EVIDENCE IN SUPPORT OF A ROLE FOR ANTI-ANGIOGENIC FACTORS IN PRETERM PRELABOR RUPTURE OF MEMBRANES

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Abstract

Objective—Vaginal bleeding, placental abruption and defective placentation are frequently observed in patients with preterm prelabor rupture of membranes (PROM). Recently, a role of vascular endothelial growth factor (VEGF) and its receptor, VEGF receptor (VEGFR)-1 has been implicated in the mechanisms of membrane rupture. The purpose of this study was to determine whether the soluble form of VEGFR-1 and -2 concentrations in amniotic fluid (AF) change with preterm PROM, intra-amniotic infection/inflammation (IAI) or parturition.

Study Design—This cross-sectional study included 544 patients in the following groups: 1) midtrimester (MT) (n=48); 2) preterm labor (PTL) leading to term delivery (n=143); 3) PTL resulting in preterm delivery with (n=72) and without IAI (n=100); 4) preterm PROM with (n=46) and without IAI. (n=42); 5) term in labor (n=48) and 6) term not in labor (n=45). The concentrations of sVEGFR-1 and sVEGFR-2 were determined by ELISA. Non-parametric statistics and logistic regression analysis were applied.

Results—1) Preterm PROM (with and without IAI) had a lower median AF concentration of sVEGFR-1 than patients with PTL who delivered at term (p<0.001 for each comparison); 2) A decrease in AF sVEGFR-1 concentrations per each quartile was associated with PROM after adjusting for confounders (OR 1.8; 95%CI 1.4-2.3); 3) IAI, regardless of the membrane status, was not associated with a change in the median AF concentrations of sVEGFR-1 and sVEGFR-2 (p>0.05 for each comparison); and 4) Spontaneous term and preterm labor did not change the median sVEGFR-1 and sVEGFR-2 concentrations (p>0.05 for each comparison).

Conclusion—1) This is the first evidence that preterm PROM is associated with a lower AF concentration of sVEGFR-1 than patients with PTL intact membranes. These findings cannot be attributed to gestational age, labor, or IAI; and 2) AF concentrations of sVEGFR-2 did not change with preterm PROM, IAI or labor at term and preterm.

Keywords

sVEGFR-1; sflt-1; sKDR; sVEGFR-2; intraamniotic infection; intraamniotic inflammation; parturition; microbial invasion of the amniotic cavity; MIAC; chorioamnionitis; prematurity; parturition; preterm labor; term labor; preterm PROM; angiogenesis; amniotic fluid

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Introduction

Preterm prelabor rupture of the membranes (PROM) is responsible for 30–40% of preterm deliveries and therefore is a leading cause of preterm birth, as well as a major contributor to perinatal morbidity and mortality worldwide [1–12]. A previous study examining the placental lesions from patients with preterm PROM reported that such placentas have two major pathologic findings: acute inflammatory lesions (acute histologic chorioamnionitis and/or funisitis) and vascular lesions (multiple infarction, intervillous thrombosis, and thrombosis or narrowing of spiral arteries) [13]. The mechanisms by which microbial invasion of the amniotic cavity and inflammation lead to membrane weakening and rupture have been extensively studied [5,12,14–26]. In contrast, a few studies have examined the mechanism responsible for vascular pathology [27–31].

A major risk factor for preterm PROM is vaginal bleeding [32,33]. Women presenting with preterm PROM are also at increased risk of developing placental abruption [34]. Moreover, a failure of physiologic transformation of the myometrial segment of the spiral artery, suggestive of defective placentation, is frequently observed in preterm PROM [35]. However, the precise mechanisms, by which, the vascular changes and disruption of vascular integrity lead to preterm PROM remain unknown.

Pregnancy is a state that requires extensive angiogenesis in both maternal and fetal compartments [36–38]. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. Its primary site of action is vascular endothelium and its main function is to promote angiogenesis, a process by which new vessels form from preexisting vasculature [39], through endothelial cell proliferation, migration [40,41], and prevention of endothelial cell apoptosis [42]. VEGF exerts its action through binding to specific receptors i.e. VEGF receptor-1 and -2 (VEGFR-1 and VEGFR-2) [43–47]. VEGFR-2 regulates endothelial cell division and migration, whereas the function of VEGFR-1 remains unclear and is thought to be a decoy receptor for VEGF. Both receptors of VEGF have soluble forms which contain an extracellular domain without intracellular portion of these proteins. The soluble form of VEGFR-1 has been found to have anti-angiogenic activity by binding to VEGF and preventing VEGF from binding to VEGFR-2 [48,49]. Similarly, under experimental conditions, the recombinant sVEGFR-2 has anti-angiogenic activity [50,51].

Normal pregnancy requires a balance between angiogenic and anti-angiogenic factors. The loss of only one copy of the VEGF gene is uniformly lethal during embryonic life [52,53]. An imbalance of angiogenic and anti-angiogenic factors of VEGF-signaling system in maternal circulation has been implicated in the pathophysiology of several obstetrical syndromes including preeclampsia [54–67], pregnancies with small for gestational age fetuses [66–69], placental abruption [70], fetal death [71,72], twin to twin transfusion syndrome [72], "mirror syndrome" [73], and spontaneous preterm parturition [74].

Both VEGF and their receptors (VEGFR-1 and -2) are expressed in the human amnion. [75,76] The precise role of VEGF and its receptors in the amnion is unknown, however, they have been implicated in the regulation of amniotic fluid volume [77,78]. Recently, VEGF and VEGFR-1 in the fetal membranes have also been suggested to play a role in the pathophysiology of preterm PROM [75]. Since VEGF and sVEGFR-1 are present in human amniotic fluid [59,79,80], it is likely that sVEGFR-1 and sVEGFR-2, the natural antagonists of VEGF, are involved in the regulation of VEGF activity in amniotic fluid cavity and play a role in the maintenance of normal pregnancy. The purpose of this study was to determine whether sVEGFR-1 and sVEGFR-2 concentrations in amniotic fluid change with gestational

age, preterm PROM, intra-amniotic infection/inflammation (IAI) or preterm and term parturition.

Materials and Methods

Study design and population

A cross sectional study was designed by searching our clinical database and bank of biological samples, and included 544 patients in the following groups: 1) women in the midtrimester of pregnancy (14–18 weeks) who underwent amniocentesis for genetic indications and delivered a normal neonate at term (n=48); 2) patients with an episode of spontaneous preterm labor (PTL) and intact membranes who delivered at term (n=143) 3) patients with PTL and intact membranes who delivered preterm (< 37 weeks gestation) with IAI (n=72) and without IAI (n=100); 4) patients with preterm PROM with IAI (n=46) and without IAI (n=42); 5) normal pregnant women at term with spontaneous labor ($n = 48$) and 6) normal pregnant women at term without spontaneous labor ($n = 45$).

All women provided written informed consent prior to collection of amniotic fluid. The collection and utilization of the samples were approved by the Human Investigation Committee of the Sotero del Rio Hospital, Santiago, Chile (a major affiliate of the Catholic University of Santiago), the Institutional Review Boards of the Wayne State University, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS). Many of these samples have been used in previous studies.

Definitions

Preterm PROM was diagnosed by sterile speculum examination confirming pooling of amniotic fluid in the vagina in association with positive nitrazine and ferning tests when necessary, before 37 weeks of gestation and in the absence of labor. Spontaneous preterm labor was defined by the presence of regular uterine contractions occurring at a frequency of at least two every 10 minutes associated with cervical change before 37 completed weeks of gestation that required hospitalization. Intra-amniotic infection was defined as a positive amniotic fluid culture for micro-organisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid interleukin (IL)-6 concentration ≥2.6 ng/ml [82]. Patients were considered to have a normal pregnancy outcome if they did not have any medical, obstetrical, or surgical complications, and delivered a term neonate (≥37weeks) of appropriate birth weight for gestational age [83,84] without complications. Small for gestational age was defined as birthweight below the 10th percentile according to the reference range proposed by Alexander et al [85] or Gonzalez et al.[86] depending on ethnicity.

Sample collection

Amniotic fluid samples were obtained by transabdominal amniocentesis performed for genetic indications, evaluation of microbial status of the amniotic cavity and/or assessment of fetal lung maturity in patients approaching term. Women at term in labor consisted of women who were admitted for suspected PTL because of uncertain dates and had an amniocentesis for the assessment of fetal lung maturity. The criteria for considering that these patients were at term in labor was derived retrospectively, if the following criteria were met: 1) spontaneous labor; 2) delivery within 24h from amniocentesis; 3) analysis of amniotic fluid consistent with fetal lung maturity; 4) birthweight >2500 g; 5) absence of respiratory distress syndrome or other complications of prematurity; and 6) physical examination of the newborn by the pediatricians consistent with a term neonate. Samples of amniotic fluid were transported to the laboratory in a sterile capped syringe and cultured for aerobic/anaerobic bacteria and genital mycoplasmas. White blood cell count, glucose concentration and gram-stain were also performed shortly after collection as previously

described [87–89]. The results of these tests were used for clinical management. Amniotic fluid IL-6 concentrations were used only for research purposes. Amniotic fluid not required for clinical assessment was centrifuged for 10 min at 4°C and the supernatant was stored at −70°C until analysis.

Determination of soluble VEGFR-1 and VEGFR-2 immunoassay in amniotic fluid

Amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 were determined by sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN, USA). The immunoassay systems were validated for using human amniotic fluid prior to conduction of this study. Briefly, the immunoassay utilized the quantitative sandwich enzyme immunoassay technique and their concentrations were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation for sVEGFR-1 were 1.4% and 3.9% respectively; and for sVEGFR-2, 2% and 4%, respectively. The sensitivities of the assays were 0.016 ng/ml for sVEGFR-1, and 0.019 ng/ml for sVEGFR-2.

Statistical analysis

The normality of the data was tested using Shapiro-Wilk and Kolmogorov-Smirnov tests. Because amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 were not normally distributed, even after logarithmic transformation, non-parametric statistics were used for analysis. Comparisons between proportions were performed with contingency tables, Chi-square and Fisher's Exact test. Kruskal-Wallis and Mann-Whitney U tests were used to determine the differences of the median among and between groups, respectively. Multivariate logistic regression (step-wise) was applied to examine the association between amniotic fluid concentrations of sVEGFR-1 and the presence of preterm PROM while adjusting for potential confounders. Spearman rank correlation was utilized to assess correlations 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

Demographic and clinical characteristics of the study population

Table I presents the demographic and clinical characteristics of patients in the midtrimester, term not in labor and term in labor groups. Table II and III display the demographic and clinical characteristics of patients with spontaneous PTL and intact membranes and those with preterm PROM, respectively. Among patients with PTL with intact membranes, those with IAI had a significantly lower median gestational age at amniocentesis than those without IAI who delivered preterm and those who delivered at term (p<0.001 for all; Table II). Similarly, the median gestational age at amniocentesis of patients with preterm PROM with IAI was lower than that of those without IAI (p=0.02; Table III).

Changes of amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 in pregnancy

While sVEGFR-1 was detected in all samples, sVEGFR-2 was detected in 99% (541/544) of patients. Three amniotic fluid samples (two from patients with PTL who delivered at term and one from a patient at term in labor) had sVEGFR-2 concentrations below the limit of detection of the immunoassay. Women with PTL and intact membranes who delivered at term had a significantly higher median amniotic fluid sVEGFR-1 concentration (median 101 ng/ml; range 0.1-595.6 ng/ml) than those in the midtrimester (median 33 ng/ml; range 10.7-74.3 ng/ml; p<0.001) and than those at term not in labor (median 64 ng/ml; range 10.6-373.4 ng/ml; p=0.01; Figure 1). Similar to sVEGFR-1, the changes of amniotic fluid concentrations of sVEGFR-2 as a function of gestational age followed the same trend as sVEGFR-1. Women with PTL who delivered at term had a significantly higher median

amniotic fluid sVEGFR-2 concentration (median 0.7 ng/ml; range 0–3.7 ng/ml) than those in the midtrimester (median 0.4 ng/ml; range 0.1–1.5 ng/ml; p=0.002) and than those at term not in labor (median 0.5 ng/ml; range 0.1–9.3 ng/ml; p=0.03; Figure 2). Collectively, the amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 increased from the midtrimester to approximately 24–28 weeks of gestation (sVEGFR-1: median 140.6 ng/ml; range 1.4–454 ng/ml; and for sVEGFR-2: median 1.2 ng/ml; range 0.2–3.9 ng/ml), and then declined until term.

Preterm PROM (with and without IAI) is associated with a decrease in amniotic fluid concentrations of sVEGFR-1

There was no significant difference in the median gestational age at amniocentesis between patients with PTL who delivered at term and those with preterm PROM with and without IAI (p>0.05 for each comparison). Patients with preterm PROM, regardless of IAI, had a significantly lower median amniotic fluid concentration of sVEGFR-1 than those with PTL who delivered at term (preterm PROM without IAI: median 56 ng/ml; range: 8.9–398.2 ng/ml, preterm PROM with IAI: median 45 ng/ml; range: 1.4–255.6 ng/ml and PTL who delivered at term: median 101 ng/m; range: 0.1–595.6 ng/ml; p=0.002 and p<0.001, respectively; Figure 3). In contrast, there was no significant difference in the median amniotic fluid sVEGFR-2 concentrations among the three groups (preterm PROM without IAI: 0.7 ng/ml; range: 0.1–2.0 ng/ml, preterm PROM with IAI: 0.6 ng/ml; range: 0.1–3.7 ng/ml, and PTL who delivered at term: 0.7 ng/ml; range: 0.0–3.7 ng/ml; p=0.8 and p=0.5, respectively; Figure 4).

Among patients with preterm gestations (PTL and preterm PROM), the frequency of preterm PROM increased as amniotic fluid concentrations of sVEGFR-1 decreased from the 4th to the 1st quartile (Q4: 9.9% (10/101), Q3: 11.8% (12/102), Q2: 28% (28/100) and Q1: 38% (38/99); chi-square for trend p<0.001). The association between a decrease in amniotic fluid sVEGFR-1 concentrations per quartile and preterm PROM remained significant after adjusting for gestational age at amniocentesis (weeks), maternal age (years), smoking, nulliparity, the presence of small-for-gestational age neonates, amniotic fluid concentrations of IL-6 (ng/ml), and duration of sample storage (years) (OR 1.8; 95% CI 1.4–2.4).

Intra-amniotic infection/inflammation is not associated with changes in amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2

Intra-amniotic infection/inflammation in patients with preterm PROM did not significantly change the median amniotic fluid concentration of sVEGFR-1 (p=0.3; Figure 3) or that of sVEGFR-2 (p=0.5; Figure 4). Similarly, among patients with PTL who delivered preterm, there was no significant difference in the median amniotic fluid concentration of sVEGFR-1 or sVEGFR-2 between patients with and without IAI (sVEGFR-1; PTL without IAI: median 99.4 ng/ml; range: 0.7–459.0 ng/ml vs. PTL with IAI: median 98.7 ng/ml; range: 2.0–544.8 ng/ml; p=0.1; Figure 5; and sVEGFR-2; PTL without IAI: median 0.6 ng/ml; range: 0.2–3.3 ng/ml vs. PTL with IAI: median 0.8 ng/ml; range: 0.1–3.9 ng/ml; p=0.2; Figure 6). There was no association between amniotic fluid concentrations of sVEGFR-1 or sVEGFR-2 with IAI in either patients with PTL or those with preterm PROM after adjusting for gestational age at amniocentesis by logistic regression analysis (p>0.05).

Labor in preterm or term gestation is not associated with changes in amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2

There was no significant difference in the median amniotic fluid concentration of sVEGFR-1 between women with PTL who delivered preterm and those who delivered at term (p=0.08; Figure 5). Similar results were observed for sVEGFR-2. (p=0.8; Figure 6).

There was no significant difference in the median amniotic fluid concentration of sVEGFR-1 and sVEGFR-2 between women at term with and without labor (sVEGFR-1; term in labor 65 ng/ml range 4.7-243.8 ng/ml vs. term not in labor median 64 ng/ml, range 10.6-373.4 ng/ml; p=0.6; Figure 7 and for sVEGFR-2; term in labor median 0.7 ng/ml, range 0.0–7.9 ng/ml vs. term not in labor median 0.5 ng/ml, range 0.1–9.3 ng/ml; p=0.5; Figure 8).

Discussion

Principal findings of this study

1) Preterm PROM, regardless of the presence or absence of IAI was associated with lower median amniotic fluid concentrations of sVEGFR-1, but not sVEGFR-2 than pregnancies with PTL with intact membranes; 2) this association had a dose-response relationship as described by an increased risk of preterm PROM with descending quartiles of sVEGFR-1 concentrations in amniotic fluid; 3) the soluble forms of VEGFR-1 and VEGFR-2 are physiologic constituents of amniotic fluid and their concentrations change with gestational age, with the highest concentration observed at 24-28 weeks of gestation; and 4) amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 did not change with intra-amniotic infection/inflammation or spontaneous labor in preterm or term gestation.

Changes of amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 in pregnancy

In this study we described the changes of sVEGFR-1 and sVEGFR-2 concentrations in amniotic fluid as a function of gestational age. The concentrations of both soluble receptors increased from midtrimester until 24-28 weeks, and then decreased as the gestational age approached term. Since both soluble forms of VEGF can bind to and inhibit VEGF, the changes of amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 with gestational age could reflect the changes of VEGF concentrations in the amniotic fluid cavity. The role of VEGF in amniotic fluid cavity during pregnancy remains unclear but could be related to fetal growth, placental development [90] and the regulation of amniotic fluid volume [91].

The sources of sVEGFR-1 and sVEGFR-2 in amniotic fluid are unknown. The concentration of sVEGFR-1 in normal pregnancy at term was the highest in the amniotic fluid (median 51,040 pg/ml) compared to those observed in maternal blood (median 3,417 pg/ml) [92] with the lowest concentration observed in umbilical artery serum (mean 188 pg/ml) [93]. The results for sVEGFR-1 concentrations in amniotic fluid of normal pregnant women at term not in labor (median 64 ng/ml) in this study were within the same range as those reported by others [92,93]. The concentration gradients of sVEGFR-1 do not support a maternal or fetal origin for sVEGFR-1 in amniotic fluid. It is possible that sVEGFR-1 in amniotic fluid could be derived from the placenta, fetal membranes or uterine decidua. In contrast, the concentration of sVEGFR-2 in normal pregnancy at term was the highest in maternal blood (mean 6.4 ng/ml) and in umbilical vein serum (mean 6.8 ng/ml) [93] with the lowest concentration observed in the amniotic fluid (median 0.5 ng/ml at term, from our study). These observations indicate that sVEGFR-2 in amniotic fluid may be derived from different sources than those of sVEGFR-1, or that the regulation of the expression of these soluble receptors in amniotic cavity is different.

Preterm PROM is associated with decreased amniotic fluid concentrations of sVEGFR-1, but not sVEGFR-2

The findings that women with preterm PROM, regardless of IAI, had lower amniotic fluid concentrations of sVEGFR-1 than those with PTL with intact membranes are consistent with a previous study demonstrating that the expression of VEGF gene was increased in the fetal membranes and decidua of patients with preterm PROM regardless of the inflammatory status [80]. It has been proposed that localized over-expression of VEGF gene in the fetal

membranes plays a role in induction and activation of matrix metalloproteinase through tissue plasminogen activator [80]. It is possible that the decreased amniotic fluid concentrations of sVEGFR-1 led to increased free VEGF concentrations/activity in amniotic fluid cavity. VEGF, in turn, would stimulate its own expression in the fetal membranes and induce the activation of matrix-degrading enzymes leading to membrane rupture [48,94].

Alternatively, a decrease in amniotic fluid concentrations of sVEGFR-1 in patients with preterm PROM could be a consequence of degradation of sVEGFR-1 by increased concentrations of matrix metalloproteinases (MMPs), a group of enzymes implicated in the mechanism of membrane rupture. The degradation of sVEGFR-1 by MMPs has been demonstrated for MMP-7, MMP-2 and MMP-9 [15,23,26,95–97]. Previous studies have shown that preterm PROM was associated with increased amniotic fluid concentrations of MMP-1, MMP-8 and MMP-9 and decreased amniotic fluid MMP-2 concentrations [15,16,20,23,26,95–97]. However, the finding that amniotic fluid sVEGFR-1 concentrations in preterm PROM were decreased regardless of the inflammatory status of the amniotic cavity contradicts this hypothesis. Since IAI was also associated with elevated MMPs concentrations in amniotic fluid [16,23,26], and thus, preterm PROM with IAI would have lower amniotic fluid sVEGFR-1 concentrations than those without IAI. Future studies focusing on the temporal relationships between sVEGRF-1 and related MMPs in amniotic fluid could help to clarify this issue.

Intra-amniotic infection/inflammation is not associated with changes in amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2

VEGF is 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 [49]. Angiogenesis requires the participation of hematopoietic progenitors, endothelial progenitors, and inflammatory cells [49,98]. Monocytes express VEGFR-1 and the migration of monocytes in response to VEGF requires the tyrosine kinase domain of VEGFR-1 (membrane isoform) [99]. Moreover, monocytes from healthy donors can release the soluble form of VEGFR-1 [100]. Recently, angiogenic/anti-angiogenic factors have been implicated in the pathophysiology of sepsis [101–105]. In experimental models of sepsis (endotoxemia and/or cecal ligation puncture) [103–105] and observational studies in septic patients [101,102,106], an increase in the plasma concentrations of VEGF [101,104,106], placental growth factor (PlGF) [105], and sVEGFR-1 [102,103] has been reported. The changes in sVEGFR-1 are considered to be an adaptive response to infection and to have survival value [103,105]. Indeed, the administration of adenovirus encoding for sVEGFR-1 [104] or exogenous sVEGFR-1 [103] attenuates the inflammatory response and reduces morbidity/mortality in mice [103,104]. Furthermore, an 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 [107]. Collectively, these observations suggest a role of VEGF and VEGFR-1 in the mechanisms of maternal infection/inflammation. Our study did not find significant changes of amniotic fluid sVEGFR-1 and sVEGFR-2 concentrations in intra-amniotic infection/inflammation. It is possible that the amniotic fluid white blood cells (which originate from the fetus), in contrast to adult monocytes, did not release sVEGFR-1 upon stimulation with proinflammatory cytokines or endotoxin.

Spontaneous labor in preterm or term gestations is not associated with changes in amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2

VEGF has been implicated in the mechanisms of human parturition. Indeed, a previous study using microarrays demonstrated up-regulation of several angiogenic factors, including VEGF, in both membranes and choriodecidual tissue in women with term labor compared

with those without labor [108]. Another study also found the increased expression of VEGF mRNA in the fetal membranes in women at term in labor, a process thought to be an inflammatory-like condition [109]. Our study did not find significant changes of amniotic fluid sVEGFR-1 and sVEGFR-2 concentrations in human parturition both in preterm and term gestation.

Strengths and limitations

This is the first study to report the changes of amniotic fluid concentrations of sVEGFR-1 in patients with preterm PROM. Moreover, the finding of a dose-response relationship between amniotic fluid concentrations of sVEGFR-1 and preterm PROM strengthens the importance of the observation.

There are two potential limitations of this study. First, since performing amniocentesis in normal pregnant women without any indications is not ethical, the control group for preterm PROM included patients with PTL without IAI who delivered at term. This is the best control group that can be obtained in pregnant women. Second, this study is cross-sectional in nature, and thus, the temporal relationship between the change of amniotic fluid sVEGFR-1 concentrations and preterm PROM cannot be established. It remains to be determined whether or not the decrease in amniotic fluid concentrations of sVEGFR-1 precedes or follows the rupture of fetal membranes.

We conclude that preterm PROM is associated with a decrease in amniotic fluid concentrations of sVEGFR-1. The soluble form of VEGF receptors (sVEGFR-1 and 2) are physiologic constituents of human amniotic fluid cavity and their concentrations change with gestational age. However, amniotic fluid concentrations of sVEGFR-1 and sVEGFR-2 are relatively stable since they did not change with IAI or labor at term or preterm.

Acknowledgments

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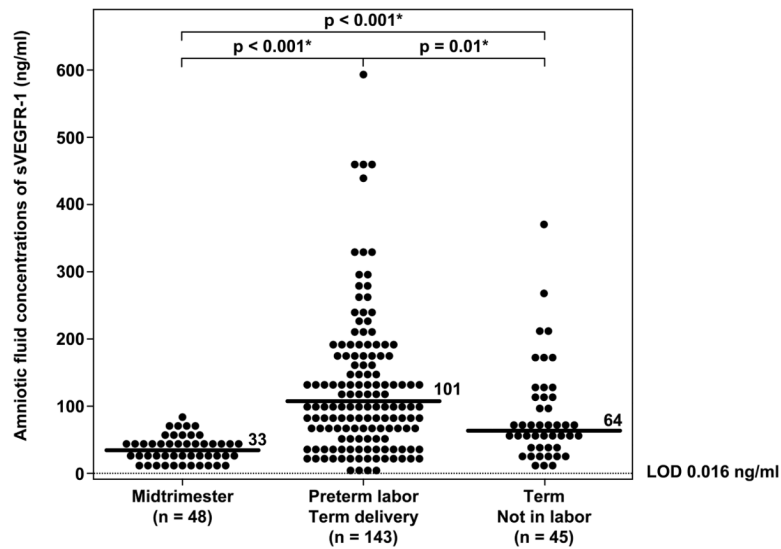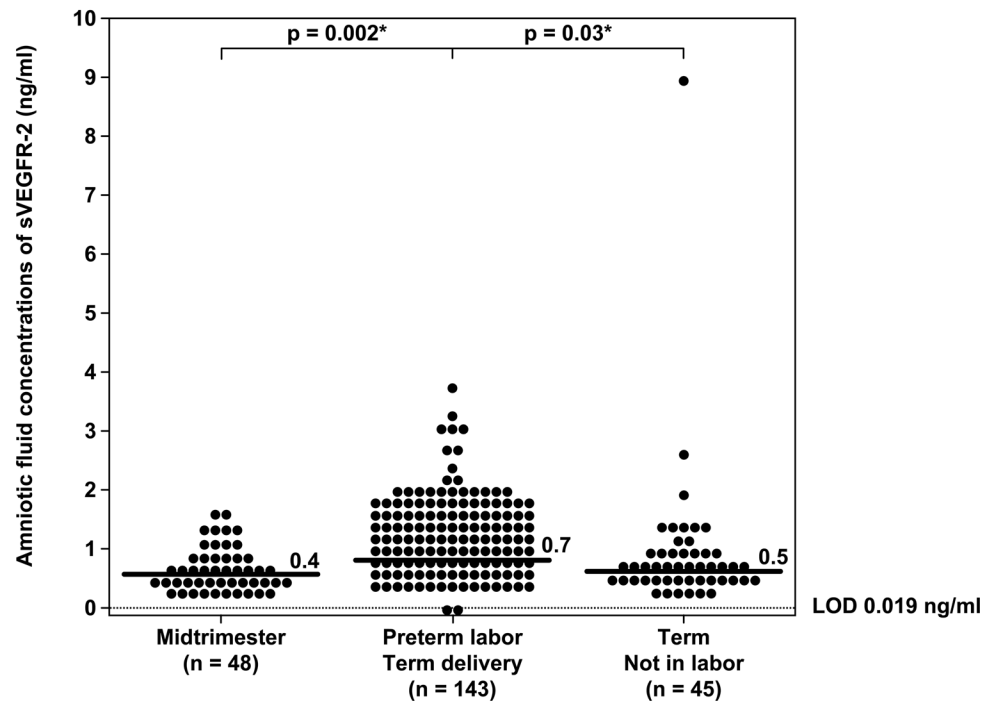
Figure 1.
Amniotic fluid concentrations of sVEGFR-1 in normal pregnant women at midtrimester, patients with preterm labor (PTL) with intact membranes who delivered at term, and women at term without labor. Patients with PTL who delivered at term (median: 101 ng/ml; range 0.1–595.6 ng/ml) had a significantly higher median sVEGFR-1 concentration in amniotic fluid than women in the midtrimester (median: 33 ng/ml; range: 10.7–74.3 ng/ml; p<0.001) and than those at term not in labor (median: 64 ng/ml; range: 10.6–373.4 ng/ml; p=0.01). Women at term not in labor had a significantly higher median amniotic fluid concentration of sVEGFR-1 than those in the midtrimester (p<0.001). LOD: limit of detection. *: p<0.05

Figure 2.
Amniotic fluid concentrations of sVEGFR-2 in normal pregnant women at midtrimester, patients with PTL with intact membranes who delivered at term, and women at term without labor. Patients with PTL who delivered at term (median: 0.7 ng/ml; range: 0–3.7 ng/ml) had a significantly higher median amniotic fluid concentration of sVEGFR-2 than women in midtrimester (median: 0.4 ng/ml; range: 0.1–1.5 ng/ml; p=0.002) and than women at term not in labor (median: 0.5 ng/ml; range: 0.1–9.3 ng/ml; p=0.03). There was no significant difference in the median amniotic fluid concentration of sVEGFR-2 between normal pregnant women at term not in labor and those in the midtrimester (p=0.4). LOD: limit of detection. *: p<0.05
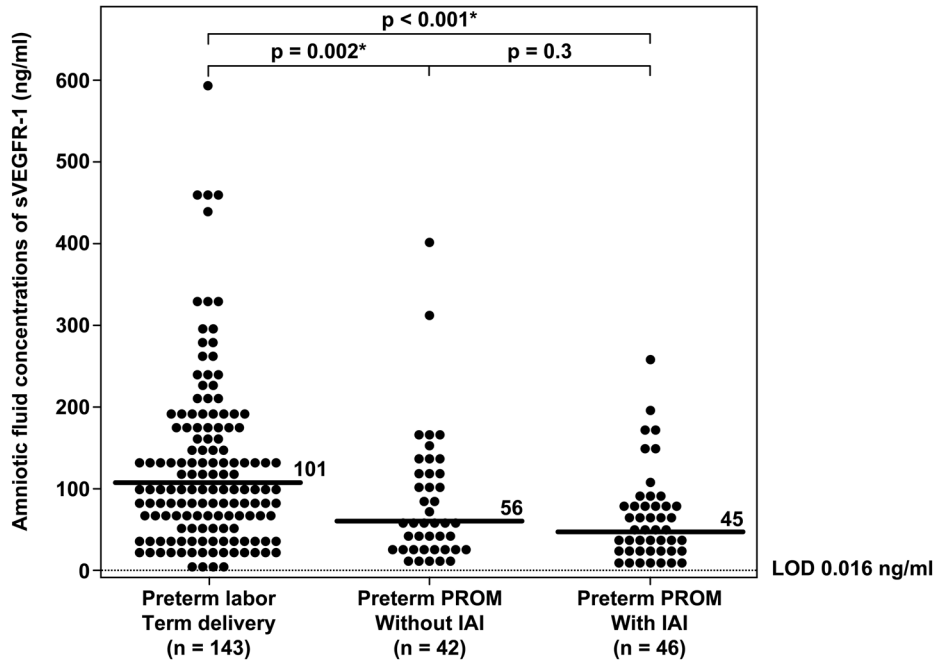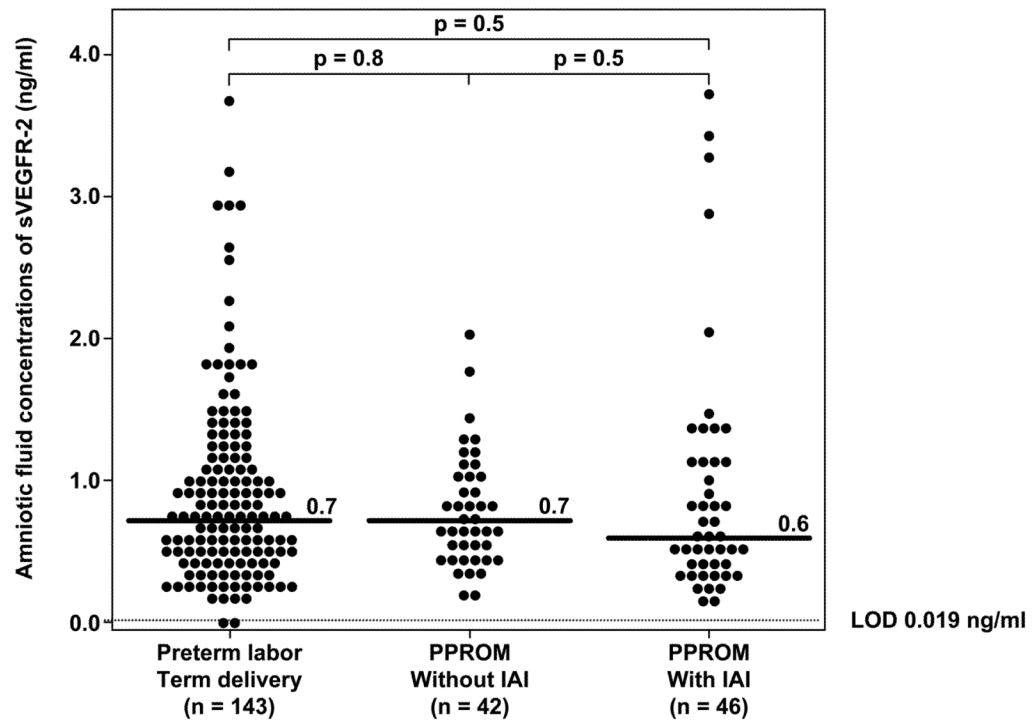
Figure 3.
Amniotic fluid concentrations of sVEGFR-1 in patients with preterm labor
(PTL) and intact membranes who delivered at term, and in those with preterm prelabor
rupture of membranes (PROM) with and without intra-amniotic infection/inflammation
(IAI). Patients with preterm PROM with (median: 45 ng/ml; range:1.4–255.6 ng/ml) and
without IAI (median: 56 ng/ml; range: 8.9–398.2 ng/ml) had a significantly lower median
amniotic fluid concentration of sVEGFR-1 than those with PTL who delivered at term
(median: 101 ng/ml; range: 0.1–595.6 ng/ml) (p<0.001 and p=0.002; respectively).
Amniotic fluid sVEGFR-1 concentrations did not change with the presence of IAI (p=0.3).
LOD: limit of detection. *: p<0.05

Figure 4.
Amniotic fluid concentrations of sVEGFR-2 in patients with preterm labor (PTL) and intact membranes who delivered at term, and in those with preterm prelabor rupture of membranes (PROM) with and without intra-amniotic infection/inflammation (IAI). There were no significant differences in the median amniotic fluid sVEGFR-2 concentrations between patients with PTL who delivered at term (median: 0.7 ng/ml; range: 0.0–3.7 ng/ml) and those with preterm PROM without IAI (median: 0.7 ng/ml; range: 0.1–2.0 ng/ml; p=0.8) and between patients with PTL and those with preterm PROM with IAI (median: 0.6 ng/ml; range: 0.1–3.7 ng/ml; p=0.5). LOD: limit of detection.
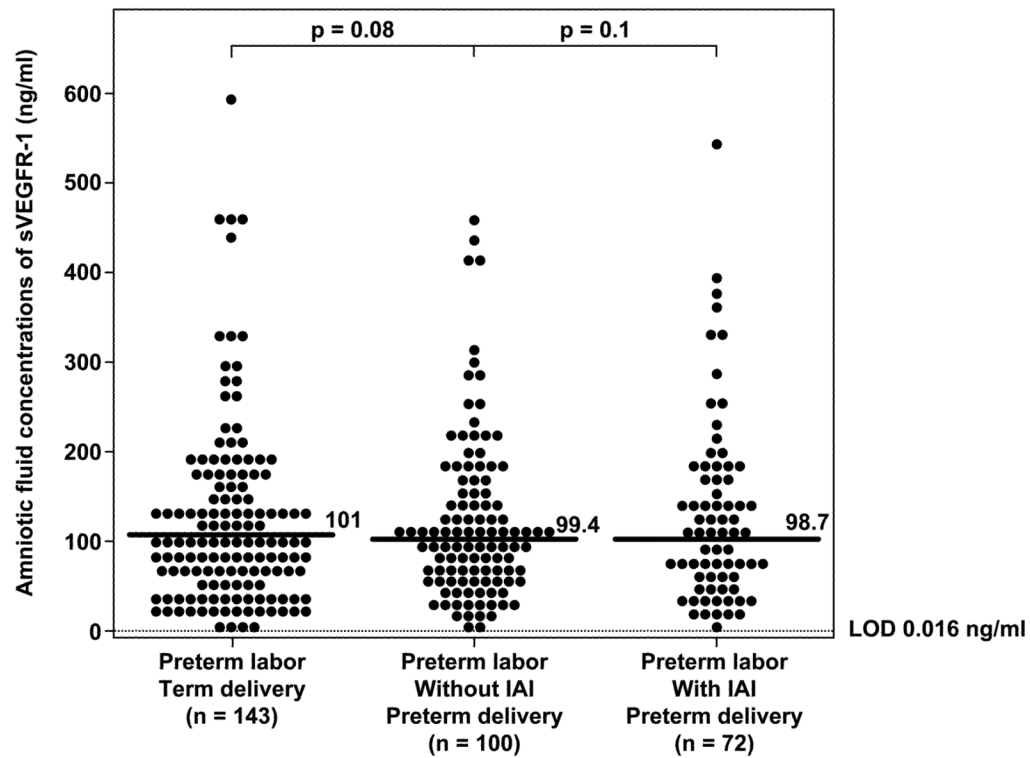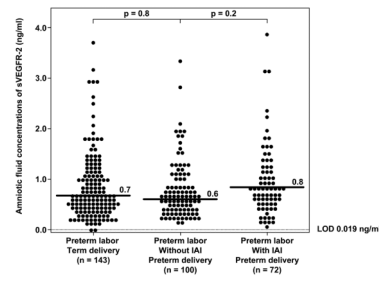
Figure 5.
Amniotic fluid concentrations of sVEGFR-1 among women with spontaneous preterm labor (PTL) and intact membranes. There were no significant differences in the median amniotic fluid sVEGFR-1 concentrations among the subgroups of patients with PTL (PTL with term delivery: median: 101 ng/ml; range: 0.1–595.6 ng/ml; PTL who delivered preterm without IAI: median: 99.4 ng/ml; range: 0.7–459 ng/ml; PTL who delivered preterm with IAI: median: 98.7 ng/ml; range: 2.0–544.8 ng/ml; all p>0.05). LOD: limit of detection.
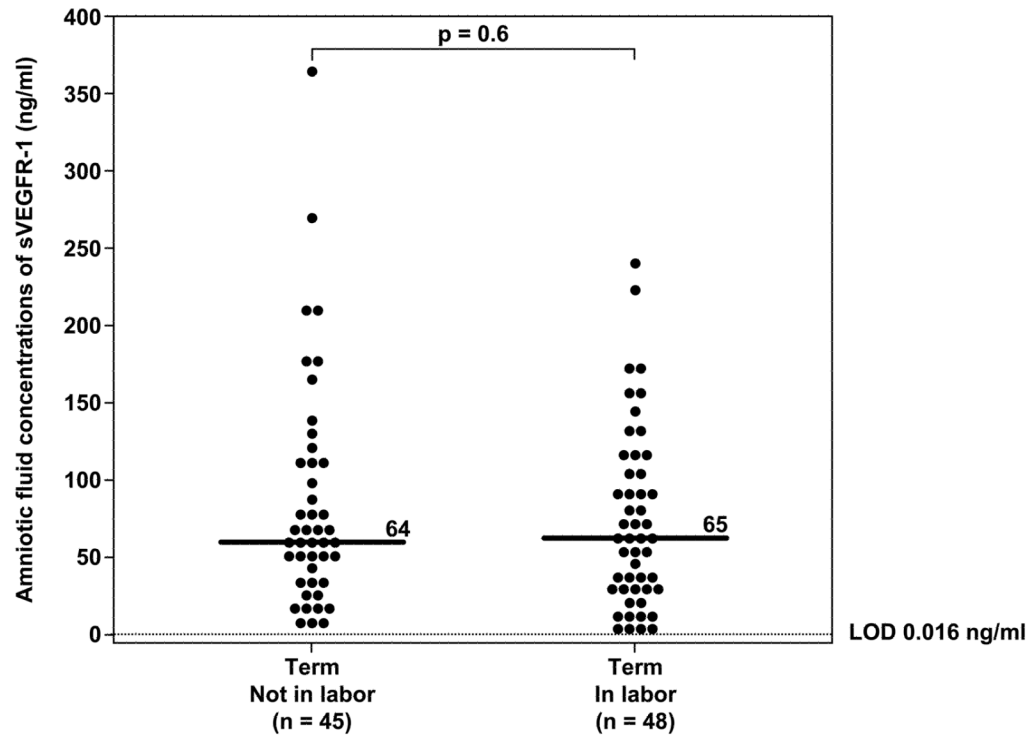
Figure 6.
Amniotic fluid concentrations of sVEGFR-2 among women with spontaneous preterm labor (PTL) and intact membranes. There were no significant differences in the median amniotic fluid sVEGFR-2 concentrations among the subgroups of patients with PTL (PTL with term delivery: median: 0.7 ng/ml; range: 0.0–3.7 ng/ml; PTL who delivered preterm without IAI: median: 0.6 ng/ml; range: 0.2–3.3 ng/ml; PTL who delivered preterm with IAI: median: 0.8 ng/ml; range: 0.1–3.9 ng/ml; all p>0.05). LOD: limit of detection.

Figure 7.
Amniotic fluid concentrations of sVEGFR-1 in normal pregnant women at term with and without labor. There was no significant difference in the median amniotic fluid sVEGFR-1 concentrations between patients with spontaneous labor and those not in labor (term in labor: median: 65 ng/ml; range: 4.7–243.8 ng/ml; vs. term not in labor: median: 64 ng/ml; range: 10.6-373.4 ng/ml; p=0.6). LOD: limit of detection.
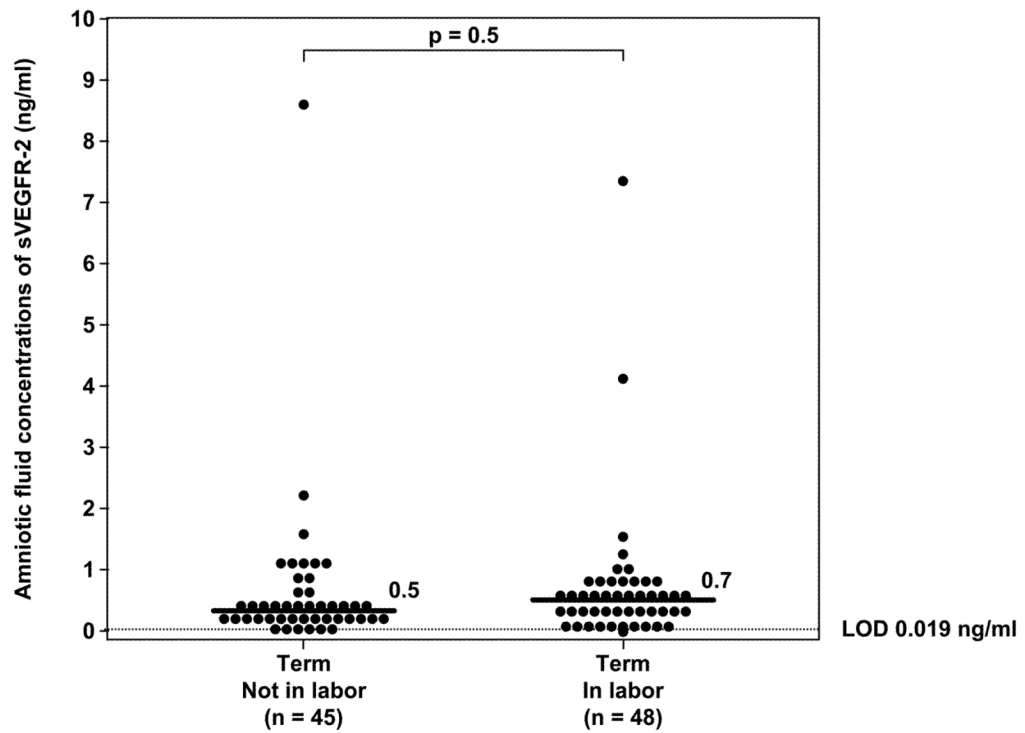
Figure 8.
Amniotic fluid concentrations of sVEGFR-2 in normal pregnant women at term with and
without labor. There was no significant difference in the median amniotic fluid sVEGFR-2
concentrations between patients with spontaneous labor at term and those at term not in
labor (term in labor: median: 0.7 ng/ml; range: 0.0-7.9 ng/ml; vs. term not in labor: median:
0.5 ng/ml; range: 0.1-9.3 ng/ml; p=0.5). LOD: limit of detection.

TABLE I

Demographic and clinical characteristics of patients in the midtrimester and those at term with and without spontaneous labor

	Midtrimester (n=48)	p[a]	Term no labor (n=45)	Term in labor (n=48)	p[b]
Maternal age(years)	36 (24–42)	<0.001	27 (14–40)	22 (16–35)	0.004
GA at amniocentesis (wks)	16 (14–18)	<0.001	38.5 (36.5–42)	38.4 (37–41)	0.7
GA at delivery(wks)	39 (37–41)	0.02	38.5 (36.5–42)	38.4 (37—41)	0.7
Birthweight (g)	3331.5 (2809–4180)	0.5	3250 (2500–4530)	3355 (2180–3930)	0.9

Values are expressed as median and range

GA: gestational age

p[a] : comparison between patients in the midtrimester and those at term not in labor

p[b] : comparison between patients at term not in labor and those at term in labor

TABLE II

Demographic and clinical characteristics of patients presenting with spontaneous preterm labor with intact membranes

	PTL without IAI term delivery (n=143)	p	PTL without IAI preterm delivery (n=100)	p^a	PTL with IAI preterm delivery (n=72)	p^b
Maternal age(years)	22 (14–42)	0.9	22 (14–40)	0.7	23 (15–41)	0.8
GA at amniocentesis (wks)	32 (20.3–36)	0.7	31.9 (23.7–34.4)	<0.001	28.2 (20.3–34.4)	<0.001
GA at delivery(wks)	38.7 (37–43)	<0.001	34.6 (26.9–36.9)	<0.001	29.2 (20.4–36)	<0.001
Birthweight (g)	3180 (2390–4750)	<0.001	2335 (800–3560)	<0.001	1190 (280–2740)	<0.001

Values are expressed as median and range

p: comparison between PTL who delivered at term and PTL withour IAI

p^a: comparison between PTL who delivered preterm without IAI and PTL with IAI

p^b: comparison between PTL who delivered at term and PTL with IAI

PTL: preterm labor; GA: gestational age;

IAI: intraamniotic infection/inflammation

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