BASIC SCIENCE: OBSTETRICS

Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia?

Yuval Bdolah, MD, MSc; Chun Lam, MD; Augustine Rajakumar, PhD; Venkatesha Shivalingappa, PhD; Walter Mutter, MD; Benjamin P. Sachs, MB, BS, DPH; Kee Hak Lim, MD; Tali Bdolah-Abram, MA; Franklin H. Epstein, MD; S. Ananth Karumanchi, MD

OBJECTIVE: Twin pregnancies are a risk factor for preeclampsia with a reported incidence of 2-3 times higher than singleton pregnancies. Soluble fms-like tyrosine kinase 1 (sFlt1), which is a circulating antiangiogenic molecule of placental origin, plays a central role in preeclampsia by antagonizing placental growth factor (PlGF) and vascular endothelial growth factor signaling in the maternal vasculature. Increased sFlt1 and the ratio sFlt1/free PlGF have been shown to antedate clinical signs in preeclampsia. Although the cause of the upregulated sFlt1 in preeclampsia still is not understood clearly, placental ischemia with accompanying hypoxia is thought to play an important role. We therefore hypothesized that the higher risk of preeclampsia in twin pregnancies results from high sFlt1 (or sFlt1/PlGF) and that the sFlt1 upregulation was due to either relative placental hypoxia and/or increased placental mass.

STUDY DESIGN: Maternal serum samples and placentas from third-trimester twin and singleton pregnancies without preeclampsia were used. Serum samples were analyzed for levels of sFlt1 and free PlGF by enzyme-linked immunosorbent assay and reported as means (in nanograms per milliliter and picograms per milliliter, respectively). Placentas were weighed and examined for content of sFlt1 and PlGF messenger RNA (mRNA) by quantitative polymerase chain reaction and hypoxia inducible factor-1α (HIF-1α) protein by Western blot.

RESULTS: Soluble Flt1 concentrations in twin pregnancy maternal serum were 2.2 times higher than those that were measured in singleton pregnancy maternal serum samples (30.98 $\pm$ 9.78 ng/mL vs 14.14 $\pm$ 9.35 ng/mL, respectively; $P = .001$). Free PlGF concentrations were not significantly different between twin and singleton maternal serum samples, but the mean sFlt1/PlGF ratio of twin pregnancy maternal serum samples was 2.2 times higher than the equivalent ratio in singleton pregnancy samples (197.58 $\pm$ 126.86 ng/mL vs 89.91 $\pm$ 70.63 ng/mL, respectively; $P = .029$). Quantitative polymerase chain reaction for sFlt1 and PlGF mRNA revealed no significant differences between the 2 study groups. Western blot analysis of placental samples for HIF-1α revealed a mean ratio HIF-1α/actin of 0.53 vs 0.87, for the twins vs singletons placental samples respectively (twins showed lower HIF-1α, not higher). The mean weights of twin and singleton placentas were 1246 vs 716 g, respectively ($P < .001$). Importantly, the placental weights correlated very well with the circulating sFlt1 levels ($R^2 = .75$).

CONCLUSION: In twin pregnancies, circulating sFlt1 levels and sFlt1/PlGF ratios were twice as high as those in singleton pregnancies. The increased serum sFlt1 levels in twin pregnancies were not accompanied by any changes in the levels of sFlt1 mRNA and HIF-1α protein in the twin placentas but were correlated with increased placental weight. These findings suggest that the increased risk of preeclampsia in twin pregnancies may be due to increased placental mass that leads to increased circulating levels of sFlt1.

Key words: angiogenesis, HIF-1α, hypoxia, multifetal pregnancy, PlGF, preeclampsia, sFlt1, trophoblast, twin pregnancy

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From the Departments of Obstetrics & Gynecology (Drs Bdolah, Sachs, Lim, and Karumanchi) and Medicine (Drs Lam, Shivalingappa, Mutter, Epstein, and Karumanchi), Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; the Department of Obstetrics & Gynecology (Dr Bdolah and Ms Bdolah-Abram), Hadassah-Hebrew University Medical Center, Jerusalem, Israel; and the Department of Obstetrics (Dr Rajakumar), Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA.

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Reprints: S. Ananth Karumanchi, MD, Beth Israel Deaconess Medical Center, 330 Brookline Ave, RW 663, Boston, MA 02215; sananth@bidmc.harvard.edu

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Twin pregnancy is a risk factor for hypertensive disorders of pregnancy, with a reported incidence of 13%-37%, which is 2-3 times higher than the reported incidence for women with singleton pregnancies.[1-5] In a multicenter study by Sibai et al,[6] women with twin gestations had a relative risk of 2.04 for having gestational hypertension and 2.62 for having preeclampsia. Women with twin gestations also had significantly higher rates of preeclampsia-associated complications, such as preterm delivery and abruptio placentae, as did control subjects. In nulliparous women,

TABLE 1
Demographics of singleton and twin pregnancies in this study

Demographic	Pregnancy: Singleton (n = 10)	Pregnancy: Twin (n = 9)	*P* value
Maternal age (y)[a]	33.5 ± 4.7	33 ± 3.8	
Primigravidity (n)	4	3	
Race (n)			
White	7	8	
Black	1	0	
Other	2	1	
Gestational age (wk)[a]	39.1 ± 1.4	36.1 ± 3.0	.001
Birthweight (g)[a]	3419.4 ± 448.8[b]	2621 ± 722.8	.017
Current smoker (n)	1	0	
Predelivery systolic blood pressure (mm Hg)[a]	112 ± 8	113 ± 7	
Predelivery diastolic blood pressure (mm Hg)[a]	71 ± 8	69 ± 6	

[a] Data are presented as mean ± SD.

[b] Data were available for 9 of 10 subjects.

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twin gestations, when compared with singleton pregnancies, had a relative risk of 4.03 for having preeclampsia and 3.41 for having eclampsia or hemolysis, elevated liver enzymes, and low platelet count syndrome.[7] Preeclampsia also affected 24.3% of triplets and quadruplicate pregnancies, which is significantly more than in the singleton population but at a rate similar to that in twins (22.6%).[8]

We have demonstrated recently that excess placental secretion of a circulating anti-angiogenic molecule, soluble fms-like tyrosine kinase 1 (sFlt1) may have a pivotal role in the pathogenesis of the maternal syndrome in preeclampsia.[9] SFlt1 acts by antagonizing 2 proangiogenic molecules, vascular endothelial growth factor, and placental growth factor (PlGF). Several additional studies have confirmed these findings.[10-15] Furthermore, alterations in sFlt1 and free PlGF antedate the onset of clinical symptoms.[16] SFlt1 was elevated 5 weeks before clinical symptoms of preeclampsia were noted and appeared to herald the onset of clinical disease. Levels of PlGF were lower in patients who are destined to experience clinical preeclampsia than in women who did not as early as first trimester, although the dramatic reductions in PlGF were during the time when sFlt1 was elevated.[17] The ratio of sFlt1/PlGF, a biologically more reliable index of the circulating angiogenic state than either of the markers alone, was found to be a better predictor of preeclampsia risk and was particularly dramatic in patients who experienced premature preeclampsia.[18-20] More recently, another antiangiogenic factor, soluble endoglin (sEng), has been found to be elevated in patients with preeclampsia and is thought to synergize with sFlt1 in mediating the maternal syndrome.[21] These data suggest that alterations in circulating angiogenic factors play a central role in the pathogenesis of the clinical signs and symptoms of preeclampsia.[22,23]

Placental ischemia with relative hypoxia has been suggested as the primary early placental insult that leads to a cascade of events that results in preeclampsia.[23,24] Evidence for this hypothesis is further strengthened by the studies that demonstrate increased expression of hypoxia-inducible transcription factors (HIF-1α and HIF-2α)[19] and gene expression signature of hypoxia[25] in preeclamptic placentas. Moreover, hypoxia is a potent inducer of sFlt 1 in vitro.[26,27] However, because sFlt1 is a potent vasoconstrictor, it has been hypothesized that increased placental ischemia that is mediated by high levels of sFlt1 and other vasoconstrictors locally may induce placental ischemia/hypoxia as a secondary phenomenon. Experimental data in pregnant rats, in which the exogenous administration of sFlt1 and sEng induces placental infarcts/ischemia supports this hypothesis.[21] Therefore, a fundamental question in the pathophysiologic condition of preeclampsia is whether placental hypoxia is the cause or the effect of a disturbance in soluble angiogenic factors such as sFlt1. The evidence for either has been reviewed recently.[23,28]

In certain conditions, such as multifetal pregnancy, it seems possible that an excess of trophoblastic tissue per se might elevate circulating concentrations of placental products that include sFlt1. In other conditions such as hydatiform moles or trisomy 13, placental trophoblasts might be programmed to produce excessive sFlt1, even in the absence of placental hypoxia. We summarize our findings in women with twin and singleton pregnancies.

MATERIALS AND METHODS

Patients

Patients were recruited from a cohort of women in the third trimester of their pregnancy who delivered at the Beth Israel Deaconess Medical Center delivery room in the years 2002-2005. After informed consent was obtained, the placentas were collected and weighed, and fresh tissue samples were frozen in liquid nitrogen in either phosphate-based saline solution or RNA Later solution (Ambion Inc, Austin, TX). Serum samples were also obtained just before delivery (−12 hours to delivery). Obstetric history was recorded for all patients (Table 1); only placentas that had been obtained from cesarean deliveries were included in the study.

Angiogenic factor serum levels

Measurement of sFlt1 and free PlGF serum concentrations were performed by