Tumor Necrosis Factor-alpha Gene Polymorphism (C-850T) in Korean Patients with Preeclampsia

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Purpose: Preeclampsia is a multisystem human pregnancy-specific disorder. The pathophysiology of preeclampsia is linked with over-stimulation of inflammatory cytokines by placental ischemia via reduced uterine perfusion pressure during pregnancy. Although an increase in tumor necrosis factor (TNF)-alpha has been reported in preeclamptic women, there is little evidence of a relationship between *TNF-alpha* gene variations and preeclampsia. In this study, we identified a single-nucleotide polymorphism (SNP), C-850T, in the *TNF-alpha* gene promoter region in Korean preeclamptic women and investigated the association between this SNP and the development of preeclampsia.

Materials and Methods: This polymorphism was analyzed in peripheral blood samples from 198 preeclamptic pregnancies and 194 normotensive pregnancies using a SNapShot kit and an ABI Prism 3100 Genetic analyzer.

Results: Genotypes and allele frequencies for C-850T did not differ between preeclamptic and normotensive pregnancies. The distributions of genotypes (CC, CT and TT) were 74.3%, 22.2% and 3.5%, respectively, in preeclamptic pregnancies, and 71.6%, 25.8% and 2.6%, respectively, in normotensive pregnancies. The frequencies of the C and T alleles were 0.85 and 0.15 in preeclamptic pregnancies and 0.84 and 0.16 in normotensive pregnancies, respectively. There was no increased risk of preeclampsia in subjects with the CT (OR, 0.83; P=0.44) or TT genotypes (OR, 1.32; P=0.64).

Conclusion: We found no differences in the genotypes or allele frequencies of the *TNF-alpha* gene polymorphism between preeclamptic and normotensive pregnancies. This study suggests that the *TNF-alpha* gene polymorphism may be not associated with the development of preeclampsia in pregnant Korean women.

Key Words: Tumor necrosis factor-alpha, Polymorphism, Preeclampsia

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Introduction

Preeclampsia is a major obstetric problem leading to substantial maternal and perinatal morbidity and mortality. It is characterized by the onset of hypertension and proteinuria after 20 weeks of gestation and is closely

related to the endothelial dysfunction of an excessive maternal systemic inflammatory response[1].

The cytokine tumor necrosis factor alpha (TNF-alpha) is a well known member of the TNF superfamily, which consists of at least 18 ligands and 29 different receptors involved in numerous cellular processes. TNF-alpha signals regulate inflammation, survival, apoptosis, cell migration, proliferation and differentiation. Physiological levels of the cytokine are important for balancing cell fusion, for the apoptotic shedding of villous trophoblasts and for limiting trophoblast invasion into the maternal decidua. Previous studies have reported that an aberrant increase in TNF-alpha levels is closely associated with the development of preeclampsia[2-4]. However, there is little evidence of a relationship between *TNF-alpha* gene variation and preeclampsia.

The single-nucleotide polymorphism (SNP), C-850T, in the *TNF-alpha* gene promoter region has been studied in various disorders. Recently, a significant difference in the genotype distribution of the C-850T polymorphism was observed with hypertensive disorder-complicating pregnancy, suggesting that the T allele at position -850 may be a protective factor against the development of hypertensive disorder-complicating pregnancy[5]. Pazarbaşi et al. and Heiskanen et al. also reported that the frequency of the TT genotype of the C-850T polymorphism was significantly reduced in preeclamptics when compared with the control group6, [7]. In contrast, Canto-Cetina et al. reported no association between this polymorphism and the risk for preeclampsia[8]. Therefore, the nature of the relationship between *TNF-alpha* gene variation and preeclampsia is still controversial.

In this study, we evaluated the allele and genotype frequencies of the *TNF-alpha* gene polymorphism, C-850T, in preeclamptic and normotensive pregnancies to investigate whether this polymorphism is associated with susceptibility to preeclampsia in pregnant Korean women.

Materials and Methods

1. Subjects

We studied 392 pregnant women in their third trimester between 2003 and 2004 at Kwan Dong University Cheil General Hospital and Ewha Womans University MokDong Hospital in Seoul, Korea. All participants included in this study were of Korean origin. Pregnant women that developed gestational hypertension without proteinuria and pregnant 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 Committees of Cheil General Hospital and Ewha Womans University MokDong Hospital approved this study.

There were 198 preeclamptic women and 194 normotensive controls who delivered a healthy neonate at term (>37 weeks of gestation). Preeclampsia was defined as the onset of hypertension (systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg) and proteinuria (≥300 mg in a 24-hour urine collection and/or ≥1+ on dipstick testing) after 20 weeks of gestation[9-11].

2. DNA Extraction and Genotyping

Genomic DNA was extracted with DNA extraction kits (Qiagen, Valencia, CA) from the peripheral blood of all subjects and stored at -80°C until required for analysis.

The genotyping was analyzed using a single base primer extension assay using the SNapShot kit (Applied Biosystems, Foster City, CA, USA) according to our previous method[11, 12]. A polymerase chain reaction (PCR) was used to amplify the genomic DNA region containing the *TNF-alpha* gene polymorphism (-850 C>T). The primers for PCR were as follows: forward: 5'-ATGTAGCGGCTCTGAGGAATG-3' and reverse: 5'-TGACCCGGAGACTCATAATGC-3'. The PCR re-

action solution contained 30 ng genomic DNA, 1.25 pM primer pair, 250 mM dNTPs, 3 mM $MgCl_2$, 1X buffer, and 0.15 units of Taq polymerase per 10 μL of total reaction volume. Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) under touch-down conditions.

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'-GGAAGTCGAGTATGGGGACCCCCCCTTAA-3'. The primer extension reaction was performed for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. One microliter of the final reaction samples, plus extension products and GeneScan 120 Liz size-standard solution, were added to 9 μL of Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and electrophoresed on an ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program of 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 the preeclamptics and controls were compared with Student's *t*-test. The comparison of allelic and genotypic frequencies of C-850T between the two groups was performed with the Chi-square or Fisher's exact test. Hardy-Weinberg equilibrium was separately tested in patients and controls using Chi-Square analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by genotypes of C-850T. In all tests, $P<0.05$ was considered to be statistically significant. The statistical analysis was performed with SPSS 12.0 statistical software (SPSS Inc, Chicago, USA).

Results

The clinical characteristics of the study are shown in Table 1. There was no significant difference in maternal age between the preeclamptics and the controls. Gestational age at delivery and birth weight in preeclamptic patients were significantly lower than those in the controls ($P<0.001$). Proteinuria was detected only in the preeclamptic patients. The maximum systolic and diastolic blood pressures were significantly higher in preeclamptics than in the controls ($P<0.001$).

The allelic and genotypic frequencies of the C-850T polymorphism are presented in Table 2. The genotypic frequencies of C-850T in preeclamptics and controls did not indicate a departure from the Hardy-Weinberg equilibrium (controls: $\chi^2=0.2939$ and $P=0.5877$, preeclamptics: $\chi^2=0.9805$ and $P=0.3220$). Allelic and genotypic frequencies of C-850T did not differ between the two groups ($P>0.05$ in both). The proportions of each genotype (CC, CT, and TT) were 74.2%, 22.2% and 3.5% in the preeclamptic patients and were 71.6%, 25.8% and 2.6% in the controls, respectively. The frequencies of the C and T alleles were 0.86 and 0.14 in

Table 1. Clinical Characteristics of Control and Preeclamptic Pregnancies

Variable	Control (n=194)	Preeclampsia (n=198)	*P* value
Maternal Age (years)	31.2±4.0	31.2±4.4	0.806
Maximum SBP (mmHg)	112.5±9.2	160.2±16.7	<0.001
Maximum DBP (mmHg)	72.5±6.8	100.8±11.6	<0.001
24hr urine protein (mg)	0	5695.5±4314.4	-
Birth Weight of the Offspring (g)	3201.4±502.4	2415.5±924.2	<0.001
Gestational Age at Delivery (weeks)	39.2±1.6	35.5±3.6	<0.001

Abbreviations : SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.
Data were expressed as the mean±SD. A *P* value was calculated using the Student's *t*-test. Statistical significance was set at the level of $P<0.05$.

Table 2. Genotype and Allele Frequencies of the *TNF-alpha* Gene Polymorphism (C-850 T) in Control and Preeclamptic Pregnancies

	No. (%)		Univariate Analysis		
	Control (n=194)	Preeclampsia (n=198)	Odds Ratio	95% Confidence Interval	*P* value
Genotype					
CC	139 (71.6)	147 (74.2)	1.00		-
CT	50 (25.8)	44 (22.2)	0.83	0.52-1.33	0.432
TT	5 (2.6)	7 (3.6)	1.32	0.41-4.27	0.641
CT+TT	55 (28.4)	51 (25.8)	0.88	0.56-1.37	0.558
Allele					
C	328 (84.5)	338 (85.4)	1.00		-
T	60 (15.5)	58 (14.6)	0.94	0.63-1.39	0.749

Data are expressed as a number (%). The *P* values were calculated using the Chi-Square test. Statistical significance was set at the level of $P<0.05$.

preeclamptics and 0.85 and 0.15 in the controls, respectively. When the association between this polymorphism and preeclampsia was estimated, the heterozygote and/or variant genotype of C-850T was not associated with an increased risk of preeclampsia ($P>0.05$, Table 2). The rare allele (T allele) of the C-850T was also not associated with an increased risk of preeclampsia ($P>0.05$, Table 2).

Discussion

Normal pregnancy is associated with activation of peripheral blood leukocytes, a response more marked in women with preeclampsia. Inflammatory cells are activated in preeclampsia and are localized to the site of vascular injury. This inflammatory response contributes to the endothelial dysfunction and thrombotic and metabolic disturbances seen in preeclampsia[13, 14]. TNF-alpha is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The primary role of TNF-alpha is in the regulation of immune cells, though it is also involved in various pathophysiologies such as induction of apoptotic cell death, inflammation stimulation, and inhibition of tumorigenesis. Therefore, TNF-alpha related to the inflammatory response is often analyzed in studies regarding the development of preeclampsia.

Previous studies have reported an association between an excessive increase in TNF-alpha levels and the development of preeclampsia[2-4]. Moreover, Brewster et al. suggested that this increase in inflammatory responsiveness with advancing gestation may provide an explanation for the incidence of late onset preeclampsia in the absence of placental pathology, as well as serve as a potential physiological priming mechanism geared toward increasing maternal sensitivity to the fetal triggers of labor[15]. However, the relationship between *TNF-alpha* gene variation and preeclampsia remains unclear.

Recently, a significant difference in the distribution of C-850T polymorphism genotypes was observed with hypertensive disorder-complicating pregnancy, suggesting that the T allele of -850 may be a protective factor against its development[3]. Pazarbaşi et al. and Heiskanen et al. also reported that the frequency of the TT genotype of C-850T polymorphism is significantly reduced in preeclamptics when compared with that of the controls[6, 7]. In contrast, Canto-Cetina et al. reported no association between this polymorphism and the risk for preeclampsia[8]. Therefore, there is still much controversy surrounding the relationship between *TNF-alpha* gene variations and preeclampsia.

In this study, we found no differences in the genotype and allele frequencies of *TNF-alpha* gene polymorphism between preeclamptic and normotensive pregnancies. This result is similar to that of Canto-Cetina et

al., although their study examined a different race[8]. Therefore, we suggest that this *TNF-alpha* gene polymorphism may be not associated with the development of preeclampsia in pregnant Korean women. However, a larger study with ethnically diverse samples is necessary to confirm the results of this study.

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국문초록

목 적 : 자간전증은 인간의 임신 특이적 증후군으로 임신 기간 동안 감소된 자궁 관류 압에 의해 나타나는 태반 허열에 의해 시작된다. 자간전증은 염증성 싸이토카인의 비정상적인 발현과 연관되어 있는 것으로 알려져 있다. 싸이토카인 중 대표적인 종양 사멸 인자-알파(tumor necrosis factor-alpha; TNF-alpha)는 자간전증 여성에서 증가되는 것으로 보고되었다. 하지만 *TNF-alpha* 유전자 다형성과 자건전증 사이의 연관성에 관한 연구는 미비한 실정이다. 따라서 이번 연구에서는 *TNF-alpha* 유전자 프로모터 지역의 C-850T의 단일염기다형성을 한국인 자간전증 여성에서 확인하고 자간전증의 발달과의 연관성을 연구하고자 한다.

대상 및 방법 : 이 유전자 다형성은 SNapShot kit와 ABI Prism 3100 Genetic analyzer를 사용하여 198명의 자간전증 임산부와 194명의 정상 임산부의 말초 혈액에서 분석하였다.

결 과 : C-850T 유전자형과 대립유전자 빈도는 자간전증 임산부와 정상 임산부 사이에 차이가 없었다. 유전자형인 CC, CT, TT는 자간전증 임산부에서 각각 74.3%, 22.2%, 3.5%였고, 정상 임산부에서 71.6%, 25.8%, 2.6%였다. 그리고 C와 T 대립유전자 빈도는 자간전증 임산부에서 각각 0.85, 0.15였고 정상 임산부에서 0.84, 0.16였다. 자간전증 발생 위험도는 C-850T의 이종접합 유전자형(CT)이나 돌연변이 유전자형(TT)을 수반하는 그룹에서 증가되지 않았다.

결 론 : 우리는 이번 연구에서 자간전증과 정상 임신부 사이에 C-850T의 유전자형과 대립유전자 빈도는 차이가 없음을 발견했다. 따라서 이번 연구는 *TNF-alpha* 유전자 다형성인 C-850T가 한국인 임신부의 자간전증 발생과 관련이 없을 가능성을 시사한다.

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