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First Trimester Placental Growth Factor and Soluble Fms-Like Tyrosine Kinase 1 and Risk for Preeclampsia

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An imbalance of pro- and antiangiogenic factors may lead to preeclampsia (PE). In this prospective nested case-control study, we investigated whether first trimester serum levels of placental growth factor (PlGF), a potent angiogenic factor, and its soluble inhibitor, soluble fms-like tyrosine kinase 1 (sFlt1), distinguished women who developed PE (n = 40) from those who developed gestational hypertension (n = 40), delivered a small for gestational age (SGA) newborn (n = 40), or completed a full term normal pregnancy (n = 80). Compared with controls, serum PlGF levels were lower among women who developed PE (23 ± 24 pg/ml *vs.* 63 ± 145 pg/ml; $P < 0.01$) or gestational hypertension (27 ± 19 pg/ml; $P = 0.03$), or who delivered a SGA newborn (21 ± 16 pg/ml; $P < 0.01$). In contrast, serum sFlt1 levels did not markedly differ between the groups: PE, 1048 ± 657 pg/ml; gestational hypertension, 942 ± 437 pg/ml; SGA newborns, 1011 ± 479 pg/ml; and normal controls, 973 ± 490 pg/ml. Multivariable analysis adjusting for potential confounders and serum sFlt1 levels demonstrated a 3.7-fold (95% confidence interval, 1.2–12.5) increase in risk for PE for every log unit decrease in serum levels of PlGF compared with controls. Analyses for gestational hypertension and SGA were not significant. Examined in tertiles, the risk for PE was increased 28.7-fold (95% confidence interval, 2.3–351.0) in the third (<12 pg/ml) compared with the first (>39 pg/ml) PlGF tertile. First trimester serum levels of PlGF and sFlt1 may identify women at high risk for PE. (*J Clin Endocrinol Metab* 89: 770–775, 2004)

PREECLAMPSIA (PE), ONE of the most common medical complications of pregnancy, is associated with considerable maternal and neonatal morbidity and mortality (1). Prophylactic interventions including antihypertensives, calcium supplementation, and aspirin have not been shown to reduce the risk of PE (2–6), although more recently a small study suggested that intervention with antioxidants reduces the risk in high-risk women (7). Although larger studies are currently under way to verify these latter results, investigators have continued to focus on identification of noninvasive biomarkers for early and accurate prediction of PE (8–16) and to target prophylactic therapies when they become available. Ideal biomarkers for PE should be easily measured, should delineate risk well before the 20th week of gestation, and although not a prerequisite, should serve as plausible mediators of its pathogenesis. The pathogenesis of PE, however, has remained incompletely understood (17).

Angiogenesis is critical for trophoblast invasion into spiral arteries, a key process in normal placental development (18–20). Placental hallmarks for PE include incomplete trophoblast invasion, trophoblast injury, and placental ischemia (15, 20, 21), features that also characterize pregnancies complicated by small for gestational age (SGA) newborns (22–24). Potent angiogenic growth factors, namely vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), are likely responsible for normal trophoblast proliferation, migration, and invasion, and low levels of VEGF and PlGF, or antagonists to VEGF and PlGF, are plausible mediators of PE (15, 20, 25–27). Indeed, whereas some investigators have suggested that second trimester pregnancy levels of PlGF (VEGF levels are undetectable early in pregnancy; Ref. 15) are not altered among women who subsequently develop PE (28, 29), others have reported just the opposite (15, 30–33). In addition, given the overlapping features of SGA newborns and PE, however, distinguishing risk for PE from risk for SGA newborns has been difficult, and accounting for potential confounding factors has been inconsistent.

Compared with normotensive controls, placentas of women with PE produce less VEGF (34), and recently, PE placentas were found to produce higher levels of soluble fms-like tyrosine kinase 1 (sFlt1 or sVEGFR-1), a splice variant of the VEGF receptor Flt1 and an antagonist of VEGF and PlGF (20, 35). In addition, elevated levels of sFlt1 have been identified in women with PE (35, 36), and in a recent study, administration of sFlt1 protein to pregnant rats resulted in the PE phenotype (hypertension, proteinuria, and glomerular endotheliosis) (35). Therefore, the pathogenesis of PE may involve an imbalance of angiogenic molecules, and measurement of PlGF in combination with sFlt1 may distinguish women who develop PE from women who develop other

Abbreviations: CI, Confidence interval; PE, preeclampsia; PlGF, placental growth factor; sFlt1, soluble fms-like tyrosine kinase 1; SGA, small for gestational age; VEGF, vascular endothelial growth factor.

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complications of pregnancy (35). We performed a prospective nested case-control study to compare serum levels of PlGF and sFlt1 in the first trimester among women who subsequently developed PE or gestational hypertension, delivered a SGA newborn, or completed a normotensive, full-term pregnancy.

Patients and Methods

Patient population

We performed a prospective nested case-control study of patients who had enrolled in the Massachusetts General Hospital Obstetrical Maternal Study (MOMS). In brief, the MOMS cohort, which has been described previously (10, 11), was established in 1998 for the prospective study of early gestational risk factors for adverse outcomes that occur later in pregnancy. Women who receive prenatal care at Massachusetts General Hospital and affiliated health centers are eligible for inclusion in the cohort. The Massachusetts General Hospital obstetrics service provides community-based obstetrical care for women from the metropolitan Boston area as well as high-risk obstetrical care for women referred from throughout New England. The cohort represents a population of women from varied ethnic and socioeconomic backgrounds. For this study, consecutive women with singleton gestations between June 1, 2001, and May 1, 2003, who enrolled in the MOMS cohort at or before 12 wk gestation and who delivered after 20 wk were eligible for inclusion. All subjects provided written informed consent, and this study was approved by the Institutional Review Board of the Massachusetts General Hospital.

The electronic medical record, which is the medical record used by the clinical staff, provides clinical and demographic data that prospectively details the events of pregnancy through the early postpartum period. Specific information obtained from the electronic medical record included age, gestational age of blood collection (estimated from the last menstrual period and verified by ultrasound dates), race, smoking, height, weight, blood pressure collected throughout gestation, fetal gestational age and weight at delivery, pregnancy outcome, and laboratory values, including results of glucose tolerance tests. Blood pressure was measured from each subject's right arm after she was seated at rest for 3–5 min using standard sphygmomanometers. After selecting the proper cuff size based on right midarm circumference, the pressures that coincided with the timing of the first (systolic) and fifth (diastolic) Korotkoff sounds were recorded. All subjects for the current study had no history of preexisting hypertension or diabetes mellitus, initiated and completed their prenatal care and pregnancy within our network, delivered a live infant, and had no evidence of hypertension within the ensuing 6 wk after delivery.

Exposures

After providing written informed consent, eligible women had their serum samples collected at their first prenatal visit, stored on ice for less than 3 h, and then frozen at −80 C for future analysis. All serum samples were frozen for less than 2 yr, and samples were thawed only once for this study. The primary exposures were serum PlGF and sFlt1. Commercial assay ELISA kits for sFlt1 and free PlGF (R&D Systems, Minneapolis, MN) were used as previously described (35). The intraassay precision coefficients of variation for sFlt1 and PlGF were 3.5 and 5.6%, respectively. The interassay precision coefficients of variation for sFlt1 and PlGF were 8.1 and 10.9%, respectively. All samples were run in duplicate, and if more than 10% variation existed between duplicates, the assay was repeated, and averages were reported. The corresponding laboratory was blinded to case status, and all samples were randomly ordered. Additional covariates included baseline demographic variables including age, gestational age of blood collection (first prenatal visit), systolic and diastolic blood pressure, smoking status (never *vs.* past or current), race (Caucasian *vs.* other), and body mass index.

Outcomes

All pregnancy outcomes were prospectively examined and verified by detailed examination of medical records, including prenatal flow sheets and laboratory investigations. Eligible cases were consecutively identified during the study period. PE was defined as systolic blood pressure elevation of at least 140 mm Hg or diastolic blood pressure of at least 90 mm Hg after 20 wk gestation, in association with proteinuria, either 2+ or greater by dipstick or at least 300 mg/24 h in the absence of urinary tract infection (1). Gestational hypertension was defined as systolic blood pressure elevation of at least 140 mm Hg or diastolic blood pressure or at least 90 mm Hg after 20 wk gestation in the absence of significant proteinuria ($\leq$1+ by dipstick or $<$300 mg/24 h) (1). SGA newborns were defined by fetal birth weight less than the 10th percentile of the U.S. population matched for gender (37), and no women in this category developed hypertension (blood pressure $>$ 140/90 mm Hg) at any point during pregnancy. Controls (2:1) were randomly selected from women who participated in the MOMS cohort within the same time period as cases, delivered appropriate for gestational age infants, and remained normotensive and nonproteinuric throughout pregnancy. Given that cases (n = 40 for each case group) represented subjects with specific outcomes during the study period, approximately four controls were randomly identified and included from each month of the study period to ensure that controls were contemporaneous with cases. Women with a history of diabetes, thyroid, liver, or chronic renal disease, or preexisting chronic hypertension (defined as blood pressure $>$ 140/90 mm Hg or need for antihypertensive medications before pregnancy or before 20 wk gestation) were excluded.

Statistical analysis

Continuous variables were analyzed by Student's *t* test, and categorical variables were analyzed by the χ^2 test. Continuous variables that demonstrated significantly skewed distributions, namely serum PlGF levels, were log transformed to achieve normality as has been done in a previous study (26), thus allowing parametric procedures to be used for comparisons. Primary exposures were examined as continuous variables, and as quantiles based on distributions of the controls. The primary measure of association was relative risk because these data were derived from a prospective cohort study (38). Multiple regression analysis was performed using logistic regression techniques. All *P* values were two-tailed, and a P value $<$ 0.05 was considered statistically significant.

Results

Baseline characteristics of women at the first prenatal visit are shown in Table 1. Body mass index and systolic and diastolic blood pressure were higher in women who developed PE or gestational hypertension, although the results for body mass index did not reach statistical significance. Gestational age at delivery and fetal birth weight differed among women who developed PE and those who delivered SGA newborns, compared with women who developed gestational hypertension and normotensive controls.

Table 2 demonstrates levels of sFlt1 and PlGF in the four groups of women. Although there were no statistically significant differences in sFlt1 levels between the groups, women who developed PE or who delivered a SGA newborn tended to have higher serum levels of sFlt1 at this early stage of pregnancy. In contrast to serum sFlt1 levels, serum PlGF levels did differ significantly compared with controls, and thus further analysis focused on the association of PlGF and subsequent risk for the primary outcomes with and without inclusion of sFlt1 in the models.

Multiple regression analysis (adjusting for baseline age, blood pressure, race, smoking status, parity, body mass index, gestational age of blood collection, and serum sFlt1 levels) was then performed to determine the association between serum levels of PlGF and risk for the primary outcomes (Table 3). In the unadjusted model, the risk for each

TABLE 1. Baseline characteristics of the study population according to outcome of pregnancy

	Normal pregnancy (n = 80)	PE (n = 40)	Gestational hypertension (n = 40)	SGA (n = 40)
Baseline characteristics				
Age (yr)	30 ± 6	31 ± 6	31 ± 4	30 ± 6
Gestational age at first prenatal visit (wk)	10.6 ± 3.1	10.6 ± 1.9	10.7 ± 1.8	9.8 ± 1.6
Past or current smoking (%)	34	44	28	30
Caucasian race (%)	47	56	68[a]	60
Body mass index (kg/m^2)	24.9 ± 5.7	27.0 ± 5.4	25.6 ± 4.0	23.5 ± 4.0
Systolic blood pressure (mm Hg)	109 ± 9	114 ± 9[a]	119 ± 10[a]	109 ± 10
Diastolic blood pressure (mm Hg)	68 ± 6	72 ± 8[a]	74 ± 8[a]	69 ± 7
Delivery characteristics				
Gestational age at delivery (wk)	39.5 ± 1.6	37.1 ± 3.0[a]	39.4 ± 1.3	37.9 ± 3.9[a]
Birth weight (g)	3477 ± 454	2941 ± 870[a]	3284 ± 447	2553 ± 776[a]

Values are expressed as means ± SD.
[a] $P < 0.05$ compared to normal pregnancy.

TABLE 2. First trimester serum levels of sFlt1 and PlGF according to outcomes of pregnancy

	Normal pregnancy (n = 80)	PE (n = 40)	Gestational hypertension (n = 40)	SGA (n = 40)
SFlt1 (pg/ml)	973 ± 490	1048 ± 657	942 ± 437	1011 ± 479
PlGF (pg/ml)	63 ± 145	23 ± 24[a]	27 ± 19[a]	21 ± 16[a]

Values are expressed as means ± SD.
[a] $P < 0.05$ compared to normal pregnancy.

TABLE 3. Multivariate analysis of first trimester serum levels of PlGF and risk for SGA newborn, gestational hypertension, and PE

Model	Relative risk[a]	95% CI[a]
Normal Pregnancy	Ref	
PE		
PlGF	2.4	1.4–4.6
PlGF + baseline demographic covariates[b]	3.2	1.3–7.7
PlGF + baseline demographic covariates[b] + sFlt1	3.7	1.2–12.5
Gestational hypertension		
PlGF	1.7	1.0–2.9
PlGF + baseline demographic covariates[b]	1.5	0.6–3.8
PlGF + baseline demographic covariates[b] + sFlt1	1.5	0.6–4.0
SGA newborn		
PlGF	2.4	1.4–4.3
PlGF + baseline demographic covariates[b]	1.3	0.6–2.7
PlGF + baseline demographic covariates[b] + sFlt1	1.4	0.7–3.1

Ref, Reference category.
[a] Relative risk is for every one log unit decrease in serum levels of PlGF.
[b] Baseline demographic variables: age, blood pressure, gestational age at blood collection, race, smoking status, and body mass index.

adverse pregnancy outcome was significantly increased for every log unit decrease in PlGF levels. After adjustment for baseline covariates, however, PlGF levels remained significantly associated only with increased risk of subsequent PE. A notable change in the point estimate of PlGF occurred when adjusting for gestational age of blood collection, as this covariate alone mitigated the significance between PlGF and risk for either gestational hypertension or SGA deliveries. Indeed, the correlation between gestational age of blood collection and serum levels of PlGF was r = 0.74 ($P < 0.01$), suggesting adjustment for gestational age of blood collection even in this narrow window of gestation (7.3–12 wk) was important to reduce misclassification. Adjustment for gestational age of blood collection did not markedly change the point estimate seen with PE, suggesting that the increase in serum PlGF expected with increase in gestational age was blunted in this group. Thereafter, addition of sFlt1 levels into the model strengthened the association between serum PlGF and risk for PE, whereas similar models for gestational hypertension and SGA did not show notable changes. We observed a slight negative correlation between baseline body mass index and serum levels of sFlt1 (r = −0.16; $P = 0.02$), suggesting negative confounding by body mass index, and which likely explained the strengthening of the association between serum levels of PlGF and risk for PE after adjustment with serum sFlt1.

We then examined serum levels of PlGF in tertiles according to the distribution of the controls (Fig. 1). In this multivariable analysis (including all covariates from Table 3), in contrast to the risks for gestational hypertension and SGA delivery, the risk for PE substantially increased with each successive tertile such that compared with the first tertile (serum PlGF levels > 39 pg/ml), the risk in the third tertile (<12 pg/ml) was 28.7 [95% confidence interval (CI), 2.3–351.1]. Removal of sFlt1 levels from this model attenuated the relative risk for PE in the third tertile (RR, 14.1; 95% CI, 2.0–102.2), although CIs overlapped. We examined the receiver-operator-curve for this model and found that for the third tertile of serum PlGF levels, area under the curve (c statistic) was 0.88 in the full model (including sFlt1) and 0.69 in the unadjusted model, suggesting that the model fit was improved when sFlt1 was included in the model.

Within the group of women who developed PE, there was a trend toward lower first trimester serum levels of PlGF comparing nulliparous women (22 ± 25 pg/ml) with multiparous women (25 ± 21 pg/ml). There were no statistical differences in serum levels of sFlt1 between the groups at this time. When all cases and controls were pooled, nulliparous women also tended to have lower levels of PlGF (24 ± 21 pg/ml) and higher levels of sFlt1 (1047 ± 512 pg/ml) compared with multiparous women (serum PlGF levels, 68 ± 151

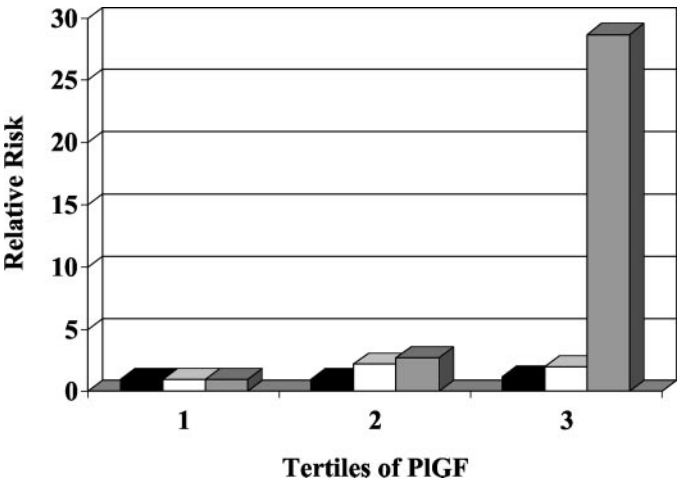

FIG. 1. Adjusted risk of gestational hypertension (*black*), delivery of a SGA newborn (*white*), and PE (*gray*) according to first trimester tertiles of PlGF. Serum PlGF tertiles are defined as follows: first tertile, more than 39 pg/ml; second tertile, 12–39 pg/ml; third tertile, less than 12 pg/ml.

pg/ml; $P < 0.01$; sFlt1, 953 ± 512 pg/ml; $P = 0.21$) with blood measurements taken at similar gestational ages. We found no significant correlation between maternal age and serum levels of PlGF (r = 0.09; $P = 0.20$), but we did find a slight positive correlation between maternal age and serum levels of sFlt1 (r = 0.15; $P = 0.03$). In a model restricted to nulliparous women and adjusted for all covariates including maternal age and serum levels of sFlt1, the risk for PE in the third compared with the first tertile of PlGF was increased 41-fold ($P < 0.01$). Finally, 33% of women who developed PE gave birth to SGA newborns. These women tended to have higher baseline levels of sFlt1 (1290 ± 881 pg/ml) compared with women who developed PE but gave birth to a newborn that was appropriate for gestational age (959 ± 326 pg/ml; $P = 0.06$). Serum PlGF levels between the two groups (PE with SGA *vs.* PE alone) did not markedly differ at this early stage of pregnancy (data not shown).

Discussion

In this prospective nested case-control study, angiogenic-related analytes measured in blood samples collected in the first trimester of pregnancy strongly identified women who developed PE later in pregnancy. Notably, low serum levels of PlGF were associated with increased risk for subsequent PE. This association was strengthened when serum levels of sFlt1 were included in the analysis, and the combination of both serum PlGF and sFlt1 distinguished women who subsequently developed PE from those who subsequently developed gestational hypertension, delivered SGA newborns, or completed a normal-term pregnancy.

In previous cross-sectional studies of women with severe PE at term, investigators noted lower serum levels of PlGF in women with PE compared with normotensive controls (26, 39, 40). Utility of serum PlGF levels before term, however, has been controversial, as some studies suggested that low levels of serum PlGF early in pregnancy were associated with subsequent PE (15, 30, 31, 33), whereas others reported no significant associations (28, 29). Discrepancies in previous studies may have been due to utilization of nonparametric methods of analysis (*e.g.* rank sum tests), which tend to have less power to detect differences compared with parametric methods (*t* tests) (41), and log transformation of PlGF levels permitted us to use the latter. We do acknowledge that even with sample sizes larger than most other studies, our CIs were wide, and thus larger studies are still needed. Another reason for discrepancies may have been due to sample degradation of serum PlGF from extended or improper storage (42). Our samples were stored at −80 C within a few hours of collection and thawed once for this study. Finally, accurate characterization of outcomes may have led to discrepant results, because women who develop PE may differ from women who deliver SGA newborns uncomplicated by hypertension, although overlapping features between these two groups may render their distinction more difficult. This latter point is important because some studies suggest that low levels of serum PlGF better identify women at risk for delivering SGA newborns and not PE (28), whereas others suggest that only women who develop PE complicated by SGA newborns exhibit significantly low levels of serum PlGF before the 20th week of gestation (15). Given recent findings suggesting that sFlt1 is a plausible mediator of PE along with evidence suggesting that sFlt1 and PlGF are intimately related (35), we found that after adjusting for potential confounders, the combination of both angiogenesis-related factors measured in the first trimester strongly identified women at subsequent risk for PE.

Gestational age of blood collection and parity are important confounders in the analysis of serum levels of PlGF and sFlt1 and risk for adverse outcomes of pregnancy. In previous studies, longitudinal measurements of serum PlGF in uncomplicated pregnancies demonstrated a steady rise in levels of PlGF early in pregnancy that peaks at 28–30 wk gestation (26). Indeed, we noted that adjustment for gestational age of blood collection (even within the narrow window of gestation our samples were collected) diminished the positive association between first trimester serum levels of PlGF and risk for gestational hypertension or delivery for SGA newborns uncomplicated by hypertension. Therefore, despite restricting a study population to a specific window of pregnancy (*e.g.* <20 wk gestation), studies focused on measuring levels of serum PlGF should adjust for gestational age of blood collection. Interestingly, adjustment for gestational age of blood collection did not markedly alter the association between serum PlGF levels and risk for PE, suggesting that in women destined to develop PE, the slope of increase in serum PlGF levels with gestational age is attenuated, as has been reported in a previous study (31). Parity also appeared to alter baseline levels of serum PlGF and sFlt1. We found that multiparous women in general tended to have higher levels of serum PlGF and lower levels of sFlt1 compared with nulliparous women when samples were measured at similar gestational ages. Although the exact reasons for these changes are unknown, it is interesting to speculate that this may explain why multiparous women are at much lower risk for developing PE compared with nulliparous women (43–45). The latter hypothesis will require testing of similar women through second pregnancies.

Our results suggest that placental compromise in PE oc-

curs early in gestation, a hypothesis that is supported by detailed work on trophoblast invasion and early uterine blood flow (22, 46, 47). Although placental compromise and, potentially, low PlGF levels may be expected in both PE and fetal growth abnormalities, our understanding of sFlt1 in the potential pathogenesis of PE (35) and the results of this current study suggest that serum sFlt1 in combination with serum PlGF will serve as important first trimester biomarkers that distinguish women who subsequently develop PE from those who develop gestational hypertension or those who deliver SGA newborns uncomplicated by hypertension. We did measure serum levels of free VEGF and found undetectable levels regardless of case status (data not shown), suggesting that free VEGF in the first trimester will not be useful at this early stage in pregnancy as has been shown (15). Our study does not specifically address the mechanism of low serum levels of PlGF during first trimester. Indeed, decreased circulating PlGF levels may be secondary to increased sFlt1 production, with an accompanying fall in free PlGF and/or decreased placental production of PlGF. This will require further study. Furthermore, additional work will be needed to address how alterations in these angiogenic factors are perhaps linked with genetic (48), metabolic (11, 49–51), inflammatory (12, 52–55), and autoimmune (16) alterations that also characterize women with PE. Finally, because of the limited number of patients in this study, we were unable to determine whether alterations in these angiogenic molecules could specifically identify the group of women at risk of developing severe, early-onset PE (<34 wk), the same group that stands to benefit the most from early disease prediction. Further studies are needed to stratify the PE group to determine whether more extreme alterations in angiogenic factors might be more strongly associated with the development of earlier and/or more severe PE.

Our findings apply to women who developed PE with appropriate-for-gestational-age and SGA newborns, although the latter group did exhibit more severe alterations in angiogenic factors even when examined in the first trimester of pregnancy. While investigators search for the origin of sFlt1 elevation in PE, further studies should not only verify our results but also consider examining the dynamic changes of sFlt1 and PlGF in a longitudinal fashion and with various outcomes of pregnancy. Furthermore, we suggest that ongoing clinical trials for PE should incorporate longitudinal measurements of serum sFlt1 and PlGF and examine how these levels correlate with potential interventions.

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