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Vascular endothelial growth factor C/A 2578 gene polymorphism and umbilical artery Doppler in preeclamptic women

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ABSTRACT

Introduction: Preeclampsia is strongly associated with placental hypoperfusion. Genetic factors have an impact on the pathogenesis of preeclampsia. The aim is to assess the association of Vascular Endothelial Growth Factor (C2578A) gene polymorphism with the occurrence and severity of preeclampsia and the umbilical artery Doppler changes among preeclamptic women.

Materials and methods: This case-control study was conducted in clinical and Chemical pathology and Obstetrics departments in Beni- Suef University, Egypt. Two hundred and ninety pregnant women above 20 weeks gestational age until delivery were divided into 2 main groups. The patient group included 145 preeclamptic women who were further sub grouped according to the severity of preeclampsia into 82 severe and 63 mild cases. Control group included 145 normotensive pregnant women. Our primary outcome was detection of VEGF C 2578 A gene mutations by a polymerase chain reaction. A secondary outcome was Doppler changes in the pulsatility index of the umbilical artery compared with VEGF genotypes.

Results: Our study showed that VEGF C 2578 A genotype and alleles frequencies were not related to the occurrence of preeclampsia (p-value 0.513 and 0.549, respectively), odds ratio (95%CI) 1.154 (0.724–1.848). Mild preeclamptic cases showed no significance comparing VEGF genotypes studied and pulsatility index of the umbilical artery. However, severe cases showed p-value < 0.0001.

Conclusion: We concluded that VEGF 2578C/A polymorphism had no association with the occurrence of preeclampsia in studied groups, whereas there was a significant relationship among severe cases between CA and CC genotypes and pulsatility index of the umbilical artery.

1. Introduction

Preeclampsia (PE) is a major cause of maternal mortality all over the world. It is also a major cause of perinatal morbidity and mortality, and it is strongly associated with placental hypoperfusion, intrauterine growth restriction and/or intrauterine fetal death [1]. Despite these effects on mother and fetus, pathophysiology of preeclampsia is still unclear [2]. PE is currently believed to occur as a result of immunologic, inflammatory, dietary and genetic factors which adversely affect normal trophoblastic invasion and remodeling of uterine spiral arteries [3]. Each of those factors showed contributing genetic explanation. Thus, many genetic researches have been established to evaluate impact of genetics in pathogenesis of preeclampsia [4,5].

Vascular endothelial growth factor (VEGF) is a signal protein that creates new blood vessels during embryonic development in a process known as vasculogenesis (the de novo formation of the embryonic circulatory system) [6]. It is a member of VEGF family which is a major angiogenic factor and potential regulator of endothelial cell proliferation [7]. It is essential for trophoblastic proliferation during pregnancy [6]. The potential role of VEGF in the pathogenesis of PE is thought to be due to over expression of the VEGF-trapping molecule, soluble fms-like tyrosine kinase receptor-1 (sFlt-1), may block podocyte-derived VEGF and induce glomerular endothelial damage, resulting in proteinuria in PE [8]. VEGF signals stimulate endothelial nitric oxide synthase (eNOS) and increase the production of nitric oxide (NO), a vasodilator, in endothelial cells. Furthermore, VEGF is a strong vascular permeability factor. Thus, a decrease in both NO and permeability via VEGF trapping by sFlt-1 may cause hypertension [2]. It was found that VEGF concentration increased in patients diagnosed later as preeclamptic before clinical onset of PE [9]. So VEGF may be useful as a

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predictive marker for PE [10]. Many studies identified polymorphisms of VEGF gene however few of them have been associated with PE and results remain controversial and inconclusive [4,5,7]. Hypertension and proteinuria were diagnosed earlier (by 1.6 weeks) in PE patients with VEGF (−2578) A allele. However, clinical impact of this finding remains to be determined [11]. Noninvasive measurements of the fetal blood flow in the umbilical artery have become widely used tools for monitoring the physiological state of the fetus [12]. Is there any relation between umbilical artery Doppler and VEGF gene polymorphism in preeclampsia? Our hypothesis is to assess significance of VEGF (C2578A) gene polymorphism in PE and its association with Doppler changes in pulsatility index of umbilical artery. This relation if present may be useful in early identification of women susceptible to preeclampsia and counseling patients on basis of clinical implications of VEGF gene polymorphism which were inconclusive in many previous studies [13–16].

2. Materials and methods

This case-control study was conducted between January 2017 and February 2018 in departments of Chemical pathology and Obstetrics and Gynecology of Beni-Suef University in Egypt. After ethical committee approval, recruitment of patients started. Three hundred and fifty pregnant women above 20 weeks gestation were approached, twenty refused to participate, thirty-five did not meet inclusion criteria and five missed follow up (consort flow diagram Fig. 1). The remaining 290 women were included after giving informed written consents. Women were divided into 2 main groups. Patient group included 145 preeclamptic women who were further sub-grouped according to severity of preeclampsia into 82 severe and 63 mild cases. Control group included 145 normotensive pregnant women. Inclusion criteria were preeclamptic pregnant women above 20 weeks. Preeclampsia was diagnosed by; systolic blood pressure (SBP) greater than or equal to 140 mm Hg or a diastolic blood pressure (DBP) greater than or equal to 90 mm Hg or higher, on two occasions at least 4 h apart in a previously normotensive patient in addition to the presence of proteinuria of greater than or equal to 0.3 g in a 24-h urine specimen, a protein (mg/dL)/creatinine (mg/dL) ratio of 0.3 or higher, or a urine dipstick protein of 1+. Severe cases were diagnosed when one or more of the following symptoms or signs are present: SBP of 160 mm Hg or higher or DBP of 110 mm Hg or higher, on two occasions at least 4 h apart while the patient is at rest and semi sitting position, impaired hepatic function as indicated by abnormally elevated blood concentrations of liver enzymes (to double the normal concentration), severe persistent upper quadrant or epigastric pain that does not respond to pharmacotherapy, progressive renal insufficiency (serum creatinine concentration > 1.1 mg/dl or a doubling of the serum creatinine concentration in the absence of other renal disease), new onset cerebral or visual disturbances, pulmonary edema, thrombocytopenia (platelet count < 100,000/μl) [17]. Exclusion criteria included patients with chronic hypertension or any renal, hepatic or cardiac disease. An abdominal ultrasound was done for all women to confirm gestational age, viability of fetus and to discover any placental abnormalities or any other obstetric complications as intrauterine growth restriction or placental hematoma. Three ml peripheral venous blood samples were withdrawn under complete aseptic conditions from each participant in the patient and control groups into a sterile EDTA vacutainer tube which was used for DNA extraction. DNA samples were stored at −20 °C. Extraction of genomic DNA from peripheral blood leucocytes was done by salting out technique using DNA purification capture column kit (Sigma Chemical Company, USA). Detection of VEGF C 2578 A gene mutations was carried out using polymerase chain reaction (PCR) amplification coupled to Restriction Fragment Length Polymorphism technique (PCR-RFLP).

Enzymatic amplification was performed by PCR using master mix, a mixture of Taq DNA polymerase (recombinant), Optimized Dream Taq Green buffer, MgCl2 and dNTPs supplied by Fermentas and two primers: Forward primer: 5′GGGCCTTAGGACACCATACC-3′ and Reverse primer: 5′-TGCCCCAGGGAACAAAGT-3′, producing amplified product at 267 pb. PCR conditions were 95 °C/3 min initial denaturation, 25–35 cycles of amplification consisting of denaturation at 95 °C for 15 s and annealing at 56 °C for 15 s, 72 °C /30 s final extension, followed by Bglll restriction enzyme digestion of the amplified product. By abolishing a Bglll restriction site, the C 2578 A mutation results in the digestion of the 267 bp amplicon into 207 and 60 bp fragments. Finally detection of the (VEGF C2578A) genotypes using agarose gel electrophoresis and ultra-violet light transillumination: Wild genotypes (2578CC) produced a single band at 267 bp and mutant heterogeneous genotypes (2578CA) produced 267, 207, and 60 bp fragments.

All women underwent an ultrasonography examination with a TUS-Xario200 diagnostic ultrasonography system (Toshiba America Medical Systems, Tustin, California, USA), which was equipped with a pulsed Doppler system for blood flow analysis. Color flow Doppler was used to identify the umbilical artery.

Doppler ultrasound was done for each woman between 34 and 36 gestational weeks to assess placental perfusion through pulsatility index of umbilical artery. The device used was TOSHIBA DIAGNOSTIC ULTRASOUND SYSTEM. MODEL TUS-X200, POWER 220-240V, 800VA, serial number 99D1615457; P/N BSM34-3218. An obstetrician with experience in maternal-fetal medicine performed ultrasound for all women.

The sample volume was applied on the examined artery with an angle of less than 45°. Diameter of umbilical artery was measured within 5 cm of umbilical cord insertion in the fetal abdomen for maximum visualization [18]. After identifying umbilical artery waveform, at least three consecutive correctly imaged waveforms were assessed. Pulsatility index (PI) (PI = PSV-EDV/TAMXV) was calculated. PI values above the 95th percentile standardized for the gestational age were considered abnormal [19].

Each woman was followed up through routine antenatal care as assigned for her condition. Hospital protocols were followed if any complication was found especially in severe preeclamptic cases.

Sample size calculation: We were planning a study of cases and controls with 1 control(s) per case. Prior data indicated that the probability of exposure among controls is 0.1 [20]. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 0.2, we needed to study 145 case patients and 145 controls (normal pregnant women) to be able to reject the null hypothesis that this odds ratio equals 1 with probability (power) 0.8. The Type I error probability associated with this test is 0.05.

The SPSS version 10.0 was used for data management and analysis. The Microsoft excel was used for charts. Parametric quantitative data were presented as mean + SD. For comparison of the two groups' means, the Student's *t*-test was used. Non-parametric quantitative data were expressed as median (percentiles). The distribution of polymorphic genotypes was assessed for deviation from the Hardy–Weinberg equilibrium; Qualitative data was expressed as frequency and percentage. Association between qualitative data was done using Chi- square test. Spearman correlation coefficient was used to correlate between quantitative variables. Risk estimate was done by odds ratio & P value was considered significant ≤ 0.05.

Ethical approval: Ethical committee of faculty of medicine Beni-Suef University approved the study on 20th of December 2016 with a registration number 1210–2016. All women gave an informed consent before study start.

3. Results

We evaluated polymorphism of vascular endothelial growth factor C/A 2578 gene in 145 women with PE and 145 controls through investigation of genotype and allele frequencies. Table 1 shows demographic variables and PE risk factors among study population.

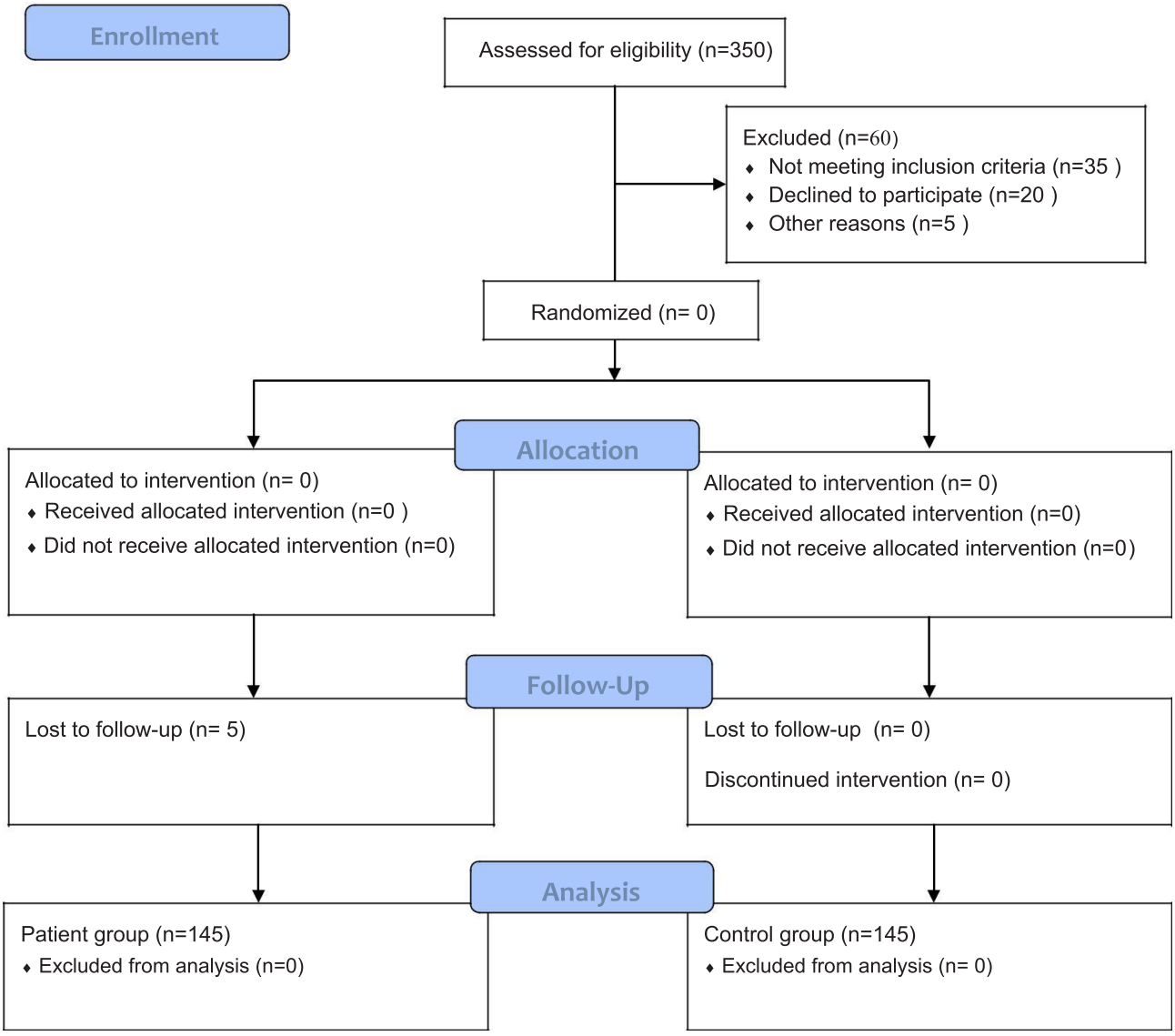

Fig. 1. CONSORT 2010 flow diagram.

Gestational age and blood pressure showed p value (< 0.001). Previous history of pre-eclampsia (p value = 0.001). In family history of pre-eclampsia, p value was 0.003.

In table 2, CA genotype was higher in the preeclamptic group than the control group 29.7% versus 26.2%.CC genotype was 70.3% in PE group compared to 73.8% in control group (p value = 0.513).

Genotype and allele frequencies of cases and controls are shown in Tables 2–5. There was no significant association in genotypic or allelic frequencies in women with pre-eclampsia relative to controls, among preeclamptic subgroups and in cases subgroups relative to controls [OR (95% CI), 1.154(0.724–1.848), 1.178(0.649–2.136), 1.137(0.655–1.973), 1.036(0.539–1.989)], respectively.

There was no significant difference between genotypes CA and CC concerning maternal and fetal complications among pre-eclamptic women as shown in Table 6.

Table 7 shows comparison between VEGF CA and CC genotypes and pulsatility index of umbilical artery in mild and severe preeclamptic cases. In mild cases p-value was 0.073 while umbilical artery PI showed significant difference in comparison with VEGF studied genotypes in severe preeclampsia (P value < 0.0001).

4. Discussion

Up to our knowledge this was the first study to investigate VEGF 2578C/A gene polymorphism in association with Doppler changes in umbilical artery and its clinical implications among preeclamptic pregnant women. However, we did not find any significant association between VEGF 2578C/A gene polymorphism and the occurrence of PE.

VEGF has an important effect in pathogenesis of pre-eclampsia and this has received great attention. Many studies have reported increased systemic VEGF levels in women with PE [21, 22, and 23]. In a cross-sectional study by Backer et al., VEGF levels were higher in pre-eclampsia group. Levels were not elevated before clinical disease. They concluded that the growth factor has a role in the endothelial cell activation that occurs in pathogenesis of the disease [21]. Another study by Kupferminc et al., who concluded that maternal plasma VEGF levels were correlated with severity of hypertension. This suggested a role of VEGF in pathogenesis of preeclampsia [22]. Hunter et al. investigated if VEGF, a vascular permeability agent, is increased in maternal serum with preclinical and clinical PE. They also evaluated how these levels change after delivery. At 30 weeks, before the onset of clinical PE, serum VEGF levels were elevated in women diagnosed as preeclampsia. Moreover, predelivery levels were significantly elevated in

Table 1
Demographic variables and risk factors among the study population.

Variable	Preeclamptic group (n = 145)	Control group (n = 145)	P value
Maternal age(y) (mean ± SD), Range	27.1 ± 4.6 (18–37)	25.6 ± 5.8 (18–37)	0.339
Primigravidity n (%)	70 (48.3%)	61(42.1%)	0.288
Gestational age (weeks) n (%)			< 0.001*
≤34 weeks	67 (46.2%)	2 (1.4%)	
> 34 weeks	78 (53.8%)	143 (98.6%)	
Previous history of PE, n (%)	14 (9.7%)	1 (0.7%)	0.001*
Family history of diabetes mellitus, n (%)	43 (29.7%)	30 (20.7%)	0.079
Family history of PE, n (%)	27 (18.6%)	10 (6.9%)	0.003*
Systolic blood pressure (mmHg), n (%)			
140–159 mmHg			< 0.001*
160–180 mmHg	120(82.8%)	0 (0%)	0
	25 (17.2%)	0 (0%)	0
Diastolic blood pressure (mmHg), n (%)			
90–109 mmHg			< 0.001*
110–120 mmHg	124 (85.5%)	0 (0%)	0
	21(14.5%)	0 (0%)	0

*Highly significant, standard deviation (SD), number (n), percent (%).

Table 2
VEGF C 2578 A genotype and alleles frequencies between study groups.

Variables	Preeclamptic group (n = 145)	Control group (n = 145)	P-value	OR(95%CI)
CA, n(%)	43 (29.7%)	38 (26.2%)		
CC, n(%)	102 (70.3%)	107 (73.8%)	0.513	1.154(0.724–1.848)
A allele, n(%)	43 (14.8%)	38 (13.1%)		
C allele, n(%)	247 (85.2%)	252 (86.9%)	0.549	

*Highly significant, number (n), percent (%), Odds Ratio (OR), confidence interval (CI).

Table 3
VEGF C 2578 A genotype and alleles frequencies between mild preeclampsia and control groups.

Variables	Mild Preeclamptic group (n = 63)	Control group (n = 145)	P-value	OR(95%CI)
CA, n(%)	19 (30.2%)	38 (26.2%)		
CC, n(%)	44 (69.8%)	107 (73.8%)	0.557	
A allele, n(%)	19 (15.1%)	38 (13.1%)		1.178(0.649–2.136)
C allele, n(%)	107 (84.9%)	252 (86.9%)	0.590	

*Highly significant, number (n), percent (%), Odds Ratio (OR), confidence interval (CI).

Table 4
VEGF C 2578 A genotype and alleles frequencies between severe preeclampsia and control groups.

Variables	Severe Preeclamptic group (n = 82)	Control group (n = 145)	P-value	OR(95%CI)
CA, n(%)	24 (29.3%)	38 (26.2%)		
CC, n(%)	58 (70.7%)	107 (73.8%)	0.619	1.137(0.655–1.973)
A allele, n(%)	24 (14.7%)	38 (13.1%)		
C allele, n(%)	140 (85.3%)	252 (86.9%)	0.648	

*Highly significant, number (n), percent (%), Odds Ratio (OR), confidence interval (CI).

Table 5
VEGF C 2578 A genotype and alleles frequencies between mild and severe preeclamptic groups.

Variables	Mild Preeclamptic group (n = 63)	Severe Preeclamptic group (n = 82)	P-value	OR(95%CI)
CA, n(%)	19 (30.2)	24 (29.3)		
CC, n(%)	44 (69.8)	58 (70.7)	0.907	1.036(0.539–1.989)
A allele, n(%)	19 (15.1)	24 (14.7)		
C allele, n(%)	107 (84.9)	140 (85.3)	0.916	

*Highly significant, number (n), percent (%), Odds Ratio (OR), confidence interval (CI).

Table 6
Maternal and fetal Complications between VEGF C 2578 A genotypes in preeclamptic patients.

Variables	CA (n = 43)	CC (n = 102)	P value
NICU n (%)	7(16%)	9(9%)	0.221
IUGR n (%)	3(7%)	5(5%)	0.633
Eclampsia n (%)	3(7%)	3(3%)	0.273
MICU n (%)	1(2%)	2(2%)	1.000

NICU: Neonatal intensive care unit, IUGR: Intrauterine growth restriction, MICU: Maternal intensive care unit.

Table 7
Comparison between VEGF CA and CC genotypes and umbilical artery pulsatility index among preeclamptic subgroups.

Mild PE	CA (n = 19)	CC (n = 44)	P-value
Pulsatility index (mean ± SD) Range	0.9 ± 0.2 (0.6–0.2)	1 ± 0.2 (0.7–1.4)	0.074
Severe PE	CA (n = 24)	CC (n = 58)	
Pulsatility index (mean ± SD) Range	0.8 ± 0.1 (0.6–0.2)	1.7 ± 0.9 (0.9–1.3)	< 0.0001*

*Highly significant, standard deviation (SD).

preeclamptic group. These findings suggest that VEGF is important in pathophysiology of PE and has the potential to act as a preclinical marker for the condition [23]. Others have reported decreased maternal serum VEGF levels in PE [24,25]. Lyall et al. evaluated wether VEGF concentrations are changed in PE. They found that in PE group, concentrations of VEGF were significantly lower than normal pregnancy group. They recommended further studies to discover mechanisms that lead to this reduction in VEGF which may provide new pathogenesis of this disorder [24]. A difference which may be due to the methodology of evaluation and different study designs [25]. Moreover, unlike non-pregnant state, most VEGF during pregnancy is bound to circulating sFlt1 due to very high levels of the latter. It has been supported that antagonism of VEGF may have a role in hypertension and proteinuria [25,26]. It has also been proved that the VEGF antagonist soluble fms-like tyrosine kinase 1 (sFlk1) does not produce the pre-eclampsia phenotype in pregnant rats [25]. Thus, there is no conclusive confirmation of the origin of elevated VEGF levels. Many conflicting results have been reported regarding the regulation of the VEGF system in PE at the transcriptional and translational level in the placenta [27–30].

Many genome-wide scans evaluated mainly maternal genotype and allele frequencies, but evidence is much on the side that the fetal gene load affects a mother's susceptibility to pre-eclampsia [31–33]. In this context, it will be more informative to measure VEGF levels in the umbilical vein and artery and investigate different maternal and fetal VEGF gene polymorphisms in further larger studies. We concluded that the VEGF 2578C/A gene polymorphism is unlikely to be major predisposing factor for preeclampsia. It has been found that lowest VEGF production was associated with the CC genotype [34]. However, taking

into consideration that VEGF has an important angiogenic role during pregnancy, we should have investigated an association between multiple common functional polymorphisms of other VEGF genotypes and PE. This might have revealed if other genotypes of VEGF represent a risk factor of PE [35]. This may be one of limitations of our work but it was related to the availability of kits and magnitude of work in our institution.

VEGF genotypes among preeclamptic women were not significantly related to maternal and fetal complications in our study. Larger research work is needed to measure VEGF genotypes polymorphisms in umbilical artery and/or vein in combination with maternal samples which may be more related to complications of PE [36]. A key metanalysis, as well as a recent Cochrane review, concluded that using umbilical artery Doppler in high risk pregnancies reduced the risk of perinatal death by 29–38%, without increasing interventions such as iatrogenic preterm delivery [37,38]. Most of the genes in placental arterial endothelial cells are associated with signal transduction and other molecular pathways including VEGF signaling. This stimulates angiogenesis and stabilizes newly formed vessels [39,40]. Thus, umbilical artery endothelial cells express more genes related to VEGF signaling [41]. That's why, in current study, authors assessed association between VEGF (C2578A) gene polymorphism and umbilical artery pulsatility index Doppler changes in preeclamptic women.

In mild PE cases of current study, pulsatility index of umbilical artery showed no significant difference compared to VEGF CA and CC genotypes. However, severe preeclamptic cases showed significant difference. This result may be attributed to, among pregnant women with severe PE; carrier state of VEGF C2578A alleles may be associated with the accelerated development of the disease. Also, progression to severe cases of PE is related to modifications in VEGF gene polymorphisms. Further studies on wide scales are needed to prove these results [11].

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Author contribution

HH Mowad and MZ Nasser: Protocol development and management of laboratory techniques.

AS Fahim and KM Abo Gabal: Data collection or management.

AS Fahim, HAA Ali, MZ Nasser and HH Mowad: Data analysis.

NAA Shehata: Manuscript writing/editing.

7. ClinicalTrials.gov ID

NCT03500588.

8. Synopsis

This case-control study investigates association of VEGF (C2578A) gene polymorphism and Umbilical artery Doppler in preeclamptic women.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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