Polymorphisms in angiotensin-converting enzyme and glutathione s-transferase genes in Turkish population and risk for preeclampsia

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Summary

Aims: This study was conducted to investigate whether insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) gene and polymorphisms in glutathione S-transferase (GST) M1 and T1 genes are associated with increased risk for preeclampsia. *Materials and Methods:* Sixty-three patients with hypertensive disorder of pregnancy and 85 controls were evaluated in a prospective case-control study. All subjects were genotyped by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. *Results:* Allele frequencies of ACE gene I/D polymorphism were found significantly different between preeclampsia and the control groups (p = 0.001). Differences in genotype frequencies of ACE gene I/D polymorphism between the two groups were statistically significant (p = 0.004). Individuals homozygous for D allele were more likely to develop preeclampsia (OR = 2.29; 95% CI, 1.39 - 3.79), whereas heterozygous individuals were not at increased risk (OR = 0.92; 95% CI, 0.56 - 1.49), compared to individuals homozygous for I allele. The differences in frequencies of functional and null alleles of GSTM1 and GSTT1 genes between the two groups were not significant (p = 0.46 and p = 0.44, respectively). *Conclusion:* ACE gene DD genotype was found to be associated with increased risk of preeclampsia development, whereas the authors did not find any significant relationship with polymorphisms of the GSTM1 and GSTT1 genes and preeclampsia.

Key words: Gene; Polymorphism; Angiotensin-converting enzyme; Glutathione s-transferase; Preeclampsia.

Introduction

The role of genetic factors in pregnancies complicated by preeclampsia is still unclear. Several lines of evidence suggest a relationship between various components of metabolizers of xenobiotics and endogenous toxins, renin-angiotensin system (RAS), and preeclampsia [1-3]. There is growing evidence that polymorphisms of genes that encode the associates of those families, could possibly be responsible from the genetic part of the pathogenesis of preeclampsia [4-6].

Deficient placentation characterized by inadequate trophoblast invasion into maternal spiral arterioles is considered one of the major pathophysiologic mechanisms of preeclampsia. Previous studies revealed that RAS mediates physiological remodeling of spiral arterioles throughout the pregnancy [7] and has a role in fluid-electrolyte balance, as well as blood pressure regulation [8]. Thereby, inappropriate activation of the RAS could play a role in the development of preeclampsia. Serum levels of angiotensin-converting enzyme (ACE), as one of the key components of the RAS, are reported to be decreased in states of preeclampsia [9]. In recent studies, differences in the ACE activity and uteroplacental circulation are found to be reliably associated with Insertion/Deletion (I/D) polymorphism of the ACE gene [10, 11]. There is conflicting data in the literature regarding the relationship between ACE gene I/D polymorphism and preeclampsia. Some of the published researches have suggested a significant interaction [6, 11, 12-14], while others could not demonstrated any connection [9, 15, 16].

Vascular endothelial damage is another important mechanism in the development of preeclampsia [17]. Significantly, lipid peroxides and endogenous toxins that are produced in vivo are responsible from this damage to the endothelium [18]. Imbalance between toxic substances and detoxifying enzymes has an increasing effect on oxidative stress and therefore, causes placental and maternal vascular endothelial damage [19]. Gluthatione S-transferases (GST) are the group of enzymes that are involved in the metabolization of toxic substances. In this regard, they are thought to play a role in the pathogenesis of preeclampsia. GST enzymes are responsible for the detoxification of wide variety of chemicals by nucleophilic addition of gluthathione to electrophilic centers of the substrates. They have an important role in protecting the tissue from oxidative damage. There are four major groups of GST enzymes-alpha (a), mu (M), pi (P), theta (T). All have been found to be genetically polymorphic. Deletion polymorphisms have been found for gluthathione S-transferase M1 (GSTM1) and gluthathione S-transferase T1 (GSTT1) genes. Approximately 45% and 20% of Caucasians bear the non-functional alleles for GSTM1 and GSTT1, respectively. The relation between polymorphisms of the both GST genes and preeclampsia has been investigated, however researchers are not yet able to find any strong evidence [20, 21].

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In the present study, the authors aimed to investigate whether genetic polymorphisms on intron 16 in the ACE gene and genetic polymorphisms in GSTM1 and GSTT1 genes are varied between preeclamptic patients and the controls.

Materials and Methods

Subjects

Sixty-three unrelated pregnant women with hypertensive disorder in pregnancy (19 patients with preeclampsia and 44 patients with severe preeclampsia) and 85 healthy pregnant unrelated controls were enrolled in the study between May 2009 and March 2010 at the Obstetrics and Gynecology Department of Uludag University. All of the participants were singletons and were in their third trimester. All participants were Caucasians with average socioeconomic status. Preeclampsia was defined as demonstration of systolic blood pressure measurement above 140 mm Hg and diastolic blood pressure reading above 90 mm Hg in at least two different occasions at more than six hours apart in a previously normotensive women after 20th gestational weeks of pregnancy, in association with proteinuria more than 300 mg/l in a 24-hour urine collection. Severe preeclampsia was defined as presence of one of the following criteria: systolic blood pressure measurement above 160 mm Hg or diastolic blood pressure measurement above 110 mm Hg on two occasions at least six hours apart, more than five grams urinary protein excretion in 24 hours or 3+ and greater random urine dipstick testing, less than 500 ml of urinary discharge in 24 hours, cerebral or visual disturbances, pulmonary edema or cyanosis, and epigastric or right upper quadrant pain. The patients of the control group were followed up with blood pressure measurements until sixth week after delivery to ensure that they did not develop preeclampsia. Women with chronic hypertension, diabetes, preexisting vascular, and chronic renal diseases were excluded from the study. The study was approved by the Ethical Committee of Uludag University. All participants were fully informed and agreed to give written informed consent.

Extraction of DNA with genomic analysis

Peripheral blood samples of 3 ml were obtained from preeclamptics and controls into tubes prepared with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Genomic DNA was extracted from circulating leucocytes with salting out procedure and was stored at –20°C until studied. All of the DNA samples collected from participants that met the criteria of the study were examined and included in the present report without any elimination. Genotyping for insertion (I) and deletion (D) alleles of the ACE gene, and deletion polymorphisms of GSTM1 and GSTT1 genes was carried out on the basis of amplification with polymerase chain reaction (PCR) technique. DNA isolation was performed with DNA isolation kit (Dr. Zeydanlı Laboratories Ltd., Ankara, Turkey) according to the instructions of the manufacturer. Laboratory work was conducted blinded to the clinical status of the subjects.

I/D polymorphism on intron 16 of the ACE gene was determined by using a respective primer pair. As PCR is known to have a tendency to preferentially amplify the shorter D allele rather than the other longer I allele when both alleles are present, confirmation was done by a second PCR amplification with insertion of specific primer pair that recognizes insertion-specific sequence for subjects who are homozygous for D allele. This avoided misdiagnosing of ID genotypes as DD and increased the specificity for DD genotyping. Primer pair sequences were shown in Table 1.

Table 1. — *Primer sequences*.

ACE I/D allele on intron 16
Forward 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'
Backward 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'
ACE (I) allele specific primer
Forward 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3'
Backward 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3'
GSTM1
Forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3'
Backward 5'-GTT GGG CTC AAA TAT ACG GTG G-3'
GSTT1
Forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3'
Backward 5'-TCA CCG GAT CAT GGC CAG CA-3'
Albumin
Forward 5'-GCC CTC TGC TAA CAA GTC CTA C-3'
Backward 5'-GCC CTA AAA AGA AAA TCC CCA ATC-3'

PCR was conducted by using 100 ng genomic DNA, 500 mmol of each primer, 0.5 mmol/l each of the four dNTPs, one unit of Taq DNA polymerase, three mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris HCl, 0.001% gelatin, pH 8.3 in a total volume of 25 µl. Thermocycling procedure was performed by first denaturation for five minutes at 94°C, followed by a second denaturation for 30 seconds in 94°C. Afterwards it was continued by 35 cycles that consisted of primer annealing for one minute at 57°C (in second procedure where DD genotype verified, was set to 63°C), extension step for two minutes at 72°C. The procedure ended with final extension for ten minutes at 72°C. The bands standing for amplified I and D alleles of ACE gene were separated on 2% agarose gel, and then stained by ethidium bromide. As a result, amplification band of 190 bp which stands for DD genotype, bands of 490 bp and 190 bp representing ID genotype, and 490 bp band of ID genotype were formed. In the second analysis used as confirmation, amplification band of 335 bp was observed which represented the presence of insertion allele.

The method performed to detect deletion polymorphisms of GSTM1 and GSTT1 genes was the same as described above. Primer pairs for albumin are co-amplified as internal controls. PCR yielded 350 bp, 219 bp, and 459 bp products representing albumin, GSTM1 and GSTT1 genes, respectively.

Statistical analysis

Statistics were performed using Statistical Package for the Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). Mean values with standard deviations were calculated for the descriptive variables of the subjects. Student's t test was used to compare characteristics between study and control groups for the continuous variables that are normally distributed. Chi-square test with continuity correction was conducted for the categorical variables. Hardy-Weinberg equilibrium was tested for allele and genotype frequencies of ACE gene I/D polymorphism for the both groups. After obeying the equilibrium, binary logistic regression model was applied to calculate odds ratios (OR) of specific ACE gene polymorphic variants. The level of statistical significance was defined as $p < 0.05$.

Table 2. — *Clinical characteristics and demographical data of patients with preeclampsia and healthy pregnant women.*

Specification	Preeclampsia (n = 63)	Controls (n = 85)	*p*
Age (years)	29.11 ± 5.47 (18 - 40)	29.35 ± 5.26 (18 - 42)	ns
Gravidity (n)	2.21 ± 1.74 (1 - 11)	2.06 ± 1.42 (1 - 9)	ns
Parity (n)	0.73 ± 1.11 (0 - 9)	0.76 ± 1.09 (0 - 7)	ns
Gestational age at admission (days)	231.14 ± 28.36 (168 - 288)	267.74 ± 17.04 (161 - 289)	< 0.001
Body mass index	24.84 ± 4.76 (1.65 - 3.98)	23.54 ± 3.6 (1.72 - 2.35)	ns
SBP at admission (mm Hg)	157.85 ± 15.9 (130 - 220)	109.85 ± 10.48 (80 - 130)	< 0.001
DBP at admission (mm Hg)	103.57 ± 11.86 (90 - 130)	72.33 ± 8.34 (50 - 90)	< 0.001

Data are given as means ± standard deviations (min - max); ns = not significant; SBP = systolic blood pressure; DBP = diastolic blood pressure.

Table 3. — *Frequencies of the study population and control subjects.*

	Preeclamptics n (%)	Controls n (%)	*p*	OR	(95% CI)
ACE					
Genotype frequencies					
DD Genotype	32 (50.8%)	22 (25.9%)	0.001	2.29	(1.39-3.79)[¥]
ID Genotype	25 (39.7%)	43 (50.6%)	0.73	0.92	(0.56-1.49)[¥]
II Genotype	6 (9.5%)	20 (23.5%)	–[§]	–[§]	
ACE					
Allele frequencies					
D Allele	89 (70%)	87 (51%)	0.001	2.29	(1.41-3.73)
I Allele	37 (30%)	83 (49%)			
GSTM1					
Present	38 (56%)	38 (47%)	0.46	1.40	(0.685-2.862)
Null	30 (44%)	42 (53%)			
GSTT1					
Present	49 (73%)	53 (65%)	0.44	1.46	(0.671-3.185)
Null	18 (27%)	28 (35%)			

[*] Chi square test was applied; OR = odds ratio; [¥]binary logistic model was applied; [§]the group that is assumed to bear no risk (OR = 1.0) in binary logistic model.
CI = confidence interval.

Results

The clinical characteristics and demographic data of 63 women with preeclampsia and 85 control subjects were presented in Table 2. Mean values of maternal age, gravidity, parity, and body mass index (BMI) of the preeclamptic women did not differ significantly from the control individuals ($p > 0.05$). Mean values of gestational age, and systolic and diastolic blood pressure were significantly lower in the preeclamptic group than in the control group.

Distribution of the polymorphic variants of the ACE gene I/D polymorphism was found significantly different between the preeclampsia and the control groups (Table 3). Frequencies of the genotypes in study group were in Hardy-Weinberg equilibrium ($\chi^2 = 0.118$, $p = 0.73$), as well as in the control group ($\chi^2 = 0.013$, $p = 0.91$). Of the total 65 preeclamptic women, 32 (50.8%) were homozygous DD genotype, 25 (39.7%) were heterozygous, and six (9.5%) were homozygous II genotype. In the control group, 22 (25.9%) of them were homozygous for DD genotype, 43 (50.6%) were heterozygous, and 20 (23.5%) were homozygous for II genotype. DD genotype predominated in preeclamptics than in controls, the difference was statistically significant ($p = 0.004$). The authors calculated an OR of 2.29 for the ACE gene DD carriers for the risk to develop preeclampsia compared to homozygous II carriers (95% CI: 1.39 - 3.79, $p = 0.001$). In the group of preeclamptics, frequency of D allele was 89 (70%), whereas 87 cases (51%) had D allele amongst controls. This increase in frequency of D allele detected in preeclamptic group was statistically significant ($p = 0.001$; OR = 2.29; 95% CI, 1.41 - 3.73). Frequency of I allele in study and control groups were 37 (30%) and 83 (49%), respectively. Compared to the control individuals, frequency of I allele was significantly lower in preeclamptic cases.

The genotype distribution of GSTM1 and GSTT1 genes in patients and controls were presented in Table 3. Patients having functional GSTM1 gene were 56% and GSTT1 gene were 73%. These findings did not differ statistically from that of controls, in which 47% and 65% had a functional glutathione S-transferase M1 and T1 gene, respectively. Of the patients with preeclampsia, 44% lacked a functional GSTM1 gene, and 27% lacked a functional GSTT1 gene. Similar to those findings, 53% and 35% of the individuals in control group lacked a functional GSTM1 or GSTT1 gene, respectively. The difference in frequencies of both alleles of GSTM1 genes between the two groups were found statistically insignificant, and presence of non-functional gene was found to have no effect in susceptibility to preeclampsia (OR = 1.40; 95% CI, 0.685 - 2.862). The authors could not find any association between non-functional GSTT1 gene and increased risk for preeclampsia (OR = 1.46; 95% CI, 0.671 - 3.185).

Discussion

Preeclampsia, which is the leading cause of maternal and fetal mortality, has been suggested to have a potent familial basis. Although the exact genetic nature remains unknown, susceptibility to preeclampsia is thought to result from complex interactions between maternal and fetal genotypes, maternal predisposing factors, and environmental factors. Polygenic and multifactorial penetrances have been reported, as well as genomic imprinting with preferential expression of the maternal alleles. In many researches regarding genetics of preeclampsia, several gene loci have been explored [5, 13].

Due to the central role of RAS on body fluid-electrolyte regulation and vascular remodeling of the placenta in pregnancy, it is thought to account for a major role in the etiology of the genetics of preeclampsia. ACE gene as a member of RAS which catalyses the conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor, has been investigated recently. From the studies in Chinese population, it was found that genetic variation in ACE gene locus is related with increased risk of pregnancy-induced hypertension and preeclampsia [12, 22].

Mello and co-workers were in agreement with the previous studies and reported diminished uteroplacental blood supply in preeclamptic Italian women that bear ACE DD genotype in contrast to patients with ID and II genotypes [11]. Beyond this statement, they found DD genotype in relation with the risk of recurrent preeclampsia and fetal growth restriction.

In the present study, the authors found that frequency of D allele is significantly higher in preeclamptic cases rather than in control subjects. Data from the present study suggests that individuals who are homozygous for D allele have increased risk for developing preeclampsia. Nevertheless, the authors could not demonstrate in this study that individuals who are heterozygous for D allele were at increased risk to develop preeclampsia. The results regarding allele frequencies and genotype frequencies were in concordance with each other, representing an increased risk of preeclampsia with the homozygosity for D allele. The current study provides evidence that there is a relationship between ACE I/D polymorphic variants and preeclampsia. However, the latter two results from this study have clinical importance in understanding possible genetic transmission model of ACE I/D polymorphism in preeclampsia.

In the current study, the authors did not find any statistically significant difference in frequencies of GSTM1 and GSTT1 polymorphisms between normal and preeclamptic subjects. These results were in corroboration with previous studies. There is a high prevalence of null alleles of GSTM1 and GSTT1 genes in humans, but interestingly the presence of homozygous nonfunctional alleles appear to have no effect on corresponding activity, and thus, is not related to GSTM1 and GSTT1 related placental oxidative stress or endothelial damage in preeclampsia pathogenesis.

In conclusion, data from this study suggests that ACE gene DD genotype is related with increased risk for developing preeclampsia. This association warrants further investigation. GSTM1 and GSTT1 polymorphisms are not directly associated with preeclampsia.

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