Original Article

Association of Vascular Endothelial Growth Factor and Soluble fms-Like Tyrosine Kinase-1 Polymorphisms with Their Circulating Protein Levels in Preeclampsia

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Abstract

Introduction: Normal development of placental vascular tree requiring angiogenesis and vasculogenesis is structurally and functionally indispensable for both adequate placental growth and delivery of nutrients from mother to the fetus. Impaired placental angiogenesis has been implicated in the pathophysiology of pregnancy complications which have immediate and long-lasting effects on the mother and her child, such as preeclampsia (PE) and fetal growth restriction. The mechanisms underlying the deregulation of placental angiogenesis in PE include a misbalance between the secretion and activity of pro-angiogenic (vascular endothelial growth factor [VEGF]) and anti-angiogenic (soluble fms-like tyrosine kinase-1 [sFlt-1]) factors. Considering the important roles of VEGF and sflt1 in pregnancy, functional polymorphisms in these genes may be potentially important as genetic markers for susceptibility to PE. Thus, the aim of the study was to screen for the presence of *VEGF* and *sFlt-1* gene polymorphisms and to measure their levels in PE patients and controls of Indian origin. **Material and Methods:** Fifty each of clinically diagnosed patients and gestational and maternal age-matched normotensive, nonproteinuric controls were recruited after taking informed consent. DNA isolated from blood samples was processed for polymerase chain reaction amplification followed by restriction fragment length polymorphism to screen for the presence of *VEGF* + 936C/T, *sFlt-1* (+4244G/A, −4771G/T, −523C/G) polymorphisms. Serum levels of VEGF-A and sFlt-1 were measured by Sandwich enzyme-linked immunosorbent assay (ELISA). **Results:** Decreased frequency of wild type genotype with respect to *VEGF* + 936C/T and *sFlt-1* (+4244G/A, −4771G/T, and −523C/G) polymorphisms was seen in patients. ELISA results showed lower VEGF-A (198.43 ± 14.63 pg/ml vs. 235.08 ± 16.72 pg/ml [mean ± standard error of mean]) and higher sFlt-1 levels (2932.81 [1802.33–5760.46] pg/ml vs. 1114.94 [655.03–2694.35] Median [Range]; $P < 0.05$) in patients as compared to controls. Preeclamptic women with increased frequency of *VEGF* + 936CT genotype had lower serum levels of VEGF-A. However, preeclamptic women with increased frequency of GA, AA, GT, TT, CG, and GG genotypes of sFlt-1 (+4244G/A, −4771G/T, −523C/G) polymorphisms had increased serum levels of sFlt-1. **Discussion and Conclusion:** The present study shows, for the first time, a possible association of *VEGF* and *sFlt-1* polymorphisms with gene expression and altered protein levels in preeclamptic patients of Indian origin.

Keywords: *Preeclampsia, sFlt-1, single nucleotide polymorphism, vascular endothelial growth factor*

Introduction

Preeclampsia (PE) is a life-threatening pregnancy-specific syndrome characterized by hypertension and proteinuria after the 20th week of gestation.[1] It is one of the four leading causes of maternal mortality and morbidity worldwide.[2] In developing countries, the prevalence of PE is much higher due to the lack of prenatal care, access to hospital care, resources, appropriate diagnosis and management of patients.[2] The cause of PE remains unclear but is believed to result from a combination of insufficient blood flow to the uterus, damage to the blood vessels, a problem with the immune system, dietary and genetic factors that lead to the failure of normal trophoblastic invasion and improper remodeling of the uterine spiral arteries.[3] The process of PE may thus begin with impaired trophoblast invasion which produces an increase in oxidative stress that results in a systemic inflammatory response and endothelial dysfunction.[4,5] The inadequate placentation

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is followed by an increase in the placenta-derived anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1).[6] sFlt1, a splice variant of VEGF receptor Flt1(VEGFR-1) lacking the transmembrane and cytoplasmic domains, acts as a potent VEGF and placental growth factor (PlGF) antagonist.[6] Vascular endothelial growth factor (VEGF) is a major angiogenic factor and a prime regulator of endothelial cell proliferation. It plays a crucial role in physiological vasculogenesis and vascular permeability.[7] Soluble Flt-1 binds VEGF-A with a higher affinity resulting in extremely low circulating levels of free VEGF-Ato bind with the VEGFR-1 thus reducing the beneficial effects of VEGF. Studies have shown that the imbalance in angiogenic/anti-angiogenic factors affects vasculogenesis, angiogenesis, and placental development and is strongly associated with signs and symptoms of PE.[8-11] Family studies have shown that genetic factors play a role in PE, but the exact inheritance pattern is still unknown.[12,13] Candidate genes (*VEGF, Flt-1*) have been studied worldwide for PE, and single nucleotide polymorphisms (SNPs) in these genes have been found to be associated with PE. Various genetic studies conducted worldwide suggest the polymorphisms of *VEGF* and sFlt-1to be significantly associated with PE.[14-19] However, there is no data available on the *VEGF* and sFlt-1 polymorphisms and their association with protein levels in PE mothers of Indian origin. Thus, the present study was planned to screen for the presence of *VEGF* (+936 C/T) and *sFlt-1* ((+4244G/A,-4771G/T,-523C/G) gene polymorphisms and also measure their protein levels in PE patients and healthy controls.

Material and Methods

Subjects

It was designed as a case–control study where clinically diagnosed patients (n = 50) and gestational and maternal age-matched normotensive, nonproteinuric controls (n = 50) from the antenatal clinic and the inpatient ward of the Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India, were recruited in the study after taking written, informed consent. A protocol of the study was approved by the Institute Ethics Committee. PE was defined according to ACOG guidelines: Blood pressure (BP) ≥140 mm Hg systolic and ≥ 90 mm Hg diastolic (mild) or ≥160 mm Hg systolic and ≥110 mm Hg diastolic (severe) on 2 occasions at least 4 h apart after 20 weeks of gestational age in women with a previously normal BP, confirmed within a short interval to facilitate timely antihypertensive therapy; proteinuria >300 mg/24 h urine collection or protein/creatinine ratio >0.3 mg/dl or dipstick reading of >1+ or in the absence of proteinuria, newonset hypertension with new onset of one or more of the following: thrombocytopenia: platelet count <100,000/µl, renal insufficiency: serum creatinine >1.1 mg/dl or doubling of serum creatinine in the absence of other renal disease, impaired liver function: elevated blood levels of liver transaminases to twice normal concentrations, pulmonary edema and cerebral edema. Pregnant women with chronic hypertension, chorioamnionitis, diabetes, renal disease, and cardiac disease were excluded from the study.

Clinical information from all the patients was recorded on a predesigned questionnaire and detailed pedigree information up to at least three generations was taken.

Sample collection

A volume of 5 ml peripheral blood was drawn (2.5 ml in ethylenediaminetetraacetic acid [EDTA] and 2.5 ml in serum vials) under aseptic conditions. The blood specimen was given a personal identifier number that was used to link and maintain the biological information derived from cases as well as controls. The study was done in two parts.

Part I

Blood sample (2.5 ml) in EDTA vial was used for DNA isolation using the salting-out method (Sambrook *et al*., 1989). Genomic DNA was amplified by polymerase chain reaction which was carried out in 25 µl volume and included 80–120 ng/µl DNA, 1X buffer (Thermo), 1.5 mM $MgCl_2$(Thermo), 10 µM DNTPs (Thermo), 0.5 U Taq polymerase (Thermo). 20 pM each of forward and reverse primers were used (Sigma) [Table 1]. The conditions for PCR amplification of *VEGF* (+936 C/T) were initial denaturation at 95°C for 7 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. The conditions for PCR amplification of *sFlt-1* (+4244G/A, −4771G/T, −523C/G) were initial denaturation at 95°C for 7 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min using a Thermal Cycler (BIO-RAD, California, USA). PCR products were separated by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide (EtBr) staining under UV (Syngene USA Inc.). The amplified products were digested using the different restriction enzymes and incubated overnight at 37°C (restriction fragment length polymorphism). The amplified products of *VEGF* (+936 C/T) were digested with the enzyme Nla III, whereas for *sFlt-1* (+4244G/A), *sFlt-1* (−4771G/T), *sFlt-1* (−523C/G), Ava II, Taq 1 and BsiHKai restriction enzymes (New England Biolabs) were used. Fragments were separated on 3% agarose gel and visualized by EtBr staining under ultraviolet light.

Part II

Serum was separated from the other 2.5 ml venous blood, aliquoted, and stored at −80°C until analysis.

The serum levels of VEGF-A and sFlt-1 proteins were measured by sandwich enzyme-linked immunosorbent assay (ELISA) (R and D Systems Inc., Minneapolis, MN, USA). The optical density was read on ELISA reader at 450 nm (TECAN, Mannedorf, Switzerland).

Statistical analysis

Data were analyzed by STATA 14.0 software (Texas, USA) and Graph Pad Prism 7. Data were presented as mean ± standard deviation, mean ± standard error of mean and median (range) as appropriate. The genotypic frequencies were analyzed/compared using McNemar's test. Protein levels of VEGF and sFlt-1 were analyzed by paired *t*-test. $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the preeclamptic patients and gestational and maternal age-matched normotensive, nonproteinuric controls are presented in Table 2.

Results of the screening of vascular endothelial growth factor + 936C/T (rs 3025039), *sFlt-1* [+4244G/A (rs 722503),-4771G/T (rs 7335588),-523C/G (rs 12584067)] polymorphisms

The analysis of *VEGF* + 936C/T showed the CC (wild type) genotype to be significantly higher in the controls while the CT (heterozygous) genotype was higher in the patients.

Genotyping of *sFlt1* + 4244G/A (rs722503) showed GG (wild type) genotype to be more in controls whereas both GA (heterozygous) and AA (homozygous) genotypes were found to be more in patients while analysis of *sFlt-1*-4771G/T (rs 7335588) showed GG genotype was found to be more in controls and GT, TT genotypes were found to be more in patients. However, these differences were not statistically significant.

Genotyping of *sFlt-1*-523C/G (rs 12584067) showed CC (wild type) genotype was more in controls and CG, GG genotypes were more in patients showing a significant difference.

Table 1: Polymerase chain reaction primer sequences of the polymorphisms

SNPs	Forward primer	Reverse primer	Reference
VEGF (+936 C/T)	AAGGAAGAGGAGACTCTGCGCAGAGC	TAAATGTATGTATGTTG GGTGTGTCTACAGG	NCBI
sFlt-1 (+4244G/A)	CCCATTCATTGGTTTCCTGT	GTCCTTGCCCTATCCTCTCC	NCBI
sFlt-1 (−4771G/T)	CCTTCAAGCAGCCAAGAATC	TGGTGGAATCAGAAGGGAAA	NCBI
sFlt-1 (−523C/G)	GGCAGTAAAGCTGGCAGAAG	TGCCTTCTCCCCACAAGTAA	NCBI

SNPs: Single nucleotide polymorphisms, VEGF: Vascular endothelial growth factor, sFlt-1: Soluble fms-like tyrosine kinase-1, NCBI: National Center for Biotechnology Information

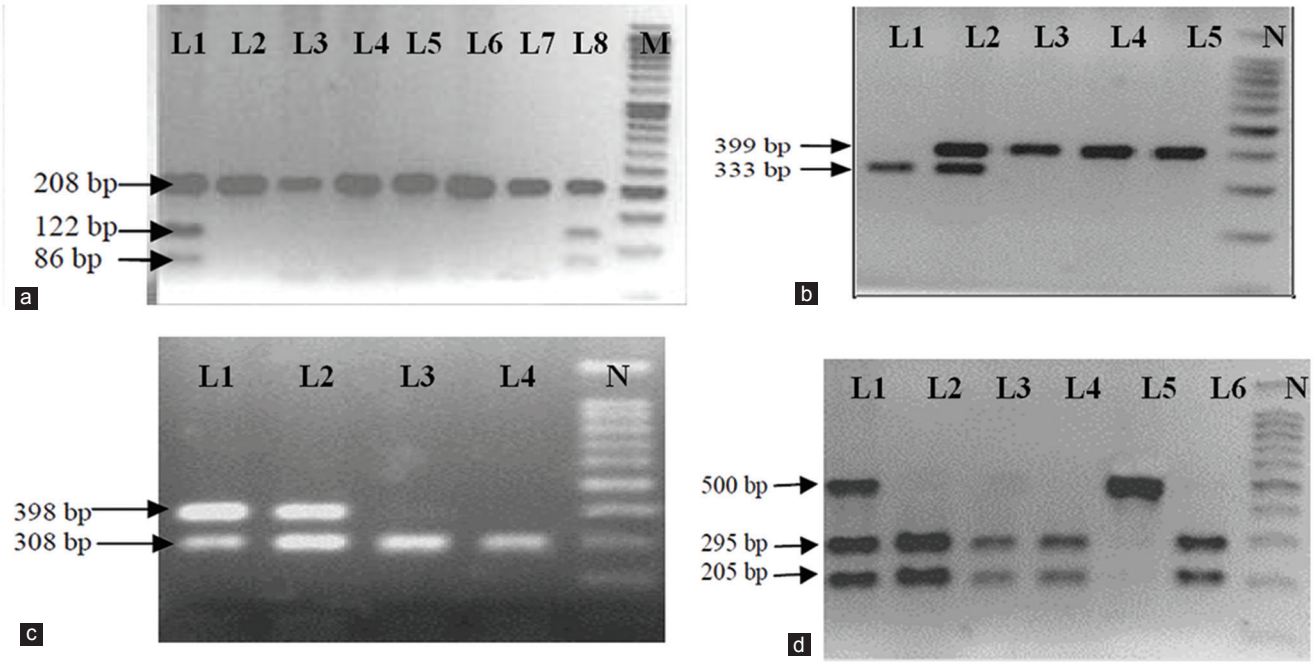

Figure 1: RFLP electrophoretogram with respect to (a) *VEGF* + 936C/T (L1-L8) (b) *sFlt-1* + 4244G/A (L1-L5) (c) *sFlt-1* -4771G/T (L1-L4) (d) *sFlt-1* -523C/G (L1-L6) showing the respective genotypes. Lanes (l): samples, M: 50bp DNA ladder, N: 100bp DNA ladder

Table 2: Clinical characteristics of the study participants

Clinical features	Mean±SD		*P*
	Patients (*n*=50)	Controls (*n*=50)	
Maternal age (years)	30.53±6.58	29.6±3.34	>0.05
Gestational age (weeks)	30.56±3.51	31.36±6.25	>0.05
Systolic BP (mm Hg)	158.9±11.88	117.8±7.34	<0.0001*
Diastolic BP (mm Hg)	101.43±8.39	74.2±6.39	<0.0001*
Intrauterine growth restriction, *n* (%)	5 (10)	1 (2)	<0.05
Proteinuria (dipstick), *n* (%)	3+=18 (36) 2+=22 (44) 1+=10 (20)	Nil or in traces	Not applicable

**P*<0.0001 and **P*<0.05 showed statistical significance SD: Standard deviation, BP: Blood pressure

Table 3: Distribution and frequency of four loci with susceptibility to preeclampsia (*n*=50)

Polymorphism	Genotype	Patients, *n* (%)	Controls, *n* (%)	Mc Nemar's test *P*
VEGF +936 C/T	CC	40 (80)	48 (96)	<0.0001*
	CT	10 (20)	2 (4)	(STATA 14.0)
	TT	0	0	
sFlt-1 +4244G/A	GG	19 (38)	25 (50)	0.5044
	GA	22 (44)	17 (34)	(STATA 14.0)
	AA	9 (18)	8 (16)	
sFlt-1 −4771G/T	GG	17 (34)	24 (48)	0.5044
	GT	32 (64)	26 (52)	(STATA 14.0)
	TT	1 (2)	0	
sFlt-1 −523C/G	CC	38 (76)	43 (86)	0.0013*
	CG	10 (20)	7 (14)	(STATA 14.0)
	GG	2 (4)	0	

**P*<0.0001 showed statistical significance VEGF: Vascular endothelial growth factor, sFlt-1: Soluble fms-like tyrosine kinase-1, STATA: Statistics and data science

Table 4: Maternal serum levels of vascular endothelial growth factor-A and soluble fms-like tyrosine kinase-1 in preeclamptic and normotensive, nonproteinuric pregnant women

Study groups	Patients	Controls	*P* (paired *t*-test)
VEGF (ρg/ml), mean±SEM	198.43±98.43	235.08±35.08	0.0645
sFlt-1 (ρg/ml), median (range)	2932.81 (1802.33-5760.46)	1114.94 (655.03-2694.35)	<0.05*

p<0.05* showed the difference in sFlt-1 concentration among patients and controls was statistically significant, VEGF: Vascular endothelial growth factor, sFlt-1: Soluble fms-like tyrosine kinase-1, SEM: Standard error of mean

Reduced vascular endothelial growth factor-A and elevated sFlt-1 levels in preeclamptic patients sera

A decrease in VEGF-A levels in PE patients (198.43 ± 14.63) pg/ml was observed as compared to normotensive, nonproteinuric controls (235.08 ± 16.72) pg/ml.

The sFlt-1 levels in patients' era were 2932.81 (1802.33–5760.46) pg/ml whereas normotensive, nonproteinuric pregnant women had 1114.94 (655.03–2694.35) pg/ml which were significantly different ($P < 0.05$).

Analysis of vascular endothelial growth factor and *sFlt-1* polymorphisms and their levels

Patients with increased frequency of *VEGF* + 936CT genotype had lower levels of VEGF-A.

sFlt-1 + 4244G/A with the GA, AA genotype, *sFlt-1*-4771G/T with the GT, TT genotype, sFlt-1-523 C/G with the CG, GG genotype showed increased serum levels of sFlt-1 in patients than controls.

Discussion

VEGF-A promotes angiogenesis, vasodilatation, increases vascular permeability, maintains the integrity of glomerular filtration barrier, and reduces apoptosis.[20,21] VEGF and its two receptors (VEGFR-1/Flt-1 and VEGFR-2/Flk) play an essential role during placentation. sFlt-1 is the soluble form of VEGF receptor. It acts as a potent antagonist of VEGF-A and PlGF by inhibiting their binding to the intact cell surface receptors and is thus considered an anti-angiogenic factor.[22,23] Soluble Flt-1 is secreted by endothelial cells, monocytes, and the placenta.[24] A critical balance between angiogenic and anti-angiogenic factors is required for the fine-tuning of vasculogenesis and angiogenesis during pregnancy.[25,26] Considering the important roles of these two molecules (VEGF and sFlt-1) during pregnancy, the polymorphisms in their genes may be potentially important as genetic markers for susceptibility to the disease. Worldwide studies reported SNPs in*VEGF and sFlt-1* genes have been found to be associated with PE.[14-19] However, there is no data available on the *VEGF* and sFlt-1 polymorphisms and their association with protein levels in PE mothers of Indian origin. Thus, the aim of the present study was to screen for the presence of *VEGF* + 936 C/T and *sFlt-1* (+4244G/A,-4771G/T,-523C/G) polymorphisms, and to measure the levels of VEGF-A and sFlt-1 proteins and correlate the polymorphisms of VEGF and sFlt-1 with their serum levels in PE patients and healthy controls. The present study indicated a possible association between *VEGF* + 936 C/T polymorphism and PE. The frequency of CT (heterozygous) being significantly higher among patients than controls [Figure 1, Table 3], which is similar to the findings of Papazoglou *et al*.[14] Similarly, Shim *et al*. also found a higher frequency of CT genotype among Korean women with PE than controls. They reported that the 936 T was more frequent in patients than controls.[15] Although Cunha *et al*. also suggested that a higher frequency of the T allele of the *VEGF* + 936C/T polymorphism was observed in patients with PE, the difference was not significant statistically.[16] Cheng *et al*. also reported the T allele carriers to have an increased

risk of developing PE.[17] A recent study conducted by Procopciuc *et al*. reported that the risk to develop PE was higher in association with the 936 C/T-VEGF heterozygous genotype and increased further in severe PE women who were carriers of the homozygous 936-TT VEGF genotype. They also reported that pregnant women with gestational hypertension and severe PE delivered at a significantly earlier gestational age and neonates having low birth weight if both the preeclamptic mothers and their newborns were carriers of the *VEGF-T936* allele.

The analysis of *sFlt-1* + 4244G/A **(**rs722503) showed the frequency of GG (wild type) genotype was more in controls, but the frequency of both GA (heterozygous) and AA (homozygous) genotypes was found to be more in patients. Screening of *sFlt-1*-4771G/T (rs 7335588**)** showed the GG (wild type) genotype was found to be more in controls. The analysis of *sFlt-1*-523C/G (rs 12584067) showed the CC (wild type) genotype to be more in controls, but CG (heterozygous) and GG (homozygous) genotype were more in patients, and the difference was statistically significant [Figure 1, Table 3]. A case-control study on 606 American pregnant women focusing on 124 tag SNPs in 6 genes found a significant association between *sFlt-1*-523C/G and *sFlt-1*-4771G/T and PE in black women and *sFlt-1* + 4244G/A and PE in white women.[19] Our study on Indian pregnant women showed a similar trend.

We reported lower VEGF-A levels in serum samples of PE patients as compared to controls [Table 4]. Similar to the studies done by Procopciuc *et al.* and Lyall *et al*.[18,27] In contrast, several studies reported increase in VEGF-A levels in the sera of patients.[28-30] The discrepancy could be due to the type of VEGF-A (free or total) measured. The trapping of VEGF by sFlt-1 reduces the availability of free circulating VEGF, whereas the total VEGF may increase or remain the same. The present study reported significantly higher sFlt-1 levels in the sera of patients as compared to controls [Table 4]. Similar to many previous studies.[31-33] Inadequate perfusion of the fetoplacental unit causing hypoxia may lead to increase in the production of sFlt-1 by placental trophoblasts, which may later reflect as raised sFlt-1 levels in maternal circulation.[34,35] The present study points to a possible association of *VEGF* and *sFlT-1* polymorphisms with gene expression leading to altered levels in PE. However, studies on a larger sample size are required to confirm the results.

Conclusion

Increased frequency of *VEGF* + 936CT genotype had lower levels of VEGF-A and *sFlt-1* + 4244G/A with the GA, AA genotype, *sFlt-1*-4771G/T with the GT, TT genotype, *sFlt-1*-523 C/G with the CG, GG genotype showed increased serum levels of sFlt-1 in patients than controls indicating their (VEGF and sFlt-1) potential role as future biomarkers in the diagnosis of PE. However, association studies on large samples are required to validate the results.

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Conflicts of interest

There are no conflicts of interest.

References

1. Gilbert JS, Ryan MJ, LaMarca BB, Sedeek M, Murphy SR, Granger JP, *et al*. Pathophysiology of hypertension during preeclampsia: Linking placental ischemia with endothelial dysfunction. Am J Physiol Heart Circ Physiol 2008;294:H541-50.
2. Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: Implication for health system strengthening. J Pregnancy 2011;2011:481095. doi: 10.1155/2011/481095.
3. Fisher SJ. Why is placentation abnormal in preeclampsia? Am J Obstet Gynecol 2015;213:S115-22.
4. Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham MW Jr., Wallace K, *et al.* The role of inflammation in the pathology of preeclampsia. Clin Sci (Lond) 2016;130:409-19.
5. Gathiram P, Moodley J. Pre-eclampsia: Its pathogenesis and pathophysiolgy. Cardiovasc J Afr 2016;27:71-8.
6. Nikuei P, Malekzadeh K, Rajaei M, Nejatizadeh A, Ghasemi N. The imbalance in expression of angiogenic and anti-angiogenic factors as candidate predictive biomarker in preeclampsia. Iran J Reprod Med 2015;13:251-62.
7. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti- and pro-angiogenic therapies. Genes Cancer 2011;2:1097-105.
8. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, *et al.* Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649-58.
9. Buhimschi CS, Norwitz ER, Funai E, Richman S, Guller S, Lockwood CJ, *et al.* Urinary angiogenic factors cluster hypertensive disorders and identify women with severe preeclampsia. Am J Obstet Gynecol 2005;192:734-41.
10. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, *et al.* Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992-1005.
11. Ramma W, Buhimschi IA, Zhao G, Dulay AT, Nayeri UA, Buhimschi CS, *et al.* The elevation in circulating anti-angiogenic factors is independent of markers of neutrophil activation in preeclampsia. Angiogenesis 2012;15:333-40.
12. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: Possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol 1986;93:898-908.
13. Arngrimsson R, Bjornsson H, Geirsson RT. Analysis of different inheritance patterns in preeclampsia/eclapmsia syndrome. Hypertens Pregnancy 1995;14:27-38.
14. Papazoglou D, Galazios G, Koukourakis MI, Kontomanolis EN, Maltezos E. Association of -634G/C and 936C/T polymorphisms of the vascular endothelial growth factor with spontaneous preterm delivery. Acta Obstet Gynecol Scand 2004;83:461-5.
15. Shim JY, Jun JK, Jung BK, Kim SH, Won HS, Lee PR. Vascular

endothelial growth factor gene +936 C/T polymorphism is associated with preeclampsia in Korean women. Am J Obstet Gynecol 2007;197:271.e1-4.
16. Cunha VM, Grecco RL, Paschoini MC, Silva SR, Ruiz MT, Balarin MA, *et al.* Genetic polymorphisms of vascular endothelial growth factor in pre-eclampsia. Rev Bras Ginecol Obstet 2011;33:158-63.
17. Cheng D, Hao Y, Zhou W, Ma Y. Vascular endothelial growth factor +936C/T, -634G/C, -2578C/A, and -1154G/A polymorphisms with risk of preeclampsia: A meta-analysis. PLoS One 2013;8:e78173.
18. Procopciuc LM, Caracostea G, Zaharie G, Stamatian F. Maternal/newborn VEGF-C936T interaction and its influence on the risk, severity and prognosis of preeclampsia, as well as on the maternal angiogenic profile. J Matern Fetal Neonatal Med 2014;27:1754-60.
19. Srinivas SK, Morrison AC, Andrela CM, Elovitz MA. Allelic variations in angiogenic pathway genes are associated with preeclampsia. Am J Obstet Gynecol 2010;202:445.e1-11.
20. Bartlett CS, Jeansson M, Quaggin SE. Vascular growth factors and glomerular disease. Annu Rev Physiol 2016;78:437-61.
21. Bates DO. Vascular endothelial growth factors and vascular permeability. Cardiovasc Res 2010;87:262-71.
22. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029-39.
23. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993;90:10705-9.
24. Shibuya M. Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): A dual regulator for angiogenesis. Angiogenesis 2006;9:225-30.
25. Seki H. Balance of antiangiogenic and angiogenic factors in the context of the etiology of preeclampsia. Acta Obstet Gynecol Scand 2014;93:959-64.
26. Chen DB, Zheng J. Regulation of placental angiogenesis. Microcirculation 2014;21:15-25.
27. Lyall F, Young A, Boswell F, Kingdom JC, Greer IA. Placental expression of vascular endothelial growth factor in placentae from pregnancies complicated by pre-eclampsia and intrauterine growth restriction does not support placental hypoxia at delivery. Placenta 1997;18:269-76.
28. Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. Obstet Gynecol 1995;86:815-21.
29. Sharkey AM, Charnock-Jones DS, Boocock CA, Brown KD, Smith SK. Expression of mRNA for vascular endothelial growth factor in human placenta. J Reprod Fertil 1993;99:609-15.
30. Brockelsby J, Wheeler T, Anthony F. Increased circulating levels of vascular endothelial growth factor in preeclampsia. Hypertens Pregnancy 1998;17:283-90.
31. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.
32. Moore Simas TA, Crawford SL, Solitro MJ, Frost SC, Meyer BA, Maynard SE. Angiogenic factors for the prediction of preeclampsia in high-risk women. Am J Obstet Gynecol 2007;197:244.e1-8.
33. Woolcock J, Hennessy A, Xu B, Thornton C, Tooher J, Makris A. Soluble Flt-1 as a diagnostic marker of pre-eclampsia. Aust N Z J Obstet Gynaecol 2008;48:64-70.
34. Noris M, Perico N, Remuzzi G. Mechanisms of disease: Pre-eclampsia. Nat Clin Pract Nephrol 2005;1:98-114.
35. Heydarian M, McCaffrey T, Florea L, Yang Z, Ross MM, Zhou W. Novel splice variants of sFlt1 are upregulated in preeclampsia. Placenta 2009;30:250-5.