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The maternal plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated in SGA and the magnitude of the increase relates to Doppler abnormalities in the maternal and fetal circulation

TINNAKORN CHAIWORAPONGSA^{1,2}, JIMMY ESPINOZA^{1,2}, FRANCESCA GOTSCH¹, YEON MEE KIM^{1,3}, GI JIN KIM¹, LUIS F. GONCALVES^{1,2}, SAMUEL EDWIN¹, JUAN PEDRO KUSANOVIC¹, OFFER EREZ¹, NANDOR GABOR THAN¹, SONIA S. HASSAN^{1,2}, ROBERTO ROMERO^{1,4}

¹Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, MI, USA

²Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

³Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan, USA

⁴Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract

OBJECTIVE: The soluble form of vascular endothelial growth factor receptor-1 (sVEGFR-1), an antagonist to vascular endothelial growth factor and placental growth factor, has been implicated in the pathophysiology of preeclampsia. Preeclampsia and pregnancy complicated with small for gestational age (SGA) fetuses share some pathophysiologic derangements, such as failure of physiologic transformation of the spiral arteries, endothelial cell dysfunction, and leukocyte activation. The objectives of this study were to: 1) determine whether plasma concentrations of sVEGFR-1 in mothers with SGA fetuses without preeclampsia at the time of diagnosis are different from those in patients with preeclampsia or normal pregnant women; and 2) examine the relationship between plasma concentrations of sVEGFR-1 and Doppler velocimetry in uterine and umbilical arteries in patients with preeclampsia and those with SGA.

STUDY DESIGN: A cross-sectional study was conducted to determine the concentrations of the soluble form of VEGFR-1 in plasma obtained from normal pregnant women (n=135), women with SGA fetuses (n=53), and patients with preeclampsia (n=112). Patients with SGA fetuses and those with preeclampsia were sub-classified according to the results of uterine and umbilical artery Doppler velocimetry examinations. Plasma concentrations of sVEGFR-1 were determined by an ELISA. Since these concentrations change with gestational age, differences among various subgroups were statistically estimated with the delta value, defined as the difference between the observed and expected plasma sVEGFR-1 concentration. The expected values were derived from

regression analysis of plasma sVEGFR-1 concentrations in normal pregnancy. Regression analysis and univariate and multivariate analysis were employed.

RESULTS: 1) Mothers with SGA fetuses had a mean plasma concentration of sVEGFR-1 higher than normal pregnant women ($p < 0.001$), but lower than patients with preeclampsia ($p < 0.001$). 2) Among patients with SGA fetuses, only those with abnormal uterine artery Doppler velocimetry had a mean plasma sVEGFR-1 concentration significantly higher than normal pregnant women ($p < 0.001$). 3) Among mothers with SGA fetuses in whom Doppler velocimetry was performed ($n=41$), those with abnormalities in both the uterine and umbilical artery velocimetry had the highest mean delta of sVEGFR-1 plasma concentration (mean $\pm$ standard deviation (SD): 0.69 ± 0.29). Conversely, patients who had normal Doppler velocimetry in both uterine and umbilical arteries had the lowest mean delta (mean $\pm$ SD: 0.09 ± 0.29) of sVEGFR-1 plasma concentrations (ANOVA; $p < 0.001$). 4) Among patients with preeclampsia in whom Doppler velocimetry was performed ($n=69$), those with abnormalities in both the uterine and umbilical artery velocimetry had the highest mean delta sVEGFR-1 plasma concentration (mean $\pm$ SD: 1.01 ± 0.22) among all groups classified (ANOVA; $p < 0.001$). 5) Among patients with SGA and those with preeclampsia, there was a relationship (Chi-square for trend $p < 0.001$ for both) between the severity of Doppler velocimetry abnormalities and the proportion of patients who had high delta sVEGFR-1 plasma concentrations (defined as a concentration 2 SD above the mean delta of normal pregnant women). 6) Multiple regression analysis suggested that the diagnostic category (e.g. SGA or preeclampsia), Doppler abnormalities, and gestational age at blood sampling were associated with an increase in plasma sVEGFR-1 concentrations ($p < 0.001$).

CONCLUSION: These observations provide support for the participation of the soluble receptor of vascular endothelial growth factor in the pathophysiology of SGA with abnormal uterine artery Doppler velocimetry and preeclampsia. An excess of sVEGFR-1 is released into the maternal circulation of patients with preeclampsia and those with SGA fetuses, as abnormalities of impedance to blood flow involve uterine and umbilical circulation.

Keywords

Small for gestational age; preeclampsia; soluble VEGFR-1; angiogenesis; uterine artery Doppler; umbilical artery Doppler

INTRODUCTION

A central feature in the pathophysiology of a subset of pregnancies resulting in small for gestational age neonates (SGA) and preeclampsia is failure of physiologic transformation of the spiral arteries [1–10]. This lesion is thought to be responsible for increased impedance to blood flow in the uterine arteries and uteroplacental ischemia [11–17]. Uterine artery Doppler abnormalities are suggestive of increased impedance to flow in the uterine circulation [18,19] as well as failure of physiologic transformation of the spiral arteries diagnosed by examination of placental bed biopsies [20–26]. Consequently, uterine artery Doppler velocimetry was used to identify a subset of patients destined to develop preeclampsia or SGA [27–36].

In preeclampsia, an ischemic placenta is thought to release factors into the maternal circulation, causing systemic endothelial cell dysfunction [37–44], intravascular inflammation [45–49] and multiple organ damage [8,41,50]. In a subset of patients with SGA without preeclampsia, there is also evidence of endothelial cell dysfunction [51–53] and leukocyte activation [54]. Yet, there are no clinical features of hypertension, proteinuria, and derangement of organ damage. Recently, both the similarity and disparity between preeclampsia and SGA were extensively reviewed [55]. Ness and Sibai proposed that although both diseases share a similar placental pathology, the absence of a maternal metabolic syndrome (adiposity, insulin resistance/hyperglycemia, hyperlipidemia, coagulopathies) or other exogenous inflammatory signals in pregnancies complicated with SGA prevent these patients from developing the maternal syndrome of preeclampsia [55].

Soluble vascular endothelial growth factor receptor (sVEGFR)-1 (known as sFlt-1 or soluble fms-like tyrosine kinase-1) has been proposed to be a factor released by the placenta of patients with preeclampsia, responsible for an anti-angiogenic state and systemic endothelial dysfunction [56]. Soluble VEGFR-1 is generated by a splice variant of the VEGFR-1 gene and contains the extracellular ligand-binding domain, while lacking the signaling tyrosine kinase domain. Thus, this protein binds VEGF or placental growth factor (PlGF) and inhibits their biological activities [57]. Using microarray technology, Maynard and colleagues reported an increased mRNA expression of sVEGFR-1 in placentas from women with preeclampsia [58]. Plasma sVEGFR-1 concentrations are elevated in preeclampsia both prior to [59–66] and at the time of clinical diagnosis [58,59,67–71]. An increased sVEGFR-1 concentration in preeclampsia is associated with a decrease in unbound VEGF and PlGF concentrations, both of which are growth factors thought to be important for the maintenance of endothelial function [57] and survival [72]. The administration of adenovirus encoding sVEGFR-1 to pregnant animals induces hypertension, proteinuria, and glomeruloendotheliosis [58].

Conflicting results have been reported regarding plasma sVEGFR-1 concentrations in pregnancies complicated by SGA without preeclampsia. Two studies evaluating SGA patients did not include uterine artery Doppler velocimetry information. Tsatsaris and colleagues reported a higher mean sVEGFR-1 plasma concentration in women with SGA neonates than in normal pregnant women [71], while Shibata and colleagues found no such change [73]. Another report revealed a higher plasma concentration of sVEGFR-1 in patients who delivered SGA neonates. This study, however, included patients with preeclampsia together with those with SGA and did not analyze the data separately [74]. Four studies reported an increased plasma/serum concentration of sVEGFR-1 in patients with the diagnosis of SGA and abnormal uterine/umbilical artery Doppler velocimetry [69,75–77].

The objectives of this study were: 1) to determine if plasma concentrations of sVEGFR-1 in mothers with SGA fetuses without preeclampsia at the time of diagnosis are different from those in patients with preeclampsia or normal pregnant women; and 2) to examine the relationship between plasma concentration of sVEGFR-1 and the results of Doppler velocimetry in the uterine/umbilical arteries in patients with preeclampsia and those with SGA.

PATIENTS AND METHODS

Study Design:

This was a cross-sectional study focusing on singleton gestations. The following groups were examined: 1) normal pregnant women (n=135); 2) patients without preeclampsia who delivered an SGA neonate (n=53); and 3) women with preeclampsia (n=112). The study was conducted by searching the clinical database and bank of biologic samples of the Perinatology Research Branch. All patients were enrolled at Hutzel Women's Hospital, Detroit MI.

All pregnancies included in the SGA group underwent ultrasound examination for dating before 24 weeks of gestation and had no major fetal congenital anomalies. The diagnosis of SGA was based on an ultrasonographic estimated fetal weight and confirmed by a birthweight below the 10th percentile for gestational age, according to the reference range proposed by Alexander et al [78,79]. Preeclampsia was defined as hypertension (systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg on at least two occasions, 4 hours to 1 week apart) and proteinuria ($\geq$ 300 milligrams in a 24-hour urine collection or one dipstick measurement $\geq$ 2+) [80]. Patients with chronic hypertension or renal disease were excluded. Severe preeclampsia was defined as either severe hypertension (diastolic blood pressure $\geq$ 110 mm Hg) and mild to severe proteinuria, or mild hypertension plus severe proteinuria (a 24-hour urine sample containing 3.5 grams protein or urine specimen $\geq$ 3+ protein by dipstick measurement) [80]. Patients with an abnormal liver function test (aspartate aminotransferase $>$ 70 IU/L) and thrombocytopenia (platelet count $<$ 100,000/ μ L), as well as those with eclampsia, were also classified as having severe preeclampsia. Normal pregnant women, enrolled from either the labor-delivery unit (in cases of scheduled cesarean section) or the antenatal clinic, were followed until delivery. A patient was considered to have a normal pregnancy if she met the following criteria: 1) no medical, obstetrical, or surgical complications; 2) absence of labor at the time of venipuncture; and 3) delivery of a normal term ($\geq$ 37 weeks) infant whose birthweight was between the 10th and 90th percentiles for gestational age. All women provided informed consent prior to the collection of plasma samples. The collection of samples and their utilization for research purposes was approved by the IRB of the National Institute of Child Health and Human Development. Many of these samples were used previously in studies of intravascular inflammation, soluble adhesion molecules, and cytokine biology in normal and complicated pregnancies [44,46].

Doppler velocimetry:

Pulse-wave and color Doppler ultrasound examinations of the uterine and umbilical arteries were performed in a subset of patients within the preeclampsia and SGA groups (Acuson, Sequoia, Mountain View, CA). Uterine artery Doppler velocimetry [35] was defined as abnormal if the mean resistance index was above the 95th percentile for gestational age (average of right and left) [81] or in the presence of bilateral diastolic notch [36]. Umbilical artery Doppler velocimetry was defined as abnormal if either the pulsatility index (PI) was above the 95th percentile for gestational age (using the reference range proposed by Arduini

and Rizzo[82]) or in the presence of abnormal waveforms (absent or reversed end-diastolic velocities) [83–85].

Sample collection and human sVEGFR-1 immunoassay:

Maternal blood was collected in tubes containing EDTA. Samples were centrifuged and stored at -70°C . The concentrations of sVEGFR-1 were measured using an enzyme-linked immunoassay (ELISA; R&D Systems, Minneapolis, MN) as previously described [68]. The inter- and intra-assay coefficients of variation (CVs) were 4.8% and 6.9%, respectively. The sensitivity was 17.8 pg/mL.

Statistical analysis:

Kolmogorov-Smirnov tests were used to test for normal distribution of the data. After logarithmic transformation ($\log \text{sVEGFR-1} + 1$), regression analysis was utilized to determine the relationship between plasma concentrations of sVEGFR-1 and gestational age in normal pregnant women. Since maternal plasma sVEGFR-1 concentration varies as a function of gestational age, the difference (delta value) between the observed and the expected (derived from regression equation of plasma sVEGFR-1 concentration of normal pregnancy) plasma sVEGFR-1 concentration in each patient was calculated and used to examine the differences of plasma sVEGFR-1 concentration among various subgroups. Analysis of variance (ANOVA) with post-hoc (Bonferroni or Dunnett's T3) corrections for multiple comparisons or Kruskal Wallis with post-hoc Mann-Whitney U tests were utilized to determine the differences of the mean or the median among groups according to the data distribution. Multiple linear regression analysis (stepwise) was applied to examine the contribution of diagnostic group category and Doppler abnormalities on the plasma concentration of sVEGFR-1, while adjusting for gestational age, parity, and duration of sample storage. Contingency tables and Chi-square tests were employed for comparisons of proportions. Spearman's correlation was used to assess the relationship between continuous variables. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered significant.

RESULTS

Clinical characteristics of the study population:

Demographic, clinical, and obstetric characteristics are displayed in Table I. Among patients with preeclampsia, 89 (79%) were diagnosed as severe preeclampsia and 54 (48%) delivered neonates whose birthweights were below the 10th percentile for gestational age. Clinical characteristics of patients with preeclampsia are displayed in Table II. Among SGA patients, 42 (79%) delivered neonates with a birthweight less than the 5th percentile for gestational age. There was no significant difference in the median gestational age at blood sampling among normal pregnant women, patients with SGA, and those with preeclampsia ($p=0.4$; see Table I).

Plasma sVEGFR-1 concentration is higher in patients with preeclampsia than in those with SGA fetuses:

1) Plasma sVEGFR-1 concentration in normal pregnant women increased as a function of gestational age according to the equation $\log(sVEGFR-1 + 1) = 0.026(\text{gestational age in weeks}) + 2.172$ ($r=0.6$; $r^2=0.34$; $p<0.001$; Figure 1). 2) Patients with SGA and those with preeclampsia had a mean plasma concentration of sVEGFR-1 higher than normal pregnant women ($p<0.001$ for both; Figure 2). 3) Patients with preeclampsia had a mean plasma concentration of sVEGFR-1 higher than those with SGA ($p<0.001$; Figure 2). Similar findings were observed after adjusting for parity (nulliparous vs. parous), gestational age at blood sampling, and duration of sample storage. Among SGA patients without preeclampsia, the mean plasma sVEGFR-1 concentration was higher in the subgroup with abnormal uterine artery Doppler velocimetry than in the subgroup with normal uterine artery Doppler velocimetry ($p=0.04$, Figure 3). 4) In contrast, there was no significant difference in the mean plasma sVEGFR-1 concentration between patients with SGA who had a normal uterine artery Doppler velocimetry and normal pregnant women ($p=0.09$; Figure 3).

SGA, sVEGFR-1 and Doppler findings: The severity of the increase in maternal plasma sVEGFR-1 concentrations (delta sVEGFR-1) was related to the Doppler velocimetry abnormalities in the uterine/umbilical arteries.

Table III displays the clinical characteristics and plasma sVEGFR-1 concentrations in patients with SGA sub-classified according to the results of uterine and umbilical artery Doppler velocimetry ($n=41$; 10 patients had no information on both uterine and umbilical artery Doppler velocimetry, whereas 2 patients had information only for uterine, but not umbilical artery Doppler velocimetry). Patients with abnormal Doppler velocimetry in both the uterine and the umbilical artery had the lowest gestational age at blood sampling and were delivered at the earliest in gestational age (Table III). Patients with SGA fetuses who had abnormalities in both the uterine and umbilical artery Doppler velocimetry had the highest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 0.69 ± 0.29) among all groups. Patients who had normal Doppler velocimetry in both uterine and umbilical arteries had the lowest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 0.09 ± 0.29 ; ANOVA $p<0.001$). Patients with abnormal uterine but normal umbilical artery Doppler velocimetry had a mean delta plasma sVEGFR-1 concentration higher than normal pregnant women (mean $\pm$ SD: 0.31 ± 0.52 vs. mean $\pm$ sd: 0.01 ± 0.21 , respectively) The difference, however, did not reach statistical significance ($p=0.4$; ANOVA post hoc Dunnett's T3; see Table III & Figure 4).

The proportion of patients who had high delta sVEGFR-1 plasma concentrations (defined as more than 2 SD above the mean of normal pregnant women) increased in the presence of Doppler velocimetry abnormalities [normal both uterine and umbilical artery Doppler velocimetry 5.6% (1/18)]; abnormal uterine artery Doppler velocimetry alone 28.6% (4/14); abnormal umbilical artery Doppler velocimetry alone 50% (1/2); and abnormal both uterine and umbilical artery Doppler velocimetry 85.7% (6/7) (Chi-square for trend: $p<0.001$; see Table III).

Preeclampsia, sVEGFR-1, and Doppler findings: the severity of the increase in maternal plasma sVEGFR-1 concentrations (delta sVEGFR-1) was related to the Doppler velocimetry abnormalities in the uterine/umbilical arteries.

Table IV displays the clinical characteristics and plasma sVEGFR-1 concentrations in patients with preeclampsia sub-classified according to the results of uterine and umbilical artery Doppler velocimetry (n=69). Patients with preeclampsia who had abnormalities in both the uterine and umbilical artery Doppler velocimetry had the highest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 1.01 ± 0.22) among all groups. Patients who had normal Doppler velocimetry in both uterine and umbilical arteries had the lowest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 0.37 ± 0.31 ; ANOVA $p < 0.001$; see Table IV). Patients with abnormal uterine, but normal umbilical artery, Doppler velocimetry had a mean delta plasma sVEGFR-1 concentration higher than normal pregnant women (mean $\pm$ SD: 0.80 ± 0.40 vs. mean $\pm$ SD: 0.01 ± 0.21 , respectively; $p < 0.001$; ANOVA post hoc Dunnett's T3; see Table IV & Figure 5).

The proportion of patients who had high delta plasma sVEGFR-1 concentrations (defined as more than 2 SD above the mean of normal pregnant women) increased with the presence or absence of Doppler velocimetry abnormalities (normal both uterine and umbilical artery Doppler velocimetry 55% (6/11), abnormal uterine artery Doppler velocimetry alone 84% (37/44), abnormal umbilical artery Doppler velocimetry alone 100% (3/3), and both abnormal uterine and umbilical artery Doppler velocimetry 100% (11/11) (Chi-square for trend: $p < 0.001$; see Table IV).

Multiple regression analysis was used to examine the contribution of diagnostic group category (e.g.: preeclampsia or SGA) and Doppler abnormalities in the plasma concentration of sVEGFR-1, while adjusting for gestational age, parity, and duration of sample storage. Factors entered into the regression model are displayed in Table V. The final regression model suggested that the diagnostic category, Doppler velocimetry abnormalities in uterine and umbilical artery, and gestational age at blood sampling were associated with an increased plasma concentration of sVEGFR-1.

The severity of the increase in maternal plasma sVEGFR-1 concentrations (delta sVEGFR-1) is related to the clinical severity:

Among patients with SGA fetuses, there were negative relationships between delta plasma sVEGFR-1 concentrations and gestational age at delivery ($r = -0.5$; $p = 0.02$), birthweight ($r = -0.6$; $p = 0.006$), as well as adjusted birthweight for gestational age ($r = -0.5$; $p = 0.01$). In contrast, no such relationships were observed in normal pregnant women (all $p > 0.05$).

Among patients with preeclampsia, those with severe preeclampsia had a mean delta plasma sVEGFR-1 concentration higher than those with mild preeclampsia (severe; mean $\pm$ SD: 0.73 ± 0.37 vs. mild; mean $\pm$ SD: 0.55 ± 0.42 ; $p = 0.03$). A higher proportion of patients with severe preeclampsia than those with mild preeclampsia had a high delta (defined as more than 2 SD above the mean of normal pregnant women) plasma sVEGFR-1 concentration [severe, 82% (73/89) vs. mild, 57% (13/23); $p = 0.01$]. Similarly, a higher proportion of patients with preeclampsia who delivered SGA neonates had a high delta

plasma sVEGFR-1 concentration [SGA, 87% (47/54) vs. without SGA, 67% (39/58); $p = 0.01$] than those who did not.

DISCUSSION

Principal findings of this study:

1) Mothers with SGA fetuses and abnormal uterine artery Doppler velocimetry at the time of diagnosis have a higher mean plasma concentration of sVEGFR-1 than normal pregnant women, but lower than patients with preeclampsia; 2) among patients with SGA and those with preeclampsia, the severity of the increase in maternal plasma sVEGFR-1 concentrations (delta sVEGFR-1) is related to the presence or absence of Doppler velocimetry abnormalities in the uterine and umbilical arteries; and 3) plasma concentrations of sVEGFR-1 increase as a function of the severity of fetal growth restriction and the clinical severity of preeclampsia.

Previous studies of maternal plasma sVEGFR-1 in SGA:

The findings of this report may help to explain conflicting conclusions of previous studies regarding whether pregnancy resulting in SGA neonates had a mean plasma concentration of sVEGFR-1 higher than normal pregnancy [71,73]. The syndromic nature (multiple etiologies) of SGA [86–90], the different definitions of SGA [71,73], and the changes of plasma sVEGFR-1 concentration as a function of gestational age in normal pregnancy [59,62] make the study of plasma sVEGFR-1 concentration in women with SGA neonates difficult. The study of Shibata and colleagues [73], which reported no changes in plasma sVEGFR-1 concentrations in patients with SGA neonates, included patients whose neonatal birthweights were less than the 10th percentile ($n=22$), and provided umbilical artery Doppler information for only 8 patients. Among them, only 3 had abnormal umbilical artery Doppler velocimetry. The median gestational age of patients with SGA was 39 weeks. In contrast, the study of Tsatsaris and colleagues [71] defined SGA patients with more stringent criteria (birthweight less than the 3rd percentile) and, as in the case of our study, reported a higher mean plasma sVEGFR-1 concentration in women with SGA neonates than in normal pregnant women. The median gestational age of the SGA group in the Tsatsaris study was 32 weeks.

Four other studies that included patients with SGA and abnormal uterine/umbilical artery Doppler velocimetry consistently reported higher plasma/serum concentrations of sVEGFR-1 in SGA patients than in normal pregnant women [69,75–77]. Interestingly, a study conducted by Savvidou and colleagues [75] indicated that plasma sVEGFR-1 concentrations in patients with fetal growth restriction and abnormal uterine artery Doppler velocimetry are increased at the time of diagnosis, but this increase is not yet evident at 23–25 weeks of gestation in patients with impaired placentation (as determined by abnormal uterine artery Doppler velocimetry) who subsequently deliver a growth-restricted neonate. Similarly, in a longitudinal study, Wathen and colleagues reported no significant difference in the median plasma concentration of sVEGFR-1 at 12–20 weeks of gestation between patients destined to deliver an SGA neonate and normal pregnant women. SGA was diagnosed by a birthweight less than 2 SD below the mean for gestational age. There was no

Doppler information reported and the median gestational age at delivery of patients with SGA was 40 weeks [65]. Although several studies reported an elevation of plasma sVEGFR-1 concentration in patients with preeclampsia approximately 5 weeks before the diagnosis [59,62], the timeline for detecting a plasma elevation of sVEGFR-1 in SGA patients with abnormal uterine artery Doppler velocimetry remains to be determined.

Relationship between maternal plasma sVEGFR-1 and Doppler velocimetry:

Other investigators have demonstrated that patients with increased impedance to flow (determined by Doppler velocimetry) in both the uterine and umbilical arteries have a higher rate of adverse perinatal outcome than those with abnormalities restricted to one of the two circulatory systems, or those without Doppler abnormalities [85,91]. In the present study, when patients with either SGA fetuses or preeclampsia were stratified according to the results of uterine and umbilical artery Doppler, the mean delta plasma sVEGFR-1 concentration increased when abnormalities in Doppler velocimetry involved both circulations.

Consistent with this observation was the finding that the proportion of patients who had an elevated delta plasma sVEGFR-1 concentration (defined as more than 2 SD above the mean of normal pregnant women) increased as Doppler velocimetry results worsened. An abnormal uterine artery Doppler velocimetry suggests an increase impedance to blood flow in the uterine circulation or maternal side of the fetomaternal interface, as demonstrated by animal experimentation [18], mathematical modeling [19], as well as studies correlating placental bed biopsy findings with Doppler uterine artery velocimetry [20–26]. In contrast, abnormal umbilical artery Doppler results suggest an increased impedance to blood flow in placental circulation or fetal side, as shown by animal experiments [92] and studies in the villous tree model [93].

Our findings can be interpreted as suggesting that pathologic conditions, affecting both the fetal/placental and maternal circulations, represent a greater stimulus for the release of sVEGFR-1 into the maternal circulation than when only one vascular territory is affected by a pathologic process. However, it is noteworthy that plasma concentrations of sVEGFR-1 in preeclampsia were higher than in SGA, even when adjusting for the results of Doppler velocimetry. This result indicates that factors, other than the increased impedance to blood flow in the uterine or umbilical artery alone, are responsible for these findings. Six of 11 (55%) patients with preeclampsia and normal uterine/umbilical artery Doppler velocimetry had high delta plasma sVEGFR-1 concentrations.

What are the possible sources and mechanisms for the elevation of sVEGFR-1 in the maternal circulation?

The precise mechanisms responsible for the elevation of plasma sVEGFR-1 concentrations in patients with preeclampsia and in those with SGA and abnormal uterine artery Doppler velocimetry remain to be determined. A reduction in utero-placental blood flow has been implicated in the pathogenesis of both SGA [94,95] and preeclampsia [96]. Experimental observations suggest that hypoxia up-regulates VEGF, VEGFR-1, and sVEGFR-1 expression, but down-regulates PlGF expression of trophoblast cells in culture [97–101].

Thus, it is possible that reduced uterine perfusion may decrease oxygen tension in the intervillous space [102–104], as well as induce expression and release of sVEGFR-1 from villous trophoblasts into the maternal circulation [97–99]. However, if this hypothesis is correct, the phenomenon is likely to be a chronic process since the dramatic elevation of sVEGFR-1 occurs later than the increase in uterine artery impedance to flow as detected by Doppler velocimetry [62,75,105,106]. Although some studies have reported that patients destined to develop preeclampsia have higher plasma concentrations of sVEGF-1 than those who have a normal pregnancy as early as 16 to 20 weeks of gestation, this elevation is modest [61,63,107]. Furthermore, there was no significant difference in the median plasma concentration of sVEGFR-1 at 16–24 weeks of gestation between patients with and without abnormal uterine artery Doppler velocimetry [108]. Our interpretation of these results is that the increased impedance to blood flow in the uterine artery alone may not be enough to induce the release of sVEGFR-1. It is possible that sVEGFR-1 might be up-regulated and released into the maternal circulation after the hypoxic insult/injury to villous trophoblasts [109] has taken place or other mechanisms (cytokines [110,111], remodeling of villous tree [102,112,113], complement activation [114], immune mechanism [115]) have become operative. In the current study, the release of sVEGFR-1 was found to be even greater when the increased impedance to blood flow involved both the uterine and villous circulations. The primary maldevelopment of tertiary villi [93,116] or vasoconstriction of arterioles in stem villi in response to intervillous flow mismatch (between oxygen and blood flow) [117] have been proposed to be responsible for the increased impedance to blood flow in the villous circulation.

In a subset of patients with SGA, however, previous studies examining arterial and venous blood pH and gases in the uterine vein and the uterine radial arteries indicate that oxygen tension is higher in blood exiting the uterus of mothers with SGA neonates. Thus, an abnormality in oxygen-extraction rather than hypoxia at the feto-maternal interface has been proposed to be present in some patients with SGA [118,119]. Some [120,121], but not all [71,73], studies demonstrated that the mRNA and protein expression of PlGF in placentas obtained from patients with SGA were higher (reflecting a hyperoxic environment) than those from normal pregnant women [120,122]. Collectively, these observations suggest that oxygen tension in the intervillous space may not be the major mechanism responsible for the increased bioavailability of sVEGFR-1 in SGA, and that other mechanisms must therefore be operative.

Other possible sources of elevated plasma sVEGFR-1 concentration, in both preeclamptic and SGA patients, are peripheral blood mononuclear cells (PBMCs) and amniotic fluid cavity. Rajakumar and colleagues reported that PBMCs from women with preeclampsia released a higher concentration of sVEGFR-1 than those obtained from normal pregnant women, suggesting that PBMCs could be an additional source of the excess sVEGFR-1 in patients with preeclampsia [123]. Amniotic fluid concentration of sVEGFR-1 in preeclampsia at the time of diagnosis [124], but not in the second trimester [63], is higher than that in normal pregnant women. However, how sVEGFR-1 gains access from the amniotic fluid to the maternal circulation is unclear. The contribution of the fetal compartment to an elevation of maternal plasma sVEGFR-1 concentration in preeclampsia and SGA seems to be negligible. The mean serum concentration of sVEGFR-1 in patients

with preeclampsia and in those with SGA was found to be higher than that of normal pregnant women in the umbilical vein, but not in the umbilical artery [70,77,125,126]. Moreover, the mean serum concentrations of sVEGFR-1 in both the umbilical artery and vein were approximately 10 times lower than that in the maternal serum [77,125].

Strength and limitation of this study:

Previous studies of sVEGFR-1 in patients with SGA have not considered the results of Doppler velocimetry. In contrast, the current study determined plasma sVEGFR-1 concentrations in patients with SGA sub-classified into those with and those without uterine artery Doppler velocimetry abnormalities. In addition, the relationships between abnormalities of uterine/umbilical artery Doppler velocimetry and plasma concentration of sVEGFR-1 were examined in both preeclamptic and SGA patients. Multiple regression analysis was used to confirm the results by adjusting for several potentially confounding factors.

This study has two potential limitations. First, the delta values were used instead of absolute concentrations to observe the differences of plasma sVEGF-R1 concentrations among subgroups and examine the relationship between plasma sVEGF-R1 concentrations and several parameters of disease severity. Since plasma sVEGF-R1 concentrations change with gestational age, and the earlier the pathologic conditions (preeclampsia or SGA) develop the higher the plasma concentrations of sVEGFR-1 become [68], the delta values may be more interpretable than absolute in reflecting the changes of plasma sVEGF-R1 concentration from normal pregnant women, without requiring to adjust for the differences in gestational age at blood sampling among the subgroups. However, the drawback of using delta value instead of multiple of median or z-score in comparisons among subgroups is that it does not take into account the variation of plasma sVEGF-R1 concentration at each gestational age among normal pregnant women. To calculate the median or mean and standard deviation of plasma sVEGFR-1 concentrations for each gestational age group or interval in normal pregnant women would require a much larger sample size than that employed in the current study. Similar conclusions could be drawn, however, with the use of an established reference range of plasma sVEGFR-1 of various gestational ages in normal pregnant women, such as that reported by Hirashima and colleagues [127]. The proportion of patients with preeclampsia and SGA who had high plasma sVEGFR-1 concentrations (defined as more than the 95th percentile for gestational age of normal pregnant women) increased with the presence or absence of Doppler velocimetry abnormalities (see Table VI).

Another limitation of this study is that Doppler velocimetry information was available in 61% (69/112) of women with preeclampsia, 77% (41/53) of women with SGA neonates, and 0% of normal pregnant women. We assumed that healthy pregnant women with normal obstetrical outcomes would have normal uterine and umbilical artery Doppler at the time of venipuncture.

In conclusion, this study provides evidence that an increased plasma sVEGFR-1 concentration during pregnancy is not a feature specific to preeclampsia, since it is also present in SGA with abnormalities in uterine/umbilical artery Doppler, another disorder associated with problems in the supply line. However, preeclampsia had higher plasma

sVEGFR-1 concentrations than SGA, even after adjusting for abnormalities in the uterine/umbilical artery Doppler velocimetry.

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Figure 1.

Plasma sVEGFR-1 concentrations of normal pregnant women increased as a function of gestational age according to the equation: $\log (\text{sVEGFR-1} + 1) = 0.026 (\text{gestational age in weeks}) + 2.172$ ($r^2 = 0.34$; $p < 0.001$).

Figure 2.

The mean plasma sVEGFR-1 concentrations of normal pregnant women, patients with SGA, and those with preeclampsia. Patients with preeclampsia (mean $\pm$ SD: 7,958 $\pm$ 9,170 pg/mL) and those with SGA (mean $\pm$ SD: 3,603 $\pm$ 6,740 pg/mL) had a higher mean plasma concentrations of sVEGFR-1 than normal pregnant women (mean $\pm$ SD: 1,445 $\pm$ 865 pg/mL; $p < 0.001$ for both). However, patients with preeclampsia had a higher mean plasma sVEGFR-1 concentration than those with SGA ($p < 0.001$). The comparisons were performed after logarithmic transformation ($\log \text{sVEGFR-1} + 1$). The statistical test used was ANOVA with Bonferroni correction for multiple comparisons. The vertical axis in the figure is in the logarithmic scale. * $p < 0.05$.

Figure 3.

The mean plasma sVEGFR-1 concentrations of normal pregnant women and patients with SGA who had normal uterine artery Doppler velocimetry and those who had abnormal uterine artery Doppler velocimetry. Patients with abnormal uterine artery Doppler velocimetry (mean $\pm$ SD: 5359 $\pm$ 9945 pg/mL) had a higher mean plasma sVEGFR-1 concentration than those with normal uterine artery Doppler (mean $\pm$ SD: 2100 $\pm$ 1540 pg/mL) and normal pregnant women (mean $\pm$ SD: 1445 $\pm$ 865 pg/mL; $p = 0.04$ and $p < 0.001$, respectively). In contrast, there was no significant difference in the mean delta plasma sVEGFR-1 concentration between patients with SGA who had normal uterine artery Doppler and normal pregnant women ($p = 0.09$). The comparisons were performed after logarithmic transformation ($\log \text{ sVEGFR-1} + 1$). The statistical test used was ANOVA with post hoc Dunnett's T3. The vertical axis in the figure is in the logarithmic scale. * $p < 0.05$.

Figure 4.

The mean delta plasma concentrations of normal pregnant women and patients with SGA fetuses (n=41) sub-classified according to the results of uterine and umbilical artery Doppler velocimetry. Patients with SGA fetuses who had abnormalities in both the uterine and umbilical artery Doppler velocimetry had the highest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 0.69 $\pm$ 0.29) among all groups. Patients who had normal Doppler velocimetry in both uterine and umbilical arteries had the lowest mean delta plasma sVEGFR-1 concentration (mean $\pm$ SD: 0.09 $\pm$ 0.29; ANOVA; $p < 0.001$). Patients with abnormal uterine, but normal umbilical artery, Doppler had a mean delta plasma sVEGFR-1 concentration higher than normal pregnant women (mean $\pm$ SD: 0.31 $\pm$ 0.52 vs. mean $\pm$ SD: 0.01 $\pm$ 0.21). However, the difference did not reach statistical significance ($p = 0.4$; ANOVA post hoc Dunnett's T3). The means $\pm$ SD of plasma sVEGF-R1 concentrations in each group are displayed in the figure. * $p < 0.05$.

Figure 5.

The mean delta plasma concentrations of normal pregnant women and patients with preeclampsia (n=69) sub-classified according to the results of uterine and umbilical artery Doppler velocimetry. Patients with preeclampsia who had abnormalities in both the uterine and umbilical artery Doppler velocimetry had the highest mean delta plasma sVEGFR-1 concentration (mean ± SD: 1.01 ± 0.22) among all groups. Patients who had normal Doppler velocimetry in both uterine and umbilical arteries had the lowest mean delta plasma sVEGFR-1 concentration (mean ± SD: 0.37 ± 0.31; ANOVA; $p < 0.001$). Patients with abnormal uterine, but normal umbilical artery, Doppler velocimetry had a mean delta plasma sVEGFR-1 concentration higher than normal pregnant women (mean ± SD: 0.80 ± 0.40 vs. mean ± SD: 0.01 ± 0.21; $p < 0.001$; ANOVA post hoc Dunnett's T3). The means ± SD of plasma sVEGFR-1 concentrations in each group are displayed in the figure. * $p < 0.05$.

Table I.

Clinical characteristics of the study population

	Normal pregnancy n = 135	SGA n = 53	p	Preeclampsia n = 112	p
Age (y) δ	25 (17–40)	24 (15–43)	0.5	23 (13–43)	0.2
Race: African American	107 (79.3%)	46 (86.8%)	0.3	91 (81.3%)	0.6
Caucasian	15 (11.1%)	4 (7.5%)		13 (11.6%)	
Hispanic	7 (5.2%)	1 (1.9%)		5 (4.5%)	
Asian	0	1 (1.9%)		1 (0.9%)	
Other	6 (4.4%)	1 (1.9%)		2 (1.8%)	
Nulliparity	37 (27.4%)	26 (49.1%)	0.005 *	69 (61.6%)	<0.001 *
GA at blood sampling (weeks) δ	37.6 (20–41.7)	36.9 (25–39.7)	0.3	35.6 (23.4–42.4)	0.3
GA at delivery (weeks) δ	39.3 (37–42.4)	37.1 (25–39.7)	<0.001 *	35.7 (23.7–42.4)	<0.001 *
Birthweight (grams) δ	3345 (2610–4080)	2050 (300–2880)	<0.001 *	2195 (530–4460)	<0.001 *
Adjusted birthweight for GA (MOM) ϕ	-0.01 $\pm$ 0.08	-0.35 $\pm$ 0.10	<0.001 *	-0.22 $\pm$ 0.17	<0.001 *
Birthweight <5 th percentile	0	42 (79.2%)	<0.001 *	32 (28.6%)	<0.001 *

Value expressed as median (range) or number (percent) except adjusted birthweight for GA as mean $\pm$ sd

GA: gestational age; MOM: multiple of the median

* p < 0.05.

 δ = Kruskal Wallis with post hoc Mann-Whitney U tests ϕ = ANOVA with post hoc Dunnett's T3 test; p < 0.001

Table II.

Clinical characteristics of patients with preeclampsia

Blood pressure (mmHg)	
Systolic	172 ± 19
Diastolic	104 ± 11
Mean arterial pressure	127 ± 12
Aspartate aminotransferase ^a (SGOT) (U/L)	64 ± 115
Platelet count (x10 ³) (μ/L)	176 ± 54
Birthweight <10 th percentile	54 (48%)
Severe preeclampsia	89 (79%)

Value expressed as mean ± SD or number (percent)

^a_(n=104)

Table III. Obstetric characteristics and plasma sVEGFR-1 concentration of the patients with SGA according to Doppler results

	Normal pregnancy n = 135	Normal UT Normal UA n=18	p	Abnormal UT Normal UA n=14	p	Normal UT Abnormal UA n=2	p	Abnormal UT Abnormal UA n=7	p
GA at blood sampling (weeks)	37.6 (20–41.7)	38.1 (29.6–39.7)	0.4	36.5 (25.3–39.7)	0.5	30.2 (25.4–35.0)	0.2	26 (25.0–32.7)	0.003 *
GA at delivery (weeks)	39.3 (37–42.4)	38.2 (29.7–39.7)	<0.001 *	37.0 (27.8–39.7)	<0.001 *	30.9 (26.9–35.0)	0.01 *	27.6 (25.0–33.1)	<0.001 *
Birthweight (grams)	3,345 (2610–4080)	2,160 (900–2880)	<0.001 *	2,130 (700–2600)	<0.001 *	1,140 (440–1840)	0.02 *	660 (300–1320)	<0.001 *
Adjusted birthweight for GA (MOM)	-0.01 ± 0.08	-0.32 ± 0.09	<0.001 *	-0.34 ± 0.09	<0.001 *	-0.46 ± 0.16	0.4	-0.48 ± 0.09	<0.001 *
Plasma sVEGFR-1 (pg/mL)	1,445 ± 865	2,059 ± 1,524		6,139 ± 12,729		2,467 ± 2,307		4,482 ± 2,707	
Delta plasma sVEGFR-1	0.01 ± 0.21	0.09 ± 0.29	0.9	0.31 ± 0.52	0.4	0.31 ± 0.67	0.9	0.69 ± 0.29	0.006 *
Delta plasma sVEGFR-1	0.42 (2SD)	5 (3.7%)		4 (28.6%)		1 (50%)		6 (85.7%)	

All p values compared to normal pregnancy (ANOVA with Dunnett's T3 tests or Kruskal Wallis with Mann-Whitney U tests).

Values expressed as median (range), mean ± SD or number (percent).

GA: gestational age; MOM: multiple of the median; SGA: small for gestational age; UT: uterine artery Doppler velocimetry; UA: umbilical artery Doppler velocimetry

* p < 0.05.

Table IV. Obstetric characteristics and plasma sVEGFR-1 concentration of the patients with preeclampsia according to Doppler results

	Normal pregnancy n = 135	Normal UT Normal UA n=11	p	Abnormal UT Normal UA n=44	p	Normal UT Abnormal UA n=3	p	Abnormal UT Abnormal UA n=11	p
GA at blood sampling (weeks)	37.6 (20–41.7)	38.6 (34.3–41.0)	0.09	33.1 (25–40.4)	0.05	32.1 (29–32.7)	0.2	29.1 (23.4–36.0)	0.007 *
GA at delivery (weeks)	39.3 (37–42.4)	38.6 (34.4–41.1)	0.05	33.4 (25.3–40.6)	<0.001 *	32.3 (29–32.7)	0.01 *	29.1 (23.7–36)	<0.001 *
Birthweight (grams)	3,345 (2610–4080)	2,740 (1,700–3210)	<0.001 *	1,810 (620–4180)	<0.001 *	1,440 (1010–2200)	0.02 *	1,000 (530–1990)	<0.001 *
Adjusted birthweight for GA (MOM)	-0.01 ± 0.08	-0.2 ± 0.09	<0.001 *	-0.23 ± 0.17	<0.001 *	-0.23 ± 0.21	0.4	-0.35 ± 0.1	<0.001 *
Plasma sVEGFR-1 (pg/ml)	1,445 ± 865	4,228 ± 2,734		9,693 ± 11,050		8,585 ± 3,438		9,829 ± 3,736	
Delta plasma sVEGFR-1	0.01 ± 0.21	0.37 ± 0.31	0.03 *	0.80 ± 0.40	<0.001 *	0.92 ± 0.14	0.03 *	1.01 ± 0.22	<0.001 *
Delta plasma sVEGFR-1 0.42 (2SD)	5 (3.7%)	6 (54.5%)		37 (84.1%)		3 (100%)		11 (100%)	

All p value compared to normal pregnancy (ANOVA with Dunnett's T3 tests or Kruskal Wallis with Mann-Whitney U tests)

Value expressed as median (range), mean ± SD or number (percent)

GA: gestational age; MOM: multiple of the median

UT: uterine artery Doppler velocimetry; UA: umbilical artery Doppler velocimetry

* p < 0.05.

Table V.

Multiple linear regression (step-wise) model for the identification of plasma concentration of log (sVEGFR-1 + 1)

	R2	Beta (standardized)	p
Variables in the final model			
Disease category ^a	0.512	0.6	<0.001 *
Doppler abnormality ^β	0.022	0.3	0.001
GA at blood sampling (weeks)	0.024	0.2	0.001 *
Total	0.54		<0.001 *
Excluded variables			
Duration of sample storage (days)			
Nulliparity (Yes/No)			

^aDisease category: [Normal pregnancy=0; SGA=1 and Preeclampsia =2]

^βDoppler abnormality: [Normal pregnancy and Normal both uterine and umbilical artery Doppler =1; Abnormal uterine artery Doppler alone=2; Abnormal umbilical artery Doppler alone=3; and Abnormal both uterine and umbilical artery Doppler =4]

* p < 0.05.

The proportion of patients with SGA and those with preeclampsia who had plasma sVEGFR-1 concentration higher than 95th percentile of normal pregnant women for gestational age (based on a published reference range¹²⁰) classified by the presence or absence of abnormal uterine and umbilical artery Doppler velocimetry

Table VI.

	Normal UT Normal UA	Abnormal UT Normal UA	Normal UT Abnormal UA	Abnormal UT Abnormal UA	P
SGA	16.7% (3/18)	42.9% (6/14)	50% (1/2)	100% (7/7)	<0.001 *
Preeclampsia	54.5% (6/11)	88.6% (39/44)	100% (3/3)	100% (11/11)	0.008 *

Value expressed as percent (number)

UT: uterine artery Doppler velocimetry; UA: umbilical artery Doppler velocimetry

Normal pregnant women 14.8% (20/135)

* p<0.05 Chi-square for trend