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Factors associated with maternal serum C-reactive protein throughout pregnancy: A longitudinal study in women of Rio de Janeiro, Brazil

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Objectives: The aim of this study was to evaluate the longitudinal changes of C-reactive protein (CRP) concentrations during pregnancy and to assess whether socioeconomic, anthropometric, dietary, behavioral, and biochemical factors are associated with these changes.

Methods: This was a prospective cohort study of 115 adult pregnant women, followed at gestational weeks 5 to 13, 20 to 26, and 30 to 36. Serum concentrations of CRP (mg/L) were measured by the immunoturbidimetric method with ultrasensitive kits (sensitivity 0.05 mg/dL). The statistics included descriptive analysis (mean + SD) and longitudinal linear mixed-effects models, reporting the β coefficient and 95% confidence intervals (CI).

Results: Serum CRP concentrations progressively increased throughout pregnancy (β = 0.121; 95% CI, 0.071–0.171). Parity (β = 1.579; 95% CI, 0.731–2.427) and prepregnancy body mass index (BMI) (β = 0.316; 95% CI, 0.053–0.587) were positively associated and dietary glycemic load was negatively associated (β = −0.203; 95% CI, −0.380 to −0.026) with CRP concentrations in the multiple model. Prepregnancy obese women presented a more pronounced increase of CRP concentrations compared with normal weight women (β = 0.210; 95% CI, 0.059–0.360 versus 0.115, respectively; 95% CI, 0.049–0.181). A statistically significant interaction was observed between parity and gestational age (β = −0.045; 95% CI, −0.084 to −0.005), indicating that the variation of CRP throughout pregnancy differed according to parity categories.

Conclusion: CRP concentrations increased throughout pregnancy. Parity and prepregnancy BMI were positively associated and dietary glycemic load was negatively associated with concentrations of CRP.

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Introduction

C-reactive protein (CRP) is an acute-phase biomarker that has been associated with the innate immune response [1]. Although CRP synthesis by pulmonary [2] and renal cells [3] has been demonstrated, most of it is produced by hepatocytes [4]. CRP's rate of synthesis is primarily stimulated by interleukin-6, interleukin-1β, and tumor necrosis factor-α as a reaction of tissues to infection or inflammation. It is suggested that concentrations of this cytokine are low and stable in healthy individuals [5].

Different factors have been related to increased CRP concentrations, such as low socioeconomic status, advanced age, high parity, excessive body weight, excessive gestational weight gain, intake of low-quality carbohydrates, smoking, and excessive alcohol consumption [6–8]. Additionally, cardiovascular diseases were already associated with increased CRP concentrations [9–11].

One study found that CRP concentrations seemed to be higher during pregnancy. However, contradictory results about CRP concentrations throughout normal pregnancy have been found [12]. Some studies have shown no changes in CRP concentrations [12,13] but other studies have found an increase in early pregnancy and at the period near delivery, with constant values across rest of the pregnancy [14,15].

Considering the lack of consensus in the literature regarding the pattern of CRP concentrations throughout pregnancy, the present study aims to describe the longitudinal changes of maternal serum CRP concentrations and to evaluate the effect of socioeconomic, anthropometric, dietary, behavioral, and biochemical variables on the trends of this cytokine throughout healthy pregnancy.

Methods

This was a prospective cohort study (November 2009 to October 2011) with a sample composed of pregnant women who received prenatal care at a Municipal Health Center in Rio de Janeiro, Brazil, during the three trimesters of pregnancy (gestational weeks 5–13, 20–26, and 30–36).

In all, we recruited 299 pregnant women who were between 5 and 13 wk of gestation, between ages 20 and 40 y, and who did not have any noncommunicable chronic diseases (NCDs) other than obesity. After baseline clinical evaluation, women were excluded if they had a confirmed prepregnancy diagnosis of NCDs (n = 9), infectious or parasitic disease (n = 9), were expecting twins (n = 4), suffered a miscarriage before the first evaluation (n = 3), did not have blood samples collected before gestation week 13 (n = 13), did not have CRP measurements (n = 67), and reported use of anti-inflammatory drugs during pregnancy (n = 15). Additionally, women were excluded if they did not know their pregestational weight (n = 12) and if they showed differences between self-reported and measured weight until the 13th week of pregnancy outside the range of ±2 SDs in the Bland and Altman graph (n = 10). We further excluded women who had a positive result for urinary tract infection in the time period during which CRP was measured (weeks 5–13, n = 42; 20–26, n = 61; 30–36, n = 63). Therefore, the baseline sample consisted of 115 pregnant women who were followed at 20 to 26 (n = 96) and at 30 to 36 (n = 94) gestational weeks.

Blood samples were collected in all three trimesters of pregnancy after a 12-h fast. Serum and plasma were separated after 5 min of centrifugation (5000g) and stored at −196°C, initially in a liquid cryogen container. Subsequently, the samples were stored in an ultra-freezer at −80°C until analysis. Plasma with EDTA as anticoagulant was used for the determination of leptin, insulin, and adiponectin concentrations.

The CRP serum concentrations (mg/L) were measured by the immunoturbidimetric method with ultra-sensitive commercial kits (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) with sensitivity of 0.05 mg/dL. Adiponectin (μg/mL), insulin (μU/mL), and leptin (ng/dL) plasma concentrations were determined using commercial enzyme-linked immunosorbent assay kits (Millipore, St. Charles, MO, USA) with sensitivities of 0.78 μg/mL, 2 μU/mL, and 0.5 ng/dL, respectively. Blood glucose was measured by the glucose oxidase-peroxidase enzymatic colorimetric method with a Wiener Lab kit (Rosario, Argentina).

Socioeconomic, demographic, reproductive, and lifestyle variables such as age (y), education (y), monthly per-capita family income (US$), marital status (married or living in union/single), self-reported skin color (white/brown and black), parity (number of parturition), smoking habit before pregnancy (no or yes) and alcohol consumption (no or yes) were obtained with structured questionnaires administered during the first follow-up visit (5–13 wk gestation).

The self-reported pregestational weight was obtained during the first follow-up visit. Maternal body weight was measured in all follow-up visits using a digital scale (Filizzola PL 150, Filizzola Ltda. Brazil), and height was measured only in the first wave of the follow-up using a portable stadiometer attached to the wall (Seca Ltda., Hamburg, Germany). Prepregnancy body mass index (BMI) was calculated based on self-reported pregestational weight and classified as normal weight (NW: 18.5–24.9 kg/m^2), overweight (OW: 25–29.9 kg/m^2), or obese (OB: ≥30 kg/m^2) according to the World Health Organization criteria [16].

Dietary variables were obtained through a semi-quantitative food frequency questionnaire during the first follow-up visit [17]. Food frequency questionnaire items were transformed into daily portion according to the daily frequency and portion size intake using a method previously described [18]. The Brazilian Food Composition Table [19] was used to determine the composition of foods, and the US Department of Agriculture nutrient database [20] was used for foods that were lacking information in the Brazilian source. These analyses were used to determine the dietary glycemic index (GI) and the glycemic load (GL). The GI was obtained considering values of the International Tables of GI and GL [21]. If a certain value was not available in this table, the GI was retrieved from the website www.glycemicindex.com of the University of Sydney [22]. The dietary GL was calculated multiplying the carbohydrate content of one serving by the GI, thus represents the quality and quantity of carbohydrates [23].

Gestational age was calculated based on data from the first ultrasonography performed (n = 95; 82.6%). The date of the last menstrual period (n = 20) was used if the first ultrasonography was not performed before week 24 of gestation.

Statistical analysis

Means and SD were used to describe the characteristics of the sample. Longitudinal linear mixed-effects (LME) models were used to assess the changes of CRP concentrations throughout pregnancy and to evaluate which variables were associated with the trend of change (reporting coefficients [β] and their 95% confidence interval [CI]).

LME regression coefficients provide a combined estimate of the effect between and within the participants, accommodate time-dependent and time-independent covariates, and allow unbalanced time intervals [24,25]. The models were fitted using the unstructured covariance matrix, and the gestational age (in weeks) was included in all LME models as both random and fixed effects to adjust for the overall and the individual variations in CRP concentrations over time. All other variables were considered as fixed-effects only.

Variables that yielded $P < 0.20$ in the longitudinal bivariate regression models were included in the full model. Subsequently, a backward selection from the full model was employed in which the variables included were removed one by one in decreasing order of significance so that only variables with $P < 0.05$ remained in the multiple model. The interaction between the variables that remained in the multiple model and the time variable (gestational age) was tested to evaluate if the effect of gestational age on CRP varied according to these variables.

Figures presenting the scatter and longitudinal linear prediction (predicted using LME models) of CRP concentrations were constructed to illustrate the variation of CRP during pregnancy for the total sample and were also stratified according to prepregnancy BMI categories (NW, OW, OB), parity (nulliparous × multiparous), and dietary GL (above or below the median).

Statistical analyses were performed using Stata Data Analysis and Statistical Software (STATA) version 12.0 (Stata Corp., College Station, TX, USA). *P* values <0.05 were considered statistically significant.

The study was approved by the Research Ethics Committees of the Municipal Secretariat of Health and Civil Defense of the State of Rio de Janeiro (IRB no. 0012.0.249.000-09, approved on August 13, 2009). All participants signed a two-way term of consent, which was obtained freely and spontaneously, after all necessary clarifications had been provided.

Results

The sample consisted of women with a mean age of 27 (5.6) y who had 8.9 (3) years of schooling and a pregestational BMI of 24.7 (4.5) kg/m^2. At the study baseline, the mean concentration of CRP was 5.7 (4.7) mg/L, adiponectin was 6 (4.4) μg/mL, insulin was 5.9 (3.7) U/mL, and leptin was 20.5 (14.5) ng/mL (Table 1).

We found an increase in CRP concentrations throughout pregnancy (β = 0.121; 95% CI, 0.071–0.170) for the overall sample (Fig. 1).

In the bivariate models, gestational age, parity, smoking habit (ex-smokers), body weight during pregnancy, and prepregnancy BMI were positively and significantly associated with CRP, whereas dietary GL was negatively associated (Table 2). These variables and the dietary GI ($P = 0.105$) were included in the full model.

In the multiple model, parity (β = 1.579; 95% CI, 0.731–2.427) and prepregnancy BMI (β = 0.316; 95% CI, 0.053–0.587) were positively and dietary GL was negatively (β = −0.203; 95% CI, −0.380 to −0.026) associated with serum concentrations of CRP. A statistically significant interaction was observed between parity and gestational age (β = −0.045; 95% CI, −0.084 to

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