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Original Article

Postpartum alterations in circulating endothelial progenitor cells in women with a history of pre-eclampsia

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ABSTRACT

Objective: To characterize persistent postpartum maternal endothelial dysfunction following pre-eclampsia (PE) through the assessment of endothelial progenitor cells as markers of endothelial reparative capacity.

Study design: Maternal circulating endothelial progenitor cells were measured at 2 months and 6 months postpartum in women who had recently experienced PE pregnancies ($n = 17$). Normotensive controls ($n = 13$) with uncomplicated pregnancies served for comparison at the same time points. Progenitor cells were measured by flow cytometry and by colony forming units. Maternal cardiovascular risk was measured at 6 months postpartum.

Main outcome measures: Levels of maternal circulating endothelial progenitor cells and cardiovascular risk in the early postpartum period of uncomplicated and PE pregnancies.

Results: CD34 + VEGFR-2+ and CD133 + VEGFR-2+ cells were elevated in PE subjects at 2 months postpartum compared to healthy control subjects, although reduced by 6 months postpartum. PE was associated with reduced colony forming units at 2 and 6 months postpartum. Cardiovascular risk scores were increased in PE compared to normotensive controls.

Conclusions: We have demonstrated that there is a physiological alteration in the number and function of circulating progenitor cells following PE pregnancies. Furthermore, this population of women exhibited elevated cardiovascular risk profiles compared to those with uncomplicated pregnancies. Pregnancy and the development of PE identify an early window for cardiovascular risk screening in women. Cellular markers of vascular health offer an approach to the investigation of postpartum endothelial dysfunction.

Introduction

Pregnancy is now recognized as a cardiovascular stress test [1] that provides information on a woman's susceptibility to metabolic and vascular dysfunction [2]. Indeed, the development of pregnancy-related complications – including preterm birth, intrauterine growth restriction, gestational diabetes mellitus, and PE – identify women at future risk of disease [1,3]. The American Heart Association's 2011 Update for the *Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women* [4] identifies complications of pregnancy as relevant to the determination of cardiovascular risk.

Endothelial dysfunction is thought to underlie the maternal hypertension and proteinuria characteristic of PE. While maternal signs and symptoms largely dissipate

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following delivery of the feto-placental unit, impaired vascular function is known to persist from months to decades after delivery in women with both early and late-onset PE [5,6].

Maintenance of endothelial homeostasis was until recently attributed to the migration and proliferation of locally activated endothelial cells. However, evidence has since emerged for the existence of bone marrow-derived endothelial progenitor cells (EPCs), capable of incorporation into sites of active angiogenesis to promote re-endothelialization and neovascularization [7,8]. EPCs are released into circulation in response to a host of chemotactic and cytokine factors arising from vascular trauma and hypoxic stressors [9,10] and are now largely believed to be critical to the maintenance of vascular integrity and function [11,12].

As a measure of regenerative and angiogenic capacity, EPCs may represent a physiological link underlying the association between the endothelial dysfunction of PE and future cardiovascular disease (CVD). PE and CVD share common risk factors including hyperlipidemia, endothelial dysfunction and lipid deposition in blood vessel walls [13]; endothelial dysfunction and long-term risk for CVD following PE pregnancies are well described [14–16]. There exists little postpartum EPC data in healthy or clinically at-risk populations however, and it remains unclear how pregnancy may affect postpartum maternal EPC physiology. We hypothesized that differences in postpartum EPC physiology would serve as a marker of endothelial dysfunction and provide insight into the postpartum recovery following PE. The objective of this study was to assess circulating EPC number and function in women who developed PE, compared with those who did not.

Materials and methods

Study population

This study was approved by the Queen's University Research Ethics Board (OBGY-108-03). Written informed consent was obtained from all participants. Women aged 18–40 years, presenting with a singleton pregnancy were approached to participate. Normotensive women were recruited at the time of presentation to general obstetrical clinics for routine third trimester check-ups. Pre-eclamptic women were identified following delivery at Kingston General Hospital. PE was defined as maternal hypertension $\geqslant$140/90 mmHg and proteinuria $\geqslant$300 mg/24 h or $\geqslant$1+ on repeat dipstick after 20 weeks gestation.

Patients with a history of hypertension, diabetes (including the development of gestational diabetes), renal disease, or CVD were excluded. Controls with previous pregnancy complications, including PE, were also excluded.

6 month postpartum follow-up involved a physical examination (blood pressure, waist circumference, and BMI calculation) and biochemical assessment for cardiovascular risk factors in the Maternal Health Clinic at Kingston General Hospital. Blood was obtained for fasting glucose, high-sensitivity C-reactive protein (hsCRP), triglycerides, total cholesterol, low-density and high-density lipoprotein cholesterols (LDL and HDL, respectively), and first morning urine for microalbumin and creatinine. Data collected at 6-month follow-up were used to calculate the lifetime risk estimates for CVD [17] and the existence of metabolic syndrome in both normotensive and PE groups. Variables included age, total and HDL cholesterol levels, current smoking status, and blood pressure.

Blood collection

Maternal peripheral venous blood was drawn via venipuncture into ethylenediaminetetraacetic acid (EDTA) into evacuated blood collection tubes (BD Vacutainer® EDTAK2). All blood samples were processed within 2 h. Plasma was stored at −80 °C until further analysis.

Flow cytometry

Vascular endothelial growth factor receptor-2 (VEGFR-2) positive cells co-expressing either CD34 or CD133 were quantified as previously described [18,19]. Briefly, erythrocytes were eliminated from whole blood using an erythrocyte lysing solution [155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2–7.3] and leukocytes were maintained in flow cytometry buffer [2%fetal bovine serum (FBS), 2 mM EDTA in phosphate buffered saline]. Non-specific binding to the Fc receptor was prevented through incubation with Fc Receptor Blocker (Miltenyi Biotech Inc., CA, USA). Mouse anti-human monoclonal antibodies (mAbs) for flow cytometry included anti-CD45-FITC (0.625 μg/mL), anti-CD34-PerCPefluor710 (1.25 μg/mL) and anti-CD133-APC (1.25 μg/mL) all from eBioscience (San Diego, CA, USA) and anti-VEGFR-2-PE (1 μg/mL) from Miltenyi Biotech (Auburn, CA, USA). Isotype-matched mAbs diluted to equivalent immunoglobulin concentrations served as negative controls. Antibody incubation was for 15 min at 4 °C in darkness. Cells were washed and fixed in 2% paraformaldehyde (PFA). Samples were stored in darkness at 4 °C until analysis.

Cells were analyzed on a Beckman Coulter FC500 flow cytometer using CXP Software (Beckman Coulter, Mississauga, Ontario, Canada). For each sample 10^6 events were collected. CD34 + VEGFR-2+ and CD133 + VEGFR-2+ cells were determined in the mononuclear gate, where EPCs are normally found, based on CD45 antigen positivity and Forward Scatter/Side Scatter profiles. Post-acquisition analyses were performed using FlowJo software (Tree Star, Inc., Ashland, Oregon, USA). Maternal EPCs were enumerated as a percentage of total mononuclear cells.

Colony forming unit-EPC (CFU-EPC) assay

Blood samples for CFU-EPC assays were provided at 2 months (Ctrl $n = 5$, PE $n = 7$) and 6 months postpartum (Ctrl $n = 13$, PE $n = 17$) concurrent to those taken for flow cytometry assessment. Mononuclear cells were cultured by adherence depletion, as previously described [11]. Briefly, isolated peripheral blood mononuclear cells were cultured in Medium 199 (M3769, Sigma–Aldrich Canada Ltd., Oakville, ON, Canada) supplemented with 20%FBS (v/v). Cells were seeded at 5×10^6 cells/well of a Fibronectin 6-well Multiwell Plate (BD BioCoat™ Cellware) and incubated at 5% CO_2, 37 °C, for 48 h. Non-adherent cells were