

Angiogenic and Antiangiogenic Factors in Preterm Neonates Born to Mothers with and without Preeclampsia

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Abstract

Background Angiogenic and antiangiogenic factors are altered in pregnant women with preeclampsia (PE), but the pattern of expression of these factors in their newborns remains unknown.

Objective This study aims to measure vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) levels in preterm neonates born to mothers with PE.

Methods Neonates with birth weight < 2,000 g and gestational age ≤ 34 weeks were included and divided into the following two groups: born to mothers with PE and without PE. Blood was collected from neonates within the first 72 hours of life. VEGF and sFlt-1 levels were measured using the enzyme-linked immunosorbent assay method.

Results A total of 88 neonates were included (37 born to mothers with PE and 51 born to mothers without PE), with a mean gestational age of 29.12 ± 2.96 weeks and birth weight of $1,223.80 \pm 417.48$ g. In the multivariate analysis, VEGF was 80% lower and sFlt-1 was 13.48 times higher in the group with PE. sFlt-1 concentration was higher in neonates small for gestational age (SGA) than in those appropriate for gestational age.

Conclusion Higher sFlt-1 and lower VEGF levels in the group with PE, as well as higher sFlt-1 levels in SGA neonates, reflect a predominance of antiangiogenic mechanisms in PE and growth restriction.

Keywords

- ▶ angiogenic factors
- ▶ preeclampsia
- ▶ prematurity
- ▶ VEGF
- ▶ sFlt-1
- ▶ antiangiogenic factors

Preeclampsia (PE) is a multisystem disorder characterized by abnormal vascular response to placentation, which is associated with increased systemic vascular resistance and platelet aggregation and with endothelial cell dysfunction. Proper angiogenesis is essential for the development of the placental vasculature. Its regulation is associated with the balance between angiogenic and antiangiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble VEGF receptor 1 (sVEGFR-1).^{1,2}

VEGF is a glycoprotein with potent angiogenic and mitogenic activities and the ability to increase the vascular permeability of endothelial cells.³ During pregnancy, VEGF is overproduced by the placenta.⁴ PlGF is a protein in the VEGF family with similar angiogenic activity, but with weak mitogenic and chemoattractant properties as compared with VEGF. In cells expressing both VEGF and PlGF, formation of VEGF/PlGF heterodimers occurs. The VEGF/PlGF heterodimer is known to be 20- to 50-fold less potent than the VEGF homodimer.⁵ In a recent study, we found higher VEGF/PlGF

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heterodimer levels in preterm newborns of preeclamptic mothers.⁶

sVEGFR-1, also known as soluble fms-like tyrosine kinase-1 (sFlt-1), is a circulating antiangiogenic protein that binds to VEGF and inhibits its biological activities. Although sFlt-1 is expressed in endothelial cells and monocytes, it is mainly produced by the placenta during pregnancy.⁷

In the setting of PE, there is poor trophoblast invasion into the maternal spiral arteries with consequent reduction in placental perfusion, leading to placental and fetal hypoxia. In women with established PE, VEGF, and PlGF concentration are reduced and sFlt-1 concentration is elevated,^{8–14} whereas in normotensive women sFlt-1 concentration is at a lower level than in preeclamptic women.^{15–18}

A few studies have compared levels of angiogenic and antiangiogenic factors (VEGF and sFlt-1) in mothers with and without PE or in umbilical cord blood of their newborns.^{7,8} However, although it is known that VEGF and sFlt-1 levels are altered in preeclamptic pregnant women, no studies have been performed to detect or analyze the pattern of expression of these factors in peripheral blood from preterm neonates born to mothers with PE. Therefore, this study aimed to measure VEGF and sFlt-1 levels in preterm neonates born to mothers with PE.

Material and Methods

The study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre, Brazil (protocol no. 11-0281). Written informed consent was obtained from the neonates' parents or legal guardians before their inclusion in the study.

The study included neonates admitted to the neonatal intensive care unit with birth weight < 2,000 g and gestational age \leq 34 weeks, born to mothers with and without PE. Exclusion criteria were as follows: (1) the neonate was transferred from another institution after 72 hours of life; (2) the neonate died before blood collection; (3) neonates with major congenital anomalies, inborn errors of metabolism, or congenital infections (STORCH screen); (4) neonates born to HIV positive mothers; (5) multiple pregnancies; and (6) mothers with autoimmune disease.

PE was defined as hypertension (blood pressure \geq 140/90 mm Hg on two separate readings) after 20 weeks' gestation in patients with no previous history of hypertension, accompanied by proteinuria (\geq 300 mg/L).¹⁹ Neonates small for gestational age (SGA) were defined as those below the 10th percentile, according to the growth curve developed by Alexander et al.²⁰ Sepsis was defined as a positive blood culture, with clinical evidence of infection (changes in breathing pattern, hypo/hyperthermia, and circulatory or gastrointestinal symptoms). Neutropenia was defined as absolute neutrophil count less than 1,000/mm³. Retinopathy of prematurity (ROP) was classified according to Brazilian guidelines for screening and treatment of ROP.²¹ Bronchopulmonary dysplasia (BPD) was considered when the newborn was oxygen dependent for more than 28 days, and had a chest X-ray compatible with BPD.

Blood samples were collected from all neonates within the first 72 hours of life. No samples were collected exclusively for the study; an additional 1 mL of blood was collected during routine blood draws. All neonates were followed up on their development from inclusion in the study until hospital discharge or death.

Blood samples were drawn into tubes containing ethylenediaminetetraacetic acid and centrifuged at 5,000 rpm for 10 minutes at 4°C. Plasma was separated and stored at -80°C in Eppendorf tubes. VEGF and sFlt-1 measurements were performed using the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems Inc, Minneapolis, MN), as performed in previous studies.^{7,8,22} The limit of detection for VEGF was 5 pg/mL, with an intra- and interassay coefficient of variation of 6.7 and 8.8%, respectively. The limit of detection for sFlt-1 was 5 pg/mL with an intra- and interassay coefficient of variation of 3.8 and 7.0%, respectively. All measurements were performed in duplicate. All samples were processed and analyzed in the laboratory of molecular biology of the institution.

Sample size calculation was based on the study by Catarino et al.⁷ Assuming a level of significance of 5 and 95% power, a minimum sample of 60 preterm neonates was required, with 30 patients in each group (with and without PE).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$. Data are expressed as median and interquartile range or mean and standard deviation. Statistical differences between groups were determined by the chi-square test, *t* test, or Mann-Whitney test. Factors found to be significant ($p < 0.05$) in the univariate analysis were used as control variables in the multivariate analysis to compare groups. Serial VEGF and sFlt-1 measurements were compared using a generalized estimating equation with gamma distribution and a log link function (after multiple comparisons with Bonferroni correction). Graphs were presented as a bee swarm plot using R package version 0.1.1.²³

Results

A total of 88 neonates were included in the study (37 born to mothers with and 51 born to mothers without PE), with a mean gestational age of 29.12 ± 2.96 weeks and mean birth weight of $1,223.80 \pm 417.48$ g. The group with PE showed lower gestational age, shorter duration of premature rupture of membranes (PROM), and higher incidence of cesarean delivery and of SGA than the control group (**Table 1**).

Plasma VEGF levels were lower in neonates born to mothers with PE (32.45 pg/mL [6.36–85.75]) than in controls (82.38 pg/mL [35–130.03]) ($p = 0.001$) (**Fig. 1**), while sFlt-1 levels were higher in neonates born to mothers with PE (1338.57 pg/mL [418.8–3472.24]) than in controls (318.13 pg/mL [182.03–453.66]) ($p < 0.001$) (**Fig. 2**). These findings remained significant in the multivariate analysis, with VEGF levels 80% lower and sFlt-1 levels 13.48 times higher in neonates born to mothers with PE (**Tables 2 and 3**). VEGF and sFlt-1 were analyzed separately in the multivariate

Table 1 Characteristics of preterm neonates according to the presence of maternal preeclampsia

	Preeclampsia (n = 37)	Control (n = 51)	p
GA (wks) ¹	30.81 ± 2.34	29.43 ± 2.80	0.014
PROM (h) ²	0 (0–0)	0 (0–9.5)	0.001
Cesarean delivery [n (%)] ³	35 (94.6)	23 (45.1)	< 0.001
Birth weight (g)	1,215.54 ± 423.08	1,229.78 ± 417.48	0.876
Male	18 (48.6%)	24 (47.1%)	1
APGAR < 7 at 5 min	10 (27%)	7 (13.7%)	0.198
SGA	26 (70.3%)	11 (21.6%)	< 0.001
SNAPPE II	18 (2.5–47)	14 (0–27)	0.231
Use of CPAP	19 (51.4%)	21 (41.2%)	0.466
Use of MV	17 (45.9%)	26 (51%)	0.802
RDS	18 (48.6%)	23 (45.1%)	0.910
BPD	6 (20%)	17 (38.6%)	0.125
Caffeine use	7 (18.9%)	8 (15.7%)	0.912
Sepsis	1 (2.7%)	0	0.420
Use of vasopressors	2 (5.4%)	7 (13.7%)	0.293
Red blood cell transfusion	2 (5.4%)	3 (5.9%)	1
Platelets < 150.000	8 (21.6%)	7 (13.7%)	0.493
Neutropenia	5 (13.5%)	5 (9.8%)	0.736
PDA	3 (8.1%)	6 (11.8%)	0.728
Seizures	1 (2.7%)	1 (2%)	1
IVH grades 3 and 4	1 (2.7%)	5 (9.8%)	0.39
Treatable ROP	0	1 (2%)	1
Death	7 (18.9%)	11 (21.6%)	0.971

Abbreviations: BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure; GA, gestational age; IVH, intraventricular hemorrhage; MV, mechanical ventilation; PDA, patent ductus arteriosus; PROM, premature rupture of membranes; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; SGA, small for gestational age.

¹t-test for independent samples.

²Mann–Whitney test.

³Chi-square test.

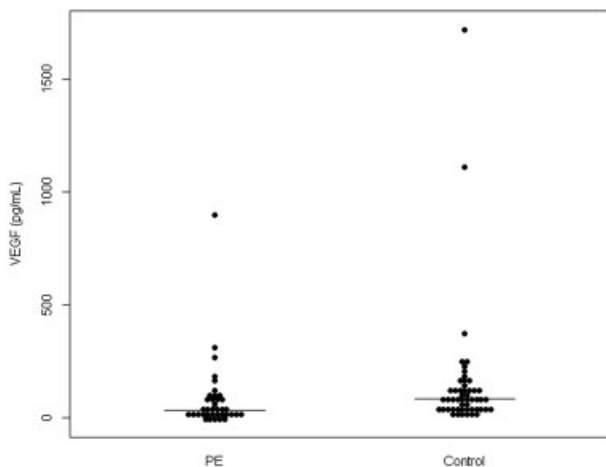


Fig. 1 Plasma VEGF levels in the groups with preeclampsia (PE) and without PE (control). The horizontal bar represents the median value ($p = 0.001$, Mann–Whitney test). VEGF, vascular endothelial growth factor.

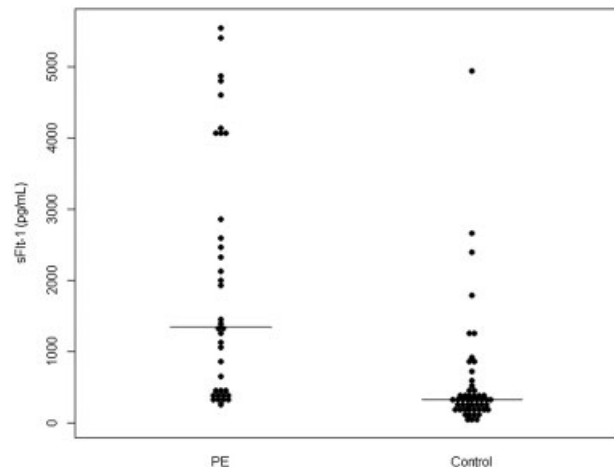


Fig. 2 Plasma sFlt-1 levels in the groups with preeclampsia (PE) and without PE (control). The horizontal bar represents the median value ($p < 0.001$, Mann–Whitney test). sFlt-1, soluble fms-like tyrosine kinase-1.

Table 2 Multivariate analysis of risk factors associated with neonates born to preeclamptic mothers, including VEGF

	OR	95% CI	<i>p</i>
GA ¹	1.10	0.85–1.44	0.47
PROM	0.95	0.78–1.00	0.48
Vaginal delivery	0.04	0.005–0.19	< 0.001
SGA	6.06	1.69–25.12	0.008
VEGF	0.20	0.04–0.76	0.02

Abbreviations: 95% CI, 95% confidence interval; GA, gestational age; OR, odds ratio; PROM, premature rupture of membranes; SGA, small for gestational age; VEGF, vascular endothelial growth factor.

¹Variables with significant differences in the univariate analysis.

model, as these two factors are closely related and could not be included in the same model.

Comparing VEGF and sFlt-1 levels regarding appropriate for gestational age (AGA) and SGA status in all population, sFlt-1 levels were higher in SGA neonates (1,044.94 pg/mL [290.64–3,472.24]) than in neonates AGA (372.67 pg/mL [236.75–860.14]) ($p = 0.013$). As for VEGF, SGA neonates had plasma levels similar to those of AGA neonates (45.64 [6.36–105.67] vs. 70.45 [29.17–126.60] pg/mL) ($p = 0.183$).

Discussion

In our study, plasma sFlt-1 levels were four times higher in preterm neonates exposed to maternal PE than in controls, whereas plasma VEGF levels were 2.5 times lower. Even after multivariate analysis, higher sFlt-1 and lower VEGF plasma concentrations were maintained in preterm exposed to maternal PE. Previous studies measuring these substances in umbilical cord blood from neonates exposed to PE showed similar results.^{7,8}

Increased sFlt-1 concentration is known to be associated with decreased biologically active VEGF concentration, as sFlt-1 binds to VEGF, leading to extremely low levels of free VEGF.² In vitro conditions, VEGF induces cytotrophoblast invasion, an effect that is blocked by addition of sFlt-1.⁴

Table 3 Multivariate analysis of risk factors associated with neonates born to preeclamptic mothers, including sFlt-1

	OR	95% CI	<i>p</i>
GA ¹	1.20	0.90–1.66	0.22
PROM	0.96	0.81–1	0.50
Vaginal delivery	0.13	0.018–0.68	0.026
SGA	4.07	1.08–17.05	0.042
sFlt-1	13.48	2.66–98.96	0.004

Abbreviations: 95%CI, 95% confidence interval; GA, gestational age; OR, odds ratio; PROM, premature rupture of membranes; SGA, small for gestational age; sFlt-1, soluble fms-like tyrosine kinase-1.

¹Variables with significant differences in the univariate analysis.

VEGF is a potent stimulator of endothelial cell proliferation, promoting angiogenesis. Its receptor, sFlt-1, binds VEGF with high affinity and inhibits its mitogenic activity.²⁴ Hypoxia increases sFlt-1 expression in trophoblasts, leading to decreased levels of free VEGF and triggering an antiangiogenic state.¹ In the setting of PE, placental cells are exposed to reduced perfusion because of inadequate trophoblast invasion, leading to elevated sFlt-1 concentration.

The placenta is the main source of sFlt-1, and studies suggest that transplacental transfer of this substance to the fetus is more important than fetal production of sFlt-1.^{7,8} As for VEGF, it is expressed at sites of active angiogenesis, as it occurs in the fetus. When comparing maternal and fetal (via umbilical cord blood) concentrations, higher fetal concentrations have been observed.⁷ We suggest that determination of those factor in umbilical blood may not reflect the actual situation in peripheral neonatal blood sample as it was described for other factors,^{25,26} once that there is neonatal participation in the production of both factors. A strong point of our study is the fact that to the best of our knowledge, no study published to date has compared VEGF and sFlt-1 levels in blood from preterm neonates born to mothers with PE.

Regarding the clinical findings, the higher incidence of cesarean delivery and, consequently, shorter duration of PROM may be attributed to an indication for pregnancy interruption in severe cases,^{27,28} although we did not classify PE in our study patients. Surprisingly, there was no difference between groups in the incidence of neutropenia, thrombocytopenia, respiratory distress syndrome, BPD, and treatable ROP, in disagreement with previous reports.^{29–36} We did not follow our patients up to discharge to determine the duration of the antiangiogenic status would last. However, our study was designed only to evaluate factors involved in angiogenesis just after birth, which is a limitation of the study.

Intrauterine growth restriction (IUGR) is characterized by an altered placental angiogenesis, leading to a reduced supply of oxygen, and nutrients to the fetus. It is described in newborns that there is an association between low sFlt-1 levels in the beginning of pregnancy followed by a significant increase along the gestation and intrauterine growth retardation.³⁷ It is possible that exposure of SGA neonates to elevated sFlt-1 concentrations leads to vascular effects that, in the long term, may play an important role in the association of low birth weight with risk of cardiovascular disease in the future.^{37,38} We found no difference in VEGF levels when SGA and AGA neonates were compared. This finding confirms previous study that showed an inversely association of umbilical cord blood VEGF levels and birth weight percentile and birth weight, but not when VEGF levels are determined in peripheral blood in term newborns.³⁹ However, sFlt-1 levels were 2.8 times higher in our SGA neonates, compared with AGA neonates, which is consistent with findings previously reported in full-term neonates.²⁴ In the setting of PE, the placenta produces high levels of sFlt-1.⁴ However, elevated sFlt-1 concentration can also be observed in cases of chronic intrauterine hypoxia without PE.²⁴

Conclusion

Higher sFlt-1 and lower VEGF levels were found in preterm neonates exposed to PE, as well as higher sFlt-1 levels in SGA delivery, reflecting a predominance of antiangiogenic mechanisms in PE and IUGR.

Conflicts of Interest

The authors declare no conflicts of interest.

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