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Low maternal concentrations of soluble vascular endothelial growth factor receptor-2 in preeclampsia and small for gestational age

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Abstract

Objectives. Preeclampsia is considered an anti-angiogenic state. A role for the anti-angiogenic factors soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and soluble endoglin in preeclampsia has been proposed. Soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) has been detected in human plasma, and the recombinant form of this protein has anti-angiogenic activity. There is a paucity of information about maternal plasma sVEGFR-2 concentrations in patients with preeclampsia and those without preeclampsia with small for gestational age (SGA) fetuses. This study was conducted to determine whether: (1) plasma sVEGFR-2 concentration changes throughout pregnancy; and (2) preeclampsia and SGA are associated with abnormalities in the maternal plasma concentration of sVEGFR-2.

Study design. This cross-sectional study included non-pregnant women ($n=40$), women with normal pregnancies ($n=135$), women with an SGA fetus ($n=53$), and women with preeclampsia ($n=112$). SGA was defined as an ultrasound-estimated fetal weight below the 10th percentile for gestational age that was confirmed by neonatal birth weight. Plasma concentrations of sVEGFR-2 were determined by ELISA.

Results. (1) There was no significant difference in the mean plasma concentration of sVEGFR-2 between non-pregnant women and those with normal pregnancies ($p=0.8$); (2) patients with preeclampsia and those without preeclampsia with SGA fetuses had a lower mean plasma concentration of sVEGFR-2 than that of women with normal pregnancies ($p < 0.001$ for both); and (3) there was no significant difference in the mean plasma concentration of sVEGFR-2 between patients with preeclampsia and those without preeclampsia with SGA ($p=0.9$).

Conclusions. Preeclampsia and SGA are associated with low plasma concentrations of sVEGFR-2. One interpretation of the findings is that plasma sVEGFR-2 concentration could reflect endothelial cell function.

Keywords: Small for gestational age, intrauterine growth restriction, preeclampsia, sVEGFR-2, KDR, pregnancy

Introduction

Preeclampsia is a syndrome characterized by hypertension and proteinuria. Mechanisms of disease implicated in this syndrome [1–4] include uteroplacental ischemia [5–11], increased trophoblast deportation [12–14] with apoptosis/necrosis [15–17], oxidative stress [18–27], and an exaggerated systemic inflammatory response [28–30]. The abnormalities observed in patients with preeclampsia include

increased insulin resistance [31–34], hyperlipidemia [35–37], excess thrombin generation [38–43], and widespread endothelial damage/dysfunction [44–46] resulting in multiple organ damage. Of interest, neonates delivered from mothers with preeclampsia are at higher risk to be small for gestational age (SGA) than those delivered from women with normal pregnancies [47,48].

SGA, one of the ‘great obstetrical syndromes’ [49], is generally diagnosed when the birth weight is

below a given threshold for gestational age, often the 10th percentile [50]. SGA is considered a syndrome because smallness at birth can be caused by many factors, including defective placentation [8,51], infection [52,53], chromosomal anomalies [54,55], genetic disorders [56–58], and environmental factors [59] such as smoking [60,61], alcohol exposure [62], and cocaine use [63]. Moreover, in a subset of patients without preeclampsia with SGA fetuses, there is also evidence of leukocyte activation [64] as well as endothelial cell dysfunction [65–67].

Recent evidence suggests that both patients with preeclampsia and those with SGA neonates have a greater risk of short- and long-term complications—especially future cardiovascular disease—than women with normal pregnancies [68–73]. Anti-angiogenic and/or insulin-resistant states may independently represent baseline factors predisposing to the development of future cardiovascular disease [33,74].

The regulation of vascular growth and remodeling (angiogenesis) is considered to be central to normal placental and fetal growth/development [75–80]. Angiogenesis is regulated by several growth factors and their receptors, such as fibroblast growth factors, transforming growth factors, hepatocyte growth factors, angiogenins, angiopoietins, ephrins, and vascular endothelial growth factors (VEGF) [75,81]. However, VEGF signaling represents a critical step in physiologic and pathologic angiogenesis [75]. There is emerging evidence that VEGF is necessary to maintain normal endothelial health in the adult vasculature, which is a role that extends beyond angiogenesis [82].

VEGF, an endothelial cell-specific growth factor, is a glycoprotein with potent angiogenic properties. Its function is to promote endothelial cell proliferation, migration [75], and survival [83]. VEGF exerts biologic effects through two high-affinity tyrosine kinase receptors: VEGFR-1 (VEGF receptor-1 or flt-1 or fms-like tyrosine kinase-1) and VEGFR-2 (KDR or kinase insert domain-containing receptor or Flk-1 or fetal liver kinase-1). While VEGFR-1 is considered a ‘decoy’ receptor, VEGFR-2 is the major mediator of the mitogenic, angiogenic, permeability-enhancing [75], and endothelial survival effects of VEGF. Both VEGFR-1 and VEGFR-2 have two isoforms: a membranous isoform and a soluble isoform.

The soluble form of VEGFR-1 (sVEGFR-1) has been proposed to be released from the placenta, leading to endothelial dysfunction in preeclampsia [84,85]. The soluble form of VEGFR-1 binds VEGF and/or placental growth factor (PlGF) and inhibits their biological activities. Though exerting its functions through VEGFR-2, VEGF binds VEGFR-1 and its soluble form (sVEGFR-1) with an affinity

that is 10 times greater than its affinity for VEGFR-2 [75]. The contribution of sVEGFR-1 to the maternal syndrome of preeclampsia is thought to be, at least in part, related to its inhibition of VEGF stimulation of the endothelium-dependent nitric oxide system (through VEGFR-2) [86]. Plasma sVEGFR-1 concentration has been found to be elevated in preeclampsia both prior to [87–94] and after clinical diagnosis [84,87,95–99]. In contrast, studies of plasma sVEGFR-1 concentration in women with SGA fetuses have yielded conflicting results—either no change [100] or an increase [99].

Recently, sVEGFR-2 has been detected in human plasma [101]. The recombinant form of this protein has anti-angiogenic activity [102]. Plasma sVEGFR-2 concentration is lower in patients with systemic lupus erythematosus (SLE) disease [103] and higher in those with acute leukemia, compared to healthy controls [104]. There is a paucity of information on plasma sVEGFR-2 concentrations in preeclampsia and SGA [105]. This study was conducted to determine whether: (1) plasma sVEGFR-2 concentration changes during pregnancy; and (2) pregnancies with preeclampsia and those without preeclampsia with SGA fetuses are associated with changes in the maternal plasma concentrations of sVEGFR-2.

Patients and methods

Study design

This cross-sectional study was conducted by searching the clinical database and bank of biologic samples of the Perinatology Research Branch. The following groups were examined: (1) non-pregnant women ($n=40$); (2) women with normal pregnancies ($n=135$); (3) patients who delivered an SGA neonate without preeclampsia ($n=53$); and (4) women with preeclampsia ($n=112$). Patients with chronic hypertension, renal disease, multiple pregnancy, and major fetal congenital anomalies were excluded.

SGA pregnancies included only patients who underwent ultrasound examination for dating before 24 weeks of gestation. The diagnosis of SGA was based on a neonatal birth weight below the 10th percentile for gestational age [50], using the reference range proposed by Alexander et al. [106]. Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two occasions, 4 hours to 1 week apart) and proteinuria (≥ 300 mg in a 24-hour urine collection or one dipstick measurement $\geq 2+$) [107]. Severe preeclampsia was defined as either severe hypertension (diastolic blood pressure ≥ 110 mmHg) and mild-to-severe proteinuria or

mild hypertension and severe proteinuria (a 24-hour urine sample containing 3.5 g protein or urine specimen $\geq 3+$ protein by dipstick measurement) [107]. Patients with abnormal liver function tests (aspartate aminotransferase > 70 IU/L) and thrombocytopenia (platelet count $< 100\,000/\mu\text{L}$), as well as those with eclampsia, were also classified as having severe preeclampsia. The non-pregnant group consisted of women who had no history of acute or chronic inflammatory conditions. Women with normal pregnancies were enrolled from either the labor-delivery unit (in cases of scheduled cesarean section) or the antenatal clinic, and 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 (≥ 37 weeks) infant whose birth weight was between the 10th and 90th percentile for gestational age.

All patients were enrolled at Hutzel Women's Hospital, Detroit, MI, and provided written informed consent prior to the collection of plasma samples. The collection of samples and their utilization for research purposes was approved by the institutional review boards of the National Institute of Child Health and Human Development and Wayne State University. Many of these samples were used previously in studies of intravascular inflammation, soluble adhesion molecules, and cytokine biology in normal and complicated pregnancies [28,46].

Doppler velocimetry

Pulse-wave and color Doppler ultrasound examinations of the uterine arteries were performed in a subset of patients with SGA (Acuson, Sequoia, Mountain View, CA, USA). Patients with SGA fetuses were classified according to the results of the uterine artery Doppler velocimetry. An abnormal uterine artery Doppler velocimetry [108] was defined as a mean resistance index above the 95th percentile for gestational age (average of right and left) [109] or the presence of bilateral diastolic notches [110].

Sample collection and human sVEGFR-2 immunoassay

Venipuncture was performed and the blood was collected into tubes containing EDTA. Samples were centrifuged and stored at -70°C . A specific and sensitive enzyme-linked immunoassay was used to determine the plasma concentration of human sVEGFR-2 (R&D Systems, Minneapolis, MN, USA). This assay employs a quantitative sandwich immunoassay technique. Briefly, recombinant human VEGFR-2 standards and maternal plasma

specimens were incubated in duplicate wells of the microtiter plates pre-coated with monoclonal antibodies specific for VEGFR-2. After an incubation period, the assay plates were subjected to a wash step to remove unbound antibody-enzyme reagent. Upon addition of a substrate solution (tetramethylbenzidine), color developed in the assay plates proportionally to the amount of VEGFR-2 bound in the initial step. The inter- and intra-assay coefficients of variation (CVs) for human sVEGFR-2 immunoassay in our laboratory were 2% and 4%, respectively. The sensitivity of the assay was 19.07 pg/mL.

Statistical analysis

Kolmogorov-Smirnov tests were used to test for normal distribution of the data. After logarithmic transformation ($\log(\text{sVEGFR-2} + 1)$), analysis of variance (ANOVA) with post-hoc tests (Dunnnett's T3) or Kruskal-Wallis with post-hoc Mann-Whitney U-tests were utilized to determine the differences in the mean and/or median among groups according to the distribution of the data. Contingency tables and Chi-square tests were employed for comparisons of proportions. Regression analysis and Pearson correlation were used to assess the relationship between two continuous variables. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL, USA). A p -value of < 0.05 was considered significant.

Results

The median maternal age of non-pregnant women was slightly higher than that of women with normal pregnancies (non-pregnant, median 26 years, range 18–40 years vs. normal pregnancy, median 25 years, range 17–40 years; $p = 0.03$). The demographic and clinical characteristics of normal pregnant women, patients with an SGA neonate, and those with preeclampsia are displayed in Tables I and II. There were no significant differences in maternal age and ethnicity distribution among the three groups (see Table I). Among patients with SGA, 79% (42/53) delivered neonates whose birth weights were below the 5th percentile for gestational age. Among patients with preeclampsia, 40% (45/112) had early-onset preeclampsia (before 34 weeks of gestation), 79% (89/112) had severe preeclampsia, and 48% (54/112) delivered SGA neonates. The clinical characteristics of patients with preeclampsia are displayed in Table II. There was no significant difference in the median gestational age at blood sampling among the three groups ($p = 0.4$; see Table I).

There was no significant difference in the mean plasma concentration of sVEGFR-2 between non-pregnant women and women with normal

Table I. Demographic and clinical characteristics of normal pregnant women, patients with SGA fetuses, and those with preeclampsia.

	Normal pregnancy <i>n</i> = 135	<i>p</i>	SGA <i>n</i> = 53	<i>p</i>	Preeclampsia <i>n</i> = 112	<i>p</i>
Age (years) [†]	25 (17–40)	0.5	24 (15–43)	0.6	23 (13–43)	0.2
Race						
African American	107 (79.3%)	0.3	46 (86.8%)	0.8	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%)	0.005*	26 (49.1%)	0.1	69 (61.6%)	<0.001*
GA at blood sampling (weeks) [†]	37.6 (20–41.7)	0.3	36.9 (25–39.7)	0.5	35.6 (23.4–42.4)	0.3
GA at delivery (weeks) [†]	39.3 (37–42.4)	<0.001*	37.1 (25–39.7)	0.3	35.7 (23.7–42.4)	<0.001*
Birth weight (grams) [†]	3345 (2610–4080)	<0.001*	2050 (300–2880)	0.03*	2195 (530–4460)	<0.001*
Birth weight <5 th percentile	0	<0.001*	42 (79.2%)	<0.001*	32 (28.6%)	<0.001*

Values expressed as median (range) or number (percent). GA, gestational age. * $p < 0.05$. [†]Kruskal–Wallis with post-hoc Mann–Whitney U-tests.

Table II. Clinical characteristics of patients with preeclampsia.

Blood pressure (mmHg)	
Systolic	172 ± 19
Diastolic	104 ± 11
Mean arterial pressure	127 ± 12
Aspartate aminotransferase [†] (SGOT) (U/L)	64 ± 115
Platelet count ($\times 10^3$) (μ L)	176 ± 54
Birth weight <10 th percentile	54 (48%)
Severe preeclampsia	89 (79%)

Values expressed as mean ± SD or number (percent). [†] $n = 132$.

pregnancies (non-pregnant, mean ± SD: 11.04 ± 3.4 ng/mL vs. normal pregnancy, mean ± SD: 11.4 ± 4.2 ng/mL; $p = 0.8$; see Figure 1). In women with normal pregnancies, the plasma concentration of sVEGFR-2 increased from 20–24 week of gestation (mean ± SD: 10.3 ± 2.7 ng/mL), peaked at approximately 28–36 weeks (mean ± SD: 14.3 ± 4.2 ng/mL), and declined as term approached (mean ± SD: 9.5 ± 3.1 ng/mL). Plasma sVEGFR-2 concentration in women with normal pregnancies changed as a function of gestational age according to the equation $\log(\text{sVEGFR-2} + 1) = -0.0018(\text{gestational age in weeks})^2 + 0.1059(\text{gestational age in weeks}) - 0.4$ ($r^2 = 0.22$; $p < 0.001$; Figure 2).

Patients with preeclampsia and those with SGA had a lower mean plasma concentration of sVEGFR-2 than women with normal pregnancies (normal pregnancy mean ± SD: 11.4 ± 4.2 ng/mL, preeclampsia mean ± SD: 8.3 ± 3.3 ng/mL, and SGA mean ± SD: 7.7 ± 1.7 ng/mL; $p < 0.001$ for both comparisons; see Figure 3). There was no significant difference in the mean plasma concentration of sVEGFR-2 between patients with preeclampsia and those with SGA ($p = 0.9$; Figure 3). Similar findings were observed after adjusting for parity, gestational age at blood sampling, and duration of sample storage; $p < 0.001$).

When patients without preeclampsia with SGA fetuses were classified according to the results of uterine artery Doppler velocimetry, there was no significant difference in the mean plasma sVEGFR-2 concentration between the two groups (normal uterine artery Doppler velocimetry mean ± SD: 7.9 ± 1.6 ng/mL vs. abnormal uterine artery Doppler velocimetry mean ± SD: 7.6 ± 2.1 ng/mL; $p = 0.9$). Both groups had a lower mean plasma concentration of sVEGFR-2 than women with normal pregnancies (ANOVA $p < 0.001$ for both).

Among patients with preeclampsia, there was no significant difference in the mean plasma concentration of sVEGFR-2 both between patients with severe and those with mild preeclampsia (severe preeclampsia, mean ± SD: 8.1 ± 3.2 ng/mL vs. mild preeclampsia, mean ± SD: 8.8 ± 3.7 ng/mL; $p = 0.5$; t -test), and between those with and without SGA (preeclampsia with SGA, mean ± SD: 7.8 ± 3.2 ng/mL vs. preeclampsia without SGA, mean ± SD: 8.7 ± 3.4 ng/mL; $p = 0.2$; t -test).

Because maternal plasma sVEGFR-1 concentration varies as a function of gestational age, the difference between the observed and the expected plasma sVEGFR-1 concentration (derived from the regression equation of plasma sVEGFR-2 concentration of normal pregnancy) in each patient (delta value) was calculated and used to examine the differences of plasma sVEGFR-2 concentration between patients with early-onset (<34 weeks) and late-onset (≥ 34 weeks) preeclampsia. Women with early-onset preeclampsia had a lower mean delta plasma concentration of sVEGFR-2 than those with late-onset disease ($p < 0.001$; Figure 4).

Among patients without preeclampsia with SGA, there was no significant difference in the mean plasma concentration of sVEGFR-2 between those with severe SGA (birth weight <5th percentile) and

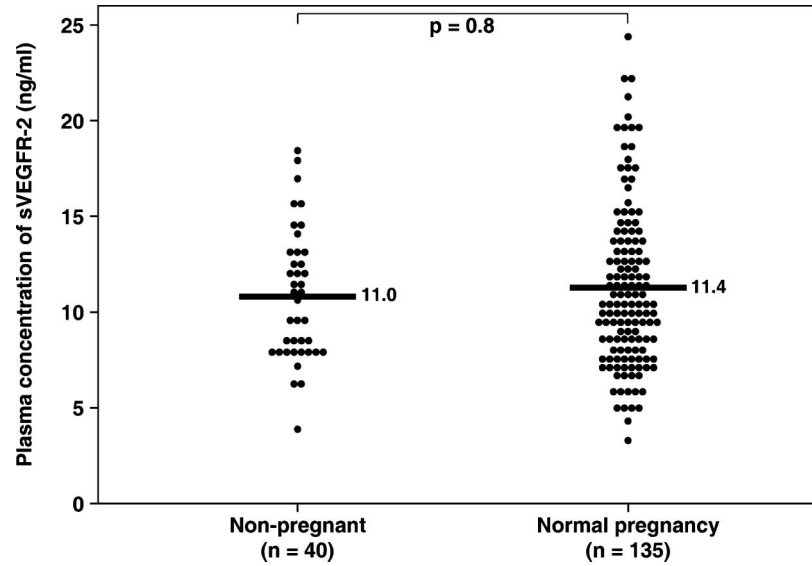


Figure 1. Mean plasma sVEGFR-2 concentration in non-pregnant and women with normal pregnancies. There was no significant difference in the mean plasma concentration of sVEGFR-2 between non-pregnant and women with normal pregnancies (non-pregnant, mean \pm SD: 11.04 ± 3.4 ng/mL vs. normal pregnancy, mean \pm SD: 11.4 ± 4.2 ng/mL; $p = 0.8$). The comparisons were performed after logarithmic transformation ($\log + 1$) of the data. The statistical test used was unpaired t -test. * $p < 0.05$.

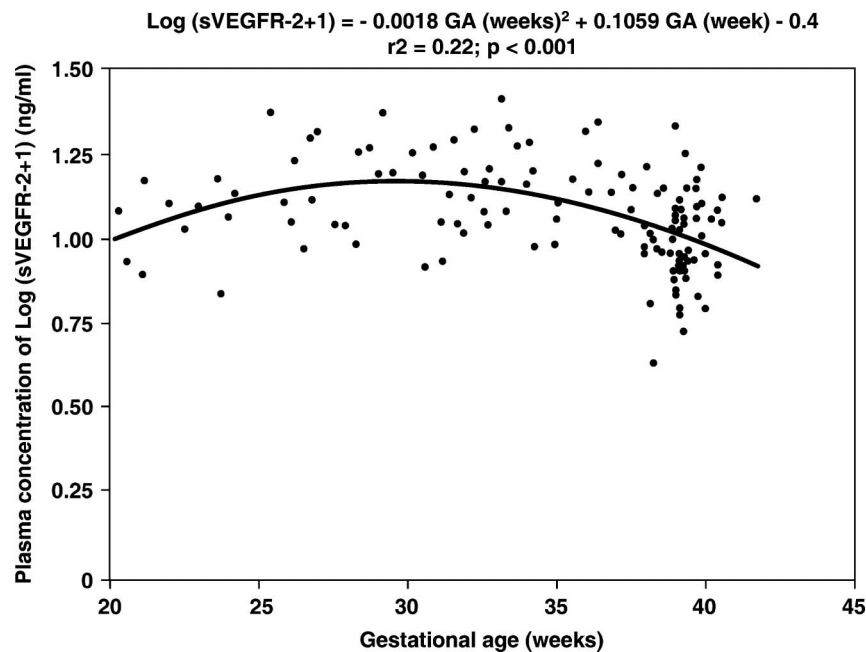


Figure 2. Plasma sVEGFR-2 concentration in women with normal pregnancies increased as a function of gestational age according to the following regression equation: $\log(sVEGFR-2+1) = -0.0018(\text{gestational age in weeks})^2 + 0.1059(\text{gestational age in weeks}) - 0.4$ ($r^2 = 0.22$; $p < 0.001$). * $p < 0.05$.

those whose neonates weighed between the 5th and 10th percentiles at birth (severe SGA ($n = 42$), mean \pm SD: 7.7 ± 1.6 ng/mL vs. mild SGA ($n = 11$), mean \pm SD: 7.9 ± 2.2 ng/mL; $p = 0.9$; t -test). There was no significant correlation between plasma concentration of sVEGFR-2 and neonatal birth weight (Pearson correlation; $p = 0.4$).

Discussion

Principal findings of this study

(1) The mean plasma sVEGFR-2 concentration in women with normal pregnancies was not different from that of non-pregnant women; (2) the maternal plasma sVEGFR-2 concentration changed as a

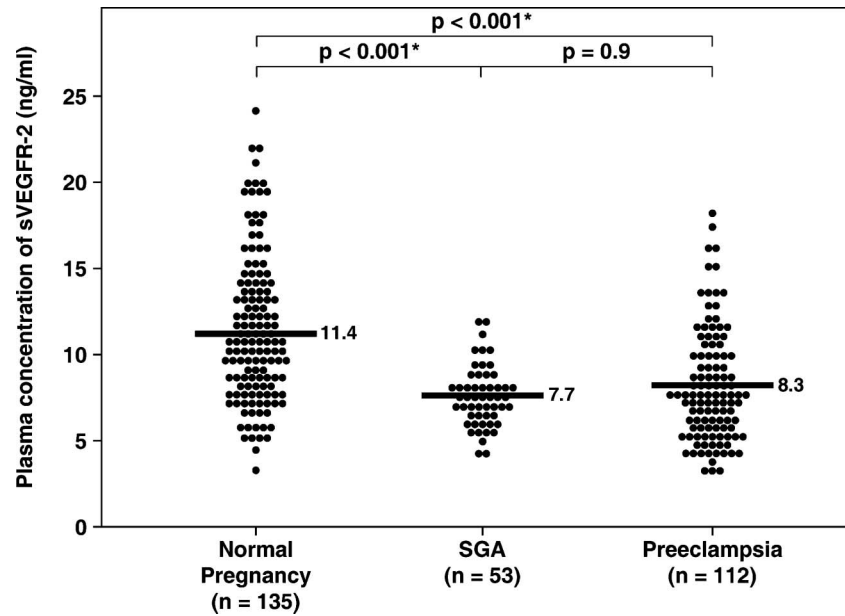


Figure 3. Mean plasma sVEGFR-2 concentration in women with normal pregnancies, patients with SGA, and patients with preeclampsia. Patients with preeclampsia and those without preeclampsia with SGA had a mean plasma concentration of sVEGFR-2 lower than women with normal pregnancies (normal pregnancy, mean \pm SD: 11.4 ± 4.2 ng/mL; preeclampsia mean \pm SD: 8.3 ± 3.3 ng/mL; SGA mean \pm SD: 7.7 ± 1.7 ng/mL; $p < 0.001$ for both). There was no significant difference in the mean plasma concentration of sVEGFR-2 between patients with preeclampsia and those with SGA ($p = 0.9$). The comparisons were performed after logarithmic transformation ($\log + 1$) of the data. The statistical test used was ANOVA with post hoc Dunnett's T3 test. * $p < 0.05$.

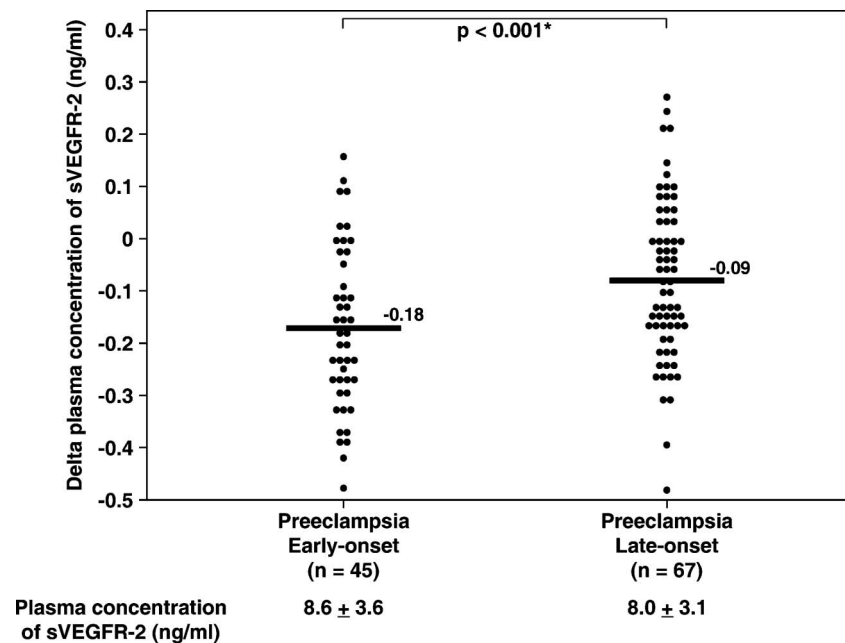


Figure 4. Mean plasma sVEGFR-2 concentration in patients with preeclampsia classified according to the onset of the diagnosis. Women with early-onset (≤ 34 weeks) preeclampsia had a mean delta plasma concentration of sVEGFR-2 lower than those with the late-onset (> 34 weeks) disease (early-onset, mean \pm SD: -0.18 ± 0.15 ng/mL vs. late-onset, mean \pm SD: -0.09 ± 0.15 ng/mL; $p < 0.001$). The statistical test used was unpaired t -test. The means \pm SD of plasma sVEGFR-2 concentrations in each group are displayed in the figure. * $p < 0.05$.

function of gestational age; (3) patients with preeclampsia and those with SGA had lower plasma sVEGFR-2 concentrations than women with normal

pregnancies; (4) there was no significant difference in the mean plasma concentration of sVEGFR-2 between patients with preeclampsia and those with

SGA; (5) among patients without preeclampsia with SGA, there was no significant difference in plasma sVEGFR-2 concentration whether or not they had normal or abnormal uterine artery Doppler velocimetry results; (6) women with early-onset preeclampsia had a mean delta plasma sVEGFR-2 concentration higher than those with the late-onset disease; and (7) the changes in plasma concentration of sVEGFR-2 in patients with preeclampsia and those with SGA, in contrast to sVEGFR-1 [87,96], are not related to the severity of the disease.

Previous studies of sVEGFR-2 in normal pregnancy and pregnancy complications

The finding that the mean plasma sVEGFR-2 concentration in women with normal pregnancies was not different from that of non-pregnant women is consistent with a previous study conducted by Masuyama et al. [105]. In the current study, we observed a change in plasma sVEGFR-2 concentration as a function of gestational age. During pregnancy, plasma concentration of sVEGFR-2 mirrored that of PlGF [87,111–113]. It increased from 20–24 weeks of gestation, peaked in the third trimester, and declined as term approached.

Similarly, the findings that plasma sVEGFR-2 concentration decreased in patients with preeclampsia and in those with SGA neonates without preeclampsia are consistent with studies conducted by both Schlembach et al. [114] and Kim et al. [115]. Both groups reported a lower serum/plasma concentration of sVEGFR-2 in patients with preeclampsia than in women with normal pregnancies. In contrast, Masuyama et al. [105] reported that there was no significant difference in the mean serum concentration of sVEGFR-2 between patients with preeclampsia and women with normal pregnancies [105]. However, only 15 patients with preeclampsia were enrolled in the study. Recently, Wallner et al. [116] also reported a lower mean serum concentration of sVEGFR-2 in patients with intrauterine growth retardation, defined as abdominal circumference below the 5th percentile (diagnosed by ultrasound examination) and confirmed with a neonatal birth weight below the 10th percentile for gestational age. Although previous studies have used either plasma or serum samples to determine sVEGFR-2 concentration, Ebos et al. [101] demonstrated that the concentration of sVEGFR-2 in plasma (EDTA, citrate, or heparin) was not significantly different from that in serum.

What are the potential sources of sVEGFR-2?

The soluble form of VEGFR-2 could be detected in conditioned media obtained from human endothelial

cells, suggesting that endothelial cells are one of the sources of plasma sVEGFR-2 [101]. Although the membranous isoform of VEGFR-2 is expressed mainly on endothelial cells, a fraction of hematopoietic cells, also known as circulating endothelial progenitor cells, express VEGFR-2 [81,117]. A low level of expression for the membranous form of VEGFR-2 is also observed in neurons, osteoblasts, pancreatic duct cells, retinal progenitor cells, and megakaryocytes [81]. There is evidence that VEGFR-2 is expressed on cytotrophoblast stem cells and cells in the proximal columns of chorionic villi during the first and second trimesters of pregnancy [118]. However, VEGFR-2 mRNA and protein are, at term, localized almost exclusively to vascular endothelial cells of the placenta (in contrast to VEGFR-1, which is expressed mainly on trophoblasts) [119,120]. There is no significant difference in the expression of VEGFR-2 mRNA [99] and protein [120] in the placenta of patients with preeclampsia and that of women with normal pregnancies. In contrast, a study using Western blot analysis of protein extracted from placentas reported a lower expression of sVEGFR-2 in patients with preeclampsia than in women with normal pregnancies [121]. The mean serum concentration of sVEGFR-2 in the umbilical vein was in the same range as in maternal blood and was reported to be lower in both patients with preeclampsia and those with SGA than in women with normal pregnancies [116].

The soluble form of VEGFR-1 can be secreted from endothelial cells, monocytes, and placenta through alternative splicing of the VEGFR-1 gene [122]. Whether the soluble form of VEGFR-2 is a result of alternative mRNA splicing, proteolytic cleavage of the membrane-bound receptor, or another mechanism is unknown [101]. While it is unclear what biological agents could stimulate sVEGFR-2 expression, the expression of the membranous isoform of VEGFR-2 is stimulated by VEGF and TNF-alpha [123,124]. Conflicting results have been reported regarding the effect of hypoxia on VEGFR-2 expression: either none or decreased or increased change, depending on the cell type (for example: either no change [125], or increased [126] in the human umbilical vein, or increased [127–129] in the lung and brain, or decreased [130] in the skin). The finding that there was no significant difference in the mean plasma sVEGFR-2 concentration among patients with SGA whether or not they had an increased impedance to blood flow in the uterine artery (as measured by uterine artery Doppler velocimetry) indicates that utero-placental ischemia may not be a major determinant of plasma sVEGFR-2 concentration.

What are the functions of sVEGFR-2?

The natural soluble form of VEGFR-2 was recently detected in both the mouse and human plasma [101]. Whether this natural sVEGFR-2 plays a significant role in VEGF signaling, as has been described for sVEGFR-1, remains to be determined. However, a previous study suggests that the soluble form of VEGFR-2 has anti-angiogenic properties. The administration of adenovirus encoding murine sVEGFR-2 to non-pregnant rats induces hypertension and proteinuria [84]. This biological effect, however, was not observed in pregnant rats, possibly owing to high levels of unopposed PlGF (which does not bind to sVEGFR-2) secreted by the placenta in the pregnant state. Another study supports the role of sVEGFR-2 as an anti-angiogenic factor derived from the observation that a local injection of recombinant sVEGFR-2 inhibits retinal neovascularization [102].

The finding that pregnant women with SGA and preeclampsia have lower plasma sVEGFR-2 concentrations than women with normal pregnancies is unexpected given that preeclampsia is considered an anti-angiogenic state [84]. On the other hand, one could speculate that the lower concentration of sVEGFR-2 in patients with preeclampsia may result from the low availability of free VEGF [84,131] to stimulate VEGFR-2 in endothelial cells. Thus, plasma sVEGFR-2 concentration could be a surrogate marker of endothelial cell function in the maternal circulation, since VEGF signaling through the membranous isoform of this protein is essential for endothelial cell function and survival [83].

Alternatively, plasma concentrations of sVEGFR-2 in patients with preeclampsia and those with SGA might reflect low endothelial cell regenerative capacity. This hypothesis is supported by two observations: (1) there is a lower number of circulating endothelial progenitor cells (as measured by colony-forming unit method), and (2) patients with preeclampsia have lower plasma concentrations of sVEGFR-2 than women with normal pregnancies [115,132]. The circulating endothelial progenitor cells, phenotypically defined by flow cytometry as cells that express CD34 and VEGFR-2 [133], are capable of mobilizing to the sites of tissue or endothelial cell injuries for repair purposes, and correlate inversely with the risk of future death in patients with coronary artery disease [134]. The numbers of these cells have been proposed to reflect endothelial cell regenerative capacity [134]. Endothelial cells play a critical role in the regulation of vascular tone, platelet activity, leukocyte adhesion, and thrombosis, and are involved in the development of atherosclerosis [116,135]. Consistent with this hypothesis, several lines of evidence suggest that

patients with preeclampsia, especially in the early-onset group, or women who delivered a low birth weight neonate have an increased risk of developing cardiovascular disease [68–73].

Future studies are required to determine when plasma sVEGFR-2 concentration in patients with preeclampsia or in those with SGA becomes lower than that of women with normal pregnancies. If plasma sVEGFR-2 concentrations in patients with preeclampsia or SGA are low in early gestation or persist in the postpartum period, it is possible that these women, with low regenerative capacity of endothelial cells, would be more susceptible to pregnancy complications and, thus, be at greater risks for cardiovascular disease. The finding that there was no correlation between the severity of preeclampsia or SGA and plasma concentration of sVEGFR-2 at the time of diagnosis suggests that other factors, (such as PlGF, sVEGFR-1, and soluble endoglin) [84,86,112] may be more directly involved in the clinical manifestations of these syndromes than sVEGFR-2.

It is possible that therapy focusing on angiogenic and anti-angiogenic processes in preeclampsia will be developed in the future [136]. Despite lower affinity to VEGF than sVEGFR-1 [81], the mean plasma concentration of sVEGFR-2 is almost 10 times higher than that of sVEGFR-1 in women with normal pregnancies [114,116]. sVEGFR-2 may be important in the future development of angiogenic therapy in preeclampsia and SGA.

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