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The Role of The ACE Gene Promoter Region in Preeclampsia in a Turkish Population

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Abstract

It is known that preeclampsia (PE), a common multisystemic and multifactorial pregnancy disease, causes 10-15% of direct maternal deaths worldwide. Many research, including whole genome sequencing studies, has been and continues to be done in search of a mutant gene that can cause the disease. According to studies, one of the candidate genes associated with PE is ACE2 gene. Therefore, in this study, it was aimed to investigate the variations in the promoter region of ACE2 gene for the first time in a Turkish population. Sequencing analysis was performed on genetic material obtained from maternal blood with samples collected from 60 PE patients and 60 healthy pregnant women with demographic data compatible with the patients. Sequencing analysis was performed with the Sanger sequencing method and ABI 3130XL instrument. According to the results of the sequence analysis, no variation was found in the studied region. However, PE disease remains a major health problem in pregnancy, and accumulating evidence indicates that ACE2 may also have effects on the disease. Therefore, improved structural and functional studies in larger populations are needed to understand these effects.

Keywords: ACE2, preeclampsia, variation

INTRODUCTION

Preeclampsia is one of the pregnancy-specific disease characterized by new-onset hypertension and proteinuria after the 20th gestational week (Hod et al., 2015). It is a common multisystemic and multifactorial disease that causes 10-15% of direct maternal deaths worldwide. PE, which affects approximately 2-8% of all pregnancies, is known to have an estimated prevalence of 14.1% (Odden et al., 2006; Duley, 2009).

In the 12th week of pregnancy, the spiral artery is reshaped with the trophoblast invasion of the spiral arteries. This situation initiates the development of PE, that is, the pathogenesis. Subsequently, abnormal placentation, which is known as a common pathology in PE, develops as a result of hypoxia and increases sFTL1 (soluble Fms-like tyrosine kinase-1) in the maternal circulation (Steegers et al., 2010; James et al, 2010). PE has three levels of pathology: (1) stress by syncyotrophoblast resulting from abnormal placental perfusion, (2) hypertension and proteinuria and (3) maternal organ dysfunction (Redman and Staff, 2015; Flint et al., 2019). However, although many factors such as oxidative stress, genetics and immunity have been shown to cause placental dysfunction, the etiology of PE is not fully known (Hiby and Walker, 2004; Mert et al., 2012).

Although secondary pathways that play a role in the development of PE are known, the main causes have not been fully elucidated. Studies including whole genome sequencing for a mutant gene that can cause the disease are ongoing, and according to the results of this research, *ACE2* may be one of the candidate genes associated with PE.

ACE2, a type of human angiotensin converting enzyme (*ACE*) homologue, is physiologically very different from *ACE* despite this high homology (40% identity and 61% similarity). As a carboxypeptidase, *ACE2* converts angiotensin-II to angiotensin (1-7) (Ang-(1-7)) (Fan et al, 2019). Studies have shown that there is an increase in Ang-(1-7) levels in the plasma and urine of healthy pregnant individuals. However, it was observed that plasma Ang-(1-7) levels were suppressed in pregnant individuals with PE compared to healthy pregnant women (Brosnihan et al., 2004). Ang-(1-7) is known to induce blood vessel growth and regulate angiogenesis, and also inhibit angiogenesis in opposition to the angiogenic effects of Ang-II. The decrease in plasma Ang-(1-7) in PE is seen as a condition that may cause an increase in blood pressure in pregnant women (Merrill et al., 2002).

There is no previous research to understand the relationship of genetic changes in the *ACE2* gene promoter region with PE.

Therefore, in this study, it was aimed to perform *ACE2* promoter region sequence analysis on genetic material to be obtained from maternal blood for the first time in a Turkish population. It was investigated whether there is a relationship between variations in the promoter region of the *ACE2* gene and PE disease.

MATERIAL AND METHOD

Subjects

The sample of this study was formed from blood samples previously collected in Sivas Cumhuriyet University Hospital Obstetrics and Gynecology Department. The blood samples of 60 PE patients and 60 normal pregnant women were selected from the blood bank in our laboratory and used. Approval for the study was obtained from the Ethics Committee of Sivas Cumhuriyet University Faculty of Medicine (ethics committee decision no: 2020-01/14).

DNA Isolation, Primer Design and PCR

Genomic DNA isolation was performed using the phenol-chloroform method (Ullrich et al., 1997) from previously collected and in our laboratory stored at -20ºC blood samples. The isolation products were stored at -20ºC until the PCR stage. For the PCR, primer design was made using the NCBI Primer BLAST, Primer3 and *in silico* programs, covering the range of the ACE2 gene promoter region -140 to +306. According to this, forward primer 5'-CTGTCCTCTCCAGGATGAACTT-3' and reverse primer 5'-TTTTCAGTTTCACGGGCAGT-3'. The annealing temperature of the primers is 60ºC and the amplicons size of the obtained PCR product is 450bp.

PCR in three amplification steps of 35 cycles, followed by a 5 minutes denaturation step at 95ºC: (1) denaturation at 95ºC for 30 seconds, (2) annealing at 60ºC for 30 seconds and (3) elongation at 72ºC for 30 seconds. The final elongation is set to 5 minutes at 72ºC.

DNA Sequencing

Sequence analysis was performed with the Sanger sequencing method and the ABI 3130XL device. The raw data of the sequence analysis were evaluated according to the electropherograms obtained from the device. Displaying the results and scanning the changes in the sequenced region was done with the Chromas Lite 2.6.6 program.

Statistical Analysis

Demographic data of patient and control group individuals were analyzed using SPSS 22.0 program. In the evaluation of the data, the ODDS ratio and 95% confidence limits were calculated by applying the Chi-Square test and $p<0.05$ was considered significant.

RESULTS

In this study, genomic DNA isolation was performed using previously collected blood samples from patients with PE and healthy pregnant individuals, followed by Sanger

sequencing analysis. The samples constituting the patient group of our study were randomly selected from the blood bank in our laboratory. The control group consisted of samples whose demographic data were compatible with the patient group. The mean ages of 60 PE patient and 60 normal pregnant women included in our study are respectively; it was found as 28.31±6.4 and 28.25±6.7. While 22 (36%) of the pregnant women in the patient group had early-onset PE, 38 (64%) had late-onset PE. When the delivery method of the pregnant in the study groups was examined, it was seen that cesarean delivery was significantly higher than normal delivery (p=0.0000001). Premature birth was observed more frequently in PE patients as the time of delivery. There was no statistically significant difference between the patient and control groups in terms of miscarriage status (p=0.8). Family history in PE patients is statistically significant compared to individuals without the disease (p=0.01). Demographic and clinical data of the samples are summarized in Table-1.

Within the scope of the study, sequence analyzes were performed for the ACE2 gene promoter region of patients diagnosed with PE and healthy control group individuals. According to the results of sequence analysis, no variation was found in the sequenced region. Since no change was observed in the sequenced region, association with demographic and clinical data could not be made. Sequence analysis results of patient and control group individuals using the Sanger sequencing method are shown in Figures 1 and 2.

Table 1: Demographic and clinical data of the patient and control groups

Character	Patient (n=60)	Control (n=60)	*p* value
Maternal age (years)	28.31±6.4	28.25±6.7	0.7
Type of delivery, n (%)			
Cesarean section	48 (80)	10 (16.6)	**0.0000001**
Vaginal delivery	12 (20)	50 (83,4)	
Nulliparous, n (%)	26 (43.33)	43 (71.66)	
Gestational age at delivery (weeks)	35±4	28±6	**0.002**
BMI*			
Before pregnancy	31.1±5.27	51.2±5.4	
After pregnancy	34.4±4.2	61.5±5.4	
Birth time, n (%)			
Preterm	30 (50)	5 (8.33)	**0.0000033**
Normal term	30 (50)	55 (91.67)	
Preeclampsia, n (%)			
Mild	28 (46.66)		
Severe	32 (53.34)		
Smoking before pregnancy, n (%)			
Present	8 (13.33)	6 (1)	0.7
Absent	52 (46.67)	54 (99)	
Onset preeclampsia, n (%)			
Late	22 (36.66)		
Early	38 (63.34)		
Abortion status, n (%)			
Present	24 (40)	19 (31.66)	0.8
Absent	36 (60)	41 (68.34)	
Family history, n (%)			
Present	6 (10)	0 (0)	**0.01**
Absent	54 (90)	60 (100)	

BMI: Body-mass index
$p<0.05$ was considered statistically significant

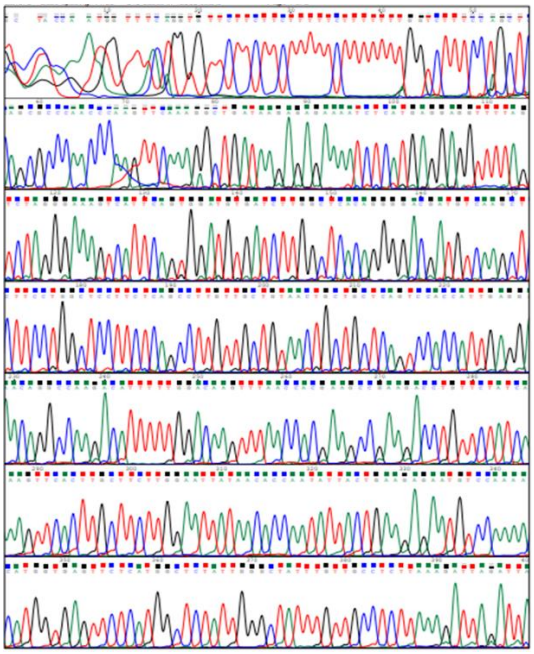

Figure 1: Sequence analysis result of the patient group

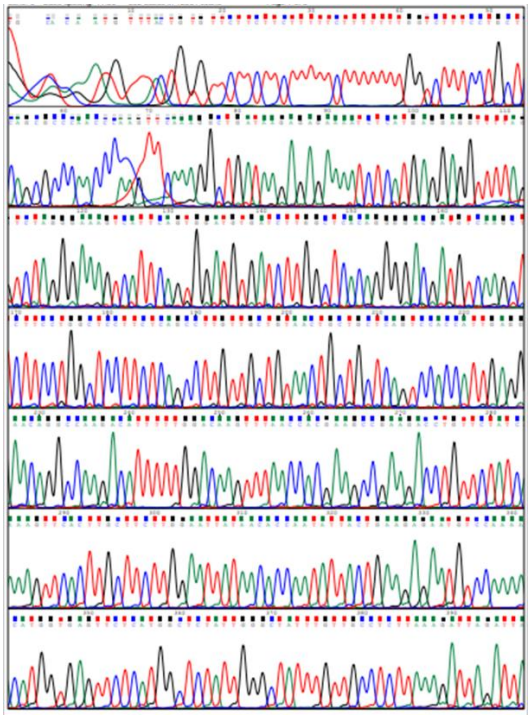

Figure 2: Sequence analysis result of the control group

DISCUSSION

The etiology of PE, which clinically starts with pregnancy and has potentially life-threatening consequences for both mother and baby, still remains unknown. However, it is known that various genetic components of both maternal and fetal origin play a role in the development of the disease. Therefore, in our study, possible variations in the range of -140 to +306, including intron, exon and promoter regions of the *ACE2* gene, which is thought to be associated with PE disease, were investigated by sequence analysis. For the study, 120 randomly selected samples from previously collected PE patient and healthy pregnant blood samples were used. No variation was observed in the region sequenced for the first time in Turkish population.

Smoking during pregnancy is thought to reduce the risk of PE by up to 50%, that is smoking has a continuous protective effect in nulliparous and multiparous, single and multi-fetal pregnancies, and mild and severe PE. Although a specific mechanism for the protective effect of smoking has not yet been supported, it is thought that smoking may have effects on angiogenic factors, endothelial dysfunction and immune system that reduce the risk of PE (England et al., 2007). When the demographic data of the sample were examined, it was seen that smoking before pregnancy increased the risk of exposure to PE compared to non-smoking pregnant women (p=0.7). This situation, which is inconsistent with the general literature, may have resulted from the sample size and/or environmental factors.

Ang-(1-7) was detected at lower levels in plasma and urine of pregnant individuals with PE compared to healthy individuals. The production of Ang-(1-7), which exerts blood vessel growth, angiogenesis and vasodilatory effects, is catalyzed by *ACE2*. The decrease in Ang-(1-7) in PE is a condition that may cause increased blood pressure in pregnant women, and it is though that a variation in *ACE2* may cause the decrease in Ang-(1-7).

Within the scope of our research, HIF-1α, a transcription factor that plays a role in the transcription of many oxygen-dependent genes related to angiogenesis and cell metabolism, binds to the region sequenced in *ACE2*. Overexpression of HIF-1α has been observed in many oxidative stress-based diseases such as PE and cancer (Rajakumar et al, 2004). A possible variation in *ACE2* could result in a change in the amount of binding of HIF-1α to *ACE2*. Therefore, this may cause a change in the expression level of *ACE2*, and this is one of the hypotheses on which our study is based.

Another starting point of our study is the renin-angiotensin system (RAS). Components of the RAS are involved in remodeling as well as insertion of spiral arteries. Because RAS polymorphisms cause changes in RAS activity, they are associated with placental abnormalities and hence PE (Yang et al., 2013). Based on this context, in this study, the relationship between PE and a possible variation in the sequenced region in *ACE2*, one of the components of RAS, was investigated. However, as a result of research, no variation was found in the sequenced region, so no relationship could be found between *ACE2* and PE. In this respect, it may be said that the sequence analyzed region has been conserved throughout the evolutionary process.

It is thought that the study population's having a similar living environment, similar pregnancy histories and the same ethnic origin had an effect on the observation of similar results on research groups. It should be noted that this study has important methodological limitations, and within the framework of these limitations, a sufficient region to investigate variation cannot be examined. It is still unknown whether there is a variation on PE disease in regions of the *ACE2* gene that were not included in our study. This result points to the need for more extensive investigation of variations to be associated with PE in different regions of the *ACE2* gene.

ACE2 is also one of the new targets for the prevention and treatment of hypertension (HT) (Velkoska et al., 2016). Although world-wide studies have been conducted to elucidate the relationship between *ACE2* and HT, the results are inconsistent. For example, in a study investigating possible

associations between single nucleotides polymorphisms in *ACE2* and organ damage due to HT in Chine, it was determined that all *ACE2* gene variations examined were associated with essential HT risk. In another study conducted in Europeans, it was determined that ACE2 gene variations were associated with blood pressure and/or blood pressure changes (Luo et al., 2019; Malard et al., 2013). In contrast to these studies, a study in Australian population found that ACE2 polymorphisms were not associated with basic HT (Benjafield, 2014).

Our study is the first to investigate the relationship between the ACE2 promoter and PE at the molecular level. However, the study result represents only a local part of Turkish population we studied. Sample size is an important factor in investigating variations in a population. Therefore, the insufficient statistical power of the sample size in this study in the Turkish population to detect a possible effect is one of the limitations of research. However, it is though that the study will contribute to the limited number of data in the literature by using it as a preliminary data for future studies. Such studies will contribute to the detection of the presence/absence of variation in related genes and the clarification of the "PE-mutant gene" relationship.

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