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Unexplained fetal death: Another anti-angiogenic state

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Abstract

Background: Pregnancy creates a unique situation in which both vasculogenesis and extensive angiogenesis are required for successful fetal and placental development. Recently, the soluble form of vascular endothelial growth factor (VEGF) receptor-1 (sVEGFR-1), an antagonist to VEGF and placental growth factor (PlGF) (two important angiogenic factors), has been implicated in the pathophysiology of preeclampsia and small for gestational age (SGA) without preeclampsia. There is, however, a paucity of information concerning plasma sVEGFR-1 concentrations in other obstetrical disorders. The purpose of this study was to determine plasma sVEGFR-1 concentrations in normal pregnancy, term gestation in labor, and in patients with pregnancy complications including spontaneous preterm labor, preterm premature rupture of the membranes (PROM), fetal death, and acute pyelonephritis.

Methods: A cross-sectional study was conducted to determine the concentrations of sVEGFR-1 in plasma obtained from 499 women in the following groups: (1) non-pregnant women (n=40); (2) pregnant women (n=135); (3) normal pregnant women at term in labor (n=60); (4) fetal death (n=60); (5) spontaneous preterm labor with intact membranes (n=102); (6) preterm PROM (n=64); and (7) pregnancy with acute pyelonephritis (n=38). Since plasma sVEGFR-1 concentration changes with gestational age, the difference between the actual and the expected plasma sVEGFR-1 concentration (derived from regression equation of normal pregnancy) for each patient (delta value) was calculated and used to examine the differences of plasma sVEGFR-1 concentrations among various groups. Plasma concentrations of sVEGFR-1 were determined by enzyme-linked immunoassay. Regression analysis and non-parametric statistics were used for analysis.

Results: (1) Normal pregnant women before term had a median plasma sVEGFR-1 concentration significantly higher than non-pregnant women ($p < 0.001$); (2) plasma sVEGFR-1 concentration increased with advancing gestational age in normal pregnancy ($r = 0.5$; $p < 0.001$); (3) there was no significant difference in the median delta plasma concentration of sVEGFR-1 between normal pregnant women at term with and without labor ($p = 0.09$); (4) patients with fetal death had a median delta plasma concentration of sVEGFR-1 significantly higher than normal pregnant women ($p = 0.001$). Among patients with fetal death, those with unexplained causes ($p = 0.04$) and those with preeclampsia ($p < 0.001$) had a significantly higher delta plasma sVEGFR-1 concentration than normal pregnant women; and (5) there was no significant difference in the median delta plasma sVEGFR-1 concentration between normal pregnancy and preterm labor with intact membranes, preterm PROM (regardless of the presence or absence of microbial invasion of the amniotic cavity), or acute pyelonephritis (all $p > 0.05$).

Conclusions: Plasma sVEGFR-1 concentration is increased in a subset of patients with fetal death, but does not change in term and preterm parturition, rupture of fetal membranes, or acute pyelonephritis.

Keywords

Preterm labor; preterm PROM; fetal death; acute pyelonephritis; great obstetrical syndrome; soluble VEGFR-1

INTRODUCTION

The development of a vascular supply is a fundamental requirement for organ development and differentiation. Two important processes are involved in these mechanisms: vasculogenesis, a process in which endothelial cells differentiate and proliferate within a previously avascular tissue, and angiogenesis, which refers to the remodeling process after the initial vascular network has been developed [1]. Therefore, vasculogenesis occurs mainly during fetal development, and angiogenesis is essential in adult life, especially for the female reproductive cycle (e.g., formation of corpus luteum, endometrial growth) and for the repair, remodeling, and regeneration of tissues (e.g., wound healing) [2]. Pregnancy creates a unique situation in which both vasculogenesis and extensive angiogenesis are required for successful fetal and placental development [3].

Several angiogenic and anti-angiogenic factors are important for successful reproductive function [4–7]. The balance between angiogenic factors such as vascular endothelial growth factor (VEGF) and its receptor, VEGFR-1, appears critical for normal pregnancy [8]. VEGF promotes endothelial cell proliferation, migration and survival of endothelial cells [9,10], and exerts its biologic effect through two high-affinity receptor tyrosine kinases: VEGFR-1 (or flt-1) and VEGFR-2 (or KDR/Flk-1) [11]. Whereas VEGFR-2 is the major mediator of the mitogenic, angiogenic, permeability-enhancing, and endothelial survival effects of VEGF, the precise function of VEGFR-1 is still subject to debate [11]. VEGFR-1 has two isoforms: transmembranous and soluble. The latter is generated by a splice variant of the VEGFR-1 gene [12], and contains the extracellular ligand-binding domain, while lacking the signaling tyrosine kinase domain. Thus, this isoform binds VEGF and inhibits its biological activities [13].

The expression of VEGFR-1 protein has been reported in endothelial and non-endothelial cells, including vascular [13] and uterine smooth muscle cells [14], renal tubular epithelium [15], decidua [16,17], amnion [17], neutrophils [18], monocytes [18,19] and trophoblasts [16,20–24]. The VEGFR-1 in monocytes is a functional ligand for VEGF and helps the migration of monocytes during the angiogenic process [18]. In contrast, the precise function of VEGFR-1 on trophoblasts remains unknown. The presence of co-localization of VEGF and VEGFR-1 proteins in the placenta and decidual tissue during the first trimester suggests that the VEGF system may participate in trophoblast growth and differentiation [20,21].

Perinatal morbidity and mortality are largely determined by five major complications of pregnancy: small for gestational age (SGA), preeclampsia, preterm labor, preterm premature rupture of membranes (PROM), and fetal death after excluding congenital anomalies. The perinatal consequences of these disorders account for the majority of infant mortality (death before the age of one year) [25]. The term ‘the great obstetrical syndromes’ has been used to refer to these conditions [26]. The key features of ‘the great obstetrical syndromes’ are: (1) multiple etiology; (2) chronicity; (3) fetal involvement; (4) their clinical manifestations are adaptive; and (5) their occurrence may be due to a gene-environment interaction [26].

Previous studies have reported an increased plasma soluble VEGFR-1 (sVEGFR-1) concentration in pregnancy complications, such as preeclampsia [8,27,28] and SGA [29]. However, information on plasma sVEGFR-1 concentration in other disease complications during pregnancy is scarce. The purpose of this study was to determine if plasma sVEGFR-1 concentration changes with advancing gestational age in normal pregnancy, in women at term in labor, and those with pregnancy complications including preterm labor, preterm PROM, fetal death, and acute pyelonephritis.

PATIENTS AND METHODS

Study Design:

A cross-sectional study was conducted by searching our clinical database and bank of biologic samples. This study included 499 women in the following groups: (1) non-pregnant women (n=40); (2) normal pregnancy (n=135); (3) normal pregnant women at term in spontaneous labor (n=60); (4) fetal death (n=60); (5) spontaneous preterm labor with intact membranes (n=102); (6) preterm PROM (n=64); and (7) pregnant women with acute pyelonephritis (n=38). The non-pregnant group consisted of women who had no history of acute or chronic inflammatory conditions. Normal pregnant women were enrolled from either a labor-delivery unit (in cases of scheduled cesarean section) or an antenatal clinic, and followed until delivery. The inclusion criteria for the normal pregnancy group included: (1) no medical, obstetrical or surgical complications; (2) not in labor; and (3) delivery of a normal term (≥ 37 weeks) infant whose birth weight was between the 10th and 90th percentile for gestational age. This group was subdivided into: (a) normal pregnancy before term (n=64), and (b) normal pregnancy at term (n=71).

Fetal death was defined as the death of the fetus after the 20th week of gestation and confirmed by ultrasound examination. This group was sub-classified into three groups, according to the causes of fetal death: (a) unexplained fetal death (n=44); (b) fetal death with

preeclampsia (n=8); and (c) fetal death with a known chromosome abnormality or major malformation (n=8). The abnormality in fetuses of the latter group included trisomy 21 (n=3), trisomy 13 (n=1), non-immune hydrops fetalis (n=3), and cardiovascular defect with single umbilical artery (n=1). Pulsed-wave and color Doppler ultrasound examination of the uterine arteries was performed in some patients in the fetal death group with a real time scanner (Acuson, Sequoia, Mountain View, CA USA) equipped with a 5-MHz probe. Abnormal uterine artery Doppler [30] was defined as either the mean resistance index from the left and right uterine artery above the 95th percentile for gestational age [31] or the presence of bilateral diastolic notch of the uterine artery Doppler waveform [32].

Preeclampsia was defined as hypertension (systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg on at least two occasions, four hours to one week apart) and proteinuria ($\geq$ 300 milligrams in a 24-hour urine collection or one dipstick measurement $\geq$ 2+) [33]. Preterm labor was defined by the presence of regular uterine contractions occurring at a frequency of at least two every ten minutes and cervical changes before 37 completed weeks of gestation. PROM was diagnosed as amniorrhexis before the onset of spontaneous labor. Membrane rupture was diagnosed with the use of vaginal pooling, ferning, or a positive nitrazine test. Women in groups 5 and 6 included only patients who had amniocentesis performed for obstetrical indications within 24 hours of blood sampling.

Women with preterm labor were subdivided into the following categories: (a) preterm labor with term delivery without microbial invasion of the amniotic cavity (MIAC) (n=24); (b) preterm labor with preterm delivery ($<$ 37 weeks) without MIAC (n=62); and (c) preterm labor and delivery with MIAC (n=16). MIAC was defined as a positive amniotic fluid culture for microorganisms. Women with preterm PROM were subdivided into PROM with (n=31) and without MIAC (n=33). Pregnant women with acute pyelonephritis (n=38) were diagnosed based on fever (temperature $\geq$ 38°C), clinical signs (e.g., back pain), pyuria, and a positive urine culture for microorganisms. This group was subdivided into those who had a positive culture for microorganisms in urine alone (n=26), and those with both positive cultures in urine and blood (n=12). All women provided written informed consent prior to the collection of plasma samples. The collection and utilization of the samples was approved by the Human Investigation Committee of Wayne State University/Hutzel Hospital and the IRB of the National Institute of Child Health and Human Development. Many of these samples were previously used in studies of soluble adhesion molecules [34].

Sample collection and human sVEGFR-1 immunoassay:

Venipuncture was performed and the blood was collected into the tubes containing EDTA. The samples were centrifuged and stored at -70° C. The concentrations of sVEGFR-1 were measured using enzyme-linked immunoassay (ELISA; R&D Systems, Minneapolis, MN, USA). This assay employs the quantitative sandwich immunoassay technique. Briefly, recombinant human VEGFR-1 standards and maternal plasma specimens were incubated in duplicate wells of the microtiter plates pre-coated with monoclonal antibodies specific for VEGFR-1. During this incubation, the immobilized antibodies in the microtiter plate bound VEGFR-1 present in both the standards and samples. After washing unbound substances, polyclonal antibodies to human VEGFR-1 conjugated to an enzyme (horseradish

peroxidase) were added to the assay wells. Once the incubation period was over, the assay plates were washed to remove unbound antibody-enzyme reagents. Upon addition of a substrate solution (tetramethylbenzidine), color developed in the assay plates proportionally to the amount of VEGFR-1 bound in the initial step. The microtiter plates were read with a programmable spectrophotometer (Ceres 900 Microplate Workstation, Bio-Tek Instruments, Winooski, VT USA). The inter- and intra-assay coefficients of variation (CVs) were 4.8% and 6.9%, respectively. The lowest maternal plasma concentration of sVEGFR-1 detectable by the immunoassay 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 plasma sVEGFR-1 concentration changes with gestational age, the difference between the actual and the expected plasma sVEGFR-1 concentration (derived from regression equation of normal pregnancy) for each patient (delta value) was calculated and used to examine the differences of plasma sVEGFR-1 concentration among various groups. KruskalWallis with post-hoc tests was utilized to determine the differences of the median among the groups. Logistic regression was used to assess the odds of the presence of each disease (compared to normal pregnancy) in relation to an increased $\log (\text{sVEGFR-1}+1)$ unit after adjusting for potential confounding factors. Contingency tables, Chi-square or Fisher's exact tests were employed for comparison of proportions. The statistics package used was SPSS 12 (SPSS Inc., Chicago, IL USA). Significance was assumed for a p value of <0.05 .

RESULTS

Clinical and obstetrical characteristics of women in each group are displayed in Tables I and II. The normal pregnancy group had the highest median gestational age among the five groups (Table II). sVEGFR-1 was detected in 94% (469/499) of plasma samples. Twenty-nine (72.5%, (29/40)) non-pregnant women and one pregnant woman with fetal death (1.7%, (1/60)) had plasma sVEGFR-1 concentrations below the detection limit of the assay (17.8 pg/mL).

Normal pregnant women before term had a higher median plasma sVEGFR-1 concentration than non-pregnant women ($p < 0.001$; Figure 1). However, normal pregnant women at term had a median plasma sVEGFR-1 concentration higher than normal pregnant women before term ($p < 0.001$; Figure 1). Plasma sVEGFR-1 concentration among normal pregnant women increased with advancing gestational age according to the equation $\log (\text{sVEGFR-1}+1) = 0.026 (\text{gestational age in weeks}) + 2.172$ ($r = 0.5$, $r^2 = 0.34$; $p < 0.001$; Figure 2).

Normal pregnant women at term in labor had a median gestational age at blood sampling higher than those without labor (term in labor: median 40 weeks, range 37–41 weeks vs. term without labor: median 39 weeks; range 37–41 weeks; $p < 0.001$). There was no significant difference in the median delta plasma sVEGFR-1 concentration between the two

groups (term in labor: median 0.08 pg/mL, range -0.4 – 0.7 vs. term without labor: median 0.03 pg/mL; range -0.4 – 0.5; $p = 0.09$).

Patients with a fetal death had a median delta plasma concentration of sVEGFR-1 significantly higher than normal pregnant women ($p = 0.001$; Figure 3). There was no significant difference in the median delta plasma sVEGFR-1 concentration between normal pregnancy and preterm labor with intact membranes, normal pregnancy and preterm PROM, as well as normal pregnancy and pregnancy with acute pyelonephritis (all $p > 0.05$; Figure 3). The proportion of patients who had a high delta plasma sVEGFR-1 concentration (defined as delta sVEGFR-1 above mean + 2SD for normal pregnant women) in each group is displayed in Table II. The odds ratio of different logistic regression models describing the relationship between each pregnancy complication and plasma sVEGFR-1 concentration (compared to normal pregnancy) after adjusting for gestational age at blood sampling and sample storage interval are shown in Table III. Only pregnancy with fetal death was associated with an increased plasma sVEGFR-1 concentration with an odds ratio of 4.9 (95% CI: 1.6–14.9).

Clinical and obstetrical characteristics of women in each subgroup of fetal death are displayed in Table IV. Among patients with fetal death, those with unexplained fetal death and fetal death with preeclampsia had a median delta plasma sVEGFR-1 concentration significantly higher than that of normal pregnant women ($p = 0.04$ and $p < 0.001$, respectively; Figure 4). There was no significant difference in median delta plasma sVEGFR-1 between patients with fetal death with a fetal anomaly and normal pregnancy ($p = 0.2$; Figure 4). Table V displays the odds ratios of different logistic regression models describing the relationship between the etiologic classification of fetal death and plasma sVEGFR-1 concentration (compared to normal pregnancy) after adjusting for gestational age at blood sampling and sample storage interval. Only pregnancies with an unexplained fetal death and fetal death with preeclampsia were associated with an increased plasma sVEGFR-1 concentration.

Among patients with unexplained fetal death, 45% (20/44) delivered SGA neonates (defined as birth weight below 10th percentile for gestational age). However, there was no significant difference in the mean delta plasma sVEGFR-1 concentration in patients with unexplained fetal death, whether they delivered SGA neonates or not (SGA mean: 0.16 ± 0.4 vs. without SGA mean: 0.16 ± 0.4 ; $p = 0.9$). The frequency of patients who had a high delta plasma sVEGFR-1 concentration (defined as delta sVEGFR-1 above mean + 2SD for normal pregnant women) in each group is displayed in Table IV. Eight (18.2%, (8/44)) patients in the unexplained fetal death group had a high delta plasma sVEGFR-1 concentration, and among them five (62.5%, (5/8)) delivered SGA neonates. Interestingly, sixteen (36.4%, (16/44)) patients in the unexplained fetal death group had an abnormal uterine artery Doppler, and among them only three (19%, (3/16)) had a high delta plasma sVEGFR-1 concentration. In contrast, most patients (87.5%, (7/8)) in the fetal death with preeclampsia group had a high delta plasma sVEGFR-1 concentration. Among patients with fetal anomalies, two (25%, (2/8)) had a high delta plasma sVEGFR-1 concentration: one with hydriops fetalis, the other with cardiovascular defects. Both had normal uterine artery

Dopplers. Only one patient (12.5%, (1/8)) in this subgroup had an abnormal uterine artery Doppler.

Clinical and obstetrical characteristics of women in each subgroup of patients with preterm labor and preterm PROM are displayed in Tables VI and VII. Among patients with preterm labor and intact membranes, there were no significant differences in the median delta plasma sVEGFR-1 concentrations among patients who delivered at term, those who delivered preterm without MIAC, and those who delivered preterm with MIAC (all $p > 0.05$; Figure 5). Three (3%) patients in this group had a high delta plasma sVEGFR-1 concentration, and none had MIAC. One patient experienced preterm labor at 28 weeks of gestation and delivered an SGA neonate at term, the other presented with preterm labor at 30 weeks of gestation and developed clinical preeclampsia three days later, and another had preterm labor at 30 weeks of gestation and delivered an appropriate for gestational age neonate one week later.

There was no significant difference in the median delta plasma sVEGFR-1 concentrations between preterm PROM patients with and without MIAC (Figure 6). Four (6%) patients had a high delta plasma sVEGFR-1 concentration and two of them delivered SGA neonates. Among patients with acute pyelonephritis, no significant difference in the median delta plasma sVEGFR-1 concentration was observed between those who had a positive blood culture for microorganisms and those who did not (positive blood culture median: 0.08 pg/mL, range: -0.4–0.5 pg/mL vs. negative blood culture median: -0.09 pg/mL, range: -0.5–0.4 pg/mL; $p = 0.3$). Only one (2.6%) patient who had a positive blood and urine culture for microorganisms had a high delta plasma sVEGFR-1 concentration.

DISCUSSION

Principal findings:

(1) The median plasma sVEGFR-1 concentration is higher in pregnant women than in non-pregnant women; (2) the maternal plasma concentration of sVEGFR-1 increases with advancing gestational age; and (3) patients with an unexplained fetal death, but not those with preterm labor, preterm PROM, or acute pyelonephritis, have a higher maternal plasma sVEGFR-1 concentration than patients with normal pregnancy (adjusted for gestational age).

sVEGFR-1 in non-pregnant and normal pregnant women:

Normal healthy non-pregnant individuals have low concentrations of VEGF [35] in the peripheral circulation. This factor is thought to be required for the maintenance of normal endothelial cell function [9]. In the present study, plasma sVEGFR-1 was undetectable in the majority of non-pregnant women (72%), but was detectable in all normal pregnant women. Of major interest, the plasma sVEGFR-1 concentration increased with advancing gestational age. We propose that this elevation of maternal plasma sVEGFR-1 during the end of gestation could serve to limit angiogenesis and blood vessel permeability [36,37] resulting from an excess of VEGF, placental growth factor (PlGF), or other ligands for VEGFR-1 [12]. The current understanding is that vascular permeability is affected by VEGF but not PlGF.

sVEGF-R1 in fetal death:

Although most of the literature has focused on the role of sVEGFR-1 in preeclampsia [8,27,28], there is evidence that this anti-angiogenic factor is also elevated in a subset of patients with SGA [29]. Moreover, there is an association between the presence of abnormal impedance to flow in the uterine and/or umbilical artery and the magnitude of the increase in plasma sVEGFR-1 concentration (Chaiworapongsa et al., unpublished observations). The current study demonstrates that unexplained fetal death is associated with an increased plasma sVEGFR-1 concentration at the time of the diagnosis. However, the magnitude of the increase observed in this condition is not as high as those observed in SGA with abnormal uterine artery Doppler velocimetry or those with preeclampsia (fetal death: median delta 0.08 vs. SGA: mean delta 0.43, mild preeclampsia: mean delta 0.55, and severe preeclampsia: mean delta 0.73; Chaiworapongsa et al., unpublished observations). Moreover, the wide range (291–14190 pg/mL) of plasma sVEGFR-1 concentrations in this subgroup suggests that fetal death is a syndrome and that only some cases have an anti-angiogenic state (defined by an elevation of sVEGFR-1).

A solid body of evidence supports an association between reduced uteroplacental perfusion, fetal growth restriction, and fetal death [38,39]. Absence of physiologic transformation of the spiral arteries had been reported in patients with second trimester spontaneous abortion [38]. Moreover, 40% of unexplained fetal deaths are SGA [39]. This is consistent with the observation that 45% (20/44) of patients with unexplained fetal deaths in our study delivered neonates with birth weights below the 10th percentile for gestational age. Of note, there was no significant difference in the median plasma sVEGFR-1 concentration in patients with unexplained fetal death between patients with and without SGA fetuses.

Among eight patients with unexplained fetal death who had high delta plasma sVEGFR-1 concentrations (defined as delta plasma sVEGFR-1 concentration greater than two standard deviations of the mean of normal pregnant women), five (62.5%) delivered SGA neonates, suggesting that the association between plasma sVEGFR-1 and SGA might exist in a subset of unexplained fetal death.

We previously found that women with SGA fetuses who had abnormal uterine artery Doppler velocimetry had an increased plasma sVEGFR-1 concentration, and 43% (10/23) had a high delta plasma sVEGFR-1 concentration (>2SD). In the current study, only 19% (3/16) of patients with unexplained fetal death with abnormal uterine artery Doppler velocimetry had a high delta plasma sVEGFR-1 concentration. From these observations, we conclude that a subset of patients with fetal death and abnormal uterine artery Doppler have normal maternal plasma sVEGFR-1 concentrations. We have proposed that an elevation of sVEGFR-1 may be a protective mechanism of the feto-placental unit. Some cases of fetal death, in which there is no elevation of VEGFR-1, may represent failure of this mechanism.

In the current study, women with fetal death and preeclampsia also had an elevation of plasma sVEGFR-1 concentration (median delta 0.78) similar to that observed in preeclampsia with live fetuses in our previous study (mild preeclampsia: mean delta 0.55 and severe preeclampsia: mean delta 0.73) (Chaiworapongsa et al., unpublished observations), suggesting that a live fetus is not required for an elevation of plasma

sVEGFR-1 in preeclampsia. This interpretation is consistent with the observation that there is no significant difference in serum concentrations of sVEGFR-1 in the umbilical vein or umbilical artery between patients with preeclampsia and normal pregnant women [40]. The lack of a gradient militates against a substantial fetal production of sVEGFR-1. Indeed, the mean concentration of serum sVEGFR-1 in the umbilical vein and artery was approximately 10 times lower than that found in maternal serum [41].

sVEGFR-1 in parturition, preterm PROM and MIAC:

VEGF is also known as a vascular permeability factor. Such activity underlies the significance of this molecule in inflammation and other pathologic conditions, such as rheumatoid arthritis and atherosclerosis [11]. Angiogenesis requires the participation of hematopoietic progenitors, endothelial progenitor, and inflammatory cells [1,11]. Monocytes express VEGFR-1 and the migration of monocytes in response to VEGF requires tyrosine kinase domain of VEGFR-1 (membrane isoform) [18]. However, monocytes can release sVEGFR-1 [19].

Recently, Daneshmand et al. [17], using *in situ* hybridization, observed an increased mRNA expression of VEGF and VEGFR-1 in the human amnion and attached decidua of patients with preterm PROM compared to that of patients with preterm labor with intact membranes. The increase of VEGF and VEGFR-1 mRNA expression is more pronounced in patients with inflammation (defined as overexpression of IL-6 mRNA and protein). In another study, the increased expression of VEGFR-1 mRNA and protein has been localized to macrophages and neutrophils infiltrating the chorionic plate of the placenta in patients with histologic chorioamnionitis [40]. Moreover, Zucker and colleagues [42] demonstrated that treatment of endothelial cells with VEGF results in activation and enhanced production of matrix-metalloproteinase (MMP), an enzyme implicated in the mechanisms of membrane rupture. Collectively, these observations suggest a role for VEGF and VEGFR-1 in the mechanisms of membrane rupture and inflammation.

VEGF has been implicated in the mechanisms of human parturition. Indeed, Marvin et al. [43], using complementary DNA array, demonstrated up-regulation of several angiogenic factors, including VEGF, in both membranes and choriodecidual tissues. The expression of VEGF mRNA in membranes is increased in labor, a process thought to be an inflammatory-like condition [44].

Our study did not find significant changes of delta plasma sVEGFR-1 concentration in term parturition, preterm labor with intact membranes, preterm PROM or MIAC. It is possible that the changes of VEGF-VEGF receptor system expression in these conditions are localized to gestational tissues and, thus, not reflected in the maternal circulation.

sVEGFR-1 in acute pyelonephritis: There is a paucity of information regarding plasma sVEGFR-1 concentration in response to sepsis or acute infection. Most studies examined VEGF and its receptor expression on endothelial cells and proposed a role for VEGF and its receptors in delayed wound healing associated with infection. Indeed, the presence of endotoxin decreased VEGF receptor density on endothelial cells as measured by flow cytometry [45]. Moreover, the mRNA expression of VEGF and VEGFR-2 in postmortem

lung tissue was reported to be lower in septic than non-septic patients [46]. However, the exposure of endothelial cell cultures to tumor necrosis factor- α , a pro-inflammatory cytokine implicated in the clinical manifestation of sepsis, has yielded conflicting results; indeed, both decreased VEGFR-1 and VEGFR-2 [47] and increased VEGFR-2 [48] have been reported. In our study population, we found no change in the median delta plasma sVEGFR-1 concentrations in pregnant patients with acute pyelonephritis.

CONCLUSIONS

This study demonstrated that plasma sVEGFR-1 concentration is higher in the pregnant than in the non-pregnant state. Moreover, an elevation in plasma sVEGFR-1 concentration was observed in a subset of patients with unexplained fetal death. However, plasma sVEGFR-1 concentration during pregnancy does not change significantly in spontaneous term and preterm parturition (with intact or ruptured membranes, with and without infection), or with maternal infections (MIAC and acute pyelonephritis). The current study suggests that a subset of patients with fetal death have an anti-angiogenic state. Further studies are required to determine whether the elevations in plasma sVEGFR-1 concentration precede fetal death.

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Figure 1. The mean plasma sVEGFR-1 concentration of non-pregnant women, pregnant women before term, and pregnant women at term.

Normal pregnant women before term had a median plasma sVEGFR-1 concentration higher than non-pregnant women (normal pregnant women before term: median 765 pg/mL, range 260–4,712 pg/mL vs. non-pregnant women: median 0 pg/mL, range 0–119 pg/mL; $p < 0.001$). However, normal pregnancy at term had a further increase of plasma sVEGFR-1 concentration (normal pregnant women at term: median 1,655 pg/mL, range 544–5,293 pg/mL vs. normal pregnant women before term: median 765 pg/mL, range 260–4,712 pg/mL; $p < 0.001$).

Figure 2. Plasma sVEGFR-1 concentration of normal pregnant women increased with advancing gestational age according to the equation:

$\log (sVEGFR-1+1) = 0.026 (\text{gestational age in weeks}) + 2.172$ ($r = 0.5$, $r^2 = 0.34$; $p < 0.001$).

Figure 3. The median delta plasma sVEGFR-1 concentration of normal pregnant women, patients with IUFD, preterm labor, preterm PROM, and pregnant women with acute pyelonephritis (Pyelo).

Patients with fetal death had a higher median delta plasma concentration of sVEGFR-1 than normal pregnant women ($p = 0.001$). There was no significant difference in the median delta plasma sVEGFR-1 concentration between normal pregnancy and preterm labor with intact membranes ($p = 0.7$), normal pregnancy and preterm PROM ($p = 0.4$) as well as normal pregnancy and pregnant women with acute pyelonephritis ($p = 0.4$). The median and range of plasma sVEGFR-1 concentrations in each group are displayed in the figure.

Figure 4. The median delta plasma sVEGFR-1 concentration of normal pregnant women, patients with unexplained fetal death, fetal death with preeclampsia (IUFD + PE), and fetal death with abnormal chromosome or major malformation (Abn Chro/Malform). Patients with unexplained fetal death and those with fetal death in the context of preeclampsia had a median delta plasma sVEGFR-1 concentration higher than that of normal pregnant women ($p = 0.04$ and $p < 0.001$, respectively). There was no significant difference in the median delta plasma sVEGFR-1 concentration between fetal death with congenital anomaly and normal pregnancy ($p = 0.2$). The median and range of plasma sVEGFR-1 concentrations in each group are displayed in the figure.

Figure 5. The median delta plasma sVEGFR-1 concentration of patients with preterm labor with intact membranes.

There were no significant differences in the median delta plasma sVEGFR-1 concentrations among patients who delivered at term, those who delivered preterm without MIAC, and those who delivered preterm with MIAC (Kruskal-Wallis test; $p = 0.9$). The median and range of plasma sVEGFR-1 concentrations in each group are displayed in the figure.

Figure 6. The median delta plasma sVEGFR-1 concentration of patients with preterm premature rupture of membranes (PPROM).

There was no significant difference in the median delta plasma sVEGFR-1 concentrations between preterm PROM patients with and without MIAC ($p = 0.3$). The median and range of plasma sVEGFR-1 concentrations in each group are displayed in the figure.

Table I.

Clinical characteristics of the non-pregnant and normal pregnancy groups

	Non-pregnant women n = 40	$p\alpha$	Normal pregnancy before term n=64	$p\beta$	Normal pregnancy at term, no labor n = 71	$p\gamma$
Age (years)	26 (18–40)	0.001	23 (17–34)	0.002	27 (17–40)	0.5
GA at blood sampling (weeks)	--	--	30.7 (20.0–36.8)	<0.001	39.1 (37.0–41.7)	--
GA at delivery (weeks)	--	--	39.6 (37–42)	0.4	39.3 (37–42)	--
Birthweight (grams)	--	--	3325 (2610–4030)	0.3	3360 (2850–4080)	--
Adjusted birthweight for GA (MOM)	--	--	−0.04 (−0.16–0.15)	0.1	−0.01 (−0.16–0.18)	--

$p\alpha$: compared between nonpregnant women and normal pregnancy before term.

$p\beta$: compared between normal pregnancy before term and normal pregnancy at term.

$p\gamma$: compared between nonpregnant women and normal pregnancy at term.

Values are expressed as median (range).

GA: gestational age; MOM: multiple of the median

Table II.

Clinical characteristics of the study population

	Normal pregnancy n = 135	IUED n=60	p	Preterm labor n = 102	p	Preterm PROM N=64	p	Acute pyelonephritis N=38	p
Age (y)	26 (18–40)	25 (17–41)	0.6	23 (13–39)	0.002*	26 (16–38)	0.1	22 (16–41)	0.07
GA at blood sampling (weeks)	37.6 (20–41)	30.9 (20.1–40.6)	<0.001*	29.9 (20.1–33.7)	<0.001*	30.6 (21.1–33.9)	<0.001*	31.6 (20.7–42.3)	<0.001*
GA at delivery (weeks)	39.3 (37–42)	31.0 (20.1–40.7)	<0.001*	32.9 (21.0–41.4)	<0.001*	32.9 (21.0–41.4)	<0.001*	32.9 (21.0–41.4) ^a	0.7
Birthweight (grams)	3345 (2610–4080)	1390 (140–5755)	<0.001*	1870 (400–3750) ^δ	<0.001*	1580 (340–2790)	<0.001*	3175 (1080–4090) ^β	0.02*
Adjusted birthweight for GA (MOM)	−0.02 (−0.16–0.18)	−0.24 (−0.77–0.65)	<0.001*	−0.11 (−0.47–0.34) ^δ	<0.001*	−0.17 (−0.56–0.29)	<0.001*	−0.07 (−0.41–0.18) ^β	0.02*
Birthweight <10 th percentile	0	28 (47%)	<0.001*	16 (16%) ^δ	<0.001*	5 (8%)	0.003*	7 (19%) ^β	<0.001*
Plasma sVEGFR-1 (pg/ml)	1,259(260–5,293)	1,419(0–14,190)		863(189–11,810)		927(223–17,440)		891(230–3,471)	
Delta plasma sVEGFR-1	0.01(−0.46–0.65)	0.15 (−2.78–1.20)	0.001*	0.03(−0.72–1.12)	0.7	0.04(−0.56–1.30)	0.4	−0.03 (−0.48–0.47)	0.4
High Delta plasma sVEGFR-1 ^γ	5 (3.7%)	17 (28.3%)	<0.001*	3 (3%)	1.0	4 (6.3%)	0.5	1 (2.6%)	1.0

All p value compared to normal pregnancy; Value expressed as median (range) or number (percent)

GA: gestational age; MOM: multiple of the median

^δ: n=100

^a: n=37

^β: n=36

^γ: delta plasma sVEGFR-1 >mean+2sd of normal pregnant women (0.42)

Table III.

Odds ratio of the presence of disease for an each unit increase of log (sVEGFR-1+1) concentration

Model	Dependent variables (vs. normal pregnancy)	p	Odds ratio	95% CI
1	Fetal death	0.006	4.9	1.6 – 14.9
2	Preterm labor with intact membranes	0.7	1.2	0.4–4.0
3	Preterm PROM	0.2	2.5	0.7 – 9.4
4	Acute pyelonephritis	0.5	0.5	0.1 – 3.2

Adjusting for gestational age at blood sampling (weeks) and sample storage interval (days). PROM: premature rupture of the membranes.

Table IV.

Clinical characteristics of normal pregnant women and those with fetal death

	Normal pregnancy n = 135	Unexplained fetal death n = 44	p	Fetal death with PE n=8	p	Fetal death with major anomaly n = 8	p
Age (y)	26 (18–40)	25 (17–41)	0.6	22 (17–37)	0.5	27 (17–40)	0.4
GA at blood sampling (weeks)	37.6 (20–41)	31.0 (20.1–40.6)	0.001*	30.6 (25.7–39.3)	0.1	23.7 (22.0–34.6)	0.001*
GA at delivery (weeks)	39.3 (37–42)	31.0 (20.6–40.7)	<0.001*	30.6 (25.9–39.6)	<0.001*	25.4 (22.1–34.7)	<0.001*
Birthweight (grams)	3345 (2610–4080)	1408 (140–5755)	<0.001*	1830 (600–2710)	<0.001*	590 (180–1980)	<0.001*
Adjusted birthweight for GA (MOM)	−0.02 (−0.16–0.18)	−0.23 (−0.77–0.65)	<0.001*	−0.20 (−0.50–0.06)	0.004*	−0.27 (−0.73–0.49)	0.001*
Birthweight <10 th percentile	0	20 (45.5%)	<0.001*	4 (50%)	<0.001*	4 (50%)	<0.001*
Plasma sVEGFR-1 (pg/ml)	1,259(260–5,293)	1,219(291–14,190)		5,307(1,215–9,471)		1,409 (0 – 5,216)	
Delta plasma sVEGFR-1	0.01(−0.46 – 0.65)	0.08 (−0.53 – 1.19)	0.04*	0.78 (−0.13 – 1.02)	<0.001*	0.22 (−2.79 – 0.96)	0.2
High Delta plasma sVEGFR-1 ^γ	5 (3.7%)	8 (18.2%)	0.004*	7 (87.5%)	<0.001*	2 (25%)	0.05

All p values compared to normal pregnancy.

Value expressed as median (range) or number (percent)

GA: gestational age; MOM: multiple of the median

^γ: delta plasma sVEGFR-1 >mean+2sd of normal pregnant women (0.42)

Table V.

Odds ratio of the presence of disease for an each unit increase of log (sVEGFR-1+1) concentration

Model	Dependent variables (vs. normal pregnancy)	p	Odds ratio	95% CI
1	Unexplained fetal death	0.01	6.6	1.6 – 27.7
2	Fetal death with preeclampsia	<0.001	7,157	53 –965,859
3	Fetal death with fetal anomaly	0.9	0.9	0.2 – 3.5

Adjusted for gestational age at blood sampling (weeks) and sample storage interval (days).

Clinical characteristics of the study population

Table VI.

	Preterm Labor Term delivery n = 24	$p\alpha$	Preterm Labor Without IAI Preterm delivery n=62	$p\beta$	Preterm Labor With IAI Preterm delivery n = 16	$p\gamma$
Age (y)	21 (17–39)	0.7	22 (13–37)	0.4	24 (15–30)	0.2
GA at blood sampling (weeks)	31.7 (27.1–33.7)	<0.007*	29.9 (21.0–33.6)	<0.001*	24.1 (20.1–32.0)	<0.001*
GA at delivery (weeks)	38.6 (37–41.4)	<0.001*	31.8 (21.3–36.9)	<0.001*	24.6 (21.0–35.6)	<0.001*
Birthweight (grams)	3060 (2340–3750)	<0.001*	1680 (400–2760) δ	<0.001*	645 (400–2380)	<0.001*
Adjusted birthweight for GA (MOM)	−0.07 (−0.25–0.10)	0.003*	−0.18 (−0.47–0.15) δ	0.01*	−0.08 (−0.24–0.34)	0.9
Birthweight <10 th percentile	6 (25%)	0.4	10 (16.7%) δ	0.1	0	--
Plasma sVEGFR-1 (pg/ml)	1,035(189–2,491)		869(227–11,810)		710(366–1,625)	
Delta plasma sVEGFR-1	0.05(−0.72 – 0.48)	0.8	0.03(−0.60 – 1.12)	0.8	−0.03(−0.21 – 0.39)	0.6
High Delta plasma sVEGFR-1 γ	1 (4.2%)	1.0	2 (3.2%)		0	

p^{α} : compared between preterm labor who delivered at term and preterm labor without IAI who delivered preterm

p^{β} : compared between preterm labor without IAI who delivered preterm and preterm labor with IAI

p^{γ} : compared between preterm labor who delivered at term and preterm labor with IAI Value expressed as median (range) or number (percent); GA: gestational age; MOM: multiple of the median

δ : n=60

γ : delta plasma sVEGFR-1 >mean+2sd of normal pregnant women (0.42)

Table VII.

Clinical characteristics of the study population

	Preterm PROM without IAI n=33	Preterm PROM with IAI n=31	p ^b
Age (y)	26 (16–38)	25 (17–38)	0.4
GA at blood sampling (weeks)	30.7 (21.1–33.6)	30.3 (21.9–33.9)	0.5
GA at delivery (weeks)	31.7 (21.3–34.3)	31.0 (22–34.1)	0.08
Birthweight (grams)	1730 (340–2790)	1410 (400–2340)	0.02*
Adjusted birthweight for GA (MOM)	−0.14 (−0.37–0.22)	−0.19 (−0.56–0.29)	0.09
Birthweight <10 th percentile	1 (3%)	4 (12.9%)	0.2
Plasma sVEGFR-1 (pg/ml)	821 (223–17,440)	1,108 (236–6,775)	
Delta plasma sVEGFR-1	−0.03 (−0.51 – 1.32)	0.09 (−0.56 – 0.87)	0.3
High Delta plasma sVEGFR-1 γ	2 (6.1%)	2 (6.5%)	0.9

Value expressed as median (range) or number (percent)

GA: gestational age; MOM: multiple of the median

γ : delta plasma sVEGFR-1 >mean+2sd of normal pregnant women (0.42)