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Evidence of inflammation and predisposition toward metabolic syndrome after pre-eclampsia

Malia S.Q. Murphy [a], Chandrakant Tayade [a], Graeme N. Smith [a,b,*]

[a] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada
[b] Department of Obstetrics and Gynaecology, Kingston General Hospital, Kingston, ON, Canada

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ABSTRACT

Background: Pre-eclampsia (PE) is a hypertensive disorder of pregnancy characterized by exaggerated inflammatory and metabolic responses. Women with a history of PE are at increased risk of the metabolic syndrome (MetS) and cardiovascular disease although the pathophysiological underpinnings of this association remain unclear. This study aimed to compare levels of plasma immunoregulatory factors with the presence of cardiovascular and MetS risk factors in women with and without a history of PE.

Study design: Maternal plasma and general health survey data were collected from women 5 to 7 months postpartum of uncomplicated pregnancies ($n = 28$) and pregnancies complicated by PE ($n = 35$). Maternal plasma samples were analyzed for 14 immunoregulatory factors using a high-sensitivity cytokine profiling array. Cardiovascular risk profiles were compiled on each participant for comparison against cytokine data.

Results: Women with a history of PE exhibited increased blood pressure and plasma triglyceride levels compared to controls, although similar for parameters of obesity, fasting cholesterols, and glucose. While plasma levels of immunoregulatory cytokines were similar between control and PE subjects, PE subjects exhibited unique patterns of correlation between biophysical parameters and plasma cytokines. In particular, plasma IL-23, MIP-1α, IL-1β and IFN-γ levels were significantly correlated with parameters considered for MetS diagnosis in women without clinical evidence of the syndrome.

Conclusions: We report unique associations between pro-inflammatory markers and MetS criteria within a year following PE. Subclinical inflammation in women with a history of PE who are otherwise healthy may indicate a sensitization of these women toward metabolic disturbances, in particular MetS.

1. Introduction

Pre-eclampsia (PE) is a hypertensive disorder of pregnancy with substantial metabolic and cardiovascular implications to maternal physiology. In PE, factors mediating angiogenic and inflammatory disruptions within the placenta are released into the maternal circulation thereby giving rise to maternal endothelial and metabolic dysfunction [1]. Although maternal symptoms of new-onset hypertension (⩾140 mmHg systolic and/or ⩾90 mmHg diastolic blood pressures) with additional adverse complications, including, but not limited to proteinuria (⩾0.3 g/day or ⩾1+ urinary dipstick), headaches, low platelets, right upper quadrant pain and elevated liver enzymes [2] typically resolve following delivery, maternal risk of future disease is well recognized [3].

Women who develop PE are at significant risk of chronic hypertension and cardiovascular and cerebrovascular diseases compared to women with no history of obstetrical complications. The magnitude of this risk appears to be conferred by the timing of onset and severity of the maternal condition. Furthermore, risk of the metabolic syndrome (MetS), a condition defined by the presence of a constellation of risk factors that imposes significant risk of type I diabetes and cardiovascular disease is increased in women with a history of PE [4].

The maternal systemic inflammatory response to pregnancy is exacerbated in PE. Here, oxidatively stressed placentae release an excess of cytokines, apoptotic bodies and syncytiotrophoblast microparticles which contribute to maternal endothelial activation and systemic inflammatory responses that are characteristic of the disorder [5]. Leukocyte activation is described in PE, a finding that is consistent with observations involving elevated circulating

* Corresponding author at: Department of Obstetrics and Gynaecology, Queen's University, Kingston General Hospital, 76 Stuart St., Kingston, Canada.
E-mail addresses: 5mm71@queensu.ca (M.S.Q. Murphy), tayadec@queensu.ca (C. Tayade), gns@queensu.ca (G.N. Smith).

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pro-inflammatory cytokines including TNF-α, IL-6, and IL-10 in women with the condition [6–8]. Although risk factors for PE bear a striking similarity to those for cardiovascular disease, including increased BMI and dyslipidemia, endothelial dysfunction independent of these traditional risk factors suggests a subclinical vascular susceptibility. Indeed, underlying inflammation and insulin resistance substantially predispose to MetS, diabetes and cardiovascular disease and are demonstrated in women decades after PE [9,10].

The association between PE and maternal risk of disease necessitates an examination of subclinical biomarkers with the potential to identify those at highest risk of cardiometabolic outcomes. In this way targeted lifestyle and education-based interventions may be used to improve short-term and long-term health in at-risk women. In this study we aimed to examine whether detectable alterations in plasma imunoregulatory factors in women with a history of PE were associated with risk of cardiovascular disease and ATP III MetS diagnostic criteria.

2. Methods

2.1. Participant recruitment

This study was approved by the Queen's University Research Ethics Board. All study participants provided written informed consent prior to sample collection. Study participants included women with a recent history of PE, who had delivered approximately 6 months previous (27.2 ± 3.5 weeks postpartum) and postpartum control subjects with uncomplicated obstetrical histories (26.6 ± 3.5 weeks postpartum). Pre-eclampsia was defined as the *de novo* onset of maternal hypertension ($\geqslant$140 mmHg systolic blood pressure or $\geqslant$90 mmHg diastolic blood pressure) after 20 weeks gestation with new-onset proteinuria ($\geqslant$1 + urinary dipstick or $\geqslant$0.3 g/24-h) or one or more adverse conditions as outlined by the American College of Obstetricians' 2014 report on Hypertension in Pregnancy. Pre-eclamptic women were identified upon routine postpartum check-up at the Maternal Health Clinic at Kingston General Hospital [11,12]. Controls were identified based on participation in other clinical research studies associated with the Queen's University Perinatal Research Unit. PE and Control study participants were those with a singleton index pregnancy with no known history of previous hypertension, diabetes (including the development of gestational diabetes), renal disease or other cardiovascular complications. Current smokers and participants who met the criteria for the MetS postpartum were excluded from analysis.

At 6 months postpartum general health questionnaires were completed to capture demographic information, weight, blood pressures, breastfeeding status and obstetrical history. Fasting blood and urine samples were collected for measurement of glucose, lipid profiles, high-sensitivity C-reactive protein (hs-CRP) and microalbumin:creatinine ratios. Calculations were made for lifetime cardiovascular disease risk, and were based on factors including; sex, smoking, total cholesterol fasting glucose, systolic blood pressure, diastolic blood pressure and antihypertensive usage [13]. Each factor was evaluated and its value stratified by categorical risk; a cumulative lifetime cardiovascular risk estimate was generated based on the number of risk factors evaluated at each risk level; All optimal (8%), $\geqslant$1 Not optimal (27%), $\geqslant$1 Elevated (39%), 1 Major (39%) and $\geqslant$2 Major (50%). When categorized as nominal data, this generated data on low lifetime risk of CVD (<39% risk) and high lifetime risk of CVD ($\geqslant$39% risk).

2.2. Sample collection and multiplex arrays

Peripheral blood samples were collected via venipuncture into evacuated blood collection tubes with ethylenediaminetetraacetic acid anticoagulant (BD Vacutainer® EDTAK2, Franklin Lakes, NJ) and inverted 5–10 times. Samples were stored on ice and processed within 2 h of collection. Blood samples were then centrifuged at 1000×*g* for 15 min at room temperature to isolate the plasma fraction. Plasma aliquots were collected and stored at −80 °C until analysis.

Plasma levels of TNF-α, IFN-γ, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17α, IL-23, MIP-1α and Fractalkine were determined by a commercial 14-plex high-sensitivity cytokine profiling array (Eve Technologies, Calgary, Canada). Target cytokines were measured using a dual laser and flow-cytometry based system. Concentrations of target cytokines were determined based on dual binding to capture antibodies coupled with colour-coded polystyrene beads and streptavidin–phycoerythrin florescent conjugates. Individual fluorophore signatures for each target cytokine were distinguished based on bead colouration using a Bio-Plex 200 bead analyzer (Eve Technologies, Calgary, Canada). In brief, one laser was used to excite the analyte-specific fluorescent dye within the beads, and a second laser was used to excite the fluorescent conjugate bound to the beads during the assay. The amount of conjugate detected by the analyzer is in direct proportion to the amount of the target cytokine. Cytokine levels were determined by comparison against a standard curve and values were recorded in pg/mL. All samples were run in duplicate.

2.3. Statistical analysis

Demographic variables are presented as mean ± standard deviation unless otherwise indicated. Continuous data were compared by means of an unpaired *t*-test or a one-way analysis of variance (ANOVA) with Bonferroni post hoc tests. Mid-P exact tests were used for comparison of categorical data. All data were analysed using GraphPad Prism 6.04 (GraphPad Software, La Jolla CA).

3. Results

3.1. Participant characteristics

A summary of pre-pregnancy and postpartum characteristics of study participants is provided in Table 1. Pre-eclamptic subjects were more likely to be primiparous upon the index pregnancy and deliver earlier in gestation than their control counterparts. Pre-pregnancy and postpartum body mass indices (BMI) and waist circumferences were similar between comparison groups, although fasting blood work and urinalysis indicated elevated triglycerides and microalbumin:creatinine levels 5–7 months after PE. While PE subjects exhibited higher systolic and diastolic blood pressures postpartum compared to controls, none met the criteria for a diagnosis of malignant hypertension or were taking antihypertensive medications at the time of assessment.

Composite risk analyses completed at the Maternal Health Clinic demonstrated that PE and control subjects exhibited similar distributions of lifetime cardiovascular risk scores. The distribution of MetS parameters (0, 1 or 2 criteria met) was also similar between comparison groups and was limited to low HDL, elevated blood pressure and increased waist circumference.

3.2. Plasma cytokine levels are correlated to MetS criteria after PE

Analysis of 14 plasma cytokines at 6 months postpartum revealed no significant differences in plasma cytokine levels between control and PE groups. Rather, women with a history of PE exhibited unique patterns of correlation between metabolic syndrome criteria and plasma cytokines that were not present in controls. Triglyceride levels were elevated postpartum of PE