Polymorphisms of the VEGF gene On Pregnant ladies Confounded for Pre-eclampsia Homing

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الخلاصة:

مقدمه: يعتبر تسمم الحمل من الامراض الشائعة لدى النساء الحوامل ، و يتصف بارتفاع ضغط الدم واكثر من ٩٠/١٤٠ و ارتفاع البروتين يوريا اكثر من ٣٠٠ ملي غرام ل٢٤ ساعه ابتداء من الاسبوع العشرون من الحمل والذي يصاحبه ضهور بعض الاعراض كضهور انتفاخ في الاطراف ،الالام الراس ، تغييرات في الرؤيا بالإضافة الى الالم البطن و الثرمبو سايتو بينيا. هدف الدراسة : لمعرفة العلاقة بين تعدد اشكال النيكليوتيدات لعامل نمو بطانة الأوعية الدموية و بدية ضهور الاعراض و خطورتها. طرق العمل: تمت مضاعفة الدي ان أي المستخلص من ٦٠ سيده حامل مشخصه بتسمم الحمل و ٦٠ سيده من ذوات الحمل الطبيعي لغرض الكشف عن تعدد اشكال النيوكليوتيدات لعامل نمو بطانة الأوعية الدموية باستخدام تقنية RFLP. لتحليا الاحصائي استخدم OR لكل البيانات باستخدام برنامج SPSS. النتائج: نتائج الانماط الجينية لعامل نمو بطانة الأوعية الدموية كانت كل الاتي النمط الجيني CC يعتبر النمط الحامي للمرض مع وجود فوارق إحصائية معنويه (٠,٠٤) و عامل وقائي (٠,٦٥٤)،بينما النمط CG يزيد من احتمالية تطور المرض ب ٢,٤١ مره ولكن للأسف لا توجد أي فوارق إحصائية معنويه بالإضافة الى امتلاكها عامل مسبب للمرض و الذي يتمثل ب(٠,٥٨٥) ، و اخير النمط الجيني GG يزيد من احتمالية تطور المرض ب ١,٢١ مره كع عدم وجود أي فوارق إحصائية معنويه مع امتلاكها لعامل مسبب للمرض و الذي يتمثل ب (٠,١٧٣). بالنسبة للاليل يعتبر C هو اليل الحامي و الذي يقلل من نسبة تطور المرض بينما G اليل يعتبر اليل المسبب للمرض. الاستنتاج: يعتبر G اليل هو العامل المسبب لمرض تسمم الحمل بينما C اليل يحمي من تطور المرض لدى النساء الحوامل .

Abstract

Background: Pre-eclampsia (PE) is a relatively common, systemic pregnancy disorder characterized by the development of concurrent hypertension (> 140/90 mmHg) and proteinuria (> 300 mg/24 h) at ≥ 20 weeks of gestation, that may also be associated with a myriad of other symptoms such as edema, headache, blurred vision, irritability, abdominal pain, and thrombocytopenia. **Aim :** Identify the association between single nucleotide polymorphisms (SNP) of VEGF and the onset and severity of the disease. **Methods:** The extracted DNA was amplified for VEGF RFLP in 60 clinically diagnosed preeclampsia pregnant women and 60 normotensive pregnant women. For statistical significance, OR was measured and all data were processed by using SPSS. **Results:** VEGF genotyping expressed the following results CC genotype considered as protective genotype with P value of (0.04) and protective factor of (0.654) while the CG genotype have 2.41 fold increase risk of having PE with unfortunately non- significant statistical analysis and etiological factor of (0.585).GG

genotype have 1.21 fold increase risk of having PE with non-significant statistical analysis and etiological factor of (0.173). Allele C considered as protective allele while G allele considered as etiological allele. **Conclusion**: VEGF G allele considered as significant risk factor for having preeclampsia. The presence of VEGF C alleles protects against having preeclampsia.

Key words: preeclampsia, VEGF RFLP, VEGF and Preeclampsia.

Introduction

Preeclampsia (PE) is a pregnancy-specific syndrome that usually occurs after 20 weeks gestation. It is determined by increased blood pressure accompanied by proteinuria. The incidence of preeclampsia ranges from 6% to 8% of all pregnancies worldwide **(1)**. As one of the leading causes of maternal and perinatal morbidity and mortality, PE is a severe threat to maternal and infant health. Although numerous studies on the etiology and pathogenesis of PE have been reported, such as inadequate trophoblast invasion **(2,3)**, placental and endothelial dysfunction **(4)**, immune maladaptation and exaggerated systemic inflammatory response **(5)**. PE displays multi-factoral inheritance and both environmental risk factors and genetic components influence the development of PE. Although various candidate genes, such as *IFN-γ*, *IL-1* and *TNF-α* **(6-8) .**

PE is a multifactorial disease characterized by systolic blood pressure P140 mmHg or diastolic P90 mmHg at bed rest, on at least two occasions, six hours apart, and proteinuria P0.3 g/24 h, measured after the 20th week of pregnancy **(9).** Symptoms frequently observed in PE include headache, blurred vision, and abdominal pain. The etiology of PE is unknown and the delivery of placenta remains the only known treatment. Clinically, it is important to diagnose the severe form of PE when hypertension and proteinuria are even higher. This disease can progress to eclampsia (characterized by seizures as a sign of affection of the cerebral vessels), syndrome HELLP (hemolysis, elevated liver enzyme, low platelets) or disseminated intravascular coagulation **(10).** PE is associated with placental disorder, endothelial cell dysfunction and systemic vasospasm. The events leading to these alterations remain unclear, but it seems that abnormal immune system activation plays a relevant role in PE development **(10,11).**

Several studies have been performed to elucidate its significance in PE. Some authors observed high levels of VEGF in PE that correlate with the severity of the disease. Several groups have demonstrated that circulating free VEGF concentrations are significantly lower in women with PE **(13)**. Levine *et al*. demonstrated that serum concentration of soluble fms-like tyrosine kinase 1 (sFlt-1), a natural antagonist of VEGF, is increased in PE, and in parallel there is a decrease in the serum level of free VEGF **(12)**. These data suggest the potential role of VEGF in the pathogenesis of the disease. In this study, we hypothesized that a maternal genetic component may contribute to altered VEGF production. The VEGF gene is highly

polymorphic. More than 80 single-nucleotide polymorphisms (SNPs) are known in this region of the human genome (NCBI, Gene accession no.: NT 007592). Some of these such as VEGF G+405C SNP may have an impact on VEGF production in peripheral blood mononuclear cells (PBMCs) **(13)**.

Patients and methods

Patients: The current study was conducted during the period from first of February 2016 to the first of December 2016, 60 patient who attended the outpatient and inpatient department, were diagnosed by a women consultant in the Maternity and Childhood Teaching Hospital on the basis of blood pressure measurement and proteinuria elevation. All subjects within the age group of 16-40 years were selected for the study. The PE diagnosis was based on the criteria from the Report of the 'National High Blood Pressure Education Program, and 60 healthy control, were included in the study. Blood pressure was measured with a mercury sphygmomanometer and the Korotkov sound technique. Diastolic pressure was indicated by the Korotkov V. sound. All patients had proteinuria ≥3+ or 4+ tested by dipstick in at least two random urine specimens obtained at least 4 h apart. Medical history, maternal age, gestational age were recorded. Pre-pregnancy BMI was calculated as weight in kilograms divided by height in metres squared. .

Selection Criteria of Patients

Inclusion Criteria: All subjects within the age group of 16-40 years were selected for the study. PE was defined as persisting elevated diastolic blood pressure (≥90mmHg), a proteinuria (≥300 mg in a 24 urine sample) and the presence of edema. Subjects willing to participate were included in the study.

Exclusion criteria: Subjects with non-confirmed PE, essential hypertension, malaria, haemolytic anemia, any other infection such as urinary tract infection or upper respiratory tract infection, fetal death, renal disease, uterine malformation, *in vitro* fertilization treatment, placental abruption, infection, cancer, gestational diabetes mellitus, or any other systemic disease, including pre-existing hypertension, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

Selection Criteria of Control groups

Inclusion Criteria: Normotensive pregnant women had systolic/diastolic blood pressure below 120/80 mmHg and no history of hypertension or proteinuria. All pregnant women showed gestational age from 20 to 40 weeks.

Exclusion Criteria: Exclusion criteria for healthy control were chronic hypertension, haemostatic abnormalities, cancer, diabetes mellitus, cardiovascular, autoimmune, renal and hepatic diseases, and anticoagulant therapy. All normotensive pregnant women have no systemic disease or chronic disease . In addition they have no dead baby or history of dead baby.

Collection of Sample

Blood Sample: Blood samples were collected from pregnant women included in present study by venipuncture. A total six ml of blood were drawn from forearm vein , 4 ml is divided into 2 ml for (GOT, GPT and ALP) and

2 ml for blood urea and the rest 2 ml sample in EDTA tubes which was immediately deeply frozen for *VEGF* SNP analysis.

Urine Collection: For pregnant women and in most identical situations, a midstream clean-catch technique is usually adequate. Ten ml of urine for protein urea detection was required.

Genotyping: DNA was extracted with standardized DNA-extracting protocols using AccuPrep®Genomic DNA extraction kit (Bioneer. Korea). The amount of used blood samples for the DNA extraction was 200 µl. The PCRs were performed in a final volume of 50 µl containing 40 µl PCR buffer, and sense and antisense primers, 2.5 µl of each. We used 5 µl DNA per PCR reaction all mixed in master mix tubes AccuPower^{TM} PCR PreMix Taq DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) Tris-HCl pH 9.0,KCl, $MgCl_2$ Stabilizer and Tracking dye. The investigated DNA sequences were amplified by the following primers: VEGF G+405C SNP: forward: 5´-CCGACGGCTTGGGGAGATTG-3´ and reverse: 5´-CGGCGGTCACCCCCAAAAG-3´.The condition of PCRs was as follows: 20 s at 94°C (denaturing), 20 s at 60°C (annealing) and 30 s at 72°C (extension) for 40 cycles. The resultant 197 bp length product was subsequently digested by BsmF I restriction endonuclease (New England Biolabs, USA) at 60°C one hour. This resulted in two fragments, 167 and 30 bp, in the presence of VEGF+405G allele. The PCR products and digested PCR products were then run on 3% agarose gel followed by ethidium bromide staining.

Data analysis: Hardy–Weinberg equilibrium of the tested SNP were calculated. Logistic regression analysis was used for the analysis of the association between VEGF genotype and risk of PE. Multiple linear regression analysis was used to test the association between carrier state of VEGF genotypes and diagnosis of hypertension and proteinuria. The associations were adjusted for maternal age, gestational age, All calculations were performed with the statistical software package SPSS 23.

Result:

The results presented in this study were based on the analysis of a random 60 pregnant women with an established diagnosis of PE, their age ranged between 16 and 39 years with a mean of 27.75 years. Also a 60 apparently healthy pregnant as control group was selected according to maternal and gestational age of the patients group. Their age ranged between 16 and 40 years with a mean of 30.2 years. The patients group was further subdivided into two subgroups according to their gestational age at which the hypertension and proteinuria appear into early onset PE , and late onset PE. So, early onset PE started before 35week of gestation and the late onset of PE appear after 35 week of gestation, the early onset represent 30 cases with gestational age range (24 -34) weeks and mean of 27.9 while the late onset (30 cases) with gestational age range between (36- 39) week and mean of 36.7. In addition the gestational age mean of the studied group was statistically significant with p value of ($P<0.05$) **table(1)**. The frequency of PE patient who have history of PE in previous

pregnancy (45%), and history of dead baby in pervious pregnancy was (35%) which represent a high percentage of cases. Family history frequency was represent (66.6%)which have sister or mother history of PE, as in **table (2).** The diastolic Blood pressure measurement of the PE patients ranged between 90 and 150, in the early onset PE the diastolic blood pressure ranged between100 and 110mm Hg and mean of (101 mm Hg), while in the late onset PE ranged between 90 and 150mm Hg with mean of (118 mm Hg) when compared to the healthy control group the diastolic blood pressure range between 70 and 80mm Hg with mean of (75.5 mm Hg). Diastolic blood pressure mean was statistically higher in PE patient when compared to the control group with P value (0.001). Regarding the systolic blood pressure in early onset PE patients ranged between 140 and 160 mmHg with mean of (163 mmHg),while in the late onset ranged between 130 and 200 mmHg with mean of (149 mmHg). Systolic blood pressure mean was statistically higher in PE patient when compared to the control group with P value (≤ 0.01). **table(3)**.

Protein urea measurement among early and late onset of PE ranged from 300-500 mg/24hours with mean of (420) in early onset and (426.7) in late onset when compared to healthy control which still normal with statistically significant P value (<0.01) as in **table (4).**

Genetic Study:

DNA Amplification The products of successful binding between the extracted DNA and specific primers for *VEGF* gene were detected by gel electrophoresis analysis using DNA marker (2000-100 bp DNA ladder) and the products size was 197bp for both patients and control groups.

Detection of *VEGF* gene Polymorphism: The distribution of *VEGF +405* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; homozygote (GG) at 167bp, and 30bp, heterozygous (CG) at 197bp and 167bp, 30bp and wild type (CC) which still undigested197bp,Figure (2), the genotype distribution of the investigated VEGF SNPs fulfilled Hardy–Weinberg criteria in the PE and control groups and agree with report of **(13).**

As shown in **table (5),figure (1).** VEGF genotyping expressed the following results CC genotype expressed (23.3%) and considered as protective genotype with P value of (0.04)and protective factor of (0.654) while the CG genotype expressed (26.7%) and have 2.41 fold increase risk of having PE with unfortunately non- significant statistical analysis and etiological factor of (0.585).GG genotype expressed (40%) have 1.21 fold increase risk of having PE with non-significant statistical analysis and etiological factor of (0.173). Allele C considered as protective allele with P value of (0.002) and protective factor of (0.189) while G allele have 2.179 fold increase risk of having PE with P value of (0.002) and etiological factor of (0.586).

Discussion

PE is a multifactorial disease caused by environmental factors that act over a genetic base, permitting the occurrence of this disorder **(14, 15, 16)** . In this study preeclampsia term was used when systolic blood pressure and/or diastolic blood pressure are higher than 140/90 mmHg after 20 weeks of gestation and 300 mg or more of protein in a 24-hour urine collection, or when there is a + random protein strip or higher, or 24-hour protein levels higher than 300 mg/dL. The severity of the condition is based on the presence of arterial blood pressure higher than 160/110 mmHg, creatinine higher than 1.1 mg/dL, brain and visual disturbances, platelet count lower than 100.000/µL, and a 2-fold increase in liver enzymes. It should be remembered that the absence of urinary protein does not exclude the diagnosis because the diagnosis is made according to the severity of signs and symptoms even in the absence of proteinuria **(17).**

Vascular endothelial growth factor (VEGF) is a potent regulator of placental vascular function **(18)** and considered an important angiogenic factor and plays a fundamental role as a regulator in endothelial cell proliferation and vascular permeability. The gene encoding *VEGF* is highly polymorphic and its functional polymorphisms may be useful as indicators of susceptibility to PE (19). The human placenta is rich in angiogenic factors such as VEGF, which probably play an important role not only in forming placental vessels, but also in maternal vascular adaptation to pregnancy. A role for VEGF in embryonic development and trophoblast vascularization was proposed (20).

The *VEGF* genotypes were assessed for their role in predicting the risk of having preeclampsia, which compared with the healthy control group. **Table (3-10)** showed that the *VEGF* genotypes, had significant predictive power. The G allele had the strongest association p=0.002 and significantly increases the risk of having preeclampsia by 2.2 times compared to healthy control. Conversely the C allele had a protective effect and significantly reduces the risk of having preeclampsia by 1.2 times. Both the heterozygous CG and the homozygous GG genotypes increase the risk of the disease by 2.4 times and 1.2 times, respectively. While the wild CC genotype showed a statistically significant protective effect. Its occurrence reduces the risk of having preeclampsia by 2.4 times. These results were highly comparable with a study conducted by

El-Sonbati *et al.*, (2014)(22) in Egypt revealed that *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) (22) **.**

The results of **Banyasz *et al.*, (2006)(13)** in Holland disagree with the present study results and have suggested that the genetic polymorphisms of *VEGF* gene, linked to an inherited alteration of VEGF production, may contribute to the pathogenesis of PE. Also have found that carrier state of the VEGF+405G allele, which is accompanied by high VEGF-producing capability, decreases the risk of PE. VEGF has a central role in many processes that are involved in the development and progression of PE. VEGF is known to play a role in the regulation of cytotrophoblast invasion and placentation . It could be hypothesized that the observation that an inherited increase of VEGF producing ability could be protective against PE is in relation to the earlier experience that VEGF has a potential effect on placentation. Hungarian women, showed no significant difference between women with PE and control groups regarding VEGF C 405 G (13)**.** In addition, **Papazoglou *et al.*,** reported that there was no 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) (21) **.** In agreement with the present study results, **(23) 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) (23).

In some recent studies, results have also shown that this elevated concentration of serum VEGF has increased to the level similar to that of the normal pregnant females without preeclampsia, leading authors to assume that the primary source of the elevated concentration of serum VEGF lies basically in the fetus and the placenta and with removal of the placenta and the fetus the level of serum VEGF will also revert to normal. According to previous studies in which different investigators have measured the VEGF concentration in the maternal circulation during normal and preeclamptic patients, results were conflicting with variable outcomes (24). VEGF has been supposed to play an important role in both the embryogenesis and placental formation; thus, the elevated levels are expected in the pregnant females (24).

It seems likely that when one, or more likely several, combination of polymorphisms occur in the same individual, perhaps together with environmental factors, when PE is expressed. This could be an explanation of individual differences found even among apparently tightly phenotype PE sufferer. PE is a morbid condition characterized by vascular dysfunction. VEGF has an important role in angiogenesis and has been the focus in many diseases in which vasculopathy has a role in etiopathogensis . The role of VEGF in normal pregnancies and abnormalities in its function which are possibly associated with PE support the idea that genetic polymorphisms in VEGF could affect the susceptibility of developing PE (25).

The present study find an association between these gene polymorphisms and PE. Bearing in mind that ethnic differences have important implications in the development of PE and may affect cytokine gene

polymorphisms expression **(26)**. Besides gene-gene interaction, behavioral or environmental factors may influence the cytokine profile, the mediators, and the mechanism involved in PE, leading to controversial results. A limitation of this study is that it analyzed only maternal genotypes and we cannot ignore the possible contribution of fetal genotype to the development of PE.

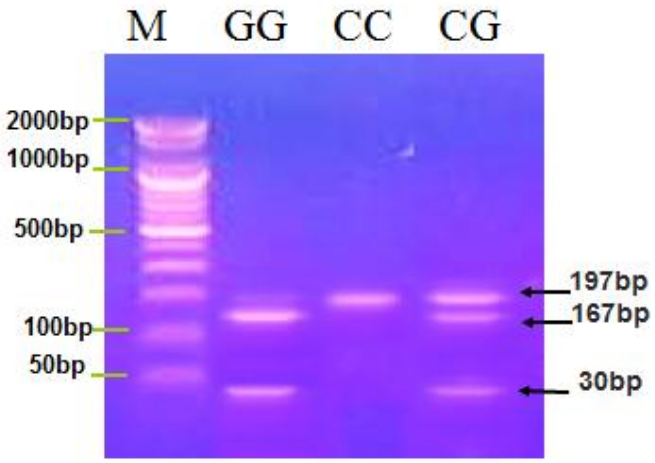

Figure (1): Agarose Gel Electrophoresis Image that Show the RFLP-PCR Product Analysis of *VEGF* Gene by Using BsmFI Restriction Enzyme from Some Blood Patient Samples and Healthy Control Sample . Where M: Marker (2000-50bp), Patient Samples as Homozygote (GG) at 167bp, 30bp. Patient Samples as Heterozygous (CG) at 197bp, 167bp, 30bp. Patient and Control that Appeared as Wild Type (CC) which still undigested.

Table (1): Description of the 3 Study Groups by Maternal Age, Gestational Age and Party.

		Preeclampsia Cases				Healthy Control		P Value
		Early Onset		Late Onset				
		No.	%	No.	%	No.	%	
1.	**Maternal Age (years)**							0.042[NS]
	16-26	11	36.7	18	60	29	48.3	
	27-37	12	40	5	16.7	13	21.7	
	≥38	7	23.3	7	23.3	18	30	
	Range	16-39		17-37		20-40		
	Mean	30.6		25.7		30.2		
	SD	7.28		7.2		7.79		
	SE	1.32		1.3		1.006		
	Total	30	100	30	100	60	100	
2.	**Gestational Age (weeks)**							< 0.05
	<35	30	100	-	-	41	86.3	
	≥35	-	-	30	100	19	31.7	
	Range	24-34		35-39		23- 39		
	Mean	27.9		36.7		31.16		
	SD	4.26		0.9		5.53		
	SE	0.78		0.2		.718		
	Total	30	100	30	100	60	100	
3.	**Parity**							0.178[NS]
	Multiparous	11	36.6	14	46.6	43	71.44	
	Uniparous	19	63.4	16	53.4	17	28.8	
	Total	30	100	30	100	60	100	

No.=Number, SD=Stander Deviation, SE=Stander Error, NS=Non-Significant.

Table (2): Frequency Distribution of Preeclampsia Cases by family history, and Patient History.

		No.	%
1.	**Family history of Preeclampsia**		
	Negative	20	33.3
	Positive	40	66.6
	Total	60	100
2.	**Patient history of Preeclampsia in previous pregnancy**		
	Negative	33	55
	Positive	27	45
	Total	60	100
3.	**History of dead baby**		
	Negative	39	65
	Positive	21	35
	Total	60	100

No.= Number.

Table (3): The Difference Between Three Study Groups in Diastolic Blood Pressure and Systolic Blood Pressure.

		early Onset	Late Onset	Healthy Control	P Value
1.	**Diastolic blood pressure mm Hg**				
	Mean	101	118	75.5	0.001
	Range	100-110	90-150	70-80	
	SD	3	26.8	5	
	SE	0.56	4.9	0.65	
	Total	30	30	60	
	Bonferonni t-test for difference in mean between:				
	Cases (PE) x Healthy Controls P Value = 0.001				
2.	**Systolic blood pressure mm Hg**				
	Mean	163	149	125.33	≤ 0.01
	Range	140-160	130-200	120-130	
	SD	27.7	5.5	5	
	SE	5	1	0.7	
	Total	30	30	60	
	Bonferonni t-test for difference in mean between:				
	Cases (PE) x Healthy Controls P Value ≤ 0.01				

SD=Stander Deviation, SE= Stander Error.

Table (4): The Case-Control Difference in Mean Concentration of Protienurea.

	Preeclampsia cases		Healthy Control	P Value
	Early Onset	Late Onset		
Protein Urea Nitrogen mg/24hour				<0.01
Range	300-500	300-500	30-100	
Mean	420	426.7	41.7	
SD	99.65	98	26	
SE	18	17.9	3.4	
Total	30	30	60	
Bonferonni t-test for difference in mean between:				
Cases (Preeclampsia) x Healthy Controls <0.01				

SD=Stander Deviation, SE= Stander Error

Table(5): VEGF (-405) Gene Polymorphism in PE Patients Compared with Healthy Control.

	PE Cases	Healthy Control	OR	Inverse OR	95% CI OR	P Value	Adjusted P	EF	PF
VEGF (-405) Polymorphism									
CC	14 (23.3)	28 (46.6)	0.35	2.9	0.2 - 0.98	0.04	0.04	***	0.654
CG	22 (26.7)	19(31.7)	2.4	0.4	0.4-1.7	[NS]	[NS]	0.585	***
GG	24 (40)	13(21.7)	1.2	0.8	0.2 -1.19	[NS]	[NS]	0.173	***
Total	60(100)	60(100)							
Allele frequency (VEGF (-405))									
C-allele	50(41.7)	75(62.5)	0.811	1.233	0.3 -0.7	0.002	0.002	***	0.189
G-allele	70(58.3)	45(37.5)	2.179	0.458	1.4-3.9	0.002	0.002	0.586	***
Total	120(100)	120(100)							

References :

1. Aiping Chena Congying Lia Jingli Wangb Han Shad Shunfu Piaoc Shiguo Liub,c. Role of Toll-Like Receptor 3 Gene Polymorphisms in Preeclampsia. Cell Physiol Biochem 2015;37:1927-1933.

2. Jia RZ, Ding GC, Gu CM, Huang T, Rui C, Wang YX, Lu Q: CDX2 enhances HTR-8/SVneo trophoblast cell invasion by altering the expression of matrix metalloproteinases. Cell Physiol Biochem 2014;34:628-636.

3. Shen Z, Wu Y, Chen X, Chang X, Zhou Q, Zhou J, Ying H, Zheng J, Duan T, Wang K: Decreased maternal serum 2-methoxyestradiol levels are associated with the development of preeclampsia. Cell Physiol Biochem 2014;34:2189-2199.

4. Liu L, Zhang X, Rong C, Rui C, Ji H, Qian YJ, Jia R, Sun L: Distinct DNA methylomes of human placentas between pre-eclampsia and gestational diabetes mellitus. Cell Physiol Biochem 2014;34:1877-1889.

5. Sibai BM: Hypertensive disorders of pregnancy: the United States perspective. Curr Opin Obstet Gynecol 2008;20:102-106.

6. Pinheiro MB, Gomes KB, Ronda CR, Guimaraes GG, Freitas LG, Teixeira-Carvalho A, Martins-Filho OA, Dusse LM: Severe preeclampsia: association of genes polymorphisms and maternal cytokines production in Brazilian population. Cytokine 2015;71:232-237.

7. Li J, Liu M, Zong J, Tan P, Wang J, Wang X, Ye Y, Liu S, Liu X: Genetic variations in IL1A and IL1RN are associated with the risk of preeclampsia in Chinese Han population. Sci Rep 2014;4:5250.

8. Zubor P, Dokus K, Zigo I, Skerenova M, Pullmann R, Danko J: TNF alpha G308A gene polymorphism has an impact on renal function, microvascular permeability, organ involvement and severity of preeclampsia. Gynecol Obstet Invest 2014;78:150-161.

9. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet 2002;2002(77):67–75.

10. Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. Am J Reprod Immunol 2011;65:377–94.

11. Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol 2010;63:534–43.

12. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH et al. (2004) Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 350,672–683.

13. Ilona Bányász , Szilvia Szabó1, Géza Bokodi1, Ádám Vannay , Barna Vásárhelyi , András Szabó , Tivadar Tulassay and János Rigó Jr. Genetic polymorphisms of vascular endothelial growth factor in severe pre-eclampsia. Molecular Human Reproduction Vol.12, No.4 pp. 233–236, 2006.

14. Wright D, Akolekar R, Syngelaki A, Poon LC, Nicolaides KH. A competing risks model in early screening for preeclampsia. Fetal Diagn Ther 2012;32(3):171-178

15. Paré E, Parry S, McElrath TF, Pucci D, Newton A, Lim KH. Clinical risk factors for preeclampsia in the 21st century. Obstet Gynecol 2014;124(4):763-770.

16. Jose Augusto, Duran Chávez, Ricardo de Carvalho Cavalli Preeclampsia: Vascular Pathophysiological Mechanism and the Basis for Early Diagnosis and Treatment. Rev. Bras. Ginecol. Obstet. vol.38 no.8 Rio de Janeiro Aug. 2016

17. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. (PDF). *Obstet Gynecol*. 122 (5): 1122–31. Nov 2013.

18. Chung, Soo-Cheon Chae. Interleukin-27 polymorphisms are associated with inflammatory bowel diseases in a Korean population. Volume 24, Issue 10 October 2009 Pages 1692–1696.

19. Valquiria Maria de Paula Cunha [I] ; Roseane Lopes da Silva Grecco [II] ; Marina Carvalho Paschoini [III] ; Sueli Riul da Silva [VI] ; Mariângela Torreglosa Ruiz [V] ; Marly Aparecida Spadotto Balarin . Genetic polymorphisms of vascular endothelial growth factor in pre-eclampsia Rev. Bras. Ginecol. Obstet. Vol.33 no.7 Rio de Janeiro July 2011

20. K. Magdoud1, M. Dendana1, V. Herbepin2, S. Hizem1, K. Ben Jazia3, S. Messaoudi1, W.Y. Almawi4, R. Touraine2, and T. Mahjoub. Identification of specific vascular endothelial growth factor susceptible and protective haplotypes associated with recurrent spontaneous miscarriages. Human Reproduction, Vol.27, No.5 pp. 1536–1541, 2012

21. Papazoglou D, Galazios G, Koukourakis MI, Panagopoulos I, Kontomanolis EN, Papatheodorou K and Maltezos E (2004) Vascular endothelial growth factor gene polymorphisms and pre-eclampsia. Mol Hum Reprod10,321–324.

22. Adel Z. EL-Sonbati, Rizk A. EL-Baz, Entstar A. Saad, Abeer S.EL-Zekred. 2014 Vascular endothelial growth factor VEGFG/C405 and C/A 2578 gene polymorphism in cases with preeclampsia. International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 6, Issue 10,

23. J. Y. Shim, J. K. Jun, B. K. Jung et al., "Vascular endothelial growth factor gene +936 C/T polymorphism is associated with preeclampsia in Korean women," *American Journal of Obstetrics & Gynecology*, vol. 197, no. 3, pp. 271.e1–271.e4, 2007.

24.Vibha Tandon, Swati Hiwale,1 Dnyanesh Amle, Tripti Nagaria, and Pradeep Kumar Patra. Assessment of Serum Vascular Endothelial Growth Factor Levels in Pregnancy-Induced Hypertension Patients. Hindawi Journal of PregnancyVolume 2017, Article ID 3179670, 5 pages

25. Surbhi Roy , Manjari Matah, Deepika Jaiswal, Kiran Singh. Role of -460C/T VEGF gene polymorphism in preeclampsia. Asian pacfic journal of reproduction.2013; 2(1): 30-33.

26. Camila S. Franchim, Nelson Sass, Rosiane Mattar, Karen P. T. Pendeloski, Lawrence Hsu Lin, Maria R. Torloni, and Silvia Daher. Inflammatory Mediators Gene Polymorphisms in Preeclampsia. *Hypertension in Pregnancy*, 30:338–346, 2011