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Periodontitis and Plasma C-Reactive Protein During Pregnancy

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Abstract

Background—Periodontitis has been associated with increased risk of adverse pregnancy outcomes and elevated C-reactive protein (CRP) concentrations in non-pregnant adults. We examined the relationship between periodontitis and CRP among women who provided dental radiographs and had blood collected during early pregnancy, excluding smokers and diabetic patients.

Methods—From Project Viva, an ongoing cohort study, we measured plasma CRP in 35 subjects with periodontitis (i.e., at least one site with ≥ 3 mm of alveolar bone loss) and a random sample of 66 periodontally healthy subjects matched on age and race/ethnicity. We performed linear regression analysis with log-transformed CRP levels as the outcome.

Results—The mean ($\pm$ SE) CRP level was 65% higher (95% confidence interval: -2% , 180% ; $P = 0.06$) in women with periodontitis (2.46 ± 0.52 mg/l) than in controls (1.49 ± 0.22 mg/l), adjusting for factors related to CRP levels, including age, race/ethnicity, pre-pregnancy body mass index, alcohol intake, education, income, and gestational age at blood collection.

Conclusions—These findings suggest that periodontitis may increase CRP levels in pregnancy. CRP could potentially mediate the association of periodontitis with adverse pregnancy outcomes.

Keywords

C-reactive protein; inflammation; periodontitis; plasma; pregnancy

C-reactive protein (CRP) is an acute-phase reactant synthesized by the liver in response to the inflammatory cytokines interleukin (IL)-6, IL-1, and tumor necrosis factor- α .¹ Circulating CRP levels are a marker of systemic inflammation and are associated with periodontal disease,^{2,3} a chronic bacterial infection associated with elevation of proinflammatory cytokines and prostaglandin.⁴ Elevated immunoglobulin G induced by bacterial species associated with destructive periodontal diseases is associated with increase in CRP.⁵ Standard non-surgical periodontal therapy resulted in a decrease in serum CRP levels.⁶

Evidence supporting the association between periodontitis and CRP is based only on studies in men and non-pregnant women. There are no studies of periodontitis and CRP in pregnant women. This is an important area for study because systemic inflammation plays a major role

in the pathogenesis of preterm delivery. CRP has been associated with adverse pregnancy outcomes, including preeclampsia,⁷ intrauterine growth restriction,⁸ and preterm delivery.⁹ Chronic infections like intrauterine infection and chorioamnionitis are linked to both preterm birth¹⁰ and elevated CRP levels.¹¹ Furthermore, periodontal disease has been associated with increased risk of preterm low birth weight,¹² low birth weight,¹³ and preterm birth.¹⁴ Therefore, CRP might be a plausible mediator of the association between periodontitis and adverse pregnancy outcomes. The purpose of this study was to examine the relationship between periodontitis and plasma CRP levels among pregnant women.

MATERIALS AND METHODS

Study Participants

Project Viva is a prospective cohort study to evaluate the roles of gestational diet and social factors on pregnancy outcomes and infant health. Between April 22, 1999 and July 31, 2002, we recruited women attending their first prenatal appointment at Harvard Vanguard Medical Associates, a multisite group practice in eastern Massachusetts. Eligibility criteria included singleton pregnancy, having initial prenatal care within the 22nd week of gestation, and fluency in English. Details of recruitment and follow-up are available elsewhere.¹⁵ In brief, 2,128 participants delivered a live infant, and 1,666 of them had responded to a dental questionnaire in the second trimester of pregnancy. Of these, 1,501 (90.1%) reported having dental radiographs taken within the past 5 years. At least 8 months after their delivery, we wrote to 1,010 of these 1,501 women still enrolled in Project Viva to seek their consent for us to obtain radiographs from their dentists. Between October 2001 and April 2003, 386 women (38.2%) provided written consent. We sent requests to the dentists and obtained radiographs for 299 of these 386 participants.

Eligible subjects for the present analysis were women who provided dental radiographs and also had plasma samples taken in early pregnancy ($N = 243$). We excluded 20 women who smoked during the 3 months before learning of pregnancy and two women who had diabetes to avoid confounding effects from these factors. Of the 221 eligible women, 35 (15.8%) had periodontitis (at least one site with ≥ 3 mm of alveolar bone loss). We included all of the periodontitis cases and a random sample of periodontally healthy controls. Controls were frequency matched on age within 5 years and race/ethnicity with one to two controls per case, resulting in 66 controls. Among the selected participants, five periodontitis cases and nine controls provided radiographs taken after the blood sample was drawn. We included these participants in the primary analysis because radiographs taken in the same individual within 5 years showed similar patterns of alveolar bone loss.¹⁶ This study was approved by the Institutional Review Boards at Harvard Medical School and Harvard Pilgrim Health Care.

Assessment of Periodontitis

One calibrated dentist (WP) read all of the radiographs using a standard periodontal probe with Williams marking, ^{††} a viewing box, and a magnifying glass to aid detailed assessment of the landmarks. We measured alveolar bone loss on all 16 posterior teeth (excluding third molars) and recorded in millimeters the distance between the cemento-enamel junction and the alveolar crest at the mesial and distal sites of each tooth. If the same site was seen in more than one radiograph, then the most severe reading was recorded. To evaluate reliability of the measurements, 10% of the radiographs were randomly read twice with an interval of at least 1 week. The Spearman correlation for intraexaminer reliability was 0.91. Radiographic bone loss ≥ 2 mm has been commonly considered evidence of periodontitis.¹⁷ We used a more stringent cutoff of ≥ 3 mm to minimize the effect of random measurement error.¹⁸

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Data Collection

Each participant provided a blood sample at the initial study visit or shortly thereafter (6.4 to 14.9 weeks of gestation). Plasma samples were stored in EDTA, refrigerated for several hours, and then transported on ice to a central laboratory, where they were centrifuged, aliquotted, and frozen in liquid nitrogen until the time of analysis. Participants provided demographic and health-related information by interview at the initial prenatal visit and/or self-administered questionnaires completed during early pregnancy.¹⁵ Pre-pregnancy body mass index (BMI) was computed by dividing pre-pregnancy weight in kilograms by height in meters squared. We estimated alcohol intake in grams per day during the first trimester of pregnancy from a food frequency questionnaire administered at study enrollment.¹⁹ Gestational age was calculated from the last menstrual period (LMP). When the LMP estimate was different from second trimester ultrasound estimate by 10 days or more, we used the ultrasound estimate.

Laboratory Analysis

Plasma samples were thawed and assayed for high-sensitivity CRP at Children's Hospital, Boston, using clinically validated immunoturbidimetric assays on an analyzer^{††} with reagents and calibrators. §§ This assay has a sensitivity of 0.03 mg/l.²⁰ The interassay coefficients of variation at concentrations of 0.91, 3.07, and 13.38 mg/l were 2.81%, 1.61%, and 1.1%, respectively. One laboratory technician who was blinded to the participants' periodontal condition and pregnancy outcome performed all assays.

Statistical Analysis

Differences between periodontitis cases and controls were compared using two sample *t* tests or Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. To evaluate the association between periodontitis and CRP, we performed multivariate linear regression analysis with log-transformed CRP levels as the outcome, adjusting for factors known to affect CRP levels, including age (continuous), race/ethnicity (white versus other), pre-pregnancy BMI (continuous), alcohol intake (continuous), completed college education (yes/no), annual household income more than \$70,000 (yes/no), and gestational age at blood collection (continuous). The adjusted means in multivariate models were computed using the analysis of variance least square means method procedures with statistical software,^{|||} holding all covariates to their average values. By taking antilogs, we transformed these means back to the original scale for easier interpretation. The SEs of the means were derived using the delta method.²¹ The percentage difference was computed from the adjusted mean on the original scale. To ensure the temporality of periodontitis and CRP association, we repeated the analysis restricted to women whose radiographs were taken before blood samples. Adjusted models were based on subjects for whom complete data were available on all covariates of interest. All statistical tests were two-tailed.

RESULTS

Due to matching, mean age and race/ethnicity distributions among periodontitis cases and non-cases were similar (Table 1). Women with periodontitis were more likely to have completed college, to have an annual household income more than \$70,000, to be leaner, and to exercise less than women without periodontitis. Mean alveolar bone loss ($\pm$ SD) was higher among periodontitis cases compared to controls (1.48 ± 0.50 mm versus 0.75 ± 0.34 mm). Participants who had dental radiographs taken before blood drawing seemed to be similar to all participants included in the study with respect to the characteristics of interest (data not shown).

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The median CRP level was 2.23 mg/l (interquartile range: 0.74 to 4.14) in pregnant women with periodontitis and 1.46 mg/l (interquartile range: 0.71 to 3.58) in those with no periodontitis. After adjusting for age, race/ethnicity, education, income, pre-pregnancy BMI, alcohol intake, and gestational age at blood collection, the mean CRP level was 65% higher (95% confidence interval [CI]: -2.2, 179.8; $P = 0.06$) in women with periodontitis (2.46 mg/l) than those without periodontitis (1.49 mg/l). Overall, this model explained 27% of the variability in CRP levels. When we restricted the analysis to only those participants who had radiographs taken before blood drawn, the results were similar, with 70% difference (95% CI: -5.7, 205.4; $P = 0.08$) between the periodontitis group (2.43 mg/l) and control group (1.43 mg/l) (Table 2).

DISCUSSION

In this study, we found 65% higher CRP levels among pregnant women with periodontitis compared to periodontally healthy women, although this association had a P value just above the traditional 0.05 cutoff point for statistical significance. To our knowledge, this is the first study to evaluate the association between periodontitis and CRP in pregnant women. The prospective design assured temporal sequence. The radiographs in this study were all taken within 5 years of delivery. We have demonstrated good concordance of radiographically diagnosed periodontitis between posterior radiographs taken 5 years apart.¹⁶ The results were essentially similar in analyses including or excluding 14 participants who provided radiographs taken after the blood sample was drawn. Misclassification of periodontitis status due to the use of preexisting radiographs or examiner's measurement error was probably random and would lead only to an underestimation of true effect. We took into account many factors that may affect CRP levels, including age, income, education, race/ethnicity, smoking, alcohol intake, BMI, diabetes, and gestational age at blood collection. Ninety percent of the Project Viva participants had dental radiographs, but we were able to obtain radiographs from 30% of those who were contacted. Participants from whom we obtained radiographs were more likely to be white and had higher socioeconomic status compared to those in the whole cohort (data not shown). It is possible that individuals with lower socioeconomic levels and African-Americans are less likely to have dental visits and therefore have radiographs and are more likely to have preterm birth and lower-birth-weight infants. However, there is no reason to expect that the association of periodontitis with CRP in our study population is different from the associations in the whole cohort or in the general population; the results of the study should therefore be generalizable.

The results are consistent with previous studies conducted among men and non-pregnant women. CRP was higher in persons with periodontal disease compared to those without disease among a representative sample of U.S. adults,^{2,22} adult U.S. urban minorities, five middle-aged adults,^{3,23,24} and hemodialysis patients.²⁵ In a study of dental patients (mean age: 42 years), the median plasma CRP levels among control subjects with localized periodontitis and subjects with generalized periodontitis were 0.90, 1.30, and 1.45 mg/l, respectively.¹⁸

Gingival bleeding and calculus were associated with CRP in another study,² but we did not assess these conditions. Using a high-sensitivity assay, we measured CRP in a single blood sample, which is reasonable as there is little seasonal²⁶ or diurnal variation²⁷ in CRP levels. In addition, most participants in the study had CRP levels below 10 mg/l, the standard threshold associated with acute-phase effects,²⁸ reducing the possibility that CRP levels were raised as a result of acute infections or trauma.

The association between CRP and periodontitis in pregnancy we observed may or may not be causal. Elevated CRP may indeed be caused by periodontal infection and inflammation. If this is the case, CRP could amplify the inflammatory response through complement activation,

tissue damage, and induction of inflammatory cytokines in monocytes¹ and therefore may mediate the relationship between periodontitis and adverse pregnancy outcomes. Alternatively, periodontal disease and CRP may share a common risk factor predisposing certain individuals to a hyperinflammatory response. The IL-1 polymorphism, which is associated with increased risk of periodontitis,²⁹ is also associated with raised CRP levels.³⁰ Fetal inflammation makes a contribution to gestational age,³¹ and the relationship between fetal CRP levels and preterm birth may be even stronger than for maternal CRP.

CONCLUSION

These results suggest that periodontitis is associated with increased plasma CRP levels in early pregnancy and raise the possibility that CRP may mediate the association of periodontitis with adverse pregnancy outcomes.

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Table 1
 Characteristics of Study Participants by Periodontitis Status (≥ 1 site with alveolar bone loss ≥ 3 mm)*

Characteristics	Periodontitis		P Value
	Yes (N = 35)	No (N = 66)	
Age (years)	35.7 $\pm$ 3.6	35.2 $\pm$ 3.8	<0.001
Race/ethnicity (%)			1.000
White	82.9	84.9	
Black	5.7	4.6	
Asian-American	8.6	7.6	
Hispanic	2.9	3.0	
Completed college (%)	97.1	80.6	0.030
Annual household income >\$70,000 [†] (%)	85.7	76.9	0.430
Employed (%) [†]	90.3	92.2	0.710
Pre-pregnancy BMI (kg/m ²)	23.0 $\pm$ 5.1	24.3 $\pm$ 4.1	<0.001
Pre-pregnancy physical activity [†] (hours/week)	7.5 $\pm$ 5.4	8.4 $\pm$ 5.6	<0.001
Alcohol consumption (g/day) [†]	2.3 $\pm$ 4.1	2.3 $\pm$ 2.7	<0.010
Gestational age at blood collection (weeks)	9.1 $\pm$ 1.5	9.8 $\pm$ 1.5	<0.001
Mean alveolar bone loss (mm)	1.48 $\pm$ 0.50	0.75 $\pm$ 0.34	<0.001
CRP level (mg/l)			0.680
Median	2.23	1.46	
Interquartile range	0.74 to 4.14	0.71 to 3.58	

* Data presented as mean $\pm$ SD unless indicated otherwise.

[†] Actual number varies somewhat due to missing data.

Multivariate Linear Regression Analysis Relating Periodontitis With Log Plasma CRP Levels (mg/l) Measured in Early Pregnancy*

Table 2

Model	Adjusted Mean CRP [†] (SE)		Difference (%) (95% CI)	P Value	R ²
	Periodontitis (≥1 site with bone loss ≥3 mm)	No Periodontitis (<1 site with alveolar bone loss ≥3 mm)			
All participants (33 cases; 63 controls)	2.46 (0.52)	1.49 (0.22)	65.4 (-2.2, 179.8)	0.06	0.27
Restricted to participants whose radiographs were taken before blood collection (28 cases; 54 controls)	2.43 (0.58)	1.43 (0.24)	69.7 (-5.7, 205.4)	0.08	0.31

* Adjusted for gestational age at blood collection (weeks), age (years), race/ethnicity (white versus other), completion of college education (yes/no), annual household income more than \$70,000 (yes/no), pre-pregnancy BMI (kg/m²), and alcohol intake at first trimester of pregnancy (g/day).

† Adjusted means in multivariate model were computed using least square means procedures, holding all covariates to their average values, and back-transformed to original scale.