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Role of -460 C/T VEGF gene polymorphism in preeclampsia

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ABSTRACT

Objective: To study association of VEGF-460C>T functional polymorphism with preeclampsia. **Methods:** The case-control study comprised of two groups: 40 pre-eclamsia patients and 45 healthy antenatal women. Genotyping for SNP -460 VEGF was done by ARMS-PCR method. For VEGF-460C>T functional polymorphism, allele and genotype distribution were evaluated using Chi-square test. **Results:** The prevalence of C allele was higher among cases compared to controls. The prevalence of CT and CC genotypes were also higher among cases compared to controls indicating that CT and CC genotypes and C allele to have a role in genetic susceptibility for preeclampsia. **Conclusions:** The carriage of VEGF-460C allele appears to be a risk factor for preeclampsia in present pilot study.

1. Introduction

Preeclampsia [PE] is a multifactorial, polygenic disorder. It has been seen that the incident risk for preeclampsia is 20 to 40 percent for daughters of preeclamptic mothers; 11 to 37 percent for sisters of preeclamptic women; and 22 to 47 percent in twin studies [1]. Preeclampsia, an enigmatic syndrome, is because of angiogenic factor imbalance and is known to be a state of endothelial dysfunction. Varying levels of VEGF can be present in preeclampsia i.e. from normal to high total VEGF levels (perhaps induced by placental hypoxia) [2, 3]. Vascular endothelial growth factor (VEGF) is a major angiogenic factor and is a prime regulator of endothelial cell proliferation. It plays a crucial role in physiological vasculogenesis and vascular permeability [4]. The gene encoding VEGF is located on chromosome 6 band p21 and comprises a 14 kb coding region with 8 exons and 7 introns [5]. It binds to sFlt-1, tyrosine kinase receptors present on endothelial cell membranes [6]. Hypoxia induces rapidly and reversible levels of VEGF mRNA both *in vitro* and *in vivo*. Also increase angiogenesis during normal physiological and pathological states leads to elevated levels of VEGF [7] .VEGF and its receptors may play a pivotal role in the altered function of PE [8]. Several groups have demonstrated that circulating total VEGF concentrations are significantly elevated in women with PE [9,10]. Free (unbound) VEGF concentrations have been reported to be significantly lower [11, 12]. This inconsistency is probably due to highly elevated levels of the soluble FMS-like tyrosine kinase 1 receptor which captures free VEGF [12]. *In vitro* study showed that the T allele of the VEGF -460 T > C polymorphism located in the promoter of the VEGF gene was associated with a decreased VEGF promoter activity [13]. Hence, the VEGF -460C allele may be associated with an increased VEGF expression, which would promote angiogenesis or preeclampsia.

The present pilot study aimed to evaluate association of -460C/T VEGF gene polymorphism with preeclampsia. Functional genetic variations in the VEGF gene may contribute to the clinical outcomes of PE.

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2. Materials and methods

Patients were recruited from the Out Patient Department

of Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University and Varanasi, India. The present case-control study consists of 85 antenatal women [age=(24.7±3.28) years] who comprise of 40 controls (normo-tensive women) and 45 with PE. All women belong to same geographical region and ethnicity. Patients underwent a standardized clinical and laboratory evaluation like urinary albumin, renal function test, liver function test, serum uric acid, ultrasound evaluation for fetal well-being. Clinical data has been summarized in Table 1. Questionnaire was maintained for each patient to record details of their lifestyle, education, habits and family history. Informed consent was obtained from every participant of each group. Approval of the University's ethical committee for research on Human material was obtained.

2.1. Genotyping of SNP -460 C/T of VEGF by ARMS-PCR

Genomic DNA was extracted from peripheral blood, using standard salting-out procedure. PCR amplification of VEGF -460 C/T mutation region was done using previously described primers by ARMS-PCR [Ref]. The PCR conditions were 30 cycles of 55 sec at 94 ℃, 1 min at 58 ℃, and 55 sec at 72 ℃. The amplified products (431bp) were separated by electrophoresis on 2% agarose gel stained with 0.5 mg/mL ethidium bromide and visualized and photographed under an ultraviolet transilluminator(Figure 1) .

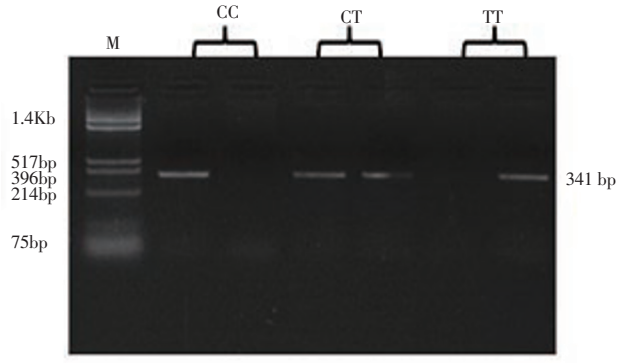

Figure 1. Agarose gel electrophoresis of PCR products after ARMS -PCR for genotyping of VEGF 460 C/T. (Lane1: Marker; Lane 2,3: CC; Lane 4,5:CT and Lane 6,7: TT)

2.2. Statistical analysis

Allele and genotype distribution among groups were evaluated using chi-square (x^2) test or fisher exact test. The difference in frequencies between the case and control groups was analyzed for statistical significance at the 95% confidence interval (CI) using x^2 test. The allele frequency of VEGF genotype was in Hardy- Weinberg equilibrium. Odds ratios (Ors) were calculated and reported within the 95% confidence limits. A P value of <0.05 was considered significant in all the analysis.

3. Results

The assessment of genetic polymorphism of the VEGF gene among cases and controls was done by ARMS PCR. The distributions of genotypes of the SNP -460 C/T of VEGF followed Hardy-Weinberg equilibrium in both the patient and control groups (P=0.85 and P=0.43 respectively). In this pilot study, comparison of allele frequency showed more prevalence of C allele indicative of the CT and CC genotypes and C allele to have a role in genetic susceptibility for preeclampsia Table 2.

In our study we further evaluated distribution of genotypes and allele frequencies of C460T SNP of VEGF amongst cases and controls with respect to adverse perinatal outcome (IUGR, IUD, Preterm Birth, Neonatal Death, HIE) and found presence of CC, CT and combined CC+CT genotypes has shown more prevalence of C allele in adverse perinatal outcome when compared to controls(Table 3).

4. Discussion

Preeclampsia is a multifactorial disease in which a woman's genetic background, her partner and her

Table 1
Distribution of clinical and demographic data between cases and controls.

Clinical parameter studied	Cases	Controls
Age (in years)	24.1±3.56	25.3±2.89
Primigravidae (%)	68%	56%
Interval between consummation of marriage and timing of conception less than 4 months in Primigravidae (%)	70.5%	50%
Multigravidae	32%	44%
Multigravidae with previous pregnancies complicated by abortion, intrauterine growth restriction, intrauterine death, neonatal death (%)*	62.5%	18.2%
Rural population (%)	74%	56%
Low socio-economic status (%)	74%	68%
Illiterate (%)*	62%	18%
Unbooked status (%)*	88%	24%
Mean gestational age at delivery(in weeks)*	34.73± 2.52	37.75± 0.97
Mean birth weight (in kg)*	2.05± 0.45	2.71± 0.27

*p-value<0.05 = statistically significant

Table 2
Distribution of genotypes and allele frequencies of C460T SNP of VEGF in the study population.

Genotype(VEGF460 C>T)	Cases (*n*=40)	Controls (*n*=45)	OR	95%CI	*P*-value
TT	12 (30.00)	17 (37.8%)	–	–	–
CT	17 (42.50%)	16 (35.6%)	1.51	0.56 to 4.07	0.46
CC	11 (27.50%)	12 (26.6%)	1.29	0.44 to 3.86	0.64
CT+CC	28 (70.00%)	28 (62.22%)	1.42	0.58 to 3.47	0.50
T	41(51.25%)	50 (55.5%)	1.19	0.65 to 2.17	0.65
C	39 (48.75%)	40 (44.5%)	–	–	–

Table 3
Distribution of genotypes and allele frequencies of C460T SNP of VEGF amongst cases and controls with respect to adverse perinatal outcome (IUGR, IUD, Preterm Birth, Neonatal Death, HIE*).

Polymorphism (VEGF460 C>T)	Cases (*n*=31)	Controls (*n*=45)	OR	95%CI	*P*-value
TT	9 (29.0%)	17 (37.8%)	–	–	–
CT	12 (38.7%)	16 (35.6%)	1.42	0.47 to 4.19	0.58
CC	10 (32.3%)	12 (26.6%)	1.57	0.49 to 4.97	0.56
CT+CC	22 (70.97%)	28 (62.22%)	1.48	0.56 to 3.90	0.47
T	30 (48.4%)	50 (55.5%)	–	–	–
C	32 (51.6%)	40 (44.5%)	1.33	0.69 to 2.54	0.41

Note: OR= odds Ratio; CI= 95% confidence interval; *P* value = 0.71(Not Significant)*HIE=Hypoxic Ischemic Encephalopathy; IUD=intra-uterine death; IUGR= intra-uterine growth restriction

environment are all interact. It seems likely that when one, or more likely several, combinations of polymorphisms occur in the same individual, perhaps together with environmental factors, then preeclampsia is expressed. This could be an explanation for individual differences found even among apparently tightly phenotyped preeclampsia sufferers. Preeclampsia is a morbid condition characterised 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 etiopathogenesis. In the present study the genotypes CT, CC and C allele were found more frequently amongst the cases with respect to controls and were also more commonly found amongst the preeclampsia cases who had adverse perinatal outcome indicating that VEGF C460T SNP may have a role in development of preeclampsia phenotype.

Previous similar studies have been done in Korean and other population [14] and a study by Shim et al [15] showed Vascular endothelial growth factor gene +936C/T polymorphism may be associated with Preeclampsia in Korean women with +936T as the risk allele. In a case-control study by Ilona *et al* [16], it was aimed to test whether VEGF genetic polymorphisms are associated with the risk of severe PE and it was observed that carriers of VEGF+405G allele may have a decreased susceptibility to PE. In another study by Dimitrios *et al* [17], it was attempted to identify associations between three functional VEGF gene polymorphisms, linked with altered VEGF gene responsiveness, and preeclampsia. Genotyping for the ±2578C/A, ±634G/C and 936C/T polymorphisms of the VEGF gene was done. No significant association between genotypic or allelic frequencies in women with Preeclampsia relative to controls was found. A statistically significant difference was found for allelic frequencies of the 936C/T polymorphism between women with severe Preeclampsia and controls (odds ratio: 2.70; 95% confidence interval: 1.09±6.63; *P*=0.019).It was observed that VEGF gene polymorphisms studied are unlikely to be major predisposing factors for Preeclampsia although the presence of the 936T allele probably has a considerable effect on disease modification.

Hence selective advantage of the VEGF 460 T allele maybe physiologically protective and presence of C allele may put an individual at risk for preeclampsia. VEGF460 TT genotype may favour normal placentation and vessel development which is crucial for a good pregnancy outcome. The patients with VEGF 460 CT and CC mutant genotypes may have compromised angiogenesis and improper placentation ultimately leading to preeclampsia and adverse pregnancy outcome. The role of VEGF in normal pregnancies and abnormalities in its function which are possibly associated with preeclampsia support the idea that genetic polymorphisms in VEGF could affect the susceptibility to the development of preeclampsia. The present study is a pilot study in north Indian population and needs further validation with larger sample size to get statistically significant results.

Conflict of interest statement

We declare that there is no conflict of interest.

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