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AT2R −1332 G:A polymorphism and its interaction with AT1R 1166 A:C, ACE I/D and MMP-9 −1562 C:T polymorphisms: Risk factors for susceptibility to preeclampsia

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ABSTRACT

The possible association of angiotensin type 2 receptor (AT2R) −1332 G:A polymorphism with susceptibility to preeclampsia was studied in 252 women consisted of 155 women with preeclampsia and 97 healthy pregnant women. Also, the interaction of this polymorphism with angiotensin type 1 receptor (AT1R) 1166 A:C, angiotensin converting enzyme insertion/deletion (ACE I/D) and also with matrix metalloproteinase-9 (MMP-9) −1562 C:T polymorphism was investigated. The AT2R −1332 G:A polymorphism was detected using PCR–RFLP method. Significantly higher frequencies of GG+GA genotype and G allele of AT2R were observed in mild (80.2%, $p = 0.003$ and 47.5%, $p = 0.012$, respectively) and severe (77.8%, $p = 0.034$ and 48.1%, $p = 0.026$, respectively) preeclampsia compared to controls (60.8% and 35.1%, respectively). The presence of G allele was associated with 1.69-fold increased risk of preeclampsia ($p = 0.005$). In severe preeclamptic women, systolic and diastolic blood pressures in the presence of GG+GA genotype were significantly higher compared to those in the presence of AA genotype. The concomitant presence of both alleles of AT2R G and AT1R C was associated with 1.3 times increased risk of mild preeclampsia ($p = 0.03$). There was an interaction between AT2R G and ACE D alleles that significantly increased the risk of mild and severe preeclampsia by 1.38- and 1.3-fold, respectively. Also, interaction between MMP-9 T and AT2R G alleles increased the risk of severe preeclampsia 1.39-fold ($p = 0.028$). Our study demonstrated that the G allele of AT2R −1332 G:A polymorphism is associated with an increased risk of preeclampsia. Also, epistatic interaction of G allele and each allele of the AT1R C, ACE D and MMP-9 T was associated with the risk of preeclampsia. Our findings suggest that the renin–angiotensin system (RAS) variants and gene–gene interactions affect the risk of preeclampsia.

1. Introduction

Preeclampsia, a complication of pregnancy, is influenced by various factors of ethnicity, parity, health status of placenta, diet and body size. The role of single or multiple genes in the pathogenesis of preeclampsia has been suggested. Renin–angiotensin system (RAS) is stimulated during pregnancy and plays a central role in the regulation of blood pressure during this period. In preeclampsia the stimulation of this system is failed and all components of the system, especially plasma renin activity are reduced (Bouba et al., 2003).

Angiotensin II is a vasoconstrictor octapeptide of RAS that is produced from decapeptide of angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE). Angiotensin II binds to several types of receptors. The angiotensin type 1 receptor (AT1R) mediates vasoconstriction and the proliferative action of angiotensin II, while the type 2 receptor (AT2R) inhibits cell proliferation and mediates apoptosis and works cardio protectively against AT1R (Abd El-Aziz et al., 2012; Alfakih et al., 2005). The cellular effects of angiotensin II in adult humans are mainly mediated by the AT1R (Abd El-Aziz et al., 2012). The RAS activity is modified by variants of the genes coding functional proteins of this pathway (Alfakih et al., 2005).

The AT2R gene is located on the chromosome X at the locus Xq23–26. The AT2R gene consists of three exons and two introns. A common AT2R −1332 G:A polymorphism (rs14035430) is located within intron 1, 29 bp before the start of exon 2, close to a region that is important for transcriptional activity. This polymorphism is designated as −1332 G:A according to the translation initiation site of the gene, although it has also been described as +1675G:A. The AT1R

Abbreviations: ACE, angiotensin converting enzyme; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; AT1R, angiotensin type 1 receptor; AT2R, angiotensin type 2 receptor; CI, confidence intervals; HDL-C, high density lipoprotein-cholesterol; I/D, insertion/deletion; LDL-C, low density lipoprotein-cholesterol; MMPs, matrix metalloproteinases; NF-κB, nuclear factor-Kappa B; OR, odds ratios; PCR, polymerase chain reaction; RAS, renin–angiotensin system; RFLP, restriction fragment length polymorphism; SPSS, software package used for statistical analysis; TC, total cholesterol; TG, triglycerides; TIMPs, tissue inhibitors matrix metalloproteinases.

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gene locates on chromosome 3q21–q25. The AT1R 1166 A:C polymorphism (rs5186) locates in the 3′-untranslated region of AT1R gene and may be involved in posttranscriptional modification of AT1R mRNA (Alfakih et al., 2005).

An insertion/deletion (I/D) polymorphism of ACE (rs1799752) has been reported. The presence of D allele of ACE I/D polymorphism has been associated with preeclampsia in some populations (Agarwal et al., 2011; Gurdol et al., 2004; Rahimi et al., 2013a; Salimi et al., 2013).

Matrix metalloproteinases (MMPs) are zinc-dependent enzymes which play a crucial role in restructuring the extracellular matrix by activating the secretion of gelatinases, collagenases and proteolytic enzymes. Functional polymorphism of −1562 C:T (rs3918242) in the promoter of MMP-9 is associated with increased MMP-9 levels (Coolman et al., 2007; Palei et al., 2008).

In one available study the association of AT2R −1332 G:A polymorphism with the risk of preeclampsia has been examined (Akbar et al., 2009). Variants of RAS genes including ACE I/D, AT1R 1166 A:C and AT2R −1332 G:A have been associated with hypertension (Akbar et al., 2009). Also, it has been reported that AT2R agonist activates MMP-1 and MMP-9 (Alfakih et al., 2005; Gard, 2010). The aim of the present study was to investigate the possible role of AT2R −1332 G:A polymorphism in the risk of preeclampsia and also, to examine its possible interaction with AT1R 1166 A:C, ACE I/D, and MMP-9 −1562 C:T polymorphisms and influence on the risk of preeclampsia in a population from Western Iran.

2. Materials and methods

One-hundred and fifty two preeclamptic patients including 101 women with mild and 54 women with severe preeclampsia and 97 women with normal pregnancy were enrolled in a case–control study. The preeclamptic women were age matched with controls. Also, mild preeclamptic women were parity matched with both severe preeclamptic patients and controls. The subjects had been admitted to the obstetric clinic of the Imam Reza Hospital of Kermanshah University of Medical Sciences. Patients were all preeclamptic women who consecutively referred to the hospital during 10 months except those with multiple-birth pregnancy, previous hypertension, diabetes, cardiac and renal diseases. All of the patients and controls were from Kermanshah with Kurdish ethnic background. There were 17 patients (14 with severe and 3 with mild preeclampsia) with early-onset preeclampsia (before 34 weeks gestation) and 138 with late onset-preeclampsia.

The criteria for diagnosis of preeclampsia were systolic blood pressure equal or higher than 140 mm Hg, diastolic blood pressure equal or higher than 90 mm Hg, presence of proteinuria by 24-hour urinary excretion exceeding 300 mg, a urine protein: creatinine ratio of >0.3, equal or higher than 30 mg/dl protein in random urine sample (1+ reaction on a standard urine dipstick). Severe preeclampsia was defined as having ≥1 of the following criteria: blood pressure equal or more than 160/110 mm Hg on 2 occasions at least 6 h apart while patient is on bed rest, proteinuria >3+ on 2 random urine samples collected at least 4 h apart, headache, visual disturbances, upper abdominal pain, serum creatinine and transaminase elevation, thrombocytopenia, and fetal-growth restriction (Sibai, 2011).

Informed written consent was obtained from each individual before participation in the study. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and was in accordance with the principles of the Declaration of Helsinki II.

2.1. Genotype analysis

Genomic DNA was extracted from peripheral blood leukocytes using the phenol–chloroform method (Rahimi et al., 2012).

The AT2R −1332 G:A polymorphism was genotyped using the primers of 5′-GGA AGG TAG AAC ATA CAT TAA ATG-3′ and 5′-AGA GAA ACA GCA GCT AAA GAA TT-3′. The polymerase chain reaction (PCR) product with 120-bp was digested with EcoRI restriction enzyme. In the presence of G allele two fragments with 91- and 29-bp fragments are produced, while in the presence of A allele the 120-bp fragment is remained intact (Zivkovic et al., 2007). The AT1R 1166 A:C polymorphism was detected by PCR-restriction fragment length polymorphism (RFLP) using Dde I restriction enzyme as previously described (Rahimi et al., 2013a). The ACE I/D polymorphism was identified using PCR as previously described (Rahimi et al., 2013a). The genotypes of MMP-9 −1562 C:T polymorphism were detected by PCR followed by digestion with Sph I restriction enzyme (Rahimi et al., 2013b).

2.2. Biochemical analysis

Using an automated RA-1000 (Technicon, CA, USA) and standard enzymatic method (Pars Azmon kit, Tehran, Iran) the levels of serum total cholesterol (TC) and triglycerides (TG) were measured. The serum low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) levels were measured using commercially available enzyme assay kits (Pars Azmon kit, Tehran, Iran).

2.3. Statistical analysis

The allelic frequencies were calculated by the chromosome counting method. The degrees of significance of differences in genotype and allele frequencies of AT2R between patients and controls were calculated using χ^2 test. Odds ratios (OR) were calculated as estimates of relative risk for the disease and 95% confidence intervals (CI) were obtained by SPSS logistic regression. The interaction between the polymorphisms of AT2R with AT1R, ACE and MMP-9 was determined using logistic regression model. The correlation values of biochemical and clinical data with the AT2R polymorphism between studied groups were calculated using linear regression and an unpaired *t* test. Two-tailed Student's *t*-test and ANOVA analysis were also used to compare quantitative data. The categorical variables among groups were compared using χ^2 test. Statistical significance was assumed at the $p < 0.05$ level. The SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 16.0 was used for the statistical analysis.

3. Results

The characteristics of patients and controls are demonstrated in Table 1. Body mass index and blood pressure (both systolic and diastolic) were significantly higher in both groups of patients than controls. Also, the blood pressure was significantly higher in severe preeclampsia compared to mild preeclampsia. However, the mean gestational age was significantly lower in both groups of patients than in controls and also was significantly lower comparing severe to mild preeclampsia. Comparing the lipid profile between patients with controls indicated in mild preeclamptic women there were a significantly higher level of serum TG (178.1 ± 29 mg/dl, $p = 0.003$) and a lower level of total cholesterol (233 ± 54.5 mg/dl, $p = 0.02$) compared with those in controls (165.4 ± 30.8 and 249.7 ± 45.2 mg/dl, respectively). In severe preeclamptic women the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was significantly higher (39.8 ± 43.8, 34.3 ± 42.4, 388.7 ± 218.6 U/L, respectively, $p < 0.001$) compared to those in controls (24.1 ± 7.8, 18.2 ± 7, 219.5 ± 67.9 U/L, respectively). Also, the activities of AST and ALT were significantly higher in severe preeclampsia than in mild preeclampsia (27 ± 20, $p = 0.013$ and 21.6 ± 27.8 U/L, $p = 0.026$, respectively) (Table 1).

Distribution of AT2R −1332 G:A genotypes and alleles was significantly different between patients and controls (Table 2).

The distribution of AT2R −1332 G:A genotypes in controls ($\chi^2 = 1.69$, $p > 0.1$) and in severe ($\chi^2 = 1.88$, $p > 0.1$) preeclamptic women was in Hardy–Weinberg equilibrium. Considering the samples were used for interaction studies revealed the frequencies of AT1R