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An elevated maternal plasma, but not amniotic fluid, soluble fms-like tyrosine kinase-1 (sFlt-1) at the time of mid-trimester genetic amniocentesis is a risk factor for preeclampsia

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KEY WORDS
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Objective: The purpose of this study was to determine if an elevated concentration of soluble fms-like tyrosine kinase-1(sFlt-1) in maternal plasma and amniotic fluid is a risk factor for the subsequent development of preeclampsia.

Study design: A case-control study was conducted to compare mid-trimester concentrations of maternal plasma and amniotic fluid sFlt-1 in patients who developed preeclampsia with those who did not. The study included 32 cases with preeclampsia (18 cases: severe preeclampsia) and 128 matched controls with normal outcomes. Patients with an abnormal fetal karyotype or major anomaly, multiple pregnancies, chronic hypertension, diabetes, and renal disease were excluded. Soluble Flt-1 concentration was measured by specific immunoassay. Nonparametric techniques were used for statistical analysis.

Results: 1) The median maternal plasma, but not amniotic fluid, sFlt-1 concentration in patients who developed preeclampsia was significantly higher than in the control cases (maternal plasma: median 730 pg/mL, range 60-3375 pg/mL vs median 441 pg/mL, range 58-1959 pg/mL, $P < .05$; amniotic fluid: median 10,504 pg/mL, range 5253-38,023 pg/mL vs median 10,236 pg/mL, range 4326-87,684 pg/mL, $P = .65$). 2) The median plasma concentration of sFlt-1 was higher in cases of severe preeclampsia than in those with mild preeclampsia without reaching statistical significance (median 762 pg/mL, range 261-3309 pg/mL vs median 334 pg/mL, range 60-3375 pg/mL; $P = .07$). However, there was no significant difference in the median amniotic fluid sFlt-1 concentrations between patients with severe preeclampsia and those with mild preeclampsia ($P = .45$). 3) An elevated maternal plasma sFlt-1 concentration (higher than 700 pg/mL) is a risk

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factor for the development of preeclampsia (OR 3.9, 95% CI 1.7-8.6) and severe preeclampsia (OR 7.4, 95% CI 2.5-22.1) after genetic amniocentesis. 4) The median interval from amniocentesis to the diagnosis of preeclampsia in patients with maternal plasma sFlt-1 concentrations higher than 700 pg/mL was 117 days (range 19-154 days).

Conclusion: An elevated concentration of sFlt-1 in maternal plasma at the time of mid-trimester amniocentesis is a risk factor for the subsequent development of preeclampsia.

Preeclampsia, a multisystem disorder characterized by the presence of hypertension and proteinuria, is thought to be caused by endothelial dysfunction and intravascular inflammation.[1-3] Some investigators proposed that placental factors released into the maternal circulation cause endothelial dysfunction.[4,5] However, the nature of the placental factor(s) remains to be determined.

A growing body of evidence indicates that soluble fms-like tyrosine kinase-1 (sFlt-1), an inhibitor of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), is involved in the pathogenesis of preeclampsia.[6] Evidence in support of this hypothesis includes: 1) sFlt-1 serum concentration[6,7] and placental content[6,8,9] is increased in patients with preeclampsia at the time of diagnosis, and also before the development of the disease[10-12]; 2) hypertension and proteinuria can be induced by the administration of anti-VEGF compounds to nonpregnant animals[13] and humans[14]; 3) administration of sFlt-1 to pregnant rats resulted in hypertension, proteinuria, and glomerular endotheliosis.[6]

Mid-trimester maternal serum testing and amniocentesis are widely used for the prenatal screening and diagnosis of fetal cytogenetic abnormalities. However, maternal blood and amniotic fluid obtained at the time of the procedure are rarely used to assess the risk of preeclampsia. Only a few studies have examined whether or not changes in the plasma/serum concentration of sFlt-1 in asymptomatic pregnant women can predict the development of the disease.[10,15] Some authors reported that serum sFlt-1 concentrations increase only 5 weeks before the onset of preeclampsia,[10] while other investigators could not demonstrate an increase in sFlt-1 in the first trimester of pregnancy in women destined to develop preeclampsia.[15]

The objective of this study was to determine if an elevated sFlt-1 concentration in maternal plasma and amniotic fluid at the time of mid-trimester genetic amniocentesis is a risk factor for the subsequent development of preeclampsia.

Material and methods

Study design

A case-control study was designed with stored amniotic fluid and maternal plasma obtained from women who underwent mid-trimester genetic amniocentesis between July 1998 and April 2004 at Seoul National University Hospital in Seoul, Korea. The case group consisted of pregnant women who subsequently developed preeclampsia. The control group consisted of women who had a normal pregnancy outcome (term gestation with a neonate with adequate weight for gestational age).

Thirty-two patients who developed preeclampsia were matched for maternal age (within 5 years), parity (nullipara vs primipara or multipara), gestational age at amniocentesis (within 2 weeks), and year of amniocentesis (within 3 years) with 128 controls with normal outcomes (ratio 1:4). Preeclampsia was defined as hypertension (systolic blood pressure $\geq$140 mm Hg or diastolic blood pressure $\geq$90 mm Hg that occurs after 20 weeks of gestation) and proteinuria ($\geq$300 mg in a 24-hour urine collection or 1 dipstick measurement $\geq$1+).[16] Preeclampsia was considered severe according to the American College of Obstetricians and Gynecologists (ACOG) practice bulletin developed by ACOG Committee.[17] Cases with an abnormal fetal karyotype, major congenital anomalies, multiple pregnancies, chronic hypertension, diabetes, or renal disease at the time of amniocentesis were excluded. The control group consisted of patients who had a mid-trimester amniocentesis who delivered a normal neonate at term without significant medical or obstetric complications, such as active vaginal bleeding, multifetal pregnancy, chronic hypertension, diabetes, renal insufficiency, low birth weight (<2500 g), congenital anomalies, or fetal demise. Written informed consent was obtained from all patients. The Institutional Review Board of Seoul National University Hospital approved the collection of these samples and the clinical information and samples for research purposes.

Measurement of sFlt-1

Amniotic fluid was retrieved by transabdominal amniocentesis and an aliquot of amniotic fluid not used for diagnostic studies was centrifuged and stored in polypropylene tubes at −70°C until assayed. Maternal blood was drawn at the time of mid-trimester genetic amniocentesis, collected into tubes containing EDTA, centrifuged, and stored at −70°C. Concentrations of sFlt-1 in amniotic fluid and maternal plasma were measured with a commercially available enzyme-linked