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Gingival changes during pregnancy: I. Influence of hormonal variations on clinical and immunological parameters

Elena Figuero, Ana Carrillo-de-Albornoz, David Herrera and Antonio Bascones-Martínez

Department of Periodontology, School of Dentistry, Complutense University of Madrid, Spain.

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Abstract

Aim: To test whether exacerbated gingival inflammation in pregnancy is associated with increased salivary hormone levels and changes in gingival crevicular fluid (GCF) interleukin-1β (IL-1β) and prostaglandin-E2 (PGE2) levels.

Material and methods: In this cohort study, 48 pregnant women without periodontitis were evaluated in the first, second, and third trimesters and at 3 months postpartum. Twenty-eight non-periodontitis non-pregnant women were evaluated twice, with a 6-month interval. Plaque and gingival indices (PlI, GI), salivary progesterone and estradiol and GCF IL-1β and PGE2 levels were determined.

ANOVA for repeated measures or Friedman's test were used for intragroup analyses. Inter-group comparisons were analysed with *t*-test or Mann–Whitney *U*-test.

Correlations were evaluated with Pearson's and Spearman's test.

Results: Pregnant women showed an increase in GI ($p<0.05$) despite maintaining low PlI values. No changes in IL-1β and PGE2 levels were observed during pregnancy. No significant correlation was found between the GI increase and salivary hormone levels. GI ($p<0.05$) and IL-1β levels ($p<0.001$) were lower in non-pregnant than in pregnant women.

Conclusions: This study confirms the presence of an exacerbated gingival inflammation during pregnancy, but this phenomenon could not be associated with an increase in progesterone or estradiol or with changes in PGE2 or IL-1β.

Key words: estradiol; gingival index; hormones; interleukin-1β (IL-1β); plaque index; post-partum; pregnancy gingivitis; progesterone; prostaglandin E2 (PGE2); saliva

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Conflict of Interest and Source of Funding Statement

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Cross-sectional and longitudinal clinical studies have reported an increase during pregnancy in the prevalence and severity of gingival inflammation (Loe & Silness 1963, Cohen et al. 1969, Hugoson 1970, Samant et al. 1976, O'Neil 1979b, Kornman & Loesche 1980, Jonsson et al. 1988, Miyazaki et al. 1991, Kinnby et al. 1996, Machuca et al. 1999, Tilakaratne et al. 2000, Yalcin et al. 2002b, Gursoy et al. 2008), which disappears postpartum with no permanent effects on periodontal attachment (Loe & Silness 1963, Hugoson 1970, Cohen et al. 1971, Lundgren & Lindhe 1971, O'Neil 1979b, Tilakaratne et al. 2000, Gursoy et al. 2008). An experimental gingivitis study also revealed more swelling, redness and bleeding during pregnancy than after delivery (Raber-Durlacher et al. 1994).

According to the currently accepted classification of periodontal diseases, pregnancy gingivitis is a gingival disease induced by plaque and modified by systemic factors (Armitage 1999). Maier & Orban (1949) studied 53 biopsies from pregnant women with gingivitis and concluded that none of their findings were exclusively characteristic of gingivitis in pregnancy.

The prevalence of pregnancy gingivitis ranges from 36% (Maier & Orban 1949) to 100% (Loe & Silness 1963), although Chaikin (1977) found that it was developed by only 0.03% of women with excellent plaque control. Furthermore, the correlation between changes in hormone levels during pregnancy and the increase in gingival inflammation remains controversial (Samant et al. 1976, O'Neil 1979b, Jonsson et al. 1988).

The precise mechanism responsible for these gingival changes is unknown but various hypotheses have been proposed (Sooriyamoorthy & Gower 1989, Mascarenhas et al. 2003, Mealey & Moritz 2003), including depression of the immune system (O'Neil 1979a, Lopatin et al. 1980, Raber-Durlacher et al. 1991, 1993), increased vascularity and vascular flow (Lindhe & Attsfrom 1967, Lindhe & Branemark 1967, Lindhe et al. 1967, Hugoson 1970, ElAttar & Hugoson 1974), cellular changes (Mariotti 1994) and changes in oral biofilms (Kornman & Loesche 1980, Jonsson et al. 1988, Raber-Durlacher et al. 1994). Hence, pregnancy gingivitis may be an exacerbated inflammatory response that results from a host–parasite imbalance (Kornman & Loesche 1980, Lopatin et al. 1980, Raber-Durlacher et al. 1993). However, it cannot be ruled out that the gingiva undergoes physiological changes during pregnancy, including an increased redness, oedema and greater bleeding tendency that clinically resemble inflammation (Raber-Durlacher et al. 1991).

Regarding these two options, it has been postulated that changes in the maternal immune system during pregnancy may contribute to a greater susceptibility to develop gingival inflammation (O'Neil 1979a, Lopatin et al. 1980, Raber-Durlacher et al. 1991). Four main research lines can be distinguished in relation to this issue. In the first, peripheral blood lymphocytes or monocytes from pregnant women were stimulated in vitro with different antigens or mitogens (O'Neil 1979a, Lopatin et al. 1980, Polan et al. 1990, Raber-Durlacher et al. 1991). Some authors reported a reduced responsiveness of maternal T-lymphocytes to antigenic stimulation (O'Neil 1979a, Lopatin et al. 1980, Polan et al. 1990) but others found no such evidence (Raber-Durlacher et al. 1991). In the second group of reports, peripheral blood lymphocytes from men and non-pregnant women were stimulated with lipopolysaccharide (LPS) and incubated with different concentrations of progesterone and estradiol (Miyagi et al. 1993, Morishita et al. 1999). Both hormones enhanced the production of prostaglandin E2 (PGE2) (Miyagi et al. 1993) and suppressed the production of interleukin-1β (IL-1β) (Morishita et al. 1999). In a third research line, Raber-Durlacher et al. (1993) induced a 14-day experimental gingivitis during pregnancy and postpartum and studied the local immune response in the gingiva, finding a decreasing number of B-cells and macrophages in consecutive gingival biopsies. Finally, human biopsies of healthy and inflamed gingiva have been incubated with estradiol and progesterone, finding that both hormones enhanced PGE2 synthesis (ElAttar & Hugoson 1974).

Only a few human studies have examined the association between increased gingival inflammation during pregnancy and changes in the local immune system. Kinnby et al. (1996) observed a higher gingival inflammatory reaction in women during pregnancy and proposed that changes in hormone levels might have a suppressive effect on local plasminogen activator inhibitor-2 (PAI-2). Yalcin et al. (2002a) found lower GCF PGE2 levels during the second and third trimester in pregnant women treated with scaling and root planing. They concluded that PGE2 levels could be used as a marker of gingival inflammation during pregnancy, although they acknowledged the lack of a control group of untreated pregnant women. Recently, Akalin et al. (2009) reported a decrease in GCF total antioxidant activity and superoxide dismutase enzyme concentrations from the first to the third trimester of pregnancy. However, although the available data indicate that IL-1β and PGE2 might play a role in gingival inflammation during pregnancy, none of these studies have evaluated their normal behaviour in pregnant women without periodontitis.

With this background, the present longitudinal investigation was designed to simultaneously evaluate the role of different aetiological pathways by means of clinical, hormonal, immunological and microbiological studies. The aim of this first part of the research was to test the hypothesis that the exacerbated gingival inflammation in pregnancy is associated with increased sex hormone levels and changes in GCF IL-1β and PGE2 levels. The specific objectives were (1) to prospectively evaluate hormonal, clinical and immunological changes in a group of pregnant women and a control group of non-pregnant women; (2) to assess whether higher gingival inflammatory reaction in pregnant women is associated with a change in GCF IL-1β or PGE2 change; and (3) to correlate clinical status with immunological and hormonal variables. In a second part (Carrillo et al., in press) microbiological results from these women will be presented.

Material and Methods

Experimental design

This was an open cohort prospective study with parallel design and a 9-month follow-up. Ethical approval was obtained from the Research and Ethics Committee of San Carlos University Hospital (Madrid). All subjects were informed of the scope of the study and their consent was obtained.

Population screening

Based on information from previous reports on pregnancy gingivitis (Cohen et al. 1969, Tilakaratne et al. 2000), a difference of 0.15 on gingival index (Löe & Silness 1963) was considered as a reference. Power calculation was done with Sample Power 2.0 programme. This analysis indicated that with 45 pregnant subjects the study would have 80% power to detect a 0.15 difference in gingival index during pregnancy with α set at 0.05.

Therefore, taking into account potential drop-outs, sample size was established as 60 pregnant women. Potential subjects were consecutively recruited from the Obstetrics Department of José Marvá Hospital (pregnant group) and from the Reception Department of the School of Dentistry of Madrid Complutense University (non-pregnant group). All subjects underwent a periodontal examination, including full-mouth evaluation of probing pocket depth, clinical attachment level and bleeding on probing (six sites per tooth). After the periodontal diagnosis was established, all subjects who fulfilled inclusion/exclusion criteria (see below) were invited to participate in the study until the desired sample sizes were reached.

Inclusion/exclusion criteria

Inclusion criteria were (i) age 20–35 years and (ii) presence of ≥ 20 natural teeth in mouth excluding third molars. Exclusion criteria were (i) diagnosis of chronic/aggressive periodontitis (Armitage 1999), (ii) presence of acute dental or periodontal disease, (iii) smoking habit, (iv) presence of systemic disease and/or medication affecting the periodontium, and (v) receipt of systemic antibiotic treatment or dental prophylaxis in previous 6 months.

In addition, to the above inclusion/exclusion criteria, pregnant women were included if they were in the 12th–14th week of pregnancy in the first visit and non-pregnant women were excluded if they were taking contraceptive drugs or if they were pregnant or planning to be so.

Study design

Data were gathered on the pregnant women at four visits: at the end of the first trimester (12–14 weeks of pregnancy), second trimester (23–25 weeks of pregnancy) and third trimester (33–36 weeks of pregnancy) and at 3 months post-delivery. Non-pregnant women received two visits 6 months apart. The hormone status of the menstrual cycle was controlled by scheduling their visits during the luteal phase (days 17–21) of the cycle. At each visit, saliva and GCF samples were collected (in this order) and a clinical examination was then performed (Fig. 1).

Patients also received oral hygiene instructions, a toothbrush (Vitis access®, Dentaid, Sant Cugat del Vallés, Spain) and a dentifrice (Colgate Total®, Colgate, Piscataway, NJ, USA). At the end of the study, all subjects received a dental prophylaxis.

Questionnaire

All women answered a structured questionnaire at the first visit. It was used to obtain information about their socioeconomic status (age, study level and profession), as well as information on their oral and periodontal status (frequency of tooth brushing, last visit to the dentist and self-evaluation of their oral status).

Clinical examination

One examiner (EF) recorded full-mouth plaque (PlI) and gingival (GI) indices at four marginal aspects of all teeth (mesial, distal, buccal and lingual) with a CPC-12 periodontal probe (Hu-Friedy, Leimen, Germany) according to Silness & Löe (1964) and Loe & Silness (1963), respectively.

Saliva sampling

Study participants were asked to rinse their mouths with water, wait for 5 min and then allow saliva to drip from their lower lip into a sterile glass tube for 2 min. Due to technical requirements, all unstimulated whole saliva samples were frozen at -20°C until further evaluation (Meulenberg & Hofman 1989, Morishita et al. 1999).

GCF sampling

A GCF sample was collected by one examiner (EF) from the mesiobuccal sulcus of each upper canine (1.3 and 2.3), using Harco Periopaper (Harco, Irvine, CA, USA) (two samples per patient and per visit). Study sites were selected based on previous reports of a greater increase in GI on anterior teeth in pregnancy gingivitis (Löe & Silness 1963). Briefly, the site was first isolated with cotton rolls and supragingival plaque was removed without touching the marginal gingiva. A gentle stream of air was directed parallel to the root surface for 5–10 s to dry the area. A periopaper strip was then inserted into the crevice until a slight resistance was felt and was left in place for 30 s (Silva et al. 2008, Yucel et al. 2008). Samples containing blood were discarded (Engebretson et al. 2002), and a new GCF sample was taken from the mesiobuccal sulcus of the adjacent upper premolar (1.4 or 2.4). If this new sample was contaminated, it was discarded and the data were recorded as missing. Each strip was measured for fluid volume with a calibrated Periotron 8000® (Harco) (Chapple et al. 1999) and placed in a sterile Eppendorf tube. GFC samples were stored at -80°C until further evaluation.

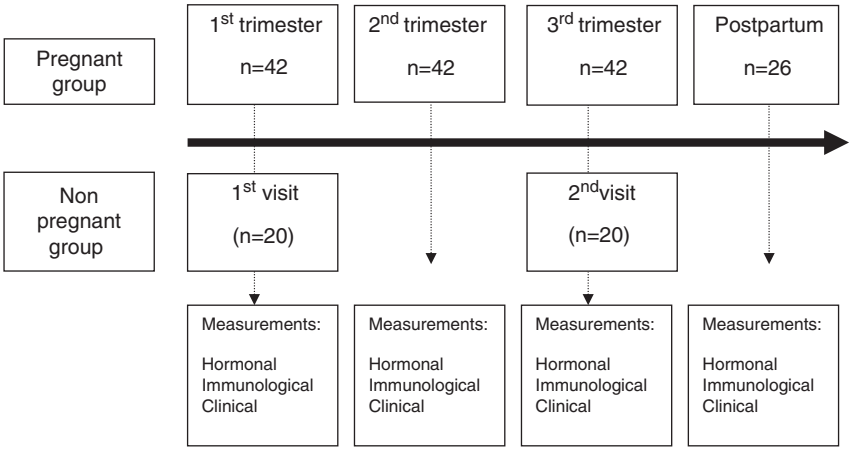

Fig. 1. Flow-chart of follow-up of pregnant group and non-pregnant groups.

Progesterone and estradiol assays

After thawing saliva samples at room temperature and centrifuging at $4000 \times g$ for 5 min, supernatants were retrieved for measurement of progesterone and estradiol by means of a competitive immunoenzymatic colorimetric method (DIA. METRA S.r.l, Foligno, Italy). Concentrations were expressed in pg/ml.

IL-1β and PGE2 assays

GCF samples were thawed at room temperature. GCF was extracted from the paper strips by eluting them with aliquots of buffer (50 mM phosphate buffer, pH 7.2, containing protease inhibitor, 0.1 mM phenylmethylsulphonyl fluoride, 50 μg/ml each of leupeptin, pepstatin and antipain) (Uematsu et al. 1996); 200 μl of this buffer was applied to each strip and kept for 20 min at 4°C. Then, the tubes were centrifuged at $19{,}000 \times g$ for 5 min. The supernatants were collected and used to determine IL-1β and PGE2 levels. In order to obtain enough levels from each inflammatory mediator, one periopaper strip was used for each of them. Therefore, the GCF sample from the upper right canine (1.3) was used to measure IL-1β and that from the upper left canine (2.3) to measure PGE2, using enzyme-linked immunosorbent assays (ELISA) (PGE2:

DRG Diagnostic, DRG Instruments GmbH, Marburg; Germany; IL-1β: BLK Diagnostics International, Badalona, Barcelona, Spain). 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-1β and PGE2 was expressed in picograms.

Data management and statistical analysis

Data were gathered for each woman on mean PlI and GI (full-mouth, anterior teeth, posterior teeth), percentage of locations with PlI of 1, 2 or 3 (plaque score) and percentage of locations with GI of 1, 2 or 3 (gingivitis score). The ratio of GI to PlI was calculated to obtain a numerical value representing the gingival reactivity to plaque (G/P) (Kinnby et al. 1996). ''High-reacting'' (HR) and ''low-reacting'' (LR) subgroups were formed according to whether G/P values in the second trimester were above or below the median, respectively (Kinnby et al. 1996). IL-1β and PGE2 levels were expressed as amounts (pg) and concentrations (ng/ml), and hormonal levels as concentrations (pg/ml).

A subject-level analysis was performed for each study parameter. Kolmogorov–Smirnov goodness-of-fit tests were computed for each variable to assess whether the variables were normally distributed. Data were expressed by mean and standard deviations (SD) for all variables.

To test the effect of ''time'' on response variables during pregnancy ANOVA for repeated measures or Friedman's test for parametric and non-parametric variables, respectively, were used. Post hoc comparisons were performed to explore intergroup differences. Correlations between pairs of variables were determined by means of Pearson's (parametric variables) and Spearman's (non-parametric variables) tests.

Analyses of the minimum detectable difference given the obtained sample size and the observed variability was calculated in those cases where non-significant difference were detected throughout pregnancy and post-partum with Sample Power 2.0 programme.

Comparisons between HR and LR groups were performed by using Mann–Whitney *U*-test for non-parametric variables.

Student's *t*-test and Mann–Whitney *U*-test, for parametric or non-parametric variables, respectively, were used to determine differences between pregnant and non-pregnant women.

Statistical significance was established at the 95% confidence level. SPSS for Windows (SPSS Inc. version 16.0) was used for all data analyses.

Results

Patients

Out of the 60 pregnant women invited to participate in the study, 48 gave their informed consent. Three of these women had a miscarriage before the second visit, one had a preterm birth, one was placed in isolation for suspicion of tuberculosis and a sixth withdrew after the second visit for personal reasons (no available time). Data from these six women were excluded from the study. The remaining 42 women complied with the first, second and third visits. After delivery, 26 of these women complied with the fourth visit (Fig. 1). The other women withdrew from the study for personal reasons (no time, loss of interest). There were no differences in any study variables between the women who remained in the study during the entire period ($n = 26$) and those who left it ($n = 16$) (repeated-measures ANOVA) (data not shown).

Out of the 30 non-pregnant women invited to participate in the study, 28 gave their informed consent. Four of these women started taking oral contraceptives after the first visit and four withdrew for personal reasons (no time, no interest). Data from these eight women were excluded from the study (Fig. 1). There were no differences in any study variable between the women who remained in the study during the entire period and those who left it (ANOVA repeated measures) (data not shown).

Evolution during pregnancy and postpartum

Steroid hormone levels in whole saliva

Table 1 shows progesterone and estradiol values in saliva over the whole study period. The concentration of both hormones significantly increased during pregnancy, reaching a peak in the third trimester ($p < 0.001$), and was markedly decreased at 3 months postpartum ($p < 0.001$).

Clinical parameters

Plaque index. The full-mouth and posterior-tooth PlIs both showed a slight decrease during pregnancy and a non-significant ($p > 0.05$) increase at 3 months postpartum (Table 2). Plaque (PlI score 1, 2 or 3) was recorded in 52.97%, 49.31% and 52.67% of sites in the first, second and third trimesters of pregnancy, respectively, compared with 57.82% at 3 months postpartum ($p < 0.05$).

Gingival index. Table 3 shows the mean GI of the pregnant group at each examination. Full-mouth GI increased from 1.01 in the first trimester to 1.13 in the second ($p < 0.05$), maintained high levels in the third trimester and then decreased to 0.98 at 3 months postpartum ($p < 0.05$). The minimum detectable difference at the post-partum visit resulted in 0.22.

Table 1. Progesterone and estradiol levels (pg/ml) in saliva of pregnant and non-pregnant women

	Pregnant group			
	first trimester ($n = 42$)	second trimester ($n = 42$)	third trimester ($n = 42$)	postpartum ($n = 26$)
Progesterone	249.75 (196.03)	545.14 (344.83)**	1543.10 (760.38)***	11.11(20.16)***
Estradiol	0.96 (2.56)	4.94 (12.35)*	24.38 (32.12)*	0.02 (0.09)***
		Non-pregnant group		
		baseline ($n = 20$)		6 months ($n = 20$)
Progesterone		20.63 (30.12)		40.03 (91.12)
Estradiol		0.01 (0.06)		0.01 (0.04)

Intragroup comparison: Friedman's test with Bonferroni corrections.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 2. Plaque index values and plaque scores (PlI = 1, 2 or 3) in pregnant and non-pregnant women (full-mouth, anterior teeth, posterior teeth)

	Pregnant group			
	first trimester ($n = 42$)	second trimester ($n = 42$)	third trimester ($n = 42$)	postpartum ($n = 26$)
Full-mouth	0.71 (0.43)	0.65 (0.33)	0.68 (0.30)	0.72 (0.28)
Anterior	0.66 (0.46)	0.60 (0.36)	0.61 (0.35)	0.68 (0.33)*
Posterior	0.75 (0.43)	0.69 (0.36)	0.73 (0.29)	0.76 (0.28)
		Non-pregnant group		
		baseline ($n = 20$)		6 months ($n = 20$)
Full-mouth		0.53 (0.31)		0.50 (0.23)
Anterior		0.50 (0.43)		0.46 (0.26)
Posterior		0.56 (0.26)		0.54 (0.25)

Intragroup comparison: ANOVA repeated measures.
*$p < 0.05$.

Table 3. Gingival index values and gingivitis scores (GI = 1, 2 or 3) in pregnant and non-pregnant women (full-mouth, anterior teeth, posterior teeth)

	Pregnant group			
	first trimester ($n = 42$)	second trimester ($n = 42$)	third trimester ($n = 42$)	postpartum ($n = 26$)
Full-mouth	1.01 (0.41)	1.13 (0.43)*	1.14 (0.44)	0.98 (0.40)
Anterior	0.95 (0.50)	1.12 (0.50)*	1.10 (0.54)	0.93 (0.39)
Posterior	1.06 (0.40)	1.14 (0.42)	1.16 (0.43)	1.02 (0.45)
		Non-pregnant group		
		baseline ($n = 20$)		6 months ($n = 20$)
Full-mouth		0.65 (0.44)		0.58 (0.28)
Anterior		0.64 (0.47)		0.47 (0.30)
Posterior		0.65 (0.43)		0.66 (0.29)

Intragroup comparison: ANOVA repeated measures.
*$p < 0.05$.

GI in anterior teeth also showed this pattern, whereas no significant change in GI was observed in posterior teeth over the study period. The percentage of sites with inflammation (GI of 1, 2 or 3) was higher ($p < 0.05$) in the second trimester (57.53%) than after delivery (49.92%).

Gingival reactivity to plaque (G/P). A significant increase in G/P in the second trimester of pregnancy was found ($p < 0.01$), meanwhile, 3 months postpartum, G/P values significantly decreased ($p < 0.001$) (Table 4).

''High-reacting'' and ''low-reacting'' subgroups were formed according to whether G/P values in the second trimester were above or below the median, respectively (Kinnby et al. 1996). G/P values in the first, second and third trimesters of pregnancy were 1.85, 2.46 and 2.17, respectively, for the high-reacting group and 1.55, 1.39 and 1.52, respectively, for the low-reacting group ($p < 0.05$ between groups for first trimester and $p < 0.01$ for second and third). At 3 months postpartum, differences in G/P values between high (1.72) and low-reacting (1.27) groups disappeared ($p = 0.484$).

Inflammatory mediators levels in GCF

Due to technical problems, PGE2 levels were only determined in 23 women during pregnancy, in 14 at 3 months postpartum and in 10 non-pregnant women.

There were no significant changes in IL-1β or in PGE2 levels (amount or concentration) during pregnancy (Tables 5 and 6). However, analyses revealed that given the obtained results, minima detectable differences for the amount of IL-1β were 10.55 pg for the increment between the first to the second term, and 9.02 pg for the second to the third term. In the case of PGE2, those values were 4.21 pg and 12.49 pg, respectively.

Three months post-partum, IL-1β levels and the concentration of PGE2 significantly decreased ($p < 0.05$). Low- and high-reacting groups did not significantly differ in IL-1β or PGE2 levels (data not shown).

Correlations during pregnancy

GI and PlI changes between the first and second trimester were positively correlated ($p < 0.01$; $r = 0.43$), i.e., the higher the rise in PlI, the higher was the increase in GI.

No correlation was found between the increase in gingival inflammation (GI, inflammation scores) and the increase in salivary hormone levels during pregnancy ($p > 0.05$).

Because no changes were observed in immunological parameters during pregnancy, they could not be correlated with the increase in gingival inflammation.

Comparison between pregnant and non-pregnant women

Questionnaire results are compiled in Table 7. The mean age was 30.15 years (range 20–35) in the pregnant group and 24.38 years (range 22–26) in the non-pregnant group ($p < 0.001$). Data on last dental visit and toothbrushing frequency were similar between the groups. However, the educational level was lower in the pregnant than in the non-pregnant group ($p < 0.001$), and most of them were employees (60.69%), whereas most of the non-pregnant women were in liberal professions (61.52%). Significant differences were also detected in self-perception of oral health status ($p < 0.001$).

In the non-pregnant group, there were no statistically significant changes in any study variable over the 6-month study period ($p > 0.05$) (Tables 1–6).

Hormonal levels

Higher salivary progesterone and estradiol concentrations were found in pregnant *versus* non-pregnant women at

Table 4. Gingival reactivity to plaque (GI/PII; mean (SD)) in pregnant and non-pregnant women

	Pregnant group			
	first trimester ($n = 42$)	second trimester ($n = 42$)	third trimester ($n = 42$)	postpartum ($n = 26$)
General	1.70 (0.86)	1.93 (0.72)**	1.85 (0.78)	1.53 (0.99)***
High reactant	1.85 (0.80)	2.46 (0.59)***	2.17 (0.85)	1.72 (1.25)*
Low reactant	1.55 (0.92)	1.39 (0.35)	1.52 (0.56)	1.27 (0.38)**

	Non-pregnant group	
	baseline ($n = 20$)	6 months ($n = 20$)
General	1.35 (0.76)	1.23 (0.54)

Intragroup comparison: Friedman's test with Bonferroni corrections.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 5. IL-1β levels (amount and concentration) in gingival crevicular fluid of pregnant and non-pregnant women

	Pregnant group			
	first trimester ($n = 42$)	second trimester ($n = 42$)	third trimester ($n = 42$)	postpartum ($n = 26$)
Amount (pg)	30.05 (27.05)	23.28 (22.98)	30.45 (24.61)	17.46 (16.64)**
Concentration (ng/ml)	73.12 (57.93)	70.01 (69.68)	85.27 (69.66)	51.78 (53.58)*

	Non-pregnant group	
	baseline ($n = 20$)	6 months ($n = 20$)
Amount (pg)	3.66 (3.39)	4.54 (5.90)
Concentration (ng/ml)	14.00 (18.13)	12.00 (10.69)

Intragroup comparison: Friedman's test with Bonferroni corrections;
*$p < 0.05$; **$p < 0.01$.

baseline and 6-month visits ($p < 0.001$). At 3 months postpartum, the differences between groups disappeared ($p > 0.05$) (Table 1).

Clinical parameters

Pregnant women tended to have higher full-mouth PII *versus* non-pregnant women, and the difference reached significance at the third-trimester visit ($p < 0.05$). Groups did not differ in anterior PII or in percentage of locations with plaque at either visit (baseline/first trimester or 6-month/third trimester) ($p > 0.05$) (Table 2).

GI values (full-mouth, anterior and posterior teeth) were higher in pregnant *versus* non-pregnant women ($p < 0.05$ for first and $p < 0.001$ for third trimester) (Table 3). Gingival reactivity to plaque was also higher in pregnant *versus* non-pregnant women ($p = 0.032$ for first and $p < 0.001$ for third trimester) (Table 4).

At 3 months postpartum, the groups significantly differed in PII ($p = 0.008$) and GI ($p = 0.001$) but not in gingival reactivity to plaque ($p > 0.05$).

Inflammatory mediators in GCF

IL-1β levels (amount and concentration) were significantly lower in non-pregnant *versus* pregnant women ($p < 0.001$) (Table 5).

PGE2 levels (amount and concentration) were similar between the groups, with no significant differences for first or third trimester ($p > 0.05$) (Table 6).

Discussion

This open cohort longitudinal study was designed to confirm reports of exacerbated gingival inflammation in pregnant women and to determine whether this reaction is associated with changes in GCF IL-1β and PGE2 levels and/or increased salivary sex hormone levels. Results obtained showed that gingival inflammation significantly increased during pregnancy, despite plaque scores remaining low or even decreasing. However, GCF IL-1β and PGE2 concentrations did not change during pregnancy and the greater gingival inflammation was not significantly correlated with higher salivary hormone levels.

The population selected for this study consisted of a group of pregnant women with no presence or history of periodontitis that was compared with a control group of non-pregnant women. Although the initial clinical examination in the selection process included evaluation of probing pocket depths and loss of attachment, these parameters were not followed up because they are known to be unaffected by pregnancy (Loe & Silness 1963, Hugoson 1970, Cohen et al. 1971, Lundgren & Lindhe 1971, O'Neil 1979b, Tilakaratne et al. 2000, Gursoy et al. 2008).

The longitudinal design of our investigation, from the first trimester through 3 months postpartum, has been used by other studies on the effect of pregnancy over the periodontium (Cohen et al. 1969, 1971, Tilakaratne et al. 2000, Gursoy et al. 2008). The ability to follow the same individuals throughout the study period improves control over possible confounding factors. A further study strength is that clinical, hormonal and immunological variables were all studied, to our knowledge for the first time together in an observational prospective study of pregnant women without periodontitis.

One of the main study weaknesses was the high incidence of dropouts. This contributed to a reduced sample size (mostly marked at the post-partum visit), which probably influenced the lack of significant differences obtained in some analyses. Therefore, the minimum detectable difference given the sample size and the observed variability was added for each analysis where no significant differences were detected.

A further limitation was the lack of homogeneity between the groups, with pregnant women being older and having received less schooling. Although these data are similar to those previously published by Machuca et al. (1999) and Yalcin et al. (2002b); they should be taken into account as they could have determined some of the differences found between them. Reports from Fransson et al. (1999), Kamma et al. (2009) and Tsalikis et al. (2002) revealed that old subjects develop more gingivitis than young subjects in

Table 6. PGE2 levels (amount and concentration) in gingival crevicular fluid of pregnant and non-pregnant women

	Pregnant group			
	first trimester ($n = 23$)	second trimester ($n = 23$)	third trimester ($n = 23$)	postpartum ($n = 14$)
Amount (pg)	19.85 (6.77)	19.46 (7.38)	21.41 (10.41)	20.71 (8.80)
Concentration (ng/ml)	67.32 (35.78)	68.75 (30.89)	69.56 (31.62)	50.36 (23.06)*
	Non-pregnant group			
	baseline ($n = 10$)		6 months ($n = 10$)	
Amount (pg)	21.88 (13.88)		16.09 (8.18)	
Concentration (ng/ml)	80.39 (69.17)		76.78 (53.38)	

Intragroup comparison: Friedman's test with Bonferroni corrections:
*$p < 0.05$.

Table 7. Demographic description of the study population

	Pregnant group	Non-pregnant group	p*
Age	30.15 years (range 20–35)	24.38 years (range 22–26)	<0.001
Education level			<0.001
None	1.9%	0%	
Primary	7.4%	0%	
Secondary	50%	3.8%	
University	40.7%	96.2%	
Profession			<0.001
Employees	60.7%	15.5%	
Liberal profession	16.1%	61.5%	
Housewife	23.2%	0%	
Student	0%	23.1%	
Frequency of tooth-brushing			NS
3 times/day	59.6%	76.9%	
2 times/day	31.6%	23.2%	
Once/day	7%	0%	
Less than once/day	1.8%	0%	
Last visit to dentist			NS
<1 year	23.1%	28.1%	
1 year	65.4%	47.4%	
>1 year	11.5%	24.6%	
Self-perception of oral health			<0.001
Poor	10.5%	3.8%	
Normal	40.4%	7.7%	
Good	49.1%	88.5%	

*χ^2 for all parameters except age (Student's t-test); NS, not statistically significant.

terms of inflammatory response to *de novo* plaque formation. However, it has to be remarked that criteria used in those reports to distinguish between old and young subjects cannot be applied to this one, as all women included here would comprise the group of young adults (20–35 years). In terms of salivary progesterone levels, Lipson & Ellison (1992) found a pattern of age variation with the highest values in the 25–34-year-old group, intermediate in the 20–24-year-old group and the lowest in 18–19- and 40–44-year-old groups. Therefore, it does not seem reasonable to explain the significant differences found here ($p < 0.001$) only in terms of age reasons.

Clinical parameters

The tendency to a decrease in plaque index levels throughout the pregnancy may be attributable to the oral health instruction at the first visit. O'Neil (1979b) and Gursoy et al. (2008) found a reduction in PlI levels in pregnant patients receiving oral health instruction at the first visit. However, other authors detected this tendency in patients who had not received this instruction (Silness & Löe 1964, Cohen et al. 1969, Hugoson 1970), hence the Hawthorne effect may also have played a role.

Most studies on pregnancy gingivitis reported an increase in gingival inflammation with a peak in the third trimester (Loe & Silness 1963, Cohen et al. 1969, Hugoson 1970, Kornman & Loesche 1980, Tilakaratne et al. 2000, Yalcin et al. 2002b), while others observed the highest value during the second trimester (Gursoy et al. 2008). The present study demonstrated elevated gingival inflammation throughout pregnancy, with a significant increase in index values from the first to the second trimesters. Although greater inflammation was observed in posterior *versus* anterior teeth, the increase in GI was only significant in anterior teeth. These data are in agreement with findings published by Loe & Silness (1963).

A significantly higher gingival reactivity to plaque was observed during than after pregnancy, as also found by Kinnby et al. (1996). We were able to differentiate two groups according to their reactivity (high and low reacting groups), as previously reported (Kinnby et al. 1996, Trombelli et al. 2004). However, we were unable to explain this difference in susceptibility to gingivitis as a function of GCF IL-1β or PGE2 levels. This is in accordance with the results obtained by Trombelli et al. (2005, 2006) or Scapoli et al. (2007), who detected no differences between groups in terms of stress (Trombelli et al. 2005), duration of exposure to plaque (Trombelli et al. 2006) or IL-6, TNF-α or lymphotoxin-α polymorphisms (Scapoli et al. 2007).

Hormone levels

Salivary hormone levels were similar to those found in previous reports on pregnant (Jonsson et al. 1988, Meulenberg & Hofman 1989) and non-pregnant women (Chatterton et al. 2005). Salivary measurement was selected because of the ease of sampling and its accuracy, since it quantifies the free and therefore active levels of steroid hormones (Meulenberg & Hofman 1989, Kaufman & Lamster 2002, Chatterton et al. 2005).

No significant correlation was found between the increase in salivary hormone levels and the increase in gingival inflammation, as also reported by other authors (O'Neil 1979b, Jonsson et al. 1988). Only one study reported a significant relationship between gingival index and sex hormone values during pregnancy (Hugoson 1970), but this was not a true correlation because they used weighted mean values for plasma estradiol and progesterone during normal pregnancy derived from two earlier studies.

IL-1β and PGE2 levels

The GCF sampling sites (mesial aspect of upper canines) were selected because greater swelling and bleeding on probing has been described in the anterior (incisor/canine) *versus* posterior (premolar/molar) region during pregnancy (Raber-Durlacher et al. 1993, 1994), with reports of a wider variation in GI for the anterior region (Loe & Silness 1963) and of a higher GI in interproximal *versus* buccal or oral areas (Hugoson 1970).

To our best knowledge, no prospective observational studies have been published on IL-1β and PGE2 levels in pregnant women without periodontitis. IL-1β is a potent pro-inflammatory cytokine that plays an important role in chronic and acute inflammation (Silva et al. 2008, Polak et al. 2009, Ren et al. 2009, Vernal et al. 2009) and has been reported to stimulate PGE2 synthesis in human fibroblasts from periodontal ligament (Saito et al. 1990). Following in vitro findings that progesterone and estradiol enhance PGE2 synthesis at local (ElAttar & Hugoson 1974) and systemic (Miyagi et al. 1993) levels, it was proposed that these increased PGE2 levels may be in part responsible for the exacerbated gingival inflammation (Sooriyamoorthy & Gower 1989, Mascarenhas et al. 2003, Mealey & Moritz 2003). In the present study, PGE2 levels remained unchanged throughout and after pregnancy and were similar to those in non-pregnant women. Therefore, this study would not support the hypothesis that locally released PGE2 is involved in the pathogenesis of pregnancy gingivitis. However these results should be interpreted with caution due to the reduced number of samples analysed, especially in the post-partum visit. Based on these data, calculation on sample size revealed that 2652 patients would have been needed to detect a minimum difference of 24.87pg/ml in PGE2 levels in GCF during pregnancy (from the first to the second term), with α set at 0.05 and a study power of 0.80.

We found higher GCF IL-1β levels in pregnant women than in non-pregnant women in luteal phase. In contrast, previous studies found lower IL-1β secretion from monocytes isolated during the third trimester of pregnancy (7 IU/ml) than from those isolated during luteal phase (52.4 IU/ml) (Polan et al. 1990), and a suppression in IL-1β production by peripheral blood lymphocytes by their stimulation with LPS and incubation with progesterone (0.2–200 ng/ml) and estradiol (20 ng/ml) (Morishita et al. 1999). The GCF IL-1β levels in our pregnant women were similar to those described in patients with chronic or experimental gingivitis (Kinane et al. 1992, Heasman et al. 1993, Johnson et al. 1997, Deinzer et al. 2007, Yucel et al. 2008). However, we found no positive correlation between the increase in gingival inflammation and IL-1β levels, in agreement with Gonzales et al. (2001), Yucel et al. (2008) and Bergmann & Deinzer (2008) but in disagreement with Kinane et al. (1992), Heasman et al. (1993) and Johnson et al. (1997). Other factors which may have an influence on the gingival inflammation in pregnant woman, such as the potential influence of genetic factors (Lang et al. 2000, Scapoli et al. 2005), has not been evaluated in the present study; therefore, its potential influence on the reported results may not be excluded.

Because the women were pregnant at the first visit in our study and already had an elevated gingival index, we cannot draw definitive conclusions about the potential influence of IL-1β on the development of gingival inflammation during pregnancy. We can only speculate that sex hormones may have no direct effect on IL-1β in vivo but rather affect the local environment, increasing oedema and tissue hypertrophy and creating a favourable environment for the overgrowth of microorganisms responsible for triggering an immune-inflammatory reaction. Further research is warranted in this line, analysing other local inflammatory mediators and applying microbiological tests to investigate the clinical relevance of these findings.

Conclusion

Within the limitations of this study, the results obtained confirm the exacerbation of gingival inflammation during pregnancy, which is specially marked in anterior teeth. This exacerbation could not be associated with increased salivary progesterone or estradiol concentrations or with changes in PGE2 or IL-1β levels. The elevated IL-1β levels observed might result from the creation of a favourable local environment rather than from the direct effects of sex hormones; however, more studies with increased sample sizes are needed within this field.

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Address:
Elena Figuero
Departamento de Estomatología III,
Facultad de Odontología
Universidad Complutense de Madrid
Plaza Ramón y Cajal S/N
28040 Madrid
Spain
E-mail: efigueruiz@gmail.com

Clinical Relevance

Scientific rationale for the study: It has been hypothesized that the increase in gingival inflammation during pregnancy might be favoured by an alteration in the maternal immune system, but no prospective observational study has addressed this issue.

Principal findings: There was an increase in the gingival reactivity to plaque during pregnancy, which was more marked in anterior teeth. IL-1β levels were higher in pregnant women but did not change during pregnancy. PGE2 levels were similar between pregnant and non-pregnant women.

Practical implications: Further studies are warranted to clarify the relevance of IL-1β in pregnancy gingivitis.