Second-trimester angiogenic factors as biomarkers for future-onset preeclampsia

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OBJECTIVE: The purpose of this study was to determine whether second-trimester soluble fms-like tyrosine kinase-1 and placenta growth factor (PlGF) are altered in patients who have preeclampsia develop compared with controls. Furthermore, soluble fms-like tyrosine kinase-1 and placenta growth factor levels in patients with chronic hypertension are described.

STUDY DESIGN: With the use of a research database, 21 patients who had severe preeclampsia develop, 34 controls, and 9 patients with chronic hypertension were enrolled. Placenta growth factor and soluble fms-like tyrosine kinase-1 serum levels were determined by enzyme-linked immunosorbent assay. Appropriate statistical tests were used and results were reported as median (quartile 1-quartile 3) in picograms per milliliter.

RESULTS: Placenta growth factor was significantly lower in patients in the second trimester who later had severe preeclampsia develop but soluble fms-like tyrosine kinase-1 was unchanged compared with healthy pregnancies. In patients with chronic hypertension, placenta growth factor and soluble fms-like tyrosine kinase-1 levels were not different compared with controls.

CONCLUSION: Second-trimester placenta growth factor levels are altered in patients who had severe preeclampsia develop.

Key words: hypertension, placenta growth factor, preeclampsia, soluble fms-like tyrosine kinase-1

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Preeclampsia is a complex disorder that affects 5-7% of all pregnancies. The disease is a leading cause of maternal and neonatal morbidity and mortality worldwide and contributes to 15% of all preterm deliveries in the United States. The first pregnancy, chronic hypertension, diabetes, and multiples are recognized clinical risk factors.[1] There is currently no effective prevention for preeclampsia and no treatment other than delivery. In clinical trials, large sample sizes are required to identify a small subset of patients with preeclampsia. In the last 3 large National Institutes of Health/National Institute of Children's Health and Human Development (NIH/NICHD) sponsored Maternal Fetal Medicine Unit Network intervention trials alone, 10,263 patients were recruited. More than 5000 subjects were exposed to treatment and only 453 had preeclampsia develop.[2-4] If patients who are destined to have preeclampsia develop during pregnancy could be identified in the first or second trimester before the onset of overt clinical disease, preeclampsia may be studied with less cost, less risk, and fewer subjects.

The pathophysiology of preeclampsia remains elusive. Recently, angiogenic factors have received considerable attention.[5-8] Circulating levels of angiogenic factors such as vascular endothelial growth factor (VEGF), placenta growth factor (PlGF), and soluble fms-like tyrosine kinase-1 (sFlt-1) were altered in patients who had preeclampsia develop. In normal pregnancy, the level of the proangiogenic factor PlGF increases during the first 2 trimesters, peaks around 29 weeks, and then decreases as pregnancy progresses to term. In patients who had preeclampsia develop, PlGF serum levels were lower than controls at the time of diagnosis. In contrast, levels of the antiangiogenic substance sFlt-1 remain stable until around 33 weeks, then increase steadily until term. In patients who had preeclampsia develop, sFlt-1 levels were higher than healthy controls at the time of diagnosis.[5] The objective of this study was to determine whether second-trimester sFlt-1 and PlGF levels are altered in the second-trimester serum of patients who later had severe preeclampsia develop compared with uncomplicated controls. A secondary objective is to describe the relationship of sFlt-1 and PlGF second-trimester maternal serum levels in patients with isolated preexisting chronic hypertension compared with healthy controls.

Materials and Methods

This case-control study was approved by the Institutional Review Board at the Medical University of South Carolina. Patients were identified by using the Perinatal Information Services (PINS) database, a research validated database. Data quality is assured by a variety of techniques to assure the production of a research quality data system. Interrater and intrarater reliability checks are performed on a 10% random sample of abstracted charts to ensure comparability between and within raters. Computer-

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TABLE 1
Demographics

Characteristics	Control (n = 34)	Preeclampsia (n = 21)	Hypertension (n = 9)	*P*-value
Gestational age at serum sample (wks)	17 (16-20)	17 (16-20)	17 (16-20)	NS*
Age (y)	24.1 (20-28)	26.3 (20-32)	31 (27-37)	< .05*
Primiparous (%)	48	43	44	NS*
Race (%)				
White	25	38	22	< .05†
African American	44	47	67	
Hispanic	31	15	11	
BMI (kg/m^2)	29 (25-35)	32 (27-35)	37 (29-42)	< .05*
Tobacco use (%)	6	5	0	NS‡

All values presented as median (quartile 1-quartile 3) except percentages.

* Kruskal-Wallis ANOVA.

† χ^2.

‡ Fisher exact test.

generated logical error checks are performed on 100% of all data entered into the data system. Validity checks are performed during a monthly conference attended by a multidisciplinary team. Severe preeclampsia is defined at our institution and subsequently in PINS by using the American College of Obstetricians and Gynecologists definition.[9] All patients enrolled with severe preeclampsia met the criteria of this definition.

Patients with a singleton pregnancy who had a serum screen performed in the second trimester (15-20 weeks' gestational age) during a 12-month period and who delivered at our institution were included in this study. All serum samples were less than 2 years of age and had been stored at −70°C. The samples of all patients with severe preeclampsia (n = 21) that were not complicated by other underlying disorders such as hypertension, diabetes, collagen vascular disease, and renal insufficiency were retrieved and included in this investigation. Healthy patients without any underlying illness or preexisting pregnancy complications and with uncomplicated pregnancy courses were used as controls (n = 34). The controls were randomly chosen by PINS through random number tables. Patients with chronic hypertension (n = 9), as definined by the National Heart, Lung, and Blood Institute working group report on hypertension in pregnancy, had prepregnancy essential hypertension, and no other disease associated with chronic hypertension such as diabetes, collagen vascular disease, or renal insufficiency.[10] Patients did not have to be taking antihypertensive medication.

Quantikine human enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) were used to measure PlGF and sFlt-1 maternal serum concentrations. Each maternal serum sample was assayed in duplicate and both PlGF and sFlt-1 concentrations were reported as the mean value in picograms per milliliter. The minimal detection, intraassay, and interassay variance of PLGF were 7 pg/mL, 7%, and 12%, respectively. The minimal detection, intraassay, and interassay variance of sFlt-1 were 5.0 pg/mL, 4%, and 8%, respectively. A high and low standard for PlGF and sFlt-1 were used between individual ELISA plates to ensure homogeneity of measurements. Laboratory personnel performing the assays were blinded to the clinical information of each subject.

With the use of data from a previous publication, a power analysis was performed to determine how many samples were needed to detect a difference in PlGF serum levels between severe preeclampsia and controls in the second trimester.[5] Thirteen samples were needed in each group. Neither the level of PlGF nor sFlt-1 has been described in patients with preexisting chronic hypertension. Categorical data were analyzed by using χ^2 or Fisher exact test where appropriate. Nonparametric continuous variables were analyzed by Mann-Whitney *U* test when 2 groups were compared or Kruskal-Wallis analysis of variance (ANOVA) by ranks when 3 groups were compared. An alpha (2-tailed) of less than .05 was considered significant.

Results

Demographic information is presented in Table 1. There were no significant differences in the gestational age at the time of serum sample, gravidity, or smoking status between controls and patients with severe preeclampsia and chronic hypertension. Although, not unexpectedly, the patients with chronic hypertension were older and had a larger body mass index (BMI) than the other 2 groups.

Outcomes are shown in Table 2. As expected, patients with severe preeclampsia and chronic hypertension delivered