Circulating Levels of the Antiangiogenic Marker Soluble FMS-Like Tyrosine Kinase 1 Are Elevated in Women With Pregestational Diabetes and Preeclampsia

Angiogenic markers in preeclampsia and preexisting diabetes

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Preeclampsia is characterized by the development of proteinuria and hypertension after 20 weeks gestation, and it is associated with maternal and fetal morbidity. Preeclampsia affects ~5% of pregnancies, though women with preexisting diabetes are three to four times more likely to develop preeclampsia (1). Preeclampsia is associated with altered angiogenic factors, including increased levels of circulating soluble FMS-like tyrosine kinase 1 (sFlt1) and reduced levels of vascular endothelial growth factor and placental growth factor (PlGF). Hypertension and proteinuria of preeclampsia may be caused by an imbalance of these angiogenic factors (2–10). Circulating sFlt1, an antiangiogenic protein, binds to proangiogenic proteins, vascular endothelial growth factor, and PlGF, preventing their interaction with endothelial cell receptors and inducing endothelial dysfunction. Rats given sFlt1 develop proteinuria, hypertension, and glomerular endotheliosis, which are hallmarks of preeclampsia (3). Alterations in sFlt1 and PlGF are observed several weeks before symptoms (2).

It is unknown whether alterations in sFlt1 and PlGF are present in women with diabetes who then develop preeclampsia or whether a different pathway is responsible. Ultimately, it would be helpful to have a way to diagnose and predict preeclampsia in women with pregestational diabetes, as they frequently have preexisting hypertension and/or proteinuria, which make it difficult to differentiate superimposed preeclampsia.

We evaluated a small group of women with diabetes and compared levels of sFlt1 and PlGF at delivery in both those who developed preeclampsia and those who did not. We hypothesized that women with preexisting diabetes who developed preeclampsia may also have elevations in sFlt1 and decreases in PlGF.

RESEARCH DESIGN AND METHODS

The population for the current study was derived from the Beth Israel Deaconess Medical Center. Serum samples were obtained at the time of delivery in women with pregestational diabetes. Specimens were frozen at −80 °C and thawed once to perform the assays. Demographic data, past medical and obstetrical history, and clinical data from prenatal visits and delivery were retrospectively collected by chart review. The protocol was approved by the institutional review board at the Beth Israel Deaconess Medical Center.

Assays

Stored serum was thawed, and commercial enzyme-linked immunosorbent assay kits were used to assay sFlt1 and PlGF (R&D Systems, Minneapolis, MN). All samples were run in duplicate by personnel who were blinded to the pregnancy outcome. The commercial kits' intra- and interassay coefficients of variation for sFlt1 and PlGF were 3.5 and 5.6 and 8.1 and 10.9, respectively.

Definition of preeclampsia

In women without baseline hypertension or proteinuria, preeclampsia was defined as the development of hypertension plus proteinuria or thrombocytopenia (11). Hypertension was defined as either a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg on two occasions, at least 4 h apart, occurring after 20 weeks gestation. Proteinuria was defined as either >300 mg of protein in a 24-h urine collection or two positive dipstick test results ≥2+ (recorded at least 4 h apart) with no evidence of a urinary tract infection. Thrombocytopenia was defined as a platelet count <100,000 per mm^3.

In women who were normotensive but proteinuric at baseline, the diagnosis of preeclampsia required the presence of thrombocytopenia, an aspartate aminotransferase >70 units/l, or hypertension accompanied by severe headaches, epigastric pain, or sudden increase in proteinuria (five times the baseline value or twice baseline if it was >5 g per 24 h). In women who had both hypertension and proteinuria at baseline, the diagnosis of preeclampsia required any of the following: thrombocytopenia, an elevated aspartate aminotransferase ≥70 units/l, or worsening hypertension (as shown by two diastolic blood pressures

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Abbreviations: PlGF, placental growth factor; sFlt1, soluble FMS-like tyrosine kinase 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Clinical characteristics and concentrations of angiogenic factors*

	Diabetic pregnancy	Diabetic pregnancy with preeclampsia
Maternal age (years)	33.4 ± 5.9	33.2 ± 2.5
Gestational age (weeks)	36.57 ± 2.03	32.14 ± 3.84
Birth weight (g)	3,761.6 ± 1,012.52	2,236.4 ± 943.88
Systolic blood pressure (mmHg)	141.4 ± 13.4	154.6 ± 16.4
Diastolic blood pressure (mmHg)	90.8 ± 5.4	95 ± 7.9
Urine protein/creatinine ratio	0.425 ± 0.39 (n = 4)	9.88 ± 11.02
Hematocrit (%)	33.48 ± 1.95	33.82 ± 4.13
Uric acid (mg/dl)	6.0 ± 0.77 (n = 4)	6.9 ± 1.11
A1C (%)	5.72 ± 0.415	6.3 ± 0.917 (n = 3)
ALT (IU/l)	11.75 ± 6.29 (n = 4)	94.25 ± 81.68 (n = 4)
sFlt1 (ng/ml)	18.13 ± 10.29	102.99 ± 39.27
PlGF (pg/ml)	353.85 ± 214.41	119.09 ± 87.30

Data are mean ± SD. n = 5 unless otherwise noted. All data were collected at time of delivery, except A1C, which was collected during the third trimester.

≥100 mmHg taken 4 h apart in the week before delivery) combined with either exacerbation of proteinuria (as above), severe headaches, or epigastric pain.

Statistical analysis

Statistical analysis was performed using the nonparametric Mann-Whitney *U* test because the distributions of sFlt1 and PlGF are highly skewed. All tests were two tailed, and $P < 0.05$ was considered statistically significant.

RESULTS — We identified five subjects with pregestational diabetes and clinical evidence of preeclampsia as well as five subjects with pregestational diabetes and no evidence of preeclampsia (Table 1). Of the five with preeclampsia, three had a prior history of hypertension and one had a history of nephropathy. The mean ± SD sFlt1 in women with diabetes and no evidence of preeclampsia was 18.13 ± 10.29 ng/ml, and the mean sFlt1 in women with diabetes and preeclampsia was 102.99 ± 39.27 ng/ml ($P = 0.01$) (Table 1). Prior studies in our laboratory with nondiabetic pregnant patients using a similar enzyme-linked immunosorbent assay procedure revealed a serum sFlt1 level of 38.71 ng/ml in those with mild preeclampsia, 65.59 ng/ml in those with severe preeclampsia, and 19.25 ng/ml in those without preeclampsia (13).

Concentrations of PlGF were also lower in diabetic patients with preeclampsia ($P = 0.03$) (Table 1). Since sFlt1 was increased and PlGF decreased, we calculated sFlt1/PlGF, an index of circulating antiangiogenic activity that has been reported to predict preeclampsia more reliably than either marker alone (13,14). The mean sFlt1/PlGF both in women with diabetes without preeclampsia and in women with diabetes and preeclampsia was 68.19 ± 58.98 and 1,782 ± 1,723.04, respectively ($P = 0.01$).

CONCLUSIONS — In this small, limited study of 10 patients, we found that women with preexisting diabetes who developed preeclampsia had elevated levels of sFlt1 and lower PlGF at time of delivery, just as women without diabetes have been shown to have in preeclampsia (2–10). Compared with levels seen in prior studies, women with diabetes who developed preeclampsia tended to have higher levels of sFlt1, in the range of severe preeclampsia. These results suggest a mechanism of preeclampsia in women with preexisting diabetes similar to that in women without preeclamsia.

A prior study (15) showed a trend toward lower PlGF levels in cord serum of diabetic pregnancies with preeclampsia, though this was cord serum and not maternal serum. There was also a study (16) in nonpregnant diabetic patients that found sFlt1 levels unaltered in patients who both did and did not have atherosclerosis. The results of these studies do not elucidate whether any alterations in sFlt1 and PlGF are present in diabetic patients who develop preeclampsia.

Larger, prospective studies of sFlt1 and PlGF levels throughout gestation in women with preexisting diabetes are needed to test the hypotheses that *1*) measurement of sFlt1 and PlGF can be used to diagnose superimposed preeclampsia in diabetic pregnancies with preexisting hypertension and proteinuria and *2*) these markers may be used to predict the onset of preeclampsia.

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