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First and second trimester immune biomarkers in preeclamptic and normotensive women

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ABSTRACT

Introduction: Circulating immune markers may be associated with preeclampsia but further investigations in early pregnancy and among preeclampsia subtypes are warranted. We examined immune markers in 208 preeclamptic women and 411 normotensive controls.
Methods: Our study was nested within the Collaborative Perinatal Project. A total of 242 women had first trimester serum samples and 392 had second trimester serum samples. Preeclampsia was defined as hypertension >20 weeks of gestation with proteinuria or pulmonary edema, oliguria, or convulsions. Preterm preeclampsia was defined as preeclampsia with delivery less than 37 weeks of gestation. Associations between immune markers RANTES, interleukin (IL)-6, IL4, IL5, IL12, IL10, IL8, IL1-beta, interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha and beta, transforming growth factor (TGF)-beta and preeclampsia were explored using a modified version of cox regression developed to address data with non-detectable levels. Models were adjusted for body mass index, gestational age of blood sampling, fetal sex, smoking, socioeconomic status and maternal age.
Results: In first trimester samples, IL-12 was associated with preeclampsia ($p = 0.0255$). IFN-gamma ($p = 0.0063$), IL1-beta ($p = 0.0006$), IL5 ($p = 0.0422$) and TNFr ($p = 0.0460$) were associated with preterm preeclampsia only. In second trimester samples, IL1-beta was associated with preeclampsia ($p = 0.0180$) and term preeclampsia ($p = 0.0454$). After correction for multiple comparisons, only IL1-beta remained associated with preterm preeclampsia in the first trimester ($p = 0.0288$).
Discussion: Elevated first trimester IL1-beta appears to be associated with preterm preeclampsia. However, few associations were observed in the second trimester. Systemic immune markers alone may not be useful for preeclampsia prediction.

1. Introduction

Despite progress in understanding preeclampsia pathogenesis, useful prediction models, treatments and specific diagnostic tests have been limited. Preeclampsia is a systemic maternal syndrome that affects 3–10% of pregnancies and is a leading cause of maternal mortality [1]. Clinically, preeclampsia is diagnosed as the new onset of hypertension ($\geqslant$140/90 mmHg) and proteinuria ($\geqslant$300 mg per 24 h urine collection) or end-stage organ failure after 20 weeks of gestation and the only treatment is delivery of the placenta [2]. The exact mechanisms that lead to preeclampsia are not completely elucidated. Furthermore, preeclampsia is hypothesized to have several subtypes which can complicate prediction. Thus, identifying biomarkers to better understand pathogenesis and differentiate subtypes will improve clinical management.

In normal pregnancy, the placenta secretes immune stimulating placental derived factors into the maternal circulation [3]. Balanced secretion of these factors may play a role in maternal immune tolerance towards the fetal allograft [4]. However, abnormal placentation could lead to increased placental shedding,

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exaggerated systemic inflammation and subsequent endothelial dysfunction, the key characteristics of preeclampsia [5,6]. This is consistent with third trimester studies reporting increased circulating pro-inflammatory markers [tumor necrosis factor alpha (TNFα), interleukin (IL)-8, IL-1β and interferon (IFN)-γ] in preeclampsia [7,8]. Similar studies have reported associations between pleiotropic or immune-modulatory markers including IL-6, IL-2, and IL-4, as well as anti-inflammatory marker IL10 [7–9]. However, studies conducted prior to the third trimester are conflicting. A second trimester study has reported lower circulating IL10, TNF-α and IFN-γ among women who develop preeclampsia [10]. While others report no significant differences in circulating immune markers [11,12]. First trimester investigations are limited. Circulating IP-10, a chemokine induced by IFN-γ, is increased in preeclampsia [13]. Additionally, first trimester elevated IL1β was shown to predict early onset preeclampsia [14].

Our own research has shown that mid-trimester systemic immune markers including IL6 and TNFβ are increased in preeclamptic women while IL1β is decreased [15]. This study conducted among 707 women from the Danish National Birth Cohort was unable to fully examine first trimester systemic immune markers. First trimester investigations are important as preeclampsia pathogenesis may begin early in pregnancy. The objective of this paper is to examine the association between first and second trimester circulating immune markers and preeclampsia. Serum immune markers including IL-6, IL-6 receptor, IL-4, IL-4 receptor, IL-5, IL-12, IL-2, TNF-α, TNF-β, TNF-receptor, IL-1β, IL-1α, IL-8, IL-10, IFN-γ, macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MIP), transforming growth factor-beta (TGF-β), and RANTES were included in our study based on previous associations with preeclampsia or their involvement in systemic inflammation or the Th1/Th17 paradigm [16–18].

2. Methods

We conducted a nested case control study of 208 singleton and primiparous preeclamptic women and 411 singleton and primiparous normotensive controls within the Collaborative Perinatal Project (CPP) [19]. Both cases and controls had no history of diabetes, cardiovascular disease or hypertension. The CPP is a longitudinal study of 55,908 pregnancies [20]. Women were enrolled between 1959 and 1965 from 12 university-affiliated medical centers in the United States (Baltimore, MD; Boston, MA; Buffalo, NY; Memphis, TN; Minneapolis, MN; New Orleans, LA; two sites in New York, NY; Philadelphia, PA; Portland, OR; Providence, RI; and Richmond, VA). Oral consent, as was standard at the time of the CPP study, was obtained from all women in the study [21]. We analyzed a total of 242 women who had first serum samples collected in the first trimester (mean gestation age 10.7 ± 1.9; range 5–13 weeks) and 392 women who had first serum samples collected in the second trimester (mean gestation age 16.5 ± 1.7; range 14–19 weeks). The study was approved by the University of Pittsburgh Institutional Review Board.

2.1. Data collection

At the first prenatal visit, all women were interviewed in person to obtain data on maternal characteristics, behavior, medical and pregnancy history. Delivery data was recorded by the attending physician. For our analysis, we considered self-reported maternal age (years), marital status (married, single), socioeconomic status, race (white, non-white), maternal smoking (yes/no), and pre-pregnancy body mass index (BMI) as potential covariates. Socioeconomic status was previously determined using a composite score that combined education, occupation and family income [22]. Pre-pregnancy BMI was determined by (weight (kg)/height (m)^2 which was reported at enrollment. Gestational week was determined by the date of delivery minus the date of last menstrual period.

2.2. Cytokine measurements

Non-fasting blood samples were obtained at the first study visit and stored at −20 °C in glass vials and monitored continuously from the time of collection. There were no recorded thaws from collection time until they were aliquoted for analysis. Each sample was labeled with a study ID and name and checked against a pull sheet at the time the samples were pulled. Our subset of samples was mailed to the Statens Serum Institute in Copenhagen where immune biomarkers were measured in duplicate with an in house multiplex flow cytometric assay system Luminex MultiAnalyte Profiling Technology (LabMap, Luminex Corporation, Austin, Texas) [23]. The calibration curves for each analyte were calculated by the Bio-Plex 3.0 software (BioRad, US). Mean intra- and interassay CVs (CV %) were 6.2% and 16%, and ranged from 6.7 (IL-4) to 13 (IL-10 and TNF-α) and 10 (IL-4) to 25 (TNF-α) [23]. Variation in precision profiles among analytes is similar to other studies [24–26]. We acknowledge that the long-term storage of CPP samples raises concerns about the measurement of the analytes. This multiplex assay has been demonstrated to be valid for the measurement from specimens obtained from long-term storage using10 anonymously collected residual dried blood spot specimens stored for 23 years at −24 °C in a national, Danish biological specimen bank [23]. In that study the measurable amounts of most cytokines were constant. Additionally, analytes measured in serum from the CPP cohort have shown to be stable in other studies [27–29]. In another CPP study, cytokines measured in the CPP were compared to fresh samples and found to be consistent across groups [30]. As IL2 and IL1α measured below the LOD for greater than 75% of patients, these biomarkers were not analyzed.

2.3. Preeclampsia definition

Preeclampsia was based on chart abstraction of blood pressure and protein levels and defined as gestational hypertension (2 or more measurements of systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg for the first time after 20 weeks of gestation) and proteinuria (2 random urine dipsticks of 1+ protein or one dipstick of 2+ protein). In the intrapartum period, the first 5 pressures obtained after hospital admission for delivery were averaged. It is accepted that preeclampsia is heterogeneous disease with subtypes (early/late onset) that have different pathophysiological pathways [31]. For our study, we classified preeclampsia resulting in either a term birth (⩾37 weeks gestation) or a preterm birth (<37 weeks gestation) as separate outcomes. Preeclampsia with preterm birth is a valid proxy for disease severity and early onset of disease. Preeclampsia with preterm birth <34 weeks of gestation was not used as the sample size was too small for analysis.

2.4. Statistical analyses

For all biomarkers, raw median levels and ranges were calculated for preeclamptic and normotensive women. Our primary analysis examined the association between immune biomarkers and preeclampsia. As circulating immune biomarkers display heterogeneity by gestational age, we stratified our analysis by first and second trimester samples. In multiplex assays, subjects frequently measure outside of the limit of detection (LOD) [32]. In general, the frequency of women who measure beyond the LOD

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