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Preeclampsia Is Associated with Lower Production of Vascular Endothelial Growth Factor by Peripheral Blood Mononuclear Cells

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Background. Recent studies show that vascular endothelial growth factor (VEGF) downregulation is implicated in preeclampsia (PE) pathophysiology. This study assessed the relationship between PE and VEGF levels produced by peripheral blood mononuclear cells (PBMCs) and their serum levels.

Methods. A cross-sectional design was performed in 36 patients who had hypertensive disorders during pregnancy. We also used a longitudinal design with 12 pregnant women with risk factors for PE development and/or abnormal uterine arteries by Doppler study. VEGF and soluble fms-like tyrosine kinase-1 (sFlt-1) levels were measured for all patients in both designs.

Results. sFlt-1 serum was higher in preeclamptic patients ($n = 26$), whereas VEGF produced by stimulated PBMCs was lower than in healthy pregnant women and VEGF levels produced by stimulated PBMCs were even lower ($p < 0.003$) in severe PE ($n = 16$). The receiver-operating characteristic curve analysis allowed establishing a cut-off value to identify patients with PE. VEGF production by PBMCs was 339.87 pg/mL. In addition, a robust linear regression model was performed to adjust the variance in VEGF levels. The patients' age decreased VEGF levels and was adjusted by weeks of gestation (WG) in our model. In the longitudinal study, 7/12 patients developed PE. VEGF produced by PBMCs cells was significantly lower in PE at 24–26 WG.

Conclusions. VEGF production by PBMCs is inhibited during PE, creating a downregulation of the microenvironment; this deficiency may contribute to the pathogenesis of disease.

Key Words: VEGF, *sFlt-1*, Preeclampsia, Hypertensive disorders of pregnancy.

Introduction

Preeclampsia (PE) is a clinical syndrome present in 5 to 8% of all pregnant women and remains as a major cause of maternal and neonatal mortality and morbidity worldwide (1–3). It is characterized by endothelial dysfunction,

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hypertension and proteinuria after 20 weeks of gestation (WG) and/or a fetal syndrome (1,2,4–6). PE also remains a leading cause of prematurity as placenta delivery is currently the only way to successfully treat the disorder. The etiology and pathogenesis of this pregnancy-specific syndrome are unclear. Accumulated evidence suggests that PE may be the result of an imbalance in angiogenic factors (7–14). Recent studies have hypothesized that vascular endothelial growth factor (VEGF) and its receptors are implicated in the pathophysiology of PE (10,12,14–18).

VEGF is a homodimeric 34–42 kDa glycoprotein with potent angiogenic activity (19). During pregnancy, VEGF is essential for normal placenta development, for trophoblast proliferation and for the development of embryonic vasculature. Three VEGF receptors have been described: VEGFR-1, also known as *fms*-like tyrosine kinase (Flt-1) (20,21); VEGFR-2, also known as kinase-insert domain-containing receptor (KDR and Flk-1) (21,22) and VEGFR-3, also known as Flt-4 (23). A soluble truncated form of the Flt-1 (soluble fms-like tyrosine kinase-1, sFlt-1) receptor produces alternative splicing and contains an extracellular domain that binds to the ligand but lacks the transmembrane and cytoplasmic domains (24), acting as a natural potent VEGF and placental growth factor (PIGF) antagonist (7) by binding them and blocking the interaction with their receptors Flt-1 and Flk-1 on the cellular membrane.

Low serum levels of free VEGF (25,26) and elevated sFlt-1 serum levels have been reported in PE (10,12,13,16,17,27). sFlt-1 elevation correlates with severity of the disease. An *in vivo* study has demonstrated that overexpression of sFlt-1 in pregnant rats produced a PE-like syndrome with hypertension, proteinuria and glomerular endotheliosis. This anti-angiogenic state can be rescued by administration of VEGF and PIGF (12). A 50% reduction of VEGF in transgenic mice resulted in a clinical entity similar to human PE, characterized by proteinuria and endotheliosis (28). Patients receiving anti-VEGF antibodies as a treatment for metastatic renal cancer developed hypertension and proteinuria (29,30). Hypertension and proteinuria decreased after therapy cessation (29).

VEGF and sFlt-1 are produced fundamentally by the placenta but these molecules and their receptors are also synthesized and secreted by endothelial cells and peripheral blood mononuclear cells (PBMCs) (24,31–33). PBMCs obtained from preeclamptic women produced significantly higher amounts of sFlt-1 under normal tissue culture conditions compared with PBMCs from normal pregnant women (33). Pregnant non-human primates undergoing a reduction in placental blood flow by induction of uteroplacental ischemia (UPI) resulted in blood pressure elevation, development of proteinuria, and histological changes, identical to human PE. UPI also resulted in an increase in circulating sFlt-1 and expressed significantly higher sFlt-1 mRNA levels in PBMCs compared with the sham group (34). On the other hand, macrophages are key regulators of the angiogenic switch in tumors; they may promote angiogenesis by producing angiogenic growth factors including VEGF (35). Macrophages produced by VEGF may recruit and interact with other cells in the tumor microenvironment (e.g., neutrophils) (35,36). A previous study reported that the proportion of T and NK cells that shows intracellular VEGF expression in the peripheral blood was markedly decreased in patients with PE when compared to healthy pregnant women (36). The cause and clinical significance of these findings remain to be determined.

Previous studies have evaluated the use of Doppler as a test to identify patients at risk of developing PE, reporting that sensitivity improves when the test is performed after 24–26 WG and when a diastolic constant notch is one of the criteria (37–41). When combining uterine artery Doppler test with maternal history and mean blood pressure, a detection rate of approximately 90 for 10% of false positives cases is achieved (42).

In this study we assessed sFlt-1 and VEGF levels in serum samples and in PBMC culture supernatant from PE patients and from patients with risk factors for PE development. We found that although VEGF levels were significantly reduced in stimulated PBMCs in PE, serum Flt-1 was significantly elevated compared with their levels in normal pregnancy. These deficient VEGF levels may contribute to the pathogenesis of PE.

Materials and Methods

Patient Details

The IMSS Scientific and Ethics Committee approved this study. Participants were informed regarding the nature of the study and written informed consent was obtained from all participants. These women were patients admitted to the Hypertensive Diseases of Pregnancy Clinic of the Gynecology Hospital ''Luis Castelazo Ayala'' of the IMSS. Study participants were at gestational age ≥20 weeks and had new-onset hypertension. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, measured twice at least 6 h apart and that returned to normal values within 3 months after delivery. Hypertensive disorder of pregnancy was defined according to the criteria of the American College of Obstetrics and Gynecology (6). Gestational hypertension (GH) was defined as isolated hypertension without significant proteinuria and mild preeclampsia (mPE) as hypertension and significant proteinuria (≥300 mg of protein in a 24 h urine specimen or a protein:creatinine ratio ≥0.30 in a random urine sample) (43,44). Severe PE (sPE) was considered when either HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), eclampsia, or PE with severe hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg on at least