groups. Offenbacher et al. (2006) observed that pregnant women with untreated periodontitis, after supra-gingival debridement and a recommendation (without instructions) to use a manual toothbrush, suffered a significant increase in IL-1β in GCF and a 2.1-fold increase in IL-6 in GCF. Conversely, the intervention group (treated with scaling and root planing, tooth polishing and instructed to use a sonic powered toothbrush) resulted in significant improvements in the clinical status. There were also significant decreases in the levels of serum IL-6 soluble receptor (IL-6sr), IL-1β in GCF and in levels of P. intermedia/nigrescens. Other studies have not found significant reductions in IL-6 levels after non surgical periodontal treatment delivered before 21 weeks of gestation, considering that the biomarker measurement was made in serum (Michalowicz et al. 2009).

TNF-α underwent a down-regulation throughout pregnancy. It was statistically significantly reduced at the third trimester, and increased after delivery. This is in agreement with previous reports that support an inhibitory effect of sex steroid hormones over TNF-α (Cohen-Solal et al. 1993, Weitzmann & Pacifici 2006, Luo et al. 2010).

Anti-inflammatory cytokines may play a key role in the survival to term of the foetal allograft, by counteracting deleterious inflammatory Th1 cytokines (Szekeres-Bartho 2002). Animal studies in mice have reported that subcutaneous injection of P. gingivalis, with the chamber model, increases maternal TNF-α and enhances adverse pregnancy outcomes (Collins et al. 1994, Lin et al. 2003). In clinical studies, limited data are available analysing local TNF-α variations on fluctuations of the sex hormone levels. In non-pregnant women with low standardized PIIL scores, Baser et al. (2009) did not observed changes in TNF-α levels in the GCF throughout menstrual cycle stages. In serum, Hasegawa et al. (2003) obtained an increased TNF-α level concomitant with significantly higher mean probing depths, while Michalowicz et al. (2009) did not observed serum changes in any evaluated biomarker (C-reactive protein, PGE2, matrix metalloproteinase-9, fibrinogen, endotoxin, IL-1β, IL-6, IL-8 and TNF-α) after non surgical periodontal treatment in pregnant women with periodontitis.

Conclusions

Within the limitations of the present study, it can be concluded that the amount of plaque deposits and the presence of P. gingivalis above culture threshold were the main implicated factors in the gingival inflammation developed throughout pregnancy, suggesting that these quantitative and qualitative differences in the dental biofilm are able to trigger the inflammatory condition. However, an indirect aetiopathogenic pathway of these factors should also be considered.

Minor significant differences were detected for GCF IL-6 and TNF-α, but these pro-inflammatory biomarkers could not be associated with the gingival inflammatory condition present during pregnancy. Further studies are needed to clarify mechanisms of pregnancy-related gingival inflammation.

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**Clinical relevance**

*Scientific rationale for study:* Different aetiological pathways have been proposed in an attempt to explain the increased gingival inflammation observed during pregnancy, but the results have been inconclusive. Simultaneous evaluation of the different potential mechanisms may provide a more thorough understanding of this gingival endocrinopathy.

*Principal findings:* Gingival inflammation during pregnancy was modulated by quantitative and qualitative variations of the supra- and subgingival biofilm.

*Practical implications:* These findings suggest that optimal plaque control may be particularly beneficial during pregnancy to control the severity of gingival inflammation.