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Renalase gene polymorphism is associated with increased blood pressure in preeclampsia

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ABSTRACT

Background: Renalase is a novel enzyme that degrades circulating catecholamines. We aimed to investigate the role of rs2576178 and rs10887800 polymorphisms of the renalase gene in preeclampsia (PE) patients
Methods: This case-control study consisted of 110 women with PE and 102 normotensive controls. PCR-RFLP method was used for determination of renalase gene polymorphisms.
Results: Allele frequency and genotype distribution of rs10887800 polymorphism were found statistically significantly higher in women with PE ($p < 0.05$). Also G allele and GG genotype of rs10887800 polymorphism were found higher in women with severe PE than that of mild PE ($p < 0.05$). There was no significant difference for rs2576178 polymorphism in terms of allele frequency and genotype distribution ($p > 0.05$). In PE patients, systolic blood pressure (SBP) means according to rs10887800 genotypes were found statistically significantly higher (GG vs AA; $p = 0.001$) and (GG vs GA; $p = 0.001$). Similarly, diastolic blood pressure (DBP) means were found statistically significantly higher in PE patients (GG vs GA: $p = 0.001$); (GG vs AA: $p = 0.004$). For rs2576178 polymorphism, SBP means were found as (GG vs AA; $p = 0.012$, GG vs GA; $p > 0.05$) in PE patients. DBP means were not significant according to rs2576178 genotypes in PE patients ($p > 0.05$).
Conclusions: The findings of the present study suggest that blood pressure may be increased by GG genotype and G allele of rs10887800 polymorphism and the polymorphism may increase the susceptibility to PE.

1. Introduction

Hypertensive disorders account for approximately 10% of all pregnancies and involve in a wide range of situations including chronic hypertension, gestational hypertension, eclampsia, hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome and preeclampsia (PE) [1–3]. PE is defined as the onset of hypertension and proteinuria after the second halfs of the pregnancy [2,4,5]. It is one of the most common medical complications of pregnancy and affects about 2–8% of all pregnancies [6,7]. Despite the developments in antenatal and neonatal care, PE continues to be one of the major causes of maternal and neonatal morbidity and mortality worldwide [8–10].

Experimental studies have provided clear evidence that eclampsia and PE are states of sympathetic hyperactivity [11,12]. Both sympathetic nervous and sympathetic adrenal activities are increased in patients with PE [12–14]. The catecholamine concentration has been found to be increased in PE and eclampsia [15,16]. Arterial epinephrine was shown to be increased in PE [12]. It was shown that increased plasma epinephrine level correlated with increased blood pressure (BP) in the development of PE [17]. In the regulation of BP, catecholamines like dopamine, norepinephrine, and epinephrine play a key role. Monoamine oxidase-A (MAO-A), monoamine oxidase-B (MAO-B) and catechol-O-methyl transferase (COMT) are the responsible intracellular enzymes for degradation of catecholamines [18]. In the last decade, researchers identified a novel soluble flavin adenine dinucleotide (FAD)-dependent amine oxidase called as renalase [19]. Renalase is mainly expressed in the kidney and also found in the heart, small intestine and skeletal muscle [19]. Renalase metabolizes catecholamines and catecholamine-like substrates [20].

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It shows significantly high activity against catecholamines such as epinephrine, dopamine and norepinephrine, but shows little or no activity towards physiologically occurring amines such as serotonin, benzylamine, tyramine, methylamine [21].

The gene encoding human renalase is called as (RNLS) and located on chromosome 10q23.33 and formed 10 exons and 309,469 base pairs (bp). The human renalase protein is 342 amino acids long, and consists of an amine oxidase domain, a FAD-binding region, and a signal peptide [19].

The kidney is primarily responsible organ for maintaining steady levels of the renalase. Renalase circulates in the blood in an inactive form called as prorenalase. Prorenalase is secreted into the blood by the kidney. In the basal state, prorenalase has no amine oxidase activity, and it is rapidly converted to renalase by increased catecholamines and heightened BP. Renalase decreases the BP by degrading the catecholamines including dopamine, epinephrine and norepinephrine [22]. Several animal experiments were designed to clarify the role of renalase in catecholamine degradation and BP regulation [20,23–25]. Recent data have indicated that deficiency of renalase associated with heightened BP and increased circulating catecholamine levels [26,27].

Two polymorphisms selected for the present study are located in the putative functional regions. rs2576178 polymorphism is located at the 5′ flanking region and rs10887800 polymorphism is located at the intron 6, near the border of exon/intron, and consequently these polymorphisms might affect regulation and expression of the RNLS gene [28]. To the best of our knowledge, polymorphisms in the renalase gene (rs2576178, rs10887800) were found to be associated with hypertension [28,29], stroke, type 2 diabetes [28], end-stage renal disease (ESRD) [30], coronary heart disease [31], and pregnancy induced hypertension (PIH) [32]. To date, there is no study investigating the role of renalase gene polymorphisms in PE patients.

Although the signs and symptoms of the disease are well known, the etiology is still unknown. Therefore it could not possible to prevent the disease [6]. Studies carried out to date have focused on the pathophysiology, prevention and treatment of the disease [6,7]. In the light of this information, we aimed to investigate the possible role of rs2576178 and rs10887800 polymorphisms of the renalase gene on the development of preeclampsia, and to show their possible associations with demographic parameters and laboratory findings, primarily blood pressure, and to find whether there is a relationship with disease severity.

2. Materials and methods

This case-control study consisted of 110 women with preeclampsia and 102 normotensive controls. All subjects had been admitted to Obstetrics & Gynecology clinic, Cumhuriyet University Hospital between April 2015 and October 2015 included in the study. All subjects of the present study were Caucasians of Turkish origin. The diagnosis of preeclampsia was determined according to the International Society for the Study of Hypertension in Pregnancy's (ISSHP) criteria [33]. The following criteria were used for the diagnosis of PE: systolic blood pressure (SBP) and diastolic blood pressure (DBP) shold be above 140/90 mmHg, at least two measurements, minimum 4 h apart, and proteinuria should be above 300 mg per 24 h. Pregnant women who did not meet these criteria were excluded from the study. The control group were recruited from the same center and consisted of normotensive volunteer pregnant women with at least one pregnancy and no history of PE. Ethics Committee of Cumhuriyet University, Faculty of Medicine approved the present study. The present study was conducted in accordance with the Declaration of Helsinki ethical principles. A written, informed consent was obtained from all subjects.

Patients with PE included in the present study were categorized into 2 groups based on the disease severity. Severe PE was defined as having one or more of the following criteria: SBP/DBP $\geqslant$ 160/110 mmHg on two occasions 4 or more hours apart in a pregnant woman on bed rest and having proteinuria with excretion $\geqslant$3 g in 24 h urine sample, visual disturbances, headache, upper abdominal pain, elevated levels of serum creatinine and transaminases, thrombocytopenia, fetal-growth restriction. Mild PE has determined as SBP/DBP between 140–160/90–110 mmHg and having proteinuria <3 g in 24 h urine sample [3].

The exclusion criteria for preeclamptic patients and controls were as follows: gestational hypertension, diabetes mellitus, gestational diabetes mellitus, multiple pregnancy, isolated proteinuria, thrombocytopenia, elevated levels of transaminases with no hypertension, maternal chronic and inflammatory disorders, stillbirth, intrahepatic cholestasis of pregnancy, and maternal hepatitis.

Demographic parameters including maternal age, gestational age, gravidity, parity, SBP and DBP, body mass index (BMI), prior PE history, family history of PE, chronic hypertension and diabetes mellitus (DM) were collected from patients and controls. Fasting blood glucose (FBG), hematocrit (HCT), hemoglobin (Hgb), creatinine, blood urea nitrogen (BUN), white blood cells count (WBC), platelet count (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urinary dipstick test were collected from routine laboratory tests of the subjects.

2.1. Genotyping

PCR-RFLP method was used for determination of renalase gene polymorphisms (rs2576178, rs10887800). Peripheral venous blood samples (3 ml) were taken into tubes with K_3EDTA from patient and control subjects. These tubes are stored −20 °C until genetic analysis time. Total genomic DNA was extracted from peripheral venous blood with according to salting out procedure [34].

Template DNAs were amplified for detection of renalase gene polymorphisms (rs2576178, rs10887800). Same PCR protocol was used for the detection of both polymorphisms. PCR were carried out in a total reaction mixture of 25 μl containing 150–300 ng of genomic DNA, 12.5 μl of PCR master mix (Fermentas, Lithuania), 9,5 μl of ddH_2O and 10 pmol of each primer (1 μl). Following primers were used for amplification reaction: for rs2576178 polymorphism, forward: 5′-AGCAGAGAAGCAGCTTAACCT-3′, reverse: 5′-TATCTGCAAGTCAGCGTAAC-3′; and for rs10887800 polymorphism, forward: 5′-CAGGAAAGAAAGAGTTGACAT-3′, reverse: 5′-AAGTTG TTCCAGCTACTGT-3′. Amplification of two polymorphism on the renalase gene was performed an initial denaturation step at 94 °C for 5 min followed by 40 cycles of denaturation step at 94 °C for 45 s, annealing step at 58 °C for 1 min, elongation step at 72 °C for 2 min and followed by a final elongation step at 72 °C for 5 min.

Restriction digestion of PCR products for rs2576178 polymorphism was performed in 10 μl volume using 5 units of MspI restriction endonuclease (New England Biolabs, UK) at 37 °C for 16 h. Digestion products were analyzed by electrophoresis on a 2% agarose gel in TAE buffer, and visualized using ethidium bromide staining. Samples with a single 525 bp band were identified as wild type AA genotype while samples with 2 bands (423, 102 bp) were identified as homozygous GG genotype and those with 3 bands (525, 423 and 102 bp) were identified as heterozygous AG genotype. Restriction digestion for rs10887800 polymorphism was performed in 10 ml volume using 10U of PstI restriction endonuclease (New England Biolabs, UK) at 37 °C for 16 h. Digestion products were electrophoresed on a 2% agarose gel in TAE buffer and visualized using ethidium bromide staining. Samples with a single 554 bp band were identified as wild type AA genotype while samples with 2 bands (415, 139 bp) were iden-