Experimental
&
Clinical Article

Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism and Its Association with the Severity of Preeclampsia

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OBJECTIVE: Pre-eclampsia is a multisystemic, idiopathic pregnancy-specific disorder. The role of inappropriate activation of renin-angiotensin system is well known. In this study it is aimed to elucidate the relationship between angiotensin converting enzyme (ACE) gene insertion/deletion polymorphism and severity of pre-eclampsia.

STUDY DESIGN: Pre-eclamptic or eclamptic 43 women who were either nulliparous or had been preeclamptic in their first pregnancy have been included to the study. It was a cross sectional observation study. Thorough obstetric examination was done for each patient. Demographic properties, laboratory findings, gestational ages and obstetric histories were recorded. Obstetric ultrasonographies were performed. Of the 43 patients included, 24 (55.8%) were suffering from severe pre-eclampsia. Other 19 (44.2%) patients were not severely ill. A correlation between preeclampsia severity and ACE gene polymorphism was not found. DD genotype was associated with higher systolic blood pressures (p=0.04).

CONCLUSION: In our study, in order to predict pre-eclampsia severity use of ACE gene polymorphism has noyt been established. DD genotype was found to be a risk factor for systolic blood pressure increment in pre-eclamptic pregnants. Randomized, prospective studies with large populations are needed on this subject.

Key Words: Angiotensin-converting enzyme, Polymorphism, Preeclampsia

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Introductıon

Preeclampsia, a syndrome of hypertension and proteinuria, is a major cause of maternal and perinatal morbidity and mortality.[1] It is presumed to be a multifactorial and multisystemic disorder with a genetic predisposition.[2] Although immune maladaptation, placental ischemia, increased oxidative stress with possible genetic implications are postulated to have roles on the pathogenesis, to date, the exact mechanisms underlying the pathogenesis still remain to be elucidated.[3] Extensive research about genetic contribution have been undertaken and evidences related with inherited predisposition are well known.[3,4] But still, the exact genetic basis remains unclear.

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Renin-angiotensin-system (RAS) has a pivotal role in regulating blood pressure. That is why, its inappropriate activation is presumed to happen during pregnancy, that is destined to result in preeclampsia.[5] Many investigators have reported data regarding the impact of genetic polymorphism in the RAS over the pathogenesis of preeclampsia. Angiotensinogen converting enzye (ACE), is one of the key components of this system.[6] A common insertion/deletion polymorphism within the ACE gene has been shown to be associated with RAS activity variability.[5] Although small in size, some studies have reported that women carrying D allele have higher ACE activity and higher resistance of uterine artery, which is considered to be a marker for future preeclampsia and intrauterine growth restriction.[5] These results have led the conclusion that, D allele might be related with the development of preeclampsia. It has been an area of interest, where the associations between preeclampsia and the genes encoding for the RAS are investigated. The studies evaluating the role of ACE -I/D polymorphism in preeclampsia yield different results. Some report an association,[7,8,9] while others not.[4,6] But to our knowledge, there has been no reports as to whether ACE-I/D polymorphisms are related with the severity of preeclampsia. That is why, this is the first study on the relationship between ACE-I/D polymorphisms and the severity of preeclampsia.

Material and Method

Subjects

Preeclamptic or eclamptic 45 women, who were either nulliparous or had been preeclamptic in their first pregnancies, have been enrolled at Zekai Tahir Burak Hospital in Turkey. This study was approved by the Human Researchs Ethics Committe of our hospital and informed consents were taken from all patients. All the subjects enrolled in the study were Caucasians. A detailed history, including demographic characteristics, past obstetric and medical histories, gestational weeks, laboratory findings were obtained from every woman. Detailed fetal anatomical surveys were carried out to exclude major fetal abnormalities and also ensure gestational weeks. Preeclampsia was defined according to National High Blood Pressure Education Program, which was; blood pressure of ≥ 140/90 mmHg on two different occasions , associated with proteinuria> 300 mg/l in a 24-h urine collection after the 20th week of pregnancy in a previously normotensive and nonproteinuric woman. At postpartum 12th week, patients were given a call to find out whether they had still high blood pressure. 2 were excluded because their blood pressure were still above 140/90 mmHg. Women with diabetes, chronic hypertension, liver and kidney disease, thrombophilias, history of thromboembolia, multiple pregnancies and pregnancies with congenitally malformed fetuses were excluded from the study.

Severe preeclampsia is defined as presence of blood pressure values ≥160/110 on two occasions > 6 h apart , associated with proteinuria >5 gr / l in a 24-h urine collection, increased liver enzymes, hemolysis and platelett counts below 100000/μl (table 1). Patients who had had eclamptic crises were also considered as severe preeclampsia and included in that group.

Sample collection

Peripheral blood samples were collected from patients in tubes containing EDTA. Genomic DNA was extracted from leucocytes. A genomic DNA fragment on intron 16 of the ACE gene was amplified by Polymerase Chain Reaction (PCR). Three types of ACE gene PCR products were identified. ACE DD, ACE II, ACE ID according to the PCR amplification of the allele with or without the insertion. Fragments with out insertion (D allele) and with insertion (I allele) were detected on 1.5% agarose gel.

Statistical analysis

Data are presented as ± S.D, percentages and numbers. Statistical analysis were carried out on SPSS for Windows. Differences between groups were tested with Mann-Whitney U test. Chi-squared test was used for the statistical evaluation of genotype and allele frequency.

Results

Of the 43 patients included, there were 24 patients (55.8 %) with severe preeclampsia, while 19 patients (44.2%) were suffering from mild disease. In severe preeclamptic group, angiotensin-converting enzme genotypes were: deletion-D (DD) in 9 women (60%), insertion-I (II) in 4 women (44%), and insertion-deletion in 11 woman (57.9%); in mild preeclamptic group (n=19), the angiotensin-converting enzme genotypes were DD in 6 women (40%), II in 5 women (55.6%), and insertion-deletion in 8 women (42.1%).

ACE genotype distribution among all patienst are as follows:15 DD (34.8%), 9 II (20.9%), 19 I/D (44.1%). The frequencies of D allele and I allele are 57% and 43% respectively. The distribution of ACE allelic frequencies is given in table 2. Clinical characteristics of both groups are presented in Table 3.

Clinical characteristics and pregnancy outcomes according to ACE genotype polymorphisms are shown in table 4.

All of the patients, except one, were pregnant. 10 of the patients with severe preclampsia, had HELLP syndrome and 3 had eclamptic seizures. There were 4 intrauterine fetal demises, all of which had happened in severe preeclamptic group. But there were no maternal deaths. Because 9 of the patients did not know the date of their last menstrual period, they could not be assessed for intrauterine growth restriction (IUGR) evaluation.

Table 1: Assesment of severity of preeclampsia

	Mild Preeclampsia	**Severe preeclampsia**
Systolic blood pressure	<160mmHg	>160mmHg
Dyastolic blood pressure	<110mmHg	>110mmHg
Proteinuria	>300mg/day	>5000mg/day
Oliguria	no	yes (<500ml/day)
Seizures	no	yes (eclampsia)
Serum creatinin levels	Normal / mildly elevated (<1,2mg/dl)	Abnormal (>1,2mg/dl)
AST, ALT	Normal / mildly elevated (<70 U/L)	Elevated (>70 U/L)
Platellet number	Normal / mildly decreased (>100 000/μL)	Decreased(<100000/ μL)
Pulmonary edema ödem or cyanosis	no	yes

Table 2: ACE gene allele frequencies in severe and mild preeclampsia groups

	Mild preeclampsia	Severe preeclampsia	Total
D allei	20 (40.8)	29 (59.2)	49 (57)
I allel	18 (48.6)	19 (51.4)	37 (43)
Total	38 (44.2)	48 (55.8)	86 (100)

Table 3: Clinical characteristics of patients in severe and mild preeclampsia

	Mild	Severe	All patients	p
Age	24+5	27+6	26+6	0.123
Gestational week	34+4	33+4	34+4	0.309
Systolic BP	148+11	168+29	160+25	0.004
Dyastolic BP	92+11	112+21	103+20	<0.001
ALT	28+31	125+130	82+110	0.001
AST	36+43	153+165	101+138	<0.001
Creatinine	0,7+0,1	0,9+0,4	0,8+0,4	0.007
Platellete (x1000)	228+101	122+69	169+99	0.001

Table 4: Clinical characteristics and pregnancy outcome according to different ACE genoytpes

	DD	ID	II	Total	p
Mild preeclampsia	6 (40)	8 (42.1)	5 (55.6)	19 (44,2)	AD
Severe preeclampsia	9 (60)	11 (57.9)	4 (44.4)	24 (55,8)	AD
Age	26.4+6.6	25.4+5.3	24.2+4.6	25,5+5,6	AD
Gestational week	3.0+5.7	33.3+3.3	33.5+5.1	33,6+4,6	AD
Systolic BP >160mmHg	8 (53.3)	5 (26.3)	1 (11.1)	13 (31,0)	0,04
Dyastolic BP>110mmHg	7 (46.7)	5 (26.3)	3 (33.3)	15 (34,9)	AD
ALT >70 U/L	4 (26.6)	7 (36.8)	3 (33.3)	14 (32,6)	AD
AST >70 U/L	5 (33.3)	6 (31.6)	4 (44.4)	14 (32,6)	AD
Creatinine >1,2mg/dL	1 (6.7)	2 (10.5)	0 (0)	3 (7,0)	AD
Platellete <100 000/ mL)	3 (20.0)	5 (25.3)	1 (11.1)	9 (20,9)	AD
IUGR (34patient)	1 (10.0)	7 (43.8)	3 (37.5)	11 (32,4)	AD
IU EX	1 (6.7)	2 (10.5)	1 (11.1)	4 (9,3)	AD
Pulmonarry Edema	1 (6.7)	1 (5.3)	0 (0)	2 (4,6)	AD
Convulsion	2 (13.3)	1 (5.3)	0 (0)	3 (7,0)	AD
Family history	1 (6.7)	3 (15.8)	2 (22.2)	6 (14)	AD

As shown in table 2 and table 4, DD and ID genotype and D allele predominate in the severe preeclampsia group, although statistically not significant. Systolic Blood Pressure was higher at patients with DD genotype and the difference was statistically significant. Even though statistically unsignificant, the diastolic blood pressure was also higher in patiens with DD genotype.

We also compared the patients with different genotypes in terms of clinical and biochemical parameters together with complications of preeclampsia as shown in Table 4 and found no sinificant difference between groups.

Comment

Despite extensive research over preeclampsia, the exact underlying mechanisms remain to be elucidated. Preeclampsia is thought to be the result of interaction between genetic and environmental factors.[5] Though, there has been a lot of data regarding genetic contribution over preeclampsia, the exact genetic basis still remains unclear.[3] Since genetic susceptibility to preeclampsia has been understood, there has been growing interest over this field to determine genetic loci, that would help to identify the risky groups. Some investigators have postulated that, inappropriate activation of RAS may have a role in the development of cardiovascular disorders including

preeclampsia.[10,11] That's why any molecular difference in the RAS may indicate genetic susceptibility.[7]

The results of the studies evaluating the relationship between ACE gene polymorphisms and preeclampsia, are contraversial. This contraversy, at some aspect, may be due to the differences in study population and geographical location.[7]

Some investigators from China,[6] from Korea,[12] and from Brazil,[4] could not show any association between ACE I/D polymorphism and preeclampsia. Mello and his friends from Italy reported that, ACE DD genotype was not associated with the risk of preeclampsia in the first pregnancy, but ACE I/D polymorphism affected uteroplacental and umblical flows and the recurrence of an adverse outcome in women with history of preeclampsia.[9] But these results are inconsistent with those of Zhou et al.[13] who had found a positive correlation between DD genotype frequency and preeclampsia . Tamura et al.[14] also reported significant association between ACE I/D polymorphism and preeclampsia. These discrepancies may be attributed to different ethnic populations and geographic locations, study design and small sample size.

To our knowledge, there has been no reports as to whether ACE I/D polymorphism is associated with the -severity- of preeclampsia. Although the number of patients with severe preeclampsia is more in women with DD genotype and D allele than the others, this can not reach the level of significance. On the other hand, systolic blood pressures of patients with DD genotypes are much more higher than the others, that is statistically significant. We have to take into consideration that, systolic blood pressure is one of the determinants of severity of preeclampsia. The number of patients included in this study is insufficient to draw strong conclusions. However, there may be a relationship between severity of preeclampsia and ACE I/D polymorphism, although we could not achieve to show it statistically, due to limited number of patients.

This is the first study on the relationship between ACE I/G polymorphism and the severity of preeclampsia. Larger studies are required to validate possible, if any, associations between ACE I/G polymorphism and the severity of preeclampsia.

Preeklampside ADE İnsersiyon/ Delesyon Gen PolimorFizmi ve Preeklampsinin Şiddeti ile İlgisi

AMAÇ: Preeklampsi sebebi bilinmeyen gebeliğe özgü bir multisistemik hastalıktır. Renin-anjiyotensin sisteminin uygunsuz aktivasyonu preeklampside rol oynadığı iyi bilinmektedir. Bu çalışmada Anjiyotensin dönüştürücü enzim geni insersiyon/delesyon polimorfizminin preeklampsi şiddeti ile ilişkisinin açığa kavuşturulması amaçlanmıştır.

GEREÇ VE YÖNTEM : Nullipar ya da ilk gebeliğinde preeklampsi hikayesi olan preeklamptik ya da eklamptik 43 gebe çalışmaya alındı. Kesitsel bir gözlem araştırması yapıldı.

BULGULARÇalışmaya dâhil edilen tüm olguların ayrıntılı obstetrik muayeneleri yapıldı. Olguların demografik özellikleri, laboratuar bulguları, gebelik haftaları, önceki gebelik öyküleri kaydedildi. Obstetrik ultrasonografileri yapıldı. Çalışmaya dâhil edilen 43 hastadan 24'ünde (%55,8) ağır preeklampsi mevcuttu. Geri kalan 19 hastada (%44,2) preeklampsi şiddetli değildi. Hastalık şiddetiyle ADE gen polimorfizmi arasında ilişki izlenmedi. DD genotipi daha yüksek sistolik kan basıncı ile ilişkili bulundu (p=0,04).

SONUÇ: Çalışmamızda preeklampsi şiddetinin tahmin edilmesinde ADE gen polimorfizminin faydası olmadığı tespit edilmiştir. DD genotipi preeklamptik gebelerde sistolik kan basıncı yüksekliği için bir risk faktörü olarak tespit edilmiştir. Bu konuda randomize, prospektif, geniş popülâsyonlu çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Anjiyotensin dönüştürücü enzim, Polimorfizm, Preeklampsi

Referances

1. Cnossen JS, AM van der Post J, Mol BWJ, Khan KS, Meads CA, ter Riet G. Prediction of pre-eclampsia: a proltocol for systematic reviews of test accuracy. BMC Pregnancy and Childbirth 2006;6:29
2. Bouba I, Makrydimas G, Kalaitzidis R, Lolis DE, Siamopoulos KC, Georgiou I. Interaction between the polymorphisms of the renin-angiotensin system in preeclampsia. European Journal of Obstetrics and Gynecology and Reproductive Biology 2003;110:8-11
3. Mütze S, Schöneborn SR, Zerres K and Rath W. Genes and the preeclampsia syndrome. J. Perinat. Med 2008;36: 38-58
4. Galao AO, de Souza LH, da Costa BEP, Scheibe RM, de Figueiredo CEP. Angiotensin-converting enzyme gene polymorphism in preeclampsia and normal pregnancy. American Journal of Obstetrics and Gynecology 2004; 191:821-4
5. Serrano NC, Diaz LA, Paez CM, Cifuentes R, Monterrosa A, Gonzales A, Smeeth L, Hingorani AD, Casas JP. Angiotensin-Converting Enzyme I/D Polymorphism and Preeclampsia Risk: Evidence of Small-Study Bias. PLOS MEDICINE 2006;3;12
6. Li H, Ma y, Fu Q, Wang L. Angiotensin-Converting Enzyme Insertion/Deletion (ACE I/D) and Angiotensin II Type 1 Receptor (AT1R) Gene Polymorphism and Its Association with Preeclampsia in Chinese Woman. Hypertension in Pregnancy
7. Gürdol F, İsbilen E, Yılmaz H, İsbir T, Dirican A. The association between preeclampsia and angiotensin-converting enzyme insertion/deletion polymorphism. Clinica Chimica Acta 2004;341:127-31.

8. Zhou N, Yu P, Chen J, Huang H, Jiang S. Detection of insertion/ deletion polymorphism of angiotensin converting enzyme gene in preeclampsia. Zhounghua Yi Xue Yi Chuan Xue Za Zhi 1999;16:29-31.
9. Mello G, Parretti E, Gensini F,Sticchi E, Mecacci F, Scarselli G, et al. Maternal-fetal flow, negative events, and preeclampsia: role of ACE I/D polymorphism. Hypertension 2003;41:932-7
10. Zaman MA, Oparil S, Calhoun DA Drugs targetting the renin-angiotensin-aldosterone system. Nat Rev Drug Discov 2002;1:621-36
11. Shah DM The role of RAS in the pathogenesis of preeclampsia. Curr Hypertens Rep 2006;8:144-152
12. Kim YJ, Pang MG, Park MY, Park MH, Kim YW, et al. A study on the association between angiotensinogen gene and angiotensin-converting enzyme gene and pregnancy-induced hypertension in Korean women. Korean J Obstet Gynecol 2001;44:1072-77
13. Zhou N, Yu Pen, Chen J, Huang H, Jiang S. Detection of insertion/deletion polymorphism of angiotensin converting enzyme gene in preeclampsia. Zhonghua Yixue Zazhi 1996;16:29-31
14. Tamura T,Johanning GL, Goldenberg R, Johnston KE, DuBard MB. Effect of angiotensin-converting enzyme gene polymorphism on pregnancy outcome. Obstet Gynecol 1996;88:497-502