Intercellular Adhesion Molecule-1 Gene Polymorphism (K469E) in Korean Preeclamptic Women

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Purpose: Preecalmpsia is a pregnancy-specific disorder that reflects widespread endothelial dysfunction resulting from increases of adhesion molecule expression. Intercellular adhesion molecule-1 (ICAM-1) is involved in the pathogenetic mechanisms responsible for preeclampsia, and ICAM-1 plasma levels and/or function is genetically influenced. Therefore, we evaluated the distribution of ICAM-1 gene K469E polymorphism in pregnant Korean women with preeclampsia and evaluated the association between this polymorphism and preeclampsia.

Methods: The K469E polymorphism was analyzed in peripheral blood samples from 197 preeclamptic pregnancies and 193 normotensive pregnancies by a SNapShot kit and an ABI Prism 3100 Genetic analyzer.

Results: Genotypic and allelic frequencies of ICAM-1 gene polymorphism (K469E) did not differ between preeclamptic and normotensive pregnancies. The distributions of the KK, KE, and EE genotypes were 40.6%, 43.7%, and 15.7%, respectively, in preeclamptic pregnancies and 38.9%, 45.1%, and 16.1%, respectively, in normotensive pregnancies. The frequencies of K and E alleles were 0.62 and 0.38, respectively, in preeclamptic pregnancies and 0.61 and 0.39, respectively, in normotensive pregnancies. By multiple logistic regression analysis, there was no increased risk of preeclampsia in subjects with ICAM-1 KE (OR, 1.08; P=0.74) or EE (OR, 1.07; P=0.88) genotypes.

Conclusion: This study suggests that the ICAM-1 gene K469E polymorphism does not associate with an increased risk of preeclampsia in pregnant Korean women.

Key Words: Intercellular adhesion molecule-1, Single-nucleotide polymorphism, Preeclampsia

Introduction

Preeclampsia (PE) is a leading cause of maternal and perinatal mortality and morbidity and is characterized by hypertension, proteinuria, and increased systemic inflammatory response[1]. Furthermore, PE is associated with an increased maternal risk of cardiovascular disease later in

life[2, 3]. Endothelial dysfunction is the most common clinical manifestation of PE, including enhanced endothelial-cell permeability and platelet aggregation[4]. Anexcessive maternal systemic inflammatory response may be responsible for the endothelial dysfunction[5]. However, the specific factors initiating endothelial dysfunction in PE are unknown.

Recently, several studies have reported that activation of peripheral blood leukocytes is higher in women with PE than in those with a normal pregnancy, and inflammatory cells were activated in women with PE. Moreover, this inflammatory cell activation was associated with higher levels of proinflammatory molecules, cytokines, and adhesion molecules. We have confirmed that maternal serum levels of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin were significantly higher in cases of severe PE than in normal pregnancies[6]. These data suggest that inflammation-mediated increases in leukocyte-endothelium adhesion may contribute to endothelial dysfunction seen in PE.

ICAM-1 is a cell-surface glycoprotein expressed on vascular endothelium, antigen presenting cells, and activated lymphocytes[7]. It plays a key role in the transendothelial migration of neutrophils to inflammatory sites. The ICAM-1 gene is a single copy gene located on chromosome 19q13.3 and has two polymorphismssuch as Gly241Arg (G241R) and Lys469Glu (K469E)[8]. ICAM-1 polymorphisms are implicated in a number of degenerative and inflammatory diseases. And the association of these polymorphisms with diseases such as celiac disease[8, 9], type 1 diabetes[10], and multiple sclerosis[11] is well studied. However, studies assessing the impact of ICAM-1 polymorphism on the risk of PE are still sparse, though only one study did report that the ICAM-1 gene K469E polymorphism did not associate with PE[12].

The aim of this study was to evaluate the allelic and genotypic frequencies of ICAM-1 gene K469E polymorphism in preeclamptic and nomortensive Korean pregnancies and to investigate whether there is an association between this polymorphism and PE in Korea.

Materials and Methods

1. Subjects

We assayed ICAM-1 gene K469E polymorphism in 390 pregnant women during the third trimester between 2003 and 2004 at Kwan Dong University Cheil General Hospital and Ewha Womans University MokDong Hospital in Seoul, Korea. All participants were of Korean origin. Women who developed gestational hypertension without proteinuria and women with an abnormal fetal karyotype, chromosomal abnormalities, chronic hypertension, diabetes, or renal disease were excluded from this study. Written informed consent was obtained from all enrolled subjects, and the Ethics Committee of Cheil General Hospital and Ewha Womans University MokDong Hospital approved this study.

The women were divided in two groups: 197 preeclamptic women and 193 normotensive controls who delivered a healthy neonate at term (>37 weeks of gestation) without significant medical or obstetric complications. Preeclampsia was defined as the new onset of hypertension (systolic blood pressure (SBP) $\geq$140 mmHg and/or diastolic blood pressure (DBP) $\geq$90 mmHg) and proteinuria ($\geq$300 mg in a 24hr urine collection and/or $\geq$ 1+ on dipstick testing) after 20 weeks of gestation, according to the Committee Terminology of the American College of Obstetricians and Gynecologists. Severe preeclampsia was characterized as either HELLP (hemolysis, elevated liver-enzyme levels, and a low platelet count) syndrome or preeclampsia with severe hypertension (DBP $\geq$110 mmHg) and/or severe proteinuria (urinary protein excretion $\geq$5 g per 24 hours and/or $\geq$3+ on dipstick testing).

2. DNA Extraction and Genotyping

Blood samples were drawn from all participants into tubes containing EDTA (Becton Dickinson, USA). Immediately after sampling, genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden Germany) following the manufacturer's protocol and was stored at −80℃ until analysis.

Genotypes were analyzed by the single base primer extension assay using the SNapShot kit (Applied Biosystems, Foster City, CA, USA) as previously reported[13]. A polymerase chain reaction (PCR) was used to amplify the region of genomic DNA containing the ICAM-1 gene K469E polymorphism. The forward primer was 5'-GCA CTTTCCCACTGCCCAT-3', and the reverse primer was 5'-GGATACAACAGGCGGTGAGG-3'. The PCR reaction contained 30 ng genomic DNA, 1.25 pM of each primer, 250 mM dNTPs, 3 mM MgCl$_2$, 1X Taq polymerase buffer, and 0.15 units of Taq polymerase in 10 µL. Amplification was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) under touch- down conditions.

After amplification, the PCR products were treated with 1 U of shrimp alkaline phosphatase (SAP) (Rhoche/Roche Diagnostic Systems, Norwalk, CT, USA) at 37℃ for 1 hr and then with 1 U of exonuclease I (USB Corporation) at 72℃ for 15 min. Primer extension reactions were performed with the SNapShot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. The extension primer was 5'-CTCAAGG GGAGGTCACCCGC-3'. One ul of the purified amplicons was added to a SNapShot Multiplex Ready reaction mixture containing 0.15 pM of internal genotyping primer. The primer extension reaction was 25 cycles of 96℃ for 10 s, 50℃ for 5 s, and 60℃ for 30 s. One ul of the final reaction samples, plus extension products and GeneScan 120 Liz size-standard solution were added to 9 µL of Hi-Di for- mamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95℃ for 5 min, followed by 5 min on ice. Samples were then electrophoresed on an ABI Prism 3100 Genetic Analyzer. The results were analyzed using the ABI Prism GeneScan and Genotyper (Applied Biosystems, Foster City, CA, USA).

3. Statistical analysis

Data are presented as the mean±SD (%). The clinical characteristics of PE patients and controls were compared using the Student's t-test and Chi-square test. Allelic and genotypic frequencies of the ICAM-1 gene K469E polymorphism between the two groups were compared with the Chi-square or Fisher's exact test. Hardy-Weinberg equilibrium was tested in patients or controls by means of the Chi-Squared analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by the ICAM-1 polymorphism. $P<0.05$ was considered statistically significant. The statistical analysis was performed with the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, USA).

Results

Table 1 presents the clinical characteristics of PE and control cases. The maternal age did not differ between PE and control cases ($P>0.05$). However, the maximum systolic and diastolic blood pressures and the body mass index at delivery were significantly higher in PE cases than in

Table 1. Clinical Characteristics of Controls and Preeclamptic Pregnancies

Variable	Con (N= 193)	PE (N= 197)	P value
Maternal Age (years)	31.3±3.9	31.1±4.4	0.55
Maximum SBP (mmHg)	122.4±10.4	159.7±16.7	<0.001
Maximum DBP (mmHg)	76.0±8.3	100.8±12.0	<0.001
24hr urine protein (mg)	0	5435.8±4794.4	–
Body Mass Index at Delivery (kg/m^2)	26.2±3.3	28.2±4.1	<0.001
Birth Weight of the Offspring (g)	3192.6±505.8	2412.2±925.6	<0.001
Gestational Age at Delivery (weeks)	39.1±1.5	35.7±3.9	<0.001

Data were expressed as the mean±standard deviation.
P value was calculated using the Student's t-test.
Significant value was taken at the level of $P<0.05$.
Abbreviations: Con, control; PE, preeclampsia; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; N, Total number

control cases ($P<0.001$). Proteinuria was detected only in cases of PE. At delivery, gestational age and birth weight were significantly lower in PE cases than in control cases ($P<0.001$).

The genotypic and allelic frequencies of K469E are presented in Table 2. K469E genotypes of PE and control cases were in Hardy-Weinberg equilibrium (control: $\chi^2=$ 0.9468 and $P=0.6228$, PE: $\chi^2=0.4637$ and $P=0.7930$). The frequencies of the K469E genotypes and alleles did not differ between the two groups ($P>0.05$ in both). The proportion of the KK, KE, and EE genotypes was 40.6%, 43.7 %, and 15.7%, respectively in PE patients. The proportion of KK, KE, and EE genotypes in controls was 38.9%, 45.0%, and 16.1%, respectively. The frequencies of the K and E alleles were 0.62 and 0.38, respectively, in PE cases and were 0.61 and 0.39, respectively, in controls. The association between this polymorphism and preeclampsia was estimated by multiple logistic regression analysis adjusting for confounding factors such as maternal age, delivery week, and birth weight. Heterozygous genotypes (KE) and/or variant genotypes (EE) of the K469E genotypes did not associate with PE ($P>0.05$, Table 2). Neither did the E allele, a rare K469E allele, associate with increased risk of PE ($P>0.05$, Table 2).

Table 2. Genotype and Allele frequencies of the ICAM-1 Gene Polymorphism (K469E) in Controls and Preeclamptic Pregnancies

	N (%)		Univariate Analysis		
	Con (n= 193)	PE (n= 197)	Odds Ratio	Confidence Interval	P value
Genotype					
KK	75 (38.9)	80 (40.6)	1.00		–
KE	87 (45.1)	86 (43.7)	1.08	0.69–1.67	0.74
EE	31 (16.1)	31 (15.7)	1.07	0.59–1.92	0.88
KE+ EE	118 (61.2)	117 (59.4)	1.08	0.72–1.61	0.76
Allele					
K	237 (0.61)	246 (0.62)	1.00		–
E	149 (0.39)	148 (0.38)	1.05	0.78–1.39	0.77

Data were expressed as number (%).
P value was calculated using the Chi-Squared test.
Significant value was taken at the level of $P<0.05$.
Abbreviations: K469E, lysine (K) substitution to glutamic acid (E) in codon 469 of ICAM-1 gene; Con, control; PE, preeclampsia; N, Number.

Discussion

ICAM-1 is a member of the immunoglobulin superfamily and is the principal ligand for leukocyte function-associated antigen-1 (LFA-1), a member of the integrin superfamily. One function of the ICAM-1/LFA-1 adhesion system is to assist leukocyte movement into tissues LFA-1-positive leukocytes adhere to ICAM-1-positive endothelia[14, 15] and pass though the basement membrane and into tissues[16, 17]. This process initiates an inflammatory response. Normal pregnancy is associated with activation of peripheral blood leukocytes, a response that is higher in women with PE[18]. Inflammatory cells are activated in PE and localize to sites of vascular injury[19]. This inflammatory response contributes to the wider syndrome of endothelial dysfunction as well as thrombotic and metabolic disturbances seen in PE. Therefore, inflammation-mediated changes in ICAM-1 expression/function have been evaluated in many PE studies. We previously reported that soluble ICAM-1 (sICAM-1) levels are significantly higher in PE patients than in normotensive pregnancies[6]. Also, higher levels of circulating ICAM-1 have been reported in PE cases[20-25]. Moreover, in animal models, ICAM-1/LFA-1 cross talk is a proximate mediator capable of disrupting successful pregnancy maintenance[26]. Therefore, ICAM-1 is implicated in the pathogenesis of PE.

The ICAM-1 gene K469E polymorphism results in an amino acid change from glutamic acid to lysine in the fifth immunoglobulin-like domain of ICAM-1. This domain is of crucial importance ICAM-1 activity because it modulates the interactions between ICAM-1 and LFA-1 and influences the adhesion of B-cells[27]. Some data suggest that this domain and/or the transmembrane domain are important for dimerization, which directly correlates with the enhancement of binding to its ligand[28]. Therefore, the ICAM-1 gene K469E polymorphism may topologically influence ICAM-1, resulting in changes of the plasma levels and/or functional activity of ICAM-1.

This study is the first to analyze the distribution and interaction of ICAM-1 gene K469E polymorphism in Korean

patients with preeclampsia. This study indicates that this polymorphism does not associate with PE in Korean populations. These results are consistent to those of Freeman et al, who examined a different race[12]. Therefore, we suggest that the ICAM-1 gene K469E polymorphism does not associate with a susceptibility of developing preeclampsia in pregnant Korean women.

Acknowledgments

: 자간전증은 임신 특이적 질환으로, 면역 반응 관련 결합 요소의 증가에 의한 광범위한 혈관내피 기능손상을 나타낸다. 자간전증을 이끄는 생태병리학적 기전에 관여하는 세포내부 결합요소-1 (intracellular adhesion molecule-1; ICAM-1)은 면역반응의 시작을 유도하고, 그것의 혈장 정도와 기능은 유전적으로 영향을 받는다. 그러므로 우리는 이번 연구에서 한국인 자간전증 임산부에서 ICAM-1의 유전자 다형성 부위인 K469E의 분포를 평가하고 자간전증과 이 유전자 다형성 사이의 연관성을 조사하였다.

: 이 유전자 다형성은 SNapShot kit와 ABI Prism 3100 Genetic analyzer를 사용하여 197명의 자간전증 임산부와 193명의 정상 임산부의 말초 혈액에서 분석하였다.

: ICAM-1 유전자 다형성인 K469E의 유전자형과 대립유전자 빈도는 자간전증 임산부와 정상 임산부 사이에 차이가 없었다. 유전자형인 KK, KE, EE는 자간전증 임산부에서 각각 40.6%, 43.7%, 15.7%였고, 정상 임산부에서 38.9%, 45.1%, 16.1%였다. 그리고 K와 E 대립유전자 빈도는 자간전증 임산부에서 각각 0.62, 0.38이었고 정상 임산부에서 0.61, 0.39였다. 다중회귀분석결과에서 자간전증 발생 위험도는 K469E의 이종접합 유전자형 (KE)이나 돌연변이 유전자형 (EE)을 수반하는 그룹에서 증가되지 않았다.

: 이 연구는 ICAM-1 유전자 다형성인 K469E가 한국인 임신부의 자간전증 발생과 연관이 없음을 나타낸다.

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