Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(1)

Association between Vascular Endothelial Growth Factor Gene Polymorphisms and the Risk of Preeclampsia in Iraqi Pregnant Women

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Abstract

Background Vascular endothelial growth factor (VEGF) is an essential factor for angiogenesis and plays important role in placental development.

Objective To investigate the association between VEGF single nucleotide polymorphisms (SNPs) +936 Cytosine/Thymine (C/T) and -634 Guanine/Cytosine (G/C) and preeclampsia risk.

Methods A total of 50 cases of pregnant women with preeclampsia and 50 healthy pregnant women (as a control) were involved in this case control study. Blood samples were collected form each woman and the Deoxyribonucleic acid (DNA) was extracted. Amplification of VEGF gene was done by conventional polymerase chain reaction (PCR) and then detection of SNPs-634 G/C and +936 C/T were carried out by restriction fragment length polymorphism PCR (PCR-RFLP).

Results The frequency of different genotypes of VEGF SNPs +936 C/T &-634 G/C are in accordance with Hardy Weinberg equilibrium. VEGF polymorphism +936 C/T appeared in 3 genotypes after digestion with restriction enzymes Cytosine Cytosine (CC), Cytosine Thymine (C/T) & Thymine Thymine (TT). VEGF SNP -634 G/C appeared in 3 genotypes after digestion with restriction enzymes; those were Guanine Guanine (GG), Guanine Cytosine (GC) and CC. The heterozygous genotype CT of polymorphism +936 C/T was more frequent among preeclamptic patients than the controls (26% versus 16%). Likewise, TT genotype was more frequent among preeclamptic patients (8% versus 2%) with no significant differences. At the allelic level, the difference was more prominent. The frequency of mutant allele (T allele) was much more frequent in preeclamptic patients than controls (21% versus 10%) with a statistically significant difference (p=0.049). Although CC genotype of polymorphism -634 G/C was more frequent among preeclamptic patients than controls (14% versus 10%), the difference was not significant (p=0.704). Likewise, there were no significant differences in allele frequency.

Conclusion The study suggested that the mutant T allele of VEGF +936 C/T polymorphism was associated with increased preeclampsia risk and disease development.

Keywords Vascular endothelial growth factor, polymorphisms, preeclampsia

Citation Khaleel MF, Al-Moayad HA. Association between vascular endothelial growth factor gene polymorphisms and the risk of preeclampsia in Iraqi pregnant women. Iraqi JMS. 2022; 20(1): 106-112. doi: 10.22578/IJMS.20.1.14

List of abbreviations: bp = Base pair, C = Cytosine, DBP = Diastolic blood pressure, DNA = Deoxy ribonucleic acid, G = Guanine, HWE = Hardy-Weinperg equilibrium, Kb = Kilo Base, P = Short arm of a chromosome, PCR = Polymerase chain reaction, PE = Preeclampsia, PIGF = Placental growth factor, PIH = Pregnancy induced hypertension, RFLP = Restriction fragment length polymorphism, SBP = Systolic blood pressure, SNP = Single nucleotide polymorphism, T = Thymine, VEGF = Vascular endothelial growth factor

Introduction

Preeclampsia (PE) has been recognized as a multifactorial disorder with great phenotypic diversity and it is caused by a complex interplay between genetic and

environmental factors [1]. PE is a leading cause of maternal death. The World Health Organization (WHO) estimates that between 50,000 and 75,000 women die globally of this condition annually [2].

PE is diagnosed as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on 2 occasions at least 4 hours apart after 20 weeks of gestation in a previously normotensive patient with the new onset of any of the following (with or without proteinuria): platelet count <100,000/µl, serum creatinine >1.1 mg/dl or doubling of the creatinine concentration in the absence of other renal disease, liver transaminases at least twice the upper limit of the normal concentrations for the local laboratory, pulmonary edema and cerebral or visual symptoms (e. g new onset and persistent headache, blurred vision, flashing lights or sparks, scotomata) [3].

Vascular endothelial growth factor (VEGF) is an essential factor for angiogenesis and plays important role in placental development [4].

VEGF gene is located on the chromosome 6 p21.3, the full length is 28kb, and encoding gene length is 14kb, consisting of 8 exons and 7 introns. The coding product is two bond linked glycoproteins, due to different splicing site of the gene, 7subtypes of the protein can be found [5]. VEGF (or VEGF-A) belongs to a gene family of those placental growth factor (PlGF), VEGF-B, VEGF-C and VEGF-D. They share structural features typical of the VEGF family, but display different biological activities, mainly owing to their different specificities for the known VEGF receptors [6].

VEGF gene polymorphisms play an important role in regulating and altering the protein expression and function, and changing the susceptibility to PE [7]. Many polymorphisms of the VEGF gene have been identified so far. Few of them have been correlated with variation in VEGF protein production [8]. Specifically, C936T located in the 39-untranslated region and -634 G/C in the 59-untranslated region [9]. The single nucleotide polymorphisms (SNP), rather than the gene, is the currency of large scale, high-throughput studies of the human genome, which are the key to complex diseases like PE [10].

The objectives of this study were to investigate the association between VEGF SNPs +936 Cytosine/Thymine (C/T) and -634 Guanine/Cytosine (G/C) and preeclampsia risk.

Methods

A case-control study was conducted at Al-Imamein Al- Kadhimein Medical City for the period from February 2019 to the end of October 2019. A total of 100 pregnant women with gestational age ≥20th weeks were included in this study. They were informed about the nature of the study and verbal with written consents were obtained from them. Eligible women were divided in to two equal groups, preeclampsia group (PG) included 50 pregnant women who had been diagnosed with PE and a control group (C) included 50 pregnant women who were normotensive with no symptoms or signs of PE & no proteinuria. Women with chronic hypertension, diabetes (type 1 or 2), chronic renal disease, autoimmune disease and rheumatic disease were excluded from the study.

DNA extraction and genotyping

Five ml of venous blood were collected from each participant. DNA was isolated from 200 µl of anticoagulated peripheral blood using a commercially available kit according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit; Qiagen Inc., USA). Amplification of the two regions of the VEGF gene containing the polymorphisms-634G/C and +936C/T were carried out in a Master cycler gradient (Hybaid/UK) thermal cycler in 20 µl reaction volumes containing 20 mmol/l Tris-HCl (pH 8. 4), $MgCl_2$, 50 mmol/l KCl, 0.2 mmol/l of each nucleotide, 20 pmol of each of the forward and reverse primers, 1 U Platinum Taq polymerase (Pioneer, Korea) and 500 ng of DNA. Following an initial denaturation step (5 min at 94°C), samples were subjected to 35 rounds of polymerase chain reaction (PCR) consisting of

94°C for 40 s, 58°C (-634G/C) or 64°C (+936C/T) for 1 min; and 72°C for 40 s with a final extension time of 5 min at 72°C. For the -34G/C the following primers amplified a fragment of 304 bp: forward 5'-ATTTATTTTTGTCTGTCTGTCTGTCCGTCA-3' and for the +936C/T the following primers amplified a fragment of 208 bp: forward 5' AAGGAA GA GGAG ACTCTGCGCAGAGC-3', reverse 5' TAAATGTATGTATGTGGGTGGGTGTG TCTACAGG-3'. The VEGF -634G/C polymorphism was analyzed by digestion of the PCR product with restriction endonuclease BsmfI (New England Biolabs, USA).

The -634G allele was cut into two fragments of 193 and 111 bp while the -634C allele remained uncut (304 bp). The VEGF 936C/T polymorphism was analyzed by digestion of the PCR product with restriction endonuclease NlaIII (New England Biolabs). The 936C allele remained uncut (208 bp), while the 936T was cut into two fragments of 122 and 86 bp.

Statistical Analysis

The statistical package for the social sciences (SPSS, version 20) was used for statistical analysis. Continuous variables were expressed as mean±standard deviation (SD). Risk association between the different genotypes of VEGF gene polymorphisms and PE susceptibility was estimated by the calculation of odd ratio (OR) and 95% confidence intervals (CI) using binary logistic regression. For this analysis, women who were homogenous for the wild genotype were considered as a reference, and polymorphisms as dependent variables. Chi square was used for testing the deviation from Hardy-Weinberg equilibrium as well as for comparing categorical variables. A p-value <0.05 was considered statistically significant.

Results

Mean age of PE patients was 26.94±4.13 years, which was slightly higher than the mean age of the controls (25.92±8.09 years) with no significant difference. Women in control group had higher mean values for parity, gestational age, and hemoglobin (Hb) (1.78±0.83, 36.24±5.15 weeks, and 11.58±1.91 g/dl respectively) than PE patients (1.12±0.65, 35.76±6.06 weeks, and10.8±1.1 g/dl, respectively); however, the differences were not significant. On the other hand, positive family history of PE was far more frequent among PE women than controls (36% versus 4%) with a highly significant difference (P value <0.001). In contrast, women in the control group showed significantly higher platelets count than PE women (236.65±58.44×10^3/mL versus 146.86±62.19 ×10^3/mL). Each of the other parameters (albumin in urine, systolic blood pressure and diastolic blood pressure), per se, were significantly higher in PE patients than controls (Table1).

The frequency of different genotypes of both VEGF +936 C/T and VEGF -634 G/C are in accordance with Hardy-Weinberg Equilibrium (HWE).

The distribution of different genotypes and allele of VEGF +936 C/T polymorphism in PE patients and controls is shown in table (2). The heterozygous genotype CT was more frequent among PE patients than control (26% vs. 16%), the difference was not significant (p= 0.16). Likewise, TT genotype was more frequent among PE patients (8% vs. 2%) with no significant difference (p=0.455). At allelic level, the difference was more frequent. The frequency of mutant allele (T allele) was much more frequent in PE patients than controls (21% vs. 10%) with a significant difference (p= 0.049).

Table 1. Demographic, reproductive and clinical characteristics preeclampsia patients and healthy controls

Variables		PE patients N=50	Controls N=50	P value
Age, years (mean ±SD)		26.94±4.13	25.92±8.09	0.236
Parity (mean ±SD)		1.12±0.65	1.78±0.83	0.136
Family history	No	32 (64%)	48 (96%)	<0.001
	Yes	18 (36%)	2 (4%)	
Gestational age (weeks) (mean±SD)		35.76±6.06	36.24±5.15	0.202
Hemoglobin (g/dL) (mean±SD)		10.8±1.1	11.58±1.91	0.072
Platelets count (×10^3/mL) (mean ±SD)		146.86±62.19	236.65±58.44	0.022
Albumin in urine (+) (mean±SD)		2.36±0.81	0.0±0.0	<0.001
SBP (mmHg) (mean ±SD)		158.5±18.23	114.6±9.6	<0.001
DBP (mmHg) (mean±SD)		101.0±12.21	70.0±8.51	<0.001

SBP: systolic blood pressure, DBP: diastolic blood pressure

Table 2. Genotypes and allele frequencies of VEGF +936C/T gene polymorphism in PE patients and controls

VEGF +936C/T		Cases n=50	Control n=50	P-value	OR (95%CI)
Genotype	CC	33 (66%)	41 (82%)	0.169	1.0
	CT	13 (26%)	8 (16%)	0.16	4.97 (0.53-46.62)
	TT	4 (8%)	1 (2%)	0.455	2.46 (0.23-26.11)
	HWE	0.126	0.432		
Allele	C	79 (79%)	90 (90%)	0.049	2.39 (1.06-5.38)
	T	21 (21%)	10 (10%)		

The distribution of different genotypes and allele of VEGF -634 G/C polymorphism showed difference between PE patients and controls, although CC genotype was more frequent among PE patients than controls (14% vs. 10%), the difference was not significant (P=0.704). Likewise, there were no significant differences in allele frequency of this polymorphism between patients and control. The frequency of mutant allele (C allele) in patients and controls was 38% and 32% respectively with a (P=0.46) as shown in table (3).

Discussion

Over the last decade, extensive research has been conducted to better understand the genetic components of the pathophysiology of PE. However, no universally accepted genetic factors can explain the onset and progression of this multifactorial disorder [(11)]. Because VEGF is known to play a role in the regulation of cytotrophoblast invasion and placentation, and there is evidence of abnormal placentation in preeclamptic placenta, it suggested that the genes related to VEGF activity would be a risk factor for PE [(12)].

Table 3. Genotypes and allele frequencies of VEGF-634G/C gene polymorphism in PE patients and controls

VEGF -634G/C		Cases n=50	Control n=50	P-value	OR (95% CI)
Genotype	GG	19 (38%)	23 (46%)	0.671	1.0
	GC	24 (48%)	22 (44%)	0.426	1.7 (0.46-6.2)
	CC	7 (14%)	5 (10%)	0.704	1.28 (0.36-4.64)
	HWE	0.896	0.938		
Allele	G	62 (62%)	68 (68%)	0.46	0.76 (0.42-1.37)
	C	38 (38%)	32 (32%)		

There are several SNPs in the VEGF gene, including +936, -634, -2578 & -1154 positions, these SNPs could alter gene expression and protein production. Representative gene mutations of VEGF such as C-936T and G-634C were associated with decreased levels of circulating VEGF [(4)]. This is suggested to be associated with increased risk of PE [(13)]. The current study evaluated the association between two common functional VEGF polymorphisms SNP +936 C/T and -634 G/C and PE risk; SNPs for which an association with PE were reported in other population and considering the potential impact on gene expression. The current study found that there is a significant association between the frequency of mutant allele (T allele) for the polymorphism +936 C/T and PE patients than controls (21 % vs 10%) (P value 0.049).

Although the genotypes (CT) and (TT) of SNP +936 C/T were more frequent among PE patients but the differences were statistically insignificant. Likewise, the frequency of CC genotype and the mutant allele (allele C) of SNP -634 G/C were higher among PE patients, the differences were not significant. Also, this study found highly significant difference of family history of PE in PE cases compared to the controls (36% vs 4%) (P value <0.001). There are several reports about the association between maternal VEGF polymorphisms and the risk of PE in different countries and ethnic groups with inconsistent results.

Papazoglou et al. involved 42 PE and 73 healthy control of Caucasians ethnicity who were genotyped for -634 G/C and +936 C/T polymorphisms of the VEGF gene. They reported no significant association between genotypic or allelic frequencies. However, with severe PE there was statistically significant difference for allelic frequencies of the +936 C/T polymorphism [(14)].

Kim et al. in a retrospective case control study in Korean pregnant women included PE cases and 237 controls healthy pregnant suggested that there is no significant difference for +936 C/T polymorphism between PE cases and controls. This result is discordant with the findings of the current study; these researchers also investigated SNP -634 G/C and found no significant association between this SNP and PE [(15)].

Garza-Veloz et al. included 78 PE cases and 86 normotensive pregnant controls of Latinos ethnicity. They found no association between VEGF allele, genotype or haplotype frequencies and PE, its severity or onset of the disease [(16)].

Cheng et al. carried a meta-analysis including 11 case-control studies with 1069 PE cases and 1315 controls with different ethnicities indicated that there is significant association between SNP +936 C/T and the risk of PE. Pregnant women carrying the T allele have significantly higher risk of PE than pregnant women carrying the +936 CC genotype [(13)].

Procopciuc et al. found in their study in Romania of 70 PE women and 94 normal pregnant found that the presence of T allele and TT genotype of SNP +936 C/T significantly increases the risk of pregnancy induced hypertension (PIH), mild and severe PE [(17)].

Keshavarzi et al. investigated the association between PE risk and -634 G/C polymorphisms in women of Asian ethnicity, they found that VEGF -634 GC and CC genotypes were significantly higher in PE pregnant women and associated with 2.6 and 2-fold higher risk of PE respectively [18].

The discrepancy between the findings of the current study and the data of some groups for VEGF polymorphisms may be explained by ethnic variation, differences of the VEGF gene polymorphisms in different regions and population, genotyping methods and gene-environmental interaction.

In conclusion, the mutant T allele of VEGF +936 C/T polymorphism was significantly associated with increased PE risk, disease development and could be a susceptibility biomarker for PE, while VEGF -634 G/C polymorphisms had no significant association with PE.

Acknowledgement

The authors would like to thank professor Dr. Haider Ahmed Shamran, Medical Genetic and Assistant Professor Dr. Kasim Shamran Haraj, Molecular Genetic for helping in genetic analysis.

Author contribution

Dr. khaleel: Scientific work, investigations, writing and editing of all data. Dr. Al-Moayad: Supervision of the study and final editing of manuscript.

Conflict of interest

Authors declare that there is no conflict of interest.

Funding

This study was supported by Al-Imamein Al-Kadhimein Medical City as research and student thesis.

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Received Jun. 13th 2021
Accepted Sep. 5th 2021