OBSTETRICS

Maternal circulating angiogenic factors in twin and singleton pregnancies

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OBJECTIVE: The purpose of this study was to compare longitudinally sampled maternal angiogenic proteins between singleton and twin pregnancies.

STUDY DESIGN: Placental growth factor (PlGF), soluble feline McDonough sarcoma (fms)-like tyrosine kinase (sFlt)-1, and soluble endoglin from healthy pregnant women were quantified at 10, 18, 26, and 35 weeks' gestation (n = 91), and during the third trimester (31-39 weeks) and at delivery (33-41 weeks; n = 41). Geometric means and 95% confidence intervals were calculated for gestational age-adjusted angiogenic protein concentrations and compared between matched twin and singleton pregnancies.

RESULTS: Maternal sFlt-1 concentrations and the sFlt-1/PlGF ratio were higher in twins than singletons across pregnancy and at delivery, with the greatest differences at week 35 (sFlt-1: 36,916 vs 10,151 pg/mL; $P < .0001$; sFlt-1/PlGF: 168.4 vs 29.0; $P < .0001$). Maternal concentrations of soluble endoglin also were higher in the third trimester and delivery. Maternal PlGF concentrations were lower in twin than singleton pregnancies at week 35 only (219.2 vs 350.2 pg/mL; $P < .0001$). Placental weight appeared to be inversely correlated with maternal sFlt-1/PlGF ratio at the end of pregnancy in both twins and singletons.

CONCLUSION: Higher maternal antiangiogenic proteins in twin than singleton pregnancies does not appear to be due to greater placental mass in the former, and may be one explanation for the increased risk of preeclampsia in women carrying multiple gestations. Determining whether women with a history of multiple gestations have an altered cardiovascular disease and breast cancer risk, like those with a history of preeclampsia, is warranted.

Key words: angiogenic balance, endoglin, pregnancy, singletons, soluble fms-like tyrosine kinase-1, twins

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Women carrying twins or other higher-order multiples are at 2-3 times the risk of developing preeclampsia, a common cause of maternal and fetal morbidity,[1] than women with singleton pregnancies.[2,3] Preeclampsia is marked by shallow trophoblast invasion into the maternal endometrium resulting in a less extensive vascular network supporting the pregnancy.[4] Alterations in angiogenic proteins, as well as inflammatory cytokines and other immune-modulating molecules, have been demonstrated in preeclamptic pregnancies,[5-13] with elevations in soluble feline McDonough sarcoma (fms)-like tyrosine kinase (sFlt)-1 and soluble endoglin (s-endoglin), 2 antiangiogenic proteins, typically preceding the clinical manifestation of maternal disease.[14-17]

Maternal angiogenic factors also appear altered in pregnancies involving multiples compared with singletons, with elevated concentrations of sFlt-1 in

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the former.[18-20] The timing of these changes in angiogenic balance may provide insight into the mechanism whereby preeclampsia risk is elevated in women carrying multiple gestations. Therefore, we followed women longitudinally through pregnancy and delivery to examine circulating maternal concentrations of placental growth factor (PlGF), sFlt-1, and s-endoglin in twin and singleton pregnancies.

Materials and Methods

Study subjects

The data for the analysis derive from 2 sources: the BIRTH cohort[21] and a study of twins at the Geisel School of Medicine (Dartmouth College). The study protocols were approved by each institutional review board and at the US National Cancer Institute, and written informed consent was obtained from all participating women.

BIRTH cohort

Participants were enrolled at 3 US tertiary care academic centers from October 2007 through June 2009. Eligible women initiated routine prenatal care at <15 weeks' gestation, were >18 years of age, and planned to deliver at the enrolling institution. Women who developed preeclampsia in the index pregnancy or a prior one (gestational hypertension defined as a blood pressure elevation of >140/90 mmhg on 2 occasions with concomitant proteinuria defined as positive urine protein test result >300 mg/24 hours or protein/creatinine >0.20 mg/mg) were excluded from the present analysis. A total of 2230 singleton and 93 twin gestations were enrolled, and 2193 and 91 singletons and twins, respectively, met the inclusion criteria for analysis.

Geisel School of Medicine twin study

Eligible were pregnant women ≥18 years of age who intended to deliver at the facility. Women carrying twin gestations and presenting for prenatal care or hospitalized for antenatal surveillance from 2003 through 2007 were approached in the third trimester of pregnancy. The next singleton pregnancy that met the eligibility criteria and could be matched to the twin pregnancy on gestational age (within 1 week), parity (nulliparous/parous), and maternal age (±5 years), in that order, was recruited for the study (prenatal controls; n = 40). Another group of women with singleton pregnancies were recruited at admission for labor and delivery (labor controls) and matched to twin mothers according to the criteria above. Five twin pregnancies and 3 singleton pregnancies were excluded because they developed preeclampsia after enrollment, leaving 41 twins and a total of 62 singleton controls (40 with blood samples in the third trimester and 52 with blood samples at labor and delivery). Placentas were routinely examined by the pathology department.

Biospecimen collection and processing

BIRTH cohort

Maternal blood samples were obtained at the following median (interquartile range) weeks of gestation: 9.7 (8.4−11.6), 17.8 (16.8−18.7), 25.9 (24.8−28.1), and 35.1 (34.6−35.9). Approximately 10 mL of blood was drawn in EDTA plasma tubes; the samples were kept at 4°C until processing for storage within 4 hours of venipuncture. The specimens were centrifuged for 20 minutes and stored at −80°C. Samples were shipped in batches on dry ice to Abbott Diagnostics (Abbott Park, IL) where they were stored at −80°C.

Geisel School of Medicine twin study

Blood samples were collected in the third trimester (31-39 weeks) and at the earliest possible time after admission for labor and before any administration of medication (33-41 weeks). A 10-mL red-top tube of whole blood was allowed to clot at room temperature, was centrifuged, and the sera were stored at −70°C. Samples were shipped on dry ice to a biorepository in Rockville, MD, where they were stored at −80°C.

Laboratory assays

BIRTH cohort

PlGF and sFlt-1 were measured with prototype Abbot Diagnostics immunoassays (Abbott Diagnostics, Abbott Park, IL). The PlGF immunoassay measures the free form of PlGF-1, with a lower limit of detection of 1 pg/mL, and a range up to 1500 pg/mL. The sFlt-1 immunoassay measures both free and bound sFlt-1, with a lower limit of detection of 0.10 ng/mL and a range up to 150 ng/mL. The combined intraassay and interassay coefficients of variation reported by the laboratory were <7% for PlGF and sFlt-1.

Geisel School of Medicine twin study

Serum levels of sFlt-1 and PlGF were determined in a blinded fashion using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) as described elsewhere.[5] Interassay CVs for the sFlt-1 kit ranged from 7.0−8.1% and for PlGF ranged from 10.9−11.8%. S-endoglin was also measured using commercially available enzyme-linked immunosorbent assay kits (R&D Systems) as described elsewhere.[5] Interassay coefficients of variation for the s-endoglin kit ranged from 6.3−6.7%.

In a subset of BIRTH cohort mothers with singleton pregnancies, PlGF and sFlt-1 concentrations were measured using both the Abbot Diagnostics immunoassay (used for study samples in the BIRTH cohort) and the R&D Systems assay (used for study samples in the Geisel School of Medicine twin study) at each time point. Pearson correlation was used to describe the concordance between logarithm-transformed values, as well as Cronbach alpha, a measure of interrater reliability. PlGF and sFlt-1 values measured by the different assays showed high concordance (Table 1). Because absolute values for the angiogenic factors differ between assays, levels between studies could not be directly compared.

Clinical data

BIRTH cohort

Maternal age, parity, conception by assisted reproductive technologies (ART), and baby's birth anthropometrics were abstracted from medical records. The participants completed a brief questionnaire that ascertained information on race/ethnicity, medical