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The Association of Prenatal C-Reactive Protein and Interleukin-8 Levels with Maternal Characteristics and Preterm Birth

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Abstract

Objective—The determinants of preterm birth remain unknown. Excessive maternal inflammation during pregnancy may play an important role in the pathogenesis of preterm birth. Our objective was to describe the association of prenatal levels of proinflammatory C-reactive protein (CRP) and interleukin-8 (IL-8) with preterm birth in participants of the Vitamin D Antenatal Asthma Reduction Trial.

Study Design—Five hundred and twenty-eight patients with available samples of both first- and third-trimester plasma were included in this analysis. CRP and IL-8 were measured from maternal prenatal samples. We examined the association between prenatal CRP and IL-8 with maternal health characteristics and the outcome of preterm birth. We also described the patterns of change in CRP and IL-8 from first to third trimester and their association with preterm birth. A subgroup analysis comparing only those with a spontaneous preterm birth phenotype to those with term birth was also performed.

Results—Maternal characteristics including lower educational attainment, higher prepregnancy body mass index, gestational diabetes, lower vitamin D, and an unhealthy diet were associated with elevated levels of prenatal CRP and IL-8. Higher third trimester CRP and an increase in CRP from first to third trimester were associated with an increased odds of preterm birth when

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Conflict of Interest

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compared to lower levels of CRP (adjusted odds ratio [aOR] = 1.49, 95% confidence interval: 1.02, 2.23, $p = 0.04$) or a decrease in CRP over pregnancy (aOR = 3.06, 95% CI = 1.31,7.55, $p = 0.01$), after adjusting for potential confounders. These associations were strengthened when comparing only patients with spontaneous preterm birth ($n = 22$) to those with term births.

Conclusion—Higher levels of the proinflammatory markers CRP and IL-8 are associated with indicators of poor maternal health and preterm birth. Prenatal CRP levels may reflect maternal prenatal health status and serve as a predictor of preterm birth, especially among those with spontaneous preterm birth.

Keywords

inflammation; preterm birth; biomarkers; maternal health; cytokine; CRP

In order to sustain a healthy term pregnancy, the maternal immune system undergoes considerable shifts throughout the prenatal period. Prior studies have described the alterations in levels of maternal serum and cervicovaginal fluid C-reactive protein (CRP), a marker of acute inflammation, and other inflammatory cytokine levels throughout pregnancy.[1,2] An excessive maternal inflammatory response has been linked to common pregnancy complications such as preterm birth that may occur in up to 10% of pregnancies.[3–6] Specifically, elevated levels of CRP and the inflammatory cytokine interleukin-8 (IL-8) have been shown in smaller cohorts to be associated with preterm birth.[6–9] Profiling of maternal CRP and IL-8 levels throughout pregnancy in preterm and term births may be important in understanding how to prevent undesirable prenatal inflammation.

Previous studies describing the changes in maternal CRP and IL-8 have been limited in sample size, cohort diversity, characterization of maternal health during pregnancy, and phenotypic characterization of preterm birth[10] or were limited to a single measurement during pregnancy. In this study, we determine the associations of CRP and IL-8 measured at two timepoints in pregnancy, with preterm birth by phenotype and maternal health characteristics and behaviors in a large, diverse, and well-characterized birth cohort. We hypothesize that poor maternal health, poor health behaviors, and preterm birth are associated with increased prenatal CRP and IL-8.

Materials and Methods

Study Design and Participants

We performed an ancillary analysis of the Vitamin D Antenatal Asthma Reduction Trial (VDAART) study, a randomized, double-blind placebo-controlled trial of 816 pregnant women randomized to 4,400IU vitamin D daily or placebo multivitamin with 400IU vitamin D daily. Study participants were aged between 18 and 39 years with a personal or biological father's history of asthma or atopy. Notable exclusion criteria included maternal chronic medical conditions, maternal smoking at enrollment, multiple gestations, and major fetal anomalies detected prior to delivery. Full details of the VDAART study design have been previously published.[11] Of the 816 women in the intention-to-treat cohort, 528 women with longitudinal plasma samples were included in this analysis. Written informed consent was

obtained, and the study was approved by the institutional review boards at each clinical center.

Ascertainment of Maternal Characteristics

Maternal characteristics assessed at baseline included age (categorized as <35 or ≥35 years), educational attainment, obstetrical history, race/ethnicity, and maternal secondhand smoke exposure. Tobacco smoke exposure during pregnancy was defined as the maternal report of any secondhand household tobacco smoke exposure assessed at enrollment or firsthand maternal smoking assessed monthly after enrollment. A short maternal health questionnaire and the Asthma Control Test (ACT) were administered monthly. The frequency of uncontrolled asthma as determined by the ACT was calculated for patients who completed >50% of the monthly surveys and was adjusted for the number of surveys completed. An electronic medical record review was also conducted monthly to monitor for pregnancy complications including preeclampsia and gestational diabetes. An extensive survey was administered in the third trimester including a validated, abbreviated 18-item food frequency questionnaire asking how often each of 18 food groups were consumed over the prior year.[12] Principal component analysis of the maternal food frequency questionnaire data was used to derive a maternal diet summary variable using the top principal component.[13] Higher top principal component score represents a less healthy diet with more red meat, processed meat, baked products, and deep-fried foods (Supplementary Table S1, available in the online version). Maternal prepregnancy and delivery body mass index (BMI) were determined from an electronic medical record review.

Prenatal Plasma Analysis: C-Reactive Protein and Interleukin-8

Maternal high-sensitivity CRP and IL-8 were measured at two prenatal timepoints: early pregnancy between 10 and 18 weeks (first trimester) of gestation and late pregnancy between 32 and 38 weeks (third trimester) of gestation. The validated Meso Scale Discovery Platform assays were employed to obtain maternal plasma CRP (in mg/L) and IL-8 (in pg/mL) levels at the Laboratory of Genital Tract Biology, Brigham and Women's Hospital, under accreditation by the College of American Pathologists.[14] Assay parameters are presented in Supplementary Table S2 (available in the online version). The same plasma samples had been used previously, to measure prenatal vitamin D [25(OH)D] levels in nanograms per milliliter with the DiaSorin LIAISON chemiluminescence immunoassay at the Channing Division of Network Medicine, Brigham and Women's Hospital.[15]

Study Outcomes: Preterm Birth

The VDAART study design included an assessment of preterm birth as a secondary pregnancy outcome and adverse event. The preterm birth occurrence was assessed monthly during gestation and again after offspring birth by electronic medical record review and defined as any live birth at less than 37 weeks of gestation. Two subgroup analyses were also performed to characterize the observed associations by preterm birth phenotype. One subgroup analysis excluding those with preterm birth with preeclampsia overlap was performed. Another subanalysis compared only those with spontaneous preterm birth at less than 37 weeks to those born at ≥37 weeks. Spontaneous preterm birth was defined as patients who delivered at <37 weeks and had spontaneous onset of labor and excluded

patients who had induction of labor at <37 weeks for maternal or fetal indications. Preterm birth phenotypes of each patient are provided in Supplementary Table S3 (available in the online version).

Statistical Analysis

All statistical analyses were performed as two-sided tests with a prespecified significance level of α equals 0.05 and using the R statistical package version 4.0.3. Reported p-values are two sided. CRP and IL-8 were log-normally distributed and transformed for analysis as appropriate. Baseline maternal characteristics were compared using Student's t-test for continuous characteristics or chi-square tests for polytomous or dichotomous characteristics.

For continuous and dichotomous maternal baseline characteristics, associations with log-transformed CRP and IL-8 were evaluated using univariate linear regression analyses. For polytomous maternal baseline characteristics such as maternal race/ethnicity and study site, association with log-transformed CRP and IL-8 was evaluated with a Kruskal–Wallis H-Test.

Wilcoxon's rank-sum tests were used to compare biomarker level group means in early and late pregnancy. We then considered the change in CRP and IL-8 over pregnancy in two ways. To describe biomarker change over pregnancy, we first categorized CRP and IL-8 values at each timepoint into tertiles and visualized the change in tertiles from early to late pregnancy with transition plots. Biomarker change over pregnancy was also examined as a dichotomous variable obtained by categorizing the difference between first and third trimester biomarker levels as positive (denoting an increase in the biomarker from early to late pregnancy) or negative (denoting a decrease in the biomarker from early to late pregnancy). When testing associations between the dichotomized biomarker change over pregnancy and maternal characteristics, an adjustment for the baseline biomarker level was included in regression models to account for regression toward the mean (i.e., [late pregnancy biomarker – early pregnancy biomarker] / early pregnancy biomarker).

Logistic regression models were used to determine associations between the binary preterm birth outcome and log-transformed CRP and IL-8 in early (10–18 weeks of gestation) and late pregnancy (32–38 weeks of gestation) as well as biomarker change over pregnancy. Based on prior knowledge, study site, maternal race/ethnicity, prepregnancy BMI, and any prenatal first and/or secondhand smoke exposure were included in models as potential confounders.[11,16,17] The six maternal race/ethnicity categories were made based on CRP distribution as Black, White, and Other to avoid overfitting the models. In the adjusted models, 81 patients with missing prepregnancy BMI and/or smoke exposure data were excluded from the analysis for a final sample size of 447 patients (n = 31 preterm births). To reduce collinearity, maternal education was not included as a potential confounder as it correlated closely with the study site (Chi-square test $p < 0.001$). Two sensitivity analyses were performed. A sensitivity analysis was performed excluding outliers with extreme IL-8 levels. Additionally, any patients whose plasma collection occurred on or after the day of delivery were excluded in a second sensitivity analysis (n = 5).

We also considered that prenatal CRP and IL-8 could partially mediate an association between prepregnancy BMI or prenatal smoke exposure and preterm birth and performed a mediation analysis using R package “mediation.”

Results

Participant Baseline Characteristics

Of the 816 patients in the original VDAART intention-to-treat study base, 528 patients had complete longitudinal prenatal plasma CRP and IL-8 data. In total, 36 of the 528 patients (7%) met the criteria for preterm delivery. A total of 445 patients (n = 30 with preterm birth) had complete covariate data and were included in the adjusted analyses. Of the patients with preterm delivery, 21 of the 30 were spontaneous preterm births and were examined in a subanalysis that excluded those with induced preterm delivery or preterm delivery by c-section without labor (Supplementary Table S3, available in the online version). Six patients had both preterm delivery and preeclampsia. Of the original 816 patients in the intention-to-treat cohort, 76 (9%) met the criteria for preterm birth. Baseline characteristics of patients who were included versus excluded based on the availability of CRP and IL-8 data were comparable except for the timing of third-trimester sample collection (included mean 34.06 weeks gestation, excluded 33.74 weeks of gestation, p = 0.01, Supplementary Table S4, available in the online version).

Baseline maternal characteristics of those with preterm delivery were comparable to those with term delivery except for differences in the study site (p = 0.02) and receipt of perinatal antibiotics (p = 0.01, Supplementary Table S5, available in the online version). Perinatal antibiotic use was more frequent among those with preterm births compared to those with term births (60.0 vs. 39.1%, p = 0.01). Vitamin D treatment assignment was balanced between those with and without preterm birth (57.1 vs. 47.0% assigned to high-dose vitamin D, p = 0.33).

Prenatal C-Reactive Protein and Interleukin-8

The median CRP level was 8.8 mg/L in the first trimester and 7.6 mg/L in the third trimester with a range from 0.14 to 99.7 mg/L. The median IL-8 level was 3.0 pg/mL in the first trimester and 2.8 pg/mL in the third trimester with a range from 0.5 to 13,300.7 pg/mL. There were three outliers with markedly elevated IL-8 levels above 10,000 pg/L, which were excluded in a separate sensitivity analysis. There was no significant correlation between first-trimester CRP and IL-8 (Spearman’s $\rho = -0.03$, p = 0.56), but there was a statistically significant albeit modest correlation between third-trimester CRP and IL-8 (Spearman’s ρ = 0.15, $p < 0.001$).

Wilcoxon’s rank-sum tests demonstrated a significant decrease from early to late pregnancy in the group means of CRP (13.7–12.1 mg/L, p = 0.001) and IL-8 (93.0–46.2 pg/mL, p = 0.02). Within individual patients, 288 out of 528 (54%) had a decrease in CRP and 298 out of 528 (56%) had a decrease in IL-8 from early to late pregnancy. When categorized by tertile, most patients remained within the same CRP tertile rather than changed tertiles between early and late pregnancy (59.0 vs. 41%), whereas the opposite result was observed

for IL-8, with more patients changing tertiles between early and late pregnancy (53 vs. 47%, Fig. 1). Of the patients who changed biomarker tertile, a minority of patients had a two-tertile change from early to late pregnancy for both CRP (4%) and IL-8 (14%).

Association of Prenatal C-Reactive Protein and Interleukin-8 with Maternal Characteristics

Significant associations between prenatal log-transformed CRP levels and maternal characteristics are displayed in Table 1 and Fig. 2. Higher levels of CRP in the first trimester and third trimester were significantly associated with lower maternal education, prior premature births, higher gravidity, and higher prepregnancy. Aninverse association was detected between first-trimester maternal 25(OH)D level and both first and third trimester CRP. Third-trimester CRP was positively associated with an increased frequency of uncontrolled asthma and unhealthy maternal diet during pregnancy. An increase in CRP over pregnancy was associated with a greater increase in BMI throughout pregnancy (adjusted odds ratio [aOR] = 1.11, $p = 0.01$) and with increased frequency of uncontrolled asthma versus controlled or no asthma (aOR = 7.77, $p = 0.02$). The association between CRP change from first to third trimester and uncontrolled asthma was preserved in an analysis limited to asthmatic mothers ($n = 194$ mothers with asthma including 52 with uncontrolled asthma, OR = 7.77, 95% confidence interval [CI] = 1.4, 53.0, $p = 0.02$). A decrease in CRP over pregnancy was associated with fewer previous pregnancies (aOR = 0.86, $p = 0.02$) and fewer living children (aOR = 0.73, $p < 0.001$).

There were fewer significant associations detected between prenatal IL-8 and maternal characteristics, and these are displayed in Table 2 and Supplementary Fig. S1 (available in the online version). Higher first-trimester IL-8 was associated with a history of prior premature births. Higher third-trimester IL-8 was associated with lower maternal education ($\beta = 0.24$, $p = 0.01$), an unhealthy maternal diet, smoke exposure during pregnancy, and higher prepregnancy BMI. Finally, increasing IL-8 over pregnancy was associated with gestational diabetes during the current pregnancy (aOR = 2.16, $p = 0.05$) and a higher prepregnancy BMI (aOR = 1.04, $p = 0.01$). Sensitivity analyses excluding the three outliers with extreme IL-8 levels produced comparable results (Supplementary Fig. S2, available in the online version).

Study site and maternal race and ethnicity were both significantly associated with first- and third-trimester prenatal biomarker levels except for a null association between first-trimester CRP and study site. We observed higher CRP levels in black Hispanic patients compared to black non-Hispanic, other Hispanic and non-Hispanic, and White Hispanic and non-Hispanic (Table 1). Higher IL-8 levels in Hispanic patients compared to non-Hispanic patients were also observed (Table 2). We found no biomarker associations with perinatal antibiotic use, mode of delivery, vitamin D treatment assignment, or third trimester 25(OH)D levels.

Association of Prenatal C-Reactive Protein and Interleukin-8 with Preterm Birth by Phenotype

In regression models adjusted for study site, maternal race/ethnicity, prepregnancy BMI, and prenatal smoke exposure, preterm birth was associated with higher third trimester

log-transformed CRP (aOR = 1.49, 95% CI = 1.02, 2.23, $p = 0.04$) and an increase in CRP from early to late pregnancy (aOR = 3.06, 95% 1.31, 7.55, $p = 0.01$, Fig. 3). Exclusion of the six patients with both preterm birth and preeclampsia produced comparable results (third trimester CRP: aOR 1.58, 95% CI = 1.05, 2.44, $p = 0.03$; CRP change: aOR = 2.77, 95% CI = 1.11,7.26, $p = 0.03$; Fig. 3; Supplementary Table S6, available in the online version). The associations observed between third-trimester CRP and an increase in CRP from early to late pregnancy were strengthened when comparing only patients with spontaneous preterm birth ($n = 22$) to those with term births (third trimester CRP: aOR = 2.05, 95% CI = 1.28, 3.39, $p < 0.001$; CRP change: aOR = 5.90, 95% CI = 2.07, 19.7, $p = 0.002$; Fig. 3; Supplementary Table S6, available in the online version). A similar trend was noted for IL-8 with higher third trimester log-transformed IL-8 (aOR = 1.20, 95% CI = 0.90, 1.54, $p = 0.18$) and an increased IL-8 from early to late pregnancy (aOR = 1.74, 95% CI = 0.77, 4.07, $p = 0.19$, Supplementary Fig. S3, available in the online version) among patients with preterm birth, but did not reach the level of significance. There were no associations between IL-8 and any of the preterm birth endotypes or after exclusion of the extreme IL-8 outliers (Supplementary Table S6 and Supplementary Fig. S4, available in the online version).

We hypothesized that in addition to confounding the association between prenatal CRP and IL-8 and pregnancy outcomes, maternal prepregnancy BMI and prenatal smoke exposure could alternatively be conceptualized as independent variables with effects on preterm birth that are partially mediated through prenatal CRP and IL-8 (Supplementary Fig. S5, available in the online version). However, mediation analyses failed to provide evidence of mediation by either CRP or IL-8 on the associations between maternal prepregnancy BMI or smoking and preterm birth (Supplementary Table S7, available in the online version). We additionally assessed for synergy between CRP and IL-8 and found that there was no significant interaction of first-trimester CRP and IL-8 or third-trimester CRP and IL-8 in the crude and adjusted models for preterm birth.

Sensitivity Analysis: Third Trimester Sample Collection Time

Three of the 528 patients had plasma samples collected 1 or 2 days after delivery instead of at the predefined 32 to 38 weeks gestation timepoint. Similarly, two patients had plasma samples collected on the day of delivery. The biomarker values for these five patients are further detailed in Supplementary Table S8 (available in the online version). Three of these five patients delivered offspring by spontaneous preterm births, and one was induced with preeclampsia (Supplementary Table S3, available in the online version). One patient delivered at full term. After exclusion of these five patients ($n = 523$), the associations with prenatal CRP and maternal characteristics were preserved, except for the association between third trimester CRP and maternal uncontrolled asthma (full cohort: $\beta = 0.75$, [95% CI = 0.08, 1.42], $p = 0.03$ vs. sensitivity analysis cohort: $\beta = 0.53$, [95% CI = 0.17, 1.23], $p = 0.13$, Supplementary Table S9, available in the online version). The direction of associations of preterm birth with third-trimester CRP and CRP change were also preserved (Supplementary Table S9, available in the online version). However, in the association of third-trimester CRP and preterm birth, the association did not reach the $\alpha = 0.05$ level of significance after the exclusion of patients with CRP measurements on or after the day of delivery (aOR = 1.38, 95% CI = 0.91, 2.1, $p = 0.13$).

Discussion

In this diverse study sample of 528 mother–child pairs, we detected a significant association between preterm birth and higher levels of third-trimester plasma CRP. Consistent with known heightened inflammation in the first trimester of pregnancy,[18,19] we found that CRP levels overall were lower in late pregnancy than in early pregnancy; however, 46% of patients exhibited an increase in CRP from early to late pregnancy, and we found an increased risk of all preterm birth phenotypes among mothers whose CRP was elevated in the third trimester or increased from first to third trimester. There was no association between first-trimester CRP and preterm birth.

While our findings are aligned with prior reports,[6,9,20] the role of prenatal CRP measurement in predicting preterm birth has been difficult to establish. This may be due to nonspecificity of CRP and the multifactorial nature of preterm birth.[10] Prior literature examining prenatal inflammatory markers and preterm birth has not distinguished between the various preterm birth phenotypes. To address this, we performed two subgroup analyses to distinguish between preterm birth phenotypes and their association with prenatal CRP. Notably, the associations we observed between prenatal CRP and preterm birth were strengthened among those with spontaneous preterm birth suggesting that prenatal inflammation plays a greater role in the pathophysiology of spontaneous preterm birth than other endotypes.

In order to better understand the mechanisms of inflammation and CRP in pregnancy outcomes, we defined relationships between CRP and several maternal characteristics that may impact birth outcomes. Maternal factors that have been previously associated with preterm birth such as smoke exposure during pregnancy, uncontrolled asthma, and an unhealthy maternal diet were associated with higher levels of CRP in our analysis.[16,21,22]

In a sensitivity analysis excluding five patients with a second CRP drawn on or within 2 days after the day of delivery, we observed an attenuated association between third-trimester CRP and preterm birth, suggesting that this association is sensitive to the timepoint of collection and that perhaps elevated CRP in these patients could have been due to other factors. While the half-life of CRP is purported to range from 19 to 46 hours,[23,24] suggesting that levels within 2 days after delivery are likely to reflect levels prior to delivery, there are other factors that could contribute to an acute rise in CRP within hours of delivery or postpartum.[25,26] In contrast, our finding that increasing CRP from early to late pregnancy is associated with preterm delivery was preserved in this sensitivity analysis, indicating that this association is robust to include only patients whose CRP measurements occurred prior to delivery.

To address the nonspecificity of CRP, we obtained measurements of prenatal IL-8, another inflammatory marker, but did not find any significant associations between prenatal IL-8 and preterm birth or any of the preterm birth phenotypes. Two studies have reported on similar associations but with positive findings. Ashford et al found elevated prenatal cervicovaginal fluid IL-8 in preterm birth, while Herrara-Munoz et al found elevated prenatal serum IL-8 in threatened preterm labor when measured between 24 and 34 weeks of gestation.[6,9] The

timing of IL-8 measurement and compartment of IL-8 measurement was not comparable to our study and may account for the contrasting findings.

Both inflammatory biomarkers of interest (CRP and IL-8) revealed similar patterns of association with lower maternal education and markers of maternal health such as higher prepregnancy BMI and a history of prior premature births. Higher first-trimester CRP was associated with the development of gestational diabetes, whereas this association was not detected for first-trimester IL-8; instead, gestational diabetes was associated with an increase in IL-8 from early to late pregnancy. Overall, more significant associations were detected between the widespread acute-phase reactant CRP with maternal characteristics and pregnancy outcomes than for IL-8, although we did observe a trend toward a positive association between IL-8 and preterm birth. Previous studies also support an association between IL-8 gene expression pathways and preterm birth and it is possible that IL-8 has more tissue-specific action or may exert its effects at different timepoints that were not captured in this analysis.[6,8,27–29]

Finally, as vitamin D was of interest in the VDAART study, prenatal plasma 25(OH)D levels were measured at the same timepoints as plasma CRP and IL-8. We found that vitamin D sufficiency in early pregnancy was associated with lower levels of CRP both in early and late pregnancy. Taken in conjunction with our findings of higher CRP in those with preterm birth, these results suggest that vitamin D sufficiency may impact maternal–fetal inflammation in pregnancy and pregnancy outcomes. This is consistent with existing literature and previously published VDAART analyses, which have detected an association between vitamin D insufficiency and dysregulation of immune response pathways and outcomes of preterm birth.[28–33] Mixed effects of high-dose vitamin D supplementation on plasma CRP in nonpregnant adults have been reported.[34] However, we did not find an association between vitamin D treatment group and either inflammatory biomarker or with preterm birth, suggesting that the inflammatory pathways may become upregulated very early in pregnancy and that improving vitamin D status prior to pregnancy is necessary to prevent harmful inflammation and would not be captured in our cohort.

There were several limitations to this study. First, the small number of patients with preterm birth in our adjusted analyses ($n = 31$) may have limited our ability to detect associations with these outcomes and differences that may exist in subcategories of these complex and heterogeneous outcomes. There were no patients included with delivery at less than 31 weeks, which limited our ability to further characterize those of the very early and early preterm birth phenotype. There was also a notably higher proportion of patients with preterm birth among patients without available prenatal biomarker data compared to patients who were included in the analysis. Although patients included versus excluded were comparable on baseline characteristics except for timepoint of third-trimester sample collection which was unlikely to be clinically significant (0.32 weeks of gestation difference in the two means), the difference in outcome prevalence may suggest potential bias. There appeared to be a degree of variability in prenatal IL-8 as we saw from our analysis of serial first and third-trimester measurements. Additional measurements throughout pregnancy may provide a more informative picture of the associations of interest and capture patterns of fluctuation important in understanding disease pathogenesis. While pregnancy can be

regarded as a systemic process, there may also be more tissue-specific IL-8 effects such as at the uteroplacental interface that is not reflected by our plasma measurements but has been reported to be elevated in placental tissue of patients with preeclampsia.[8] Given the complexity and multifactorial nature of pregnancy outcomes, there may be unknown or unexpected confounders that were not addressed.

Conclusion

Our study provides support for the role of maternal inflammation in preterm birth, especially among the phenotype of spontaneous preterm birth. Our results also suggest that prenatal CRP may be a surrogate marker of poor maternal health and poor maternal health behaviors, important modifiable determinants in pregnancy outcomes. This study adds further support for an association of elevated prenatal CRP with adverse pregnancy outcomes. Further studies to determine the appropriate measures and mediators of prenatal inflammation, including the timing of sample collection, are needed to understand their role in the prediction and treatment of preterm birth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Elevated prenatal CRP is associated with poor maternal health.
- High prenatal CRP may predict premature birth, especially spontaneous premature birth phenotypes.
- Vitamin D insufficiency may be a modifiable risk factor for prenatal inflammation.

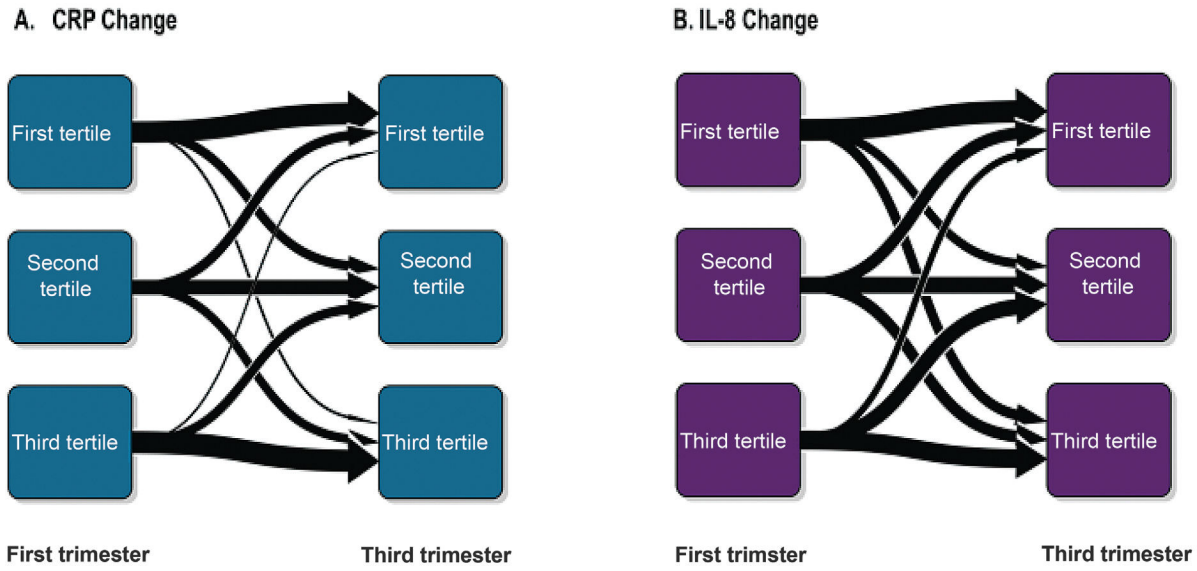

Fig. 1.

Transition plots depicting biomarker trajectory of patients categorized by CRP or IL-8 tertile in the first and third trimesters of pregnancy. Width of arrows is proportional to the number of patients in each trajectory, illustrating that more patients stay within the same tertile than shift tertiles over pregnancy. (**A**) CRP in mg/L tertile cutoffs: first trimester (0.2, 5.6, 14.5, and 82.9) and third trimester (0.1, 5.2, 12.6, and 99.7). (**B**) IL-8 in pg/mL tertiles cutoffs: first trimester (0.5, 2.3, 3.9, and 13300.7) and third trimester (0.5, 2.2, 3.7, and 11974.3). CRP, C-reactive protein; IL, interleukin.

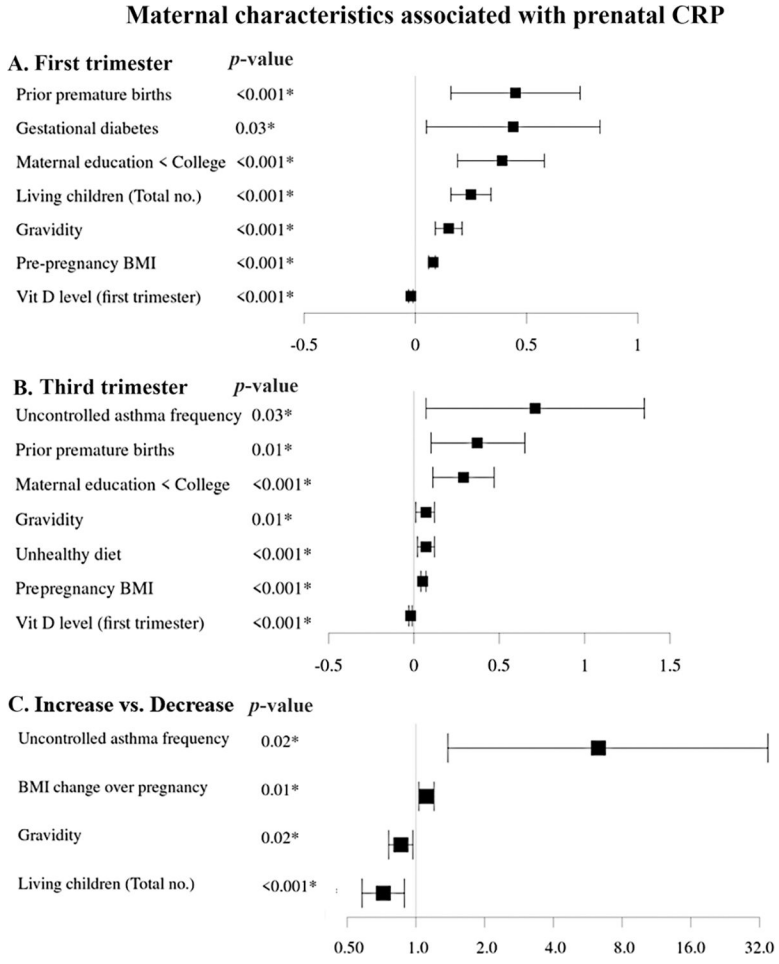

Fig. 2.

(A–C) Forest plots depicting model estimates and 95% confidence intervals for significant associations of maternal characteristics with **(A)** first trimester log(CRP) as determined by univariate linear regression, **(B)** third trimester log(CRP) as determined by univariate linear regression, and **(C)** an increase in CRP over pregnancy as determined by logistic regression adjusted for baseline CRP level. Maternal characteristics are ordered from highest to lowest beta coefficient **(A, B)** or odds ratio **(C).** An unhealthy diet was based on principal component analysis of food frequency questionnaire data with higher scores characterized by a higher consumption of red or processed meats, baked products, and deep-fried foods. *Denotes *p*-value less than the predefined level of significance, α <0.05. CRP, C-reactive protein.

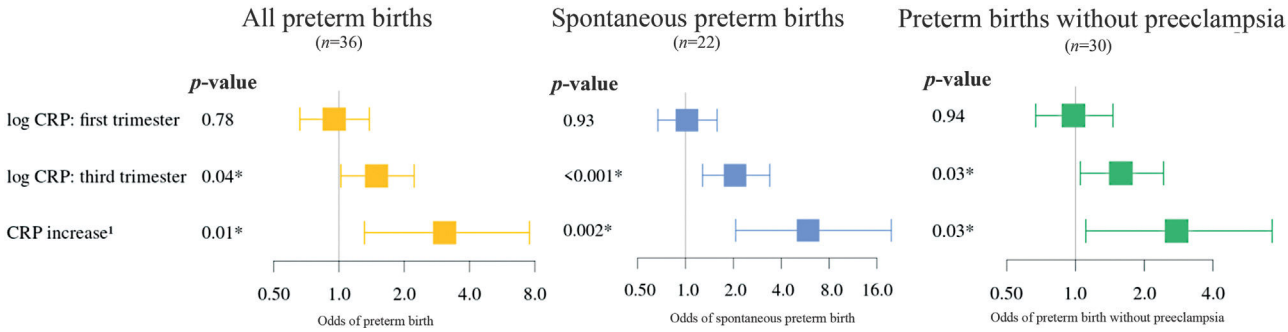

Fig. 3.

Forest plots depicting the adjusted odds ratio and 95% confidence intervals obtained from logistic regression models of associations between prenatal CRP with preterm birth phenotypes. All preterm birth patients compared to term patients are depicted in yellow, only spontaneous preterm birth patients compared to term patients are depicted in blue, and preterm birth patients excluding those with concurrent preeclampsia are depicted in green. Models were adjusted for study site, maternal race/ethnicity, prepregnancy BMI, and any prenatal smoke exposure.[1] Models for biomarker change over pregnancy were additionally adjusted for the baseline biomarker value. Denotes *p*-value less than predefined level of significance, α <0.05. BMI, body mass index; CRP, C-reactive protein.

Table 1

Associations of maternal baseline characteristics with first and third trimester log-transformed CRP

Association by linear regression model	**First trimester log (CRP)** ***n*** **= 528** ***β*** **(95% CI)**	***p*-Value**	**Third trimester log (CRP)** ***n*** **= 528** ***β*** **(95% CI)**	***p*-Value**
Maternal age ≥ 35 y	−0.09 (−0.44, 0.26)	0.62	−0.17 (−0.50, 0.16)	0.30
Maternal education: less than college	0.39 (0.19, 0.58)	<0.001[a]	0.29 (0.11, 0.47)	<0.001[a]
Treatment arm: high-dose prenatal vitamin D (4,400 IU/day)	0.02 (−0.17, 0.21)	0.84	0.05 (−0.12, 0.23)	0.55
Prepregnancy BMI	0.08 (0.06, 0.09)	<0.001[a]	0.05 (0.04, 0.07)	<0.001[a]
BMI change over pregnancy	−0.03 (−0.06, 0.00)	0.09	0.01 (−0.02, 0.03)	0.66
Gravidity (total no.)	0.15 (0.09, 0.21)	<0.001[a]	0.07 (0.01, 0.12)	0.01[a]
Prior premature births (total no.)	0.47 (0.18, 0.77)	<0.001[a]	0.37 (0.10, 0.65)	0.01[a]
Living children (total no.)	0.25 (0.16, 0.34)	<0.001[a]	0.08 (−0.01, 0.17)	0.07
Birth mode: C-section	0.18 (−0.03, 0.39)	0.10	0.18 (−0.01, 0.38)	0.07
Perinatal antibiotics	−0.13 (−0.33, 0.06)	0.19	−0.05 (−0.23, 0.13)	0.56
Unhealthy diet by PCA	0.05 (0.00, 0.10)	0.06	0.07 (0.02, 0.12)	<0.001[a]
Any smoke exposure during pregnancy	–	–	0.16 (−0.08, 0.40)	0.19
Gestational diabetes	0.44 (0.05, 0.82)	0.03[a]	−0.06 (−0.42, 0.30)	0.76
Frequency of uncontrolled asthma in pregnancy	−0.14 (−0.89, 0.61)	0.71	0.75 (0.08, 1.42)	0.03[a]
Vitamin D level (first trimester)	−0.02 (−0.03, −0.01)	<0.001[a]	−0.02 (−0.03, −0.01)	<0.001[a]
Vitamin D level (third trimester)	–	–	0.00 (−0.01, 0.00)	0.24
Association by Kruskal-Wallis *H*-test	CRP in mg/L (median, 25%ile, 75%ile)	*p*-Value	CRP in mg/L (median, 25%ile, 75%ile)	*p*-Value
Maternal race/ethnicity				
Black				
Hispanic	17.8 (12.1, 38.1)		17.3 (12.5, 26.5)	
Non-Hispanic	9.8 (3.8, 20.0)		9.5 (4.6, 18.3)	
Other				
Hispanic	10.6 (6.6, 23.3)	<0.001[a]	7.9 (4.8, 15.1)	<0.001[a]
Non-Hispanic	6.9 (3.5, 10.6)		5.6 (2.7, 11.5)	
White				
Hispanic	7.7 (5.2, 16.8)		7.2 (4.0, 12.4)	

Association by linear regression model	First trimester log (CRP) n = 528 β (95% CI)	p-Value	Third trimester log (CRP) n = 528 β (95% CI)	p-Value
Non-Hispanic	6.6 (3.1, 14.9)		6.0 (3.5, 14.8)	
Study site				
Boston	8.8 (4.2, 21.0)	0.33	8.6 (4.4, 17.9)	0.04[a]
San Diego	7.8 (3.7, 16.6)		6.6 (3.5, 12.7)	
St. Louis	10.1 (3.6, 18.6)		9.3 (4.3, 17.8)	

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; IU, international units; PCA, principal component analysis (unhealthy diet high in red meat, processed meats, baked goods, and deep-fried foods); SD, standard deviation.

Note: Missingness: pre-pregnancy BMI (n = 77), BMI change over pregnancy (n = 98), unhealthy diet and red meat consumption by PCA (n = 3), vitamin D level in third trimester (n = 2), smoke exposure during pregnancy (n = 6).

[a]Denotes p-value less than predefined level of significance, α <0.05.

Table 2

Associations of maternal baseline characteristics with first and third trimester log-transformed IL-8

Association by linear regression model	**First trimester log (IL-8)** ***n*** **= 528** ***β*** **(95% CI)**	***p*-Value**	**Third trimester log (IL-8)** ***n*** **= 528** ***β*** **(95% CI)**	***p*-Value**
Maternal age ≥ 35 years	−0.23 (−0.66, 0.20)	0.29	−0.15 (−0.49, 0.19)	0.39
Maternal education: less than college	0.14 (−0.10, 0.38)	0.24	0.24 (0.05, 0.43)	0.01[a]
Treatment arm: high-dose prenatal vitamin D (4,400 IU/d)	−0.01 (−0.25, 0.22)	0.90	−0.07 (−0.25, 0.12)	0.47
Prepregnancy BMI	0.00 (−0.01, 0.02)	0.88	0.02 (0.01, 0.04)	<0.001
BMI change over pregnancy	−0.01 (−0.04, 0.03)	0.68	−0.01 (−0.04, 0.02)	0.36
Gravidity (total no.)	−0.02 (−0.10, 0.05)	0.50	0.00 (−0.06, 0.06)	0.98
Prior premature births (total no.)	0.42 (0.06, 0.77)	0.02[a]	−0.01 (−0.29, 0.28)	0.97
Living children (total no.)	−0.03 (−0.14, 0.09)	0.64	0.01 (−0.08, 0.10)	0.87
Birth mode: C-section	−0.02 (−0.28, 0.23)	0.85	0.00 (−0.21, 0.20)	0.99
Perinatal antibiotics	−0.05 (−0.28, 0.19)	0.69	0.11 (−0.08, 0.30)	0.25
Unhealthy diet by PCA	−0.01 (−0.07, 0.05)	0.76	0.07 (0.02, 0.12)	0.01[a]
Any smoke exposure during pregnancy	–	–	0.28 (0.03, 0.53)	0.03[a]
Gestational diabetes	−0.06 (−0.53, 0.41)	0.80	0.06 (−0.31, 0.44)	0.74
Frequency of uncontrolled asthma in pregnancy	−0.19 (−1.13, 0.75)	0.69	−0.03 (−0.71, 0.65)	0.93
Vitamin D level (first trimester)	0.00 (−0.01, 0.01)	0.59	0.00 (−0.01, 0.01)	0.45
Vitamin D level (third trimester)	–	–	0.00 (−0.01, 0.00)	0.53
Association by Kruskal – Wallis *H*-test	IL-8 in pg/mL (median: 25%ile,75%ile)	*p*-Value	IL-8 in pg/mL (median: 25%ile,75%ile)	*p*-Value
Maternal race/ethnicity				
Black				
Hispanic	2.9 (2.3, 4.6)		3.2 (2.0, 5.0)	
Non-Hispanic	2.5 (1.7, 4.2)		2.3 (1.7, 4.2)	
Other				
Hispanic	3.7 (2.6, 5.1)	<0.001[a]	3.1 (2.4, 3.8)	0.03[a]
Non-Hispanic	2.9 (2.2, 3.6)		3.5 (2.3, 4.5)	
White				
Hispanic	3.8 (2.3, 4.9)		3.3 (2.3, 4.4)	

Association by linear regression model	First trimester log (IL-8) n = 528 β (95% CI)	p-Value	Third trimester log (IL-8) n = 528 β (95% CI)	p-Value
Non-Hispanic	3.1 (2.1, 4.4)		2.8 (2.1, 4.1)	
Study site				
Boston	3.5 (2.3, 5.2)	<0.001[a]	2.8 (1.9, 3.8)	0.01[a]
San Diego	3.2 (2.4, 4.3)		3.1 (2.3, 4.3)	
St. Louis	2.3 (1.6, 3.6)		2.5 (1.6, 4.8)	

Abbreviations: BMI, body mass index; CI, confidence interval; IL-8, interleukin-8; IU, international units; PCA, principal component analysis (unhealthy diet high in red meat, processed meats, baked goods, and deep-fried foods); SD, standard deviation.

Note: Missingness: prepregnancy BMI (n = 77), BMI change over pregnancy (n = 98), unhealthy diet and red meat consumption by PCA (n = 3), vitamin-D level in third trimester (n = 2), smoke exposure during pregnancy (n = 6).

[a]Denotes p-value less than predefined level of significance, α <0.05.