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Original Article

VASCULAR ENDOTHELIAL GROWTH FACTOR VEGF G/C 405 AND C/A 2578 GENE POLYMORPHISMS IN CASES WITH PRE-ECLAMPSIA

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ABSTRACT

Vascular endothelial growth factor gene plays a crucial role in physiological vasculogenesis and vascular permeability and has been implicated in the pathogenesis of pre-eclampsia (PE). VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels. This study carried on 170 pregnant women. Out of them, 100 cases with PE. Their mean age ± SD was 25.28 ± 4.59 years. Seventy nine (79%) cases had mild PE and 21 (21%) cases had severe PE. The other 70 subjects were clinically healthy pregnant women with complete successful pregnancy and no PE. VEGF C 405 G and C 2578 A polymorphisms were studied using PCR and RFLP. We found that PE among Egyptian women was strongly associated with pre-eclamptic mutations related to VEGF C 405 G and C 2578 A gene polymorphisms. There were statistically significant low frequencies of wild homozygous genotype CC and high frequencies of heterozygous mutant genotypes CG & CA and homozygous mutant genotypes GG & AA in PE cases compared to controls. The frequency of cases carrying the homozygous mutant genotype GG was higher among severe pre-eclamptic cases compared to mild pre-eclamptic cases. In addition, there were statistically significant high frequencies in PE cases compared to controls of mutant G and mutant A alleles.

Keywords: Vascular endothelial growth factor, Polymorphisms, Pre-eclampsia.

INTRODUCTION

Pre-eclampsia (PE) is a syndrome of pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation. Pregnancies with PE result in maternal and fetal complications, which include renal failure, liver failure, cerebral edema, low birth weight, prematurity and death. PE is a systemic disease that results from placental defects and occurs in about 5–8% of pregnancies worldwide. PE is a disease of many theories. Wherein investigators put forward their favorite mechanistic ideas, each with a causal appeal for the pathogenesis of PE [1-2].

The PE/eclampsia syndrome is a multisystem disorder that can include cardiovascular changes, hematologic abnormalities, hepatic and renal impairment, and neurologic or cerebral manifestations. It also can affect the eye and visual pathways. Visual symptoms concern up to 25% of patients with severe PE and 50% of patients with eclampsia [3].

Some epidemiologic studies suggest that paternal genetic contributions to the zygotic genotype in addition to maternal genes may contribute in susceptibility to PE [4]. Polymorphisms of genes involved in the regulation of blood pressure or coagulation, such as renin, angiotensinogen (T235), endothelial nitric oxide synthase (eNOS), prothrombin, factor V Leiden, and methyltetrahydrofolate (MTHF) were estimated [5-9].

The increased incidence of PE in women with chronic diseases such as diabetes and hypertension may also advance susceptibility to PE. In addition to increased vascular reactivity, the vasoconstriction appears to be mediated at least in part by alterations in local concentrations of several vasoactive molecules, including the vasoconstrictors norepinephrine, endothelin, and perhaps thromboxane, and the vasodilators prostacyclin and perhaps nitric oxide [10-11].

Proteinuria may rarely precede hypertension but usually accompanies or follows it. After pregnancy is terminated, proteinuria commonly disappears within 3 to 8 weeks, but occasionally persists for months. The quantity of protein excreted in the urine varies widely from less than one gram to 8 to 10 g per day. The urinary sediment is usually bland; red blood cells and cellular casts are rare. PE is the leading cause of nephrotic syndrome during pregnancy. Recent data suggest that a loss of both size and charge selectivity of the glomerular barrier contributes to the development of albuminuria [12].

Increased vessel formation, observed in diffusion villi from pre-eclamptic placentas, would be predicted to facilitate oxygen/ nutrient transfer between mother and fetus. However, it has been postulated that a greater number of vessels in the villi, can reduce oxygen diffusion in the placenta, due to diffusional screening, in which the oxygen delivery to the central vessels is reduced by surrounding vessels. Such reduced oxygen delivery is indicated by increased placental hypoxia inducible factor in PE. Therefore, it is feasible that the increased vessel formation in the pre-eclamptic placenta might not benefit the fetus. It is also possible that the increased angiogenesis is not sufficient to overcome both the reduced oxygen delivery that is due to the infarcted placenta and the reduced oxygen delivery that would be predicted with failed remodeling of the maternal spiral arteries supplying the intervillous space. [13-14]. The aim of this study is to investigate the association of common mutations of the vascular endothelial growth factor (VEGF) gene including C 405 G and C 2578 polymorphisms with PE among Egyptian women.

MATERIALS AND METHODS

Subjects

Cases with PE

This group consisted of 100 pregnant women suffering from PE. They were randomly selected from those attending Obstetrics and Gynecology Department at Mansoura University Hospitals, Egypt. They were recruited in the period between January 2012 and January 2013. Criteria of those PE cases are recorded in Table 1.

Controls

This group was comprised of 70 healthy pregnant women with complete successful pregnancy and no PE. Their characteristics are involved in Table 1.

Sampling

Peripheral blood samples were collected from each case and control in a tube containing ethylene diamine tetracetic acid (EDTA) solution, pH 8.0 as an anticoagulant. From this blood, DNA was

extracted and purified then used for detection of VEGF C 2578 A and C 405 G gene mutations using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique.

DNA extraction and purification

DNA extraction and purification from whole blood was performed using the generation DNA purification capture column kit (Gentra system, USA).

Detection of VEGF G 405 C gene mutations using PCR amplification-RFLP technique

The VEGF G 405 C genotype was determined using PCR-RFLP analysis. G 405 C→ VEGF variant was identified using PCR followed by restriction enzyme digestion of the amplified product. Briefly, PCR primers for amplification of VEGF mutation generate a 197 base pairs (bp) fragment. The GC substitution at bp 197 creates a BsmF1 recognition sequence. By abolishing a BsmF1 restriction site, the G 405 C mutation results in the digestion of the 197 bp amplicon into 167 and 30 bp fragments. Wild type (405CC) produced a single band at 197 bp, heterozygotes (405CG) produced 197, 167 and 30 bp fragments, and homozygous mutant (405GG) produced 167 and 30 bp fragments [15].

Primers for VEGF G 405 C: Forward Primer: 5'-CCGACGGCTTGGGGAGATTG-3' and Reverse Primer: 5'-CGGCGGTCACCCCCAAAAG-3'.

Detection of VEGF C 2578 A gene mutations using PCR amplification-RFLP technique

Similarly, the VEGF C 2578 A genotype was determined using PCR-RFLP analysis. The 2578CA→VEGF variant was identified using PCR followed by restriction enzyme digestion of the amplified product. PCR primers for amplification of the VEGF mutation generate a 267 bp fragment.

The CA→ substitution at bp 2578 creates a Bglll recognition sequence. By abolishing a Bglllrestriction site, the C 2578 A mutation results in the digestion of the 267 bp amplicon into 208 and 60 bp fragments. Wild types (2578CC) produced a single band at 267 bp, heterozygotes (2578CA) produced 267, 208, and 60 bp fragments, and homozygous mutants (2578AA) produced 208 and 60 bp fragments [15].

Primers for VEGF C 2578 A: Forward Primer 5'-GGGCCTTAGGACACCATACC-3r and Reverse Primer 5'-TGCCCCAGGGAACAAAGT-3f.

Table 1: Descriptive data of all studied subjects

Description	PE cases N = 100	Controls N = 70
Age (years)		
Mean ± SD	25.25 ± 4.50	23.73 ± 2.80
Range	20 – 40	19 – 31
Diagnosis		
Mild preeclampsia	79 (79.0%)	-
Sever preeclampsia	21 (21.0%)	-
Gravidity		
Primigravida	51 (51.0%)	40 (57.2%)
Multigravida	49 (49.0%)	30 (42.8%)
Family history of PE (positive/negative)		
Positive	41 (41.0%)	0.0 (0.0%)
Negative	59 (59.0%)	70 (100%)
Systolic blood pressure		
140 -160	69 (69.0%)	-
> 160	31 (31.0%)	-
Diastolic blood pressure		
90 – 110	45(45.0%)	-
> 110	55(55.0%)	-
Gestational age		
≤ 36 weeks	73 (73.0%)	0.0 (0.0%)
> 36 weeks	27 (27.0%)	70 (100%)

PE cases; pre-eclamptic cases, N; number of cases

Statistical analysis

The obtained data were statistically analyzed using SPSS program. The Fisher's exact test was performed with Graph Pad InStat. Power calculations were performed to give the probability of finding differences between gene frequencies. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

PE belongs to hypertensive disorders and occurs in 5-8% of all pregnancies. It is a life threatening disease associated with high mortality rate of mothers and fetuses. It is considered as a multi systematic syndrome with still unclear etiology [16].

The present study revealed a statistically significant high frequency of homozygous mutant genotype AA in Egyptian women with PE compared to controls (15% vs. 0%, P = 0.001) accompanied with low frequency of the normal wild genotype CC (30% vs. 60%, P = 0.001) of VEGF C 2578 A gene. Also, there was a statistically significant high frequency of heterozygous mutant genotype CA in cases with PE compared to control cases (55% vs. 40%, P = 0.02). In relation to VEGF C 405 G, there was statistically significant low frequency of wild homozygous genotype CC in PE cases compared to control subjects (21% vs. 65.7%, P = 0.005), high frequency in heterozygous mutant genotype CG (68% vs. 34.3%, P = 0.004) and high frequency of homozygous mutant genotype GG (11% vs. 0%, P = 0.001). In addition, there were statistically significant high frequencies in PE cases compared to controls of mutant G (45% vs. 23.8%, P = 0.002) and mutant A alleles (42.5% vs. 20%, P = 0.001) (Table 2).

In contradiction to our findings, regarding Hungarian women, Bányász et al. (2006) showed no significant difference between women with PE and control groups regarding VEGF C 2578 G homozygous mutant genotype AA (18% vs. 20%, P> 0.05) [15]. In addition, Papazoglou et al. (2004) reported that there was no any significant difference between the frequency of VEGF 405 mutant G allele in Greek women with PE compared to controls (20.2% vs. 12.3%, P> 0.05).

They added that, there was no significant association between VEGF C 405 G and VEGF C 2578 A polymorphisms and PE [17]. Furthermore, among Caucasian Brazilian women there were no

significant differences in the distribution of VEGF C 2578 A genotypes or alleles between pre-eclamptic white and non-white pregnant women and controls (P> 0.05) [18]. Another study by Garza-veloz et al. (2011) regarding Mexican women, showed no evidence of an association between homozygous mutant genotype AA of VEGF gene in women with PE compared to controls (11% vs. 11%, P = 0.31) [19].

In agreement with our results, Shim et al. (2007) stated that there was a statistically significant higher frequency of VEGF C 405 G homozygous mutant genotype GG between Korean women with PE compared to controls (6.4% vs. 1.9%, P< 0.001). Also, Korean women carrying the mutant C 405 G allele were significantly higher in frequency in PE women than in control subjects (27% vs. 15%, P< 0.05) [20].

Table 2: Frequencies of VEGF C 405 G and C 2578 A mutations among cases of PE compared to controls

Genotype	PE cases N= 100	Controls N = 70	P
VEGF C 405 G			
CC	21 (21.0%)	46 (65.7%)	0.005
CG	68 (68.0%)	24 (34.3%)	0.004
GG	11 (11.0%)	0.0 (0.00%)	0.001
G 405 C Alleles			
C. allele	110 (55.0%)	116 (76.2%)	0.001
G. allele	90 (45.0%)	48 (23.8%)	0.002
VEGF C 2578 A			
CC	30 (30.0%)	42 (60.0%)	0.001
CA	55 (55.0%)	28 (40.0%)	0.020
AA	15 (15.0%)	0.0 (0.00%)	0.001
C 2578 A Alleles			
C. allele	115 (57.5%)	112 (80.0%)	0.001
A. allele	85 (42.5%)	28 (20.0%)	0.001

N; number of cases, P; probability and P < 0.05 means significant, CC; normal homozygous wild type, CG and CA; heterozygous mutant types, GG and AA; homozygous mutant types

Based on our results, we can say that there is significant association between VEGF C 405 G and VEGF C 2578 A gene polymorphisms and PE among Egyptian women. Regarding severity of PE (Table 3), our study showed that the frequency of cases carrying the homozygous mutant genotype GG was statistically significantly higher among severe cases compared to mild cases (11.1% vs. 10.9%, P = 0.01). Statistically non-significant differences (P > 0.05) in allelic frequencies of the VEGF G 405 C gene among mild and severe cases were found. Table 3 also revealed statistically non-significant differences (P > 0.05) in VEGF C 2578 A gene genotypic and allelic frequencies among mild and severe PE cases. On contrary, the frequency of VEGF C 405 G mutant G allele in Greek women with mild PE compared to severe was significantly lower (11.4% vs. 27.5%, P = 0.019) [17].

Table 3: Frequencies of VEGF C 405 G and C 2578 A genes polymorphisms in pre-eclamptic cases according to severity of the disease

Genotype	Mild PE N = 46	Severe PE N = 54	P
VEGF C 405 G			
CC	9 (19.6%)	12 (22.2%)	0.04
CG	32 (69.6%)	36 (66.7%)	0.46
GG	5 (10.9%)	6 (11.1%)	0.01
G 405 C Alleles			
C allele	50 (54.4%)	60 (55.5%)	0.30
G allele	42 (45.6%)	48 (44.4%)	0.20
VEGF C 2578 A			
CC	18 (32.9%)	12 (19.1%)	0.06
CA	22 (50.6%)	33 (71.4%)	0.36
AA	6 (16.5%)	9 (9.50%)	0.25
C 2578 A Alleles			
C allele	58 (58.2%)	57 (54.8%)	0.73
A allele	34 (41.8%)	51 (45.2%)	0.71

N; number of cases, P; probability and P < 0.05 means significant, CC; normal homozygous wild type, CG and CA; heterozygous mutant types, GG and AA; homozygous mutant types

Many factors such as: family history of PE/eclampsia, nulliparity, previous pregnancy with PE development, multiple pregnancy, obesity, diabetes mellitus, chronic hypertension, renal and autoimmune diseases were indicated to increase the risk of PE [21]. Watson et al. (2000) stated that the VEGF C 405 G and VEGF C 2578 A polymorphisms determine the production of VEGF. The highest VEGF production was associated with the VEGF 405 GG genotype; the intermediate production was associated with the GC genotype and the lowest production with the CC genotype [22]. It was suggested that the genetic polymorphisms of VEGF gene is linked to an inherited alteration of VEGF production may contribute to the pathogenesis of PE since VEGF has a central role in many processes that are involved in the development and progression of PE [23].

CONCLUSION

There is a significant association between VEGF C 405 G and VEGF C 2578 A gene polymorphisms and PE among Egyptian women. There were statistically significant low frequencies of wild homozygous genotype CC and high frequencies of heterozygous mutant genotypes CG & CA and homozygous mutant genotypes GG & AA in PE cases compared to controls. The frequency of cases carrying the homozygous mutant genotype GG was higher among severe pre-

eclamptic cases compared to mild pre-eclamptic cases. In addition, there were statistically significant high frequencies in PE cases compared to controls of mutant G and mutant A alleles. Thus, we have to recommend routine screening for pre-eclamptic mutations for all Egyptian pregnant women in order to set up an appropriate method of prophylaxis against these pre-eclamptic disorders. Special emphasis should be directed to cases with PE that deserve serious consideration.

CONFLICT OF INTERESTS

Declared None

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