Assessment of insertion/deletion polymorphism of ACE gene as a genetic risk marker for preeclampsia in pregnant women

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Abstract

Objective: To investigate the possible associations of angiotensin-converting enzyme insertion or deletion genotypes and alleles with the risk of preeclampsia in Arab women.

Methods: The case-control study was conducted from January 2016 to December 2017 at King Abdulaziz University Hospital and Maternity & Children Hospital, Jeddah, Saudi Arabia, and comprised pregnant women with preeclampsia as cases and normal pregnant women as controls. Deoxyribonucleic acid was extracted and angiotensin-converting enzyme gene was amplified by polymerase chain reaction analysis and characterised through gel electrophoresis.

Results: Of the 162 women, 68(42%) were cases and 94(58%) controls. The mean values of age, body mass index, and systolic and diastolic blood pressure were significantly different among the cases than the controls (p<0.05), but mean gestational age did not significantly differ between the groups (p>0.05). The distribution of the polymorphic variants of the angiotensin-converting enzyme gene insertion/deletion was not significantly different between the groups (p>0.05). Also, genotype distribution and allelic frequencies were not significantly different between the groups (p>0.05).

Conclusion: For insertion/deletion polymorphism, no significant differences were detected in the genotype and allele frequencies or any of the inheritance models between preeclampsia patients and controls.

Keywords: ACE gene, Preeclampsia, Genetic risk marker, Polymorphism, Pregnant women.

Introduction

Preeclampsia (PE) is a pregnancy-specific vascular disorder manifesting in the latter part of the pregnancy; after 20 weeks of gestation. It is characterised by elevated blood pressure (BP), protein in the urine (proteinuria) and edema. Severe PE and eclampsia are rare and serious complications that cause seizures during pregnancy. PE is a multisystem disease, featuring hypertension (HTN), proteinuria, renal, hepatic and neurological involvement. According to the American Congress of Obstetricians and Gynaecologists (ACOG), HTN is defined as systolic blood pressure (SBP) ≥140mmHg and/or a diastolic blood pressure (DBP) ≥90mmHg. PE complicates around 3-5% of pregnancies worldwide. Angiotensin-converting enzyme (ACE) is a key renin-angiotensin-aldosterone system (RAAS) which plays an important role in the regulation of renal function and arterial pressure during pregnancy, and several studies have implicated the RAAS in PE pathophysiology. The gene for ACE has been mapped to chromosome 17q23 and translates into a 150 kDa protein, which belongs to the family of zinc metallopeptidases. ACE cleaves the C-terminal dipeptide His-Leu from Ang I, thus generating Ang II but Ang I is by far not the only substrate of ACE. ACE also metabolises bradykinin. A variation in the ACE gene structure consisting of the insertion (I) or deletion (D) of a 250bp deoxyribonucleic acid (DNA) fragment located in intron 16. This so-called “ACE I/D polymorphism” has been suspected to be associated with a variety of diseases. The current study was planned to investigate the possible association of ACE I/D genotype and alleles with the risk of PE in Arab women.
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Samplings and Methods
The case-control study was conducted from January 2016 to December 2017 at King Abdulaziz University Hospital and Maternity & Children Hospital, Jeddah, Saudi Arabia, and comprised pregnant women with PE and normal pregnant women as controls. After approval was obtained from the ethics committee of King Abdulaziz University Hospital and of the Saudi Ministry of Health, the sample size was calculated while adjusting the power at 80%, odds ratio (OR) of 2, two-sided significant level as 95%, and the proportion of control with exposure 10.3%.

The patients were all PE women referred to the hospital. Those with multiple-birth pregnancy, previous HTN, diabetes, cardiac and renal diseases were excluded. PE was defined as SBP equal or higher than 140mmHg, DBP equal or higher than 90mmHg, presence of proteinuria by 24-hour urinary excretion exceeding 300mg, urine protein-to-creatinine ratio >0.3 and equal or higher than 30mg/dl protein in a random urine sample (1+ reaction on a standard urine dipstick). Severe PE was defined as BP >160/110mmHg, proteinuria >3+, headache, visual disturbances, upper abdominal pain, serum creatinine and transaminase elevation, thrombocytopenia and foetal-growth restriction. The controls were at a gestational age exceeding 20 weeks, without PE symptoms, from whom a detailed history was taken, and they were subjected to a full clinical examination.

All the patients and the controls were enrolled after they signed informed written consent.

Genomic DNA was extracted from the whole blood of patients by using QIAGEN QIAamp DNA Mini kit. Blood samples were drawn from each subject into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and were stored between 4°C and -21°C.

Polymerase chain reaction (PCR) was conducted using S1000 Thermal Cycler (BIO-RAD company). Total volume of the reaction mixture was 10μl. Briefly, each 10μl reaction mixture contained 5μl of GoTaq® Green Master Mix, 2X of 1μl forward primer: 5’-CTG GAG ACC ACT CCC ATC TCT-3’ and 1μl of backward primer: 5’-GAT GTG GCC ATC ACA TTC GTC AGA T-3’ and 1μl distilled water. For amplification, denaturation was first done for 1 minute at 95°C, followed by second denaturation for 30 seconds at 95°C. It was continued for 34 cycles that consisted of primer annealing for 30 seconds at 63°C followed by extension step for 30 seconds at 72°C. The procedure ended with the final extension for five minutes at 72°C.

For gel electrophoresis, the bands of PCR products of I and D alleles of ACE gene were separated on 2% agarose gel, and then stained with ethidium bromide. The I allele was expected to produce a fragment in the of 490bp area and the D allele in the 190bp area. The ID genotype could show two separate bands in the 490bp and in the 190bp areas.

Data was analysed using SPSS 12. Chi-square with continuity correction was used to test for association between the two groups. Odds ratios (OR) of specific ACE gene polymorphic variants were calculated as estimates of relative risk (RR) for disease, and 95% confidence intervals (CI) were obtained to evaluate the strength of the association between ACE-I/D variant and PE risk. Logistic regression analysis was used to test for association between the ACE I/D polymorphisms. The level of statistical significance was defined at p<0.05.

Results
Of the 162 women, 68(42%) were cases with a mean age of 28.34±5.53 years, and 94(58%) were controls with a mean age of 28.22±5.50 years (p=0.03). There was significant difference between the groups in terms of mean age, body mass index (BMI), SBP and DBP, but the difference was not significant in the mean gestational age (Table 1).

Early onset PE before 34 weeks of gestation was diagnosed in 22(32%) in the PE group, while 46(68%) were diagnosed as late-onset PE after 34-week gestation. Distribution of the polymorphic variants of the ACE gene I/D polymorphism on preeclampsia at early onset were DD, ID, II, 12(55%), 9(40%), 1(5%), and in the late onset were 27(58%), 16(35%) and 3(7%) respectively (Table 2). Gel electrophoresis results indicated that ACE II genotype produced a 490bp fragment, DD genotype produced a 190bp fragment and ID genotype produced two fragments of 490bp and 190bp in the control group and the PE group, respectively (Figures 1-2). Distribution of the polymorphic variants of the ACE gene I/D polymorphism was not significantly different between the groups (Table 3). Stratified regression analysis indicated that the sample size of case groups was a significant source of heterogeneity, which suggested no significant association between ACE gene I/D in the two groups (p>0.05).
The genotype frequencies of DD, ID, and II genotypes were 39 (57%), 2 (37%), and 4 (6%) in the patients, and 55 (59%), 34 (36%), and 5 (5%) in the controls. In addition, each of DD and ID genotypes showed no significant relation to PE occurrence. There was no significant difference in ACE I/D genotypes between the groups (p=0.97). The frequency of D allele was 103 (76%) in patients and 144 (77%) in controls. The frequency of I allele was 44 (24%) in the patients and 44 (23%) in the controls. There was no significant relation between D allele and I allele between the groups.

The genotype model analysis of ACE I/D polymorphisms under dominant, co-dominant and recessive models did not show any kind of statistical significance (p>0.05). There was no significant association between the genotype and the severity of the disease (Table 4).

**Discussion**

In the last decade, the incidence of PE has increased...
Women with PE have an increased risk of developing cardiovascular diseases later in life.\(^6,7\) Accurate prediction and prevention of the condition at present is a very challenging task since the precise etiology of PE remains unknown.\(^7\) PE results from complex interactions between the genetic components and environmental factors.\(^8\) The familial nature of PE has been known for many years and extensive genetic research has been carried out in this area using strategies that include candidate gene studies and linkage analysis.\(^9\) Despite the numerous genetic association studies that have been performed to elucidate the genetic background of PE,\(^9\) the role of genetic factors in pregnancy complications by PE is still unclear. There is some evidence that suggests a relationship between various components of metabolisers of xenobiotics and endogenous toxins, RAAS and PE.\(^10\) Due to the central role of RASS on body fluid-electrolyte regulation and vascular remodelling of the placenta in pregnancy, it is thought to account for a major role in the etiology of the genetics of PE. ACE gene as a member of RAAS that catalyses the conversion of angiotensin I to angiotensin II has been investigated recently.\(^10\) The current study showed that more than half of PE women were diagnosed with late onset. A study by Uma et al.\(^11\) supported the fact that there were no differences in maternal or infant ACE activities in relation to PE onset. Their findings suggested an association between the DD genotype of the ACE gene and early-onset, but not late-onset PE, which may give a partial explanation for the higher recurrence risk with early-onset PE.

In the current study, there was no statistically significant difference in frequencies of I/D polymorphisms between PE patients and control women. These results are in agreement with previous studies.\(^12\) Kim et al.\(^13\) reported no association between D allele of ACE intron 16 and PE according to age. Kobashi et al.\(^14\) studied I/D polymorphisms of the ACE gene and PE in Japanese women. They found no significant differences in the frequency of the DD genotype of ACE between PE patients and controls. However, in a subgroup positive for family history of HTN, the frequency of the DD genotype tended to be higher in PE patients (25%) than in controls (8%; \(p=0.061\)). Carrying the DD genotype may have some influence on the pathogenesis of PE, perhaps through effects on placental hypoxia or the interaction of hypertensive disease and atherosclerosis, although this influence may not be strong. Additional studies using a larger number of patients and analyses that include other genetic and environmental factors are necessary to confirm these results.

In the current study, distribution of the polymorphic variants of the ACE gene I/D polymorphism was found not to be significantly different between the preeclampsia women and the control groups. These findings may indicate that each of DD and ID genotypes had no significant relation to PE occurrence. Furthermore, there was no significant relation between ACE I/D genotypes between the PE and control groups (\(p=0.97\)). The frequency of D allele was 76% in preeclampsia patients and 77% in controls. The frequency of I allele was 24% in PE patients and 23% in controls. There was no significant relation between D Allele and I Allele between the groups (\(p=0.850\)) comprising Arab women. Shaik et al.\(^15\) reviewed a meta-analysis of ACE gene polymorphisms and risk of PE in women. Overall, 1,620 PE cases and 2,158 controls were analysed for intron 16 I/D polymorphism in ACE.
gene. They found no significant PE risk in both eNOS and ACE genes with these polymorphisms. Moreover, a study on Brazilian women showed no association between ACE polymorphism and the development of PE. On the contrary, Kamha et al. studied ACE and angiotensin II type I receptor (AT1R) polymorphisms in Egyptian PE patients. The study was conducted on 65 women with severe PE and 25 healthy pregnant women who matched the study group in age and parity. They found that both DD and ID genotypes of the ACE gene showed a significant relation to the occurrence of PE in Egyptian pregnant preeclampsia women.

Additionally, DD genotype was significantly related to early PE onset. D allele showed a significant relation to the occurrence of early PE as well. The differences in the results of the current study and the earlier study might be due to the number of their controls (25) compared to the number of controls (94) in the current study, which is almost three times higher. The current study suggests that ACE gene I/D polymorphism may not be related to the risk of PE in Arab pregnant women. Large-scale multi-centre studies are needed to understand better the role of other genotypes association with PE in Arab women.

Genome-wide association studies (GWAS) represent an alternative approach to the previous hypothesis-free genome-wide linkage studies, with the ability to detect common markers with moderate effect associated with disease and could lead to an increased understanding of diverse molecular pathways underlying PE. The causal variants are rarely revealed and the statistical power to detect small gene-gene and gene-environment interactions are limited. Furthermore, variants identified in one population are not always transferable to other populations and until today, to capture information about rare variants associated with disease found very difficult, need more exploration studies in these areas.

**Conclusion**

For I/D polymorphism, no significant differences were detected in the genotype and allele frequencies or any of the inheritance models between preeclampsia patients and controls of Arab women.

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**References**


