

Tumor Necrosis Factor- α and Interleukin-1- β Polymorphisms in Pre-Eclampsia

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ABSTRACT

Background: Pre-eclampsia is the most common critical condition during pregnancy. Plasma concentrations of tumor necrosis factor-alpha (TNF- α) and interleukin-1-beta (IL-1 β) increase in pregnant women with pre-eclampsia, compared to normal pregnant women. **Objective:** To investigate the polymorphisms of IL-1 β (C+3954T), TNF- α (G-308A), and (G-238A) in preeclamptic women northeastern Iran. **Methods:** This study was conducted on 153 preeclamptic women (case group) and 150 healthy pregnant women (control group), admitted to Ghaem and Imam Reza hospitals of Mashhad, Iran. IL-1 β (C+3954T), TNF- α (G-238A) and TNF- α (G-308A) gene polymorphisms in the promoter region were screened by polymerase chain reaction. Data were analyzed, using SPSS version 16.0. **Results:** The mean age of the participants in the case and control groups was 28.2 ± 6.1 and 27.1 ± 6.3 years, respectively ($P=0.68$). The frequency of G-308A polymorphism was significantly higher in the case group, compared to the control group ($p<0.001$). However, no significant relationship was found between IL-1 β genotype and pre-eclampsia ($p=0.39$). The frequency of TNF- α (G-238A) AA genotype was significantly higher in the case group, while GG genotype was less frequently detected in the case group, compared to the control group ($p<0.001$ for both genotypes). Moreover, the frequencies of AA genotypes of -238 TNF- α and G-308A polymorphisms were significantly higher in the case group, compared to the control group ($p<0.001$). **Conclusion:** The significant correlation between inflammation promoting genotypes of TNF- α and Pre-eclampsia is noteworthy and provides evidence on the contribution of immune related genes in this disease.

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Keywords: Gene Polymorphism, Interleukin-1 β , PCR-RFLP, Pre-eclampsia, Tumor necrosis Factor- α

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INTRODUCTION

Pre-eclampsia is the most common critical condition during pregnancy, occurring in 2-5% of all pregnancies, worldwide. Pre-eclampsia is characterized by an increase in blood pressure and proteinuria. This disorder, which is defined as elevated blood pressure after 20 weeks of gestation, can be life-threatening for both the mother and fetus (1-3).

To the best of our knowledge, monitored mild inflammations in the maternal immune system are common during pregnancy. These inflammations are characterized by increased plasma concentrations of tumor necrosis factor-alpha (TNF- α) and interleukin-1-beta (IL-1 β) (4). Based on previous research, plasma concentrations of TNF- α and IL-1 β (placental pro-inflammatory cytokines) are higher in preeclamptic women, compared to normal pregnant women (5-8).

Although a number of immune-related processes are involved in pre-eclampsia, the etiology of this disorder is not fully understood. Several etiologies have been proposed for the development of pre-eclampsia. Accordingly maladaptation and overt activation of non-specific immune system in mothers may result in the occurrence of pre-eclampsia (4, 9). In fact, during pregnancy, immune cells generate several cytokines, which are frequently found in patients with pre-eclampsia; these cytokines are likely to be the cause of this disorder (10).

Lymphocytes, macrophages, and trophoblasts are responsible for the production of TNF- α , which is a T-helper-1-cell cytokine. Some functions of TNF- α such as placental invasion, damage to endothelial cells, and oxidative stress may lead to pre-eclampsia (11). In fact, researchers have noticed higher serum concentrations of TNF- α and soluble TNF receptors (TNFR) in patients with pre-eclampsia. Moreover, in preeclamptic women, expression of mRNA protein of TNF/TNFR is elevated in white blood cells and placenta (12).

Considering the inheritable nature of pre-eclampsia, several studies have been designed to determine the relationship between pre-eclampsia and different single nucleotide polymorphisms (SNPs) in the promoter sequence of TNF- α gene. The extremely polymorphic Major Histocompatibility Complex (MHC) is located on chromosome 6p21.3, which contains the TNF- α gene. Notably, the promoter region of TNF- α gene includes several SNPs.

Overall, -308 G/A SNP is the most frequently investigated polymorphism in previous studies; however, these studies have revealed inconsistent results. Many investigations on human cases have suggested a relationship between -308 G/A SNP, other TNF- α gene polymorphisms, and various inflammatory and metabolic states (13). On the contrary, based on several studies including a recent meta-analysis, polymorphism at position-308 was not associated with pre-eclampsia (14-16).

A pro-inflammatory cascade is triggered after the release of IL-1 β , and some cytokines such as TNF- α , interferon gamma (IFN- γ), interleukin 2 (IL-2), and interleukin-12 (IL-12) are produced in this cascade. In fact, IL-1 gene family is located on chromosome 2q13-14. Various diallelic polymorphisms have been identified on IL-1 β gene, given its extremely polymorphic nature (17).

Pro-inflammatory cytokines such as IL-1 β and TNF- α mediate the inflammatory response in pre-eclampsia. These cytokines draw and activate white blood cells in body tissues and trigger the release of other lymphocytotropic cytokines and catabolic enzymes, resulting in oxidative stress in pre-eclampsia (18). The aim of the present study was to investigate the relationship between pre-eclampsia and polymorphisms of

IL-1 β (C+3954T), TNF- α (G-308A), and TNF- α gene at position -238(G/A) in Khorasan Province, situated in northeast of Iran.

MATERIALS AND METHODS

This case-control study was conducted on 153 pregnant women with pre-eclampsia (case group) and 150 healthy pregnant women (control group), admitted to Ghaem and Imam Reza hospitals of Mashhad, Iran.

In this study, pre-eclampsia was defined as recently detected high blood pressure after 20 weeks of gestation, accompanied by proteinuria. Also, proteinuria was defined as the presence of protein (≥ 30) mg in the urine per day. Subjects with systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg were categorized as hypertensive. It should be mentioned that the subjects in the control group had no hypertension or proteinuria during pregnancy and gave birth to healthy neonates (with appropriate weight and size).

Subjects' blood pressure was measured in a sitting position on two different occasions within a 15-min interval. For this purpose, a standardized mercury sphygmomanometer was employed, and a suitable cuff size was used for each subject. At first, 10cc blood samples were obtained from each subject and transferred to lab tubes, containing 5% ethylenediaminetetraacetic acid (EDTA). DNA was extracted from leukocytes via salting-out method, and the purity of the final product was assessed via spectrophotometry (10).

A 71 base pair sequence of TNF- α promoter was amplified by polymerase chain reaction (PCR-RFLP), using (Biometra T3 Thermocycler, Analytik, GmbH). A primer sequence of 20 nucleotides was designed, using Gene Runner software and was compared with GenBank and European Molecular and Biology Laboratory (EMBL) libraries. The polymorphisms of IL-1 β gene (C+3954T) in exon 5, TNF- α (G-238A) and TNF- α gene (G-308A) in the promoter region were screened via a PCR-RFLP method. The following primers were used in the process:

TNF- α (-308) forward primer: 5' AGGCAATAGGTTTTGAGGGCCAT 3'

TNF- α (-308) reverse primer: 5' AACTCCCCATCCTCCCGGCT 3'

TNF- α (-238) forward primer: 5'GGT CCT ACA CAC AAA TCA GT 3'

TNF- α (-238) reverse primer: 5'CAC TCC CCA TCC TCC CTG GTC 3'

IL-1 β (+3954) forward primer: 5'GTTGTCATCAGACTTTGACC 3'

IL-1 β (+3954) reverse primer: 5'TTCAGTTCATATGGACCAGA 3'

The aliquots of PCR products were analyzed on 1.5% agarose gel, stained with ethidium bromide before digestion to verify the proper amplification of Gene fragments. The PCR products of TNF- α gene were digested with TaqI and Ava II enzyme aliquots and analyzed on 1.5% agarose gel, stained with ethidium bromide before the proper amplification of fragments. The PCR products of IL-1 β , NcoI endonuclease were subsequently analyzed.

After the control and case groups were matched in terms of age, all participants were informed about the study both verbally and in writing. Subjects who were unwilling to continue the study at any stage were excluded from the study. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences. Written informed consents were obtained from the subjects. The study conformed to the principles outlined in the Declaration of Helsinki.

Statistical Analysis. The sample size was calculated by using frequencies reported in previous studies, with a power of 80% and a minimum detectable odds ratio of 2.5. All statistical analyses were performed, using SPSS for Windows™, version 16 (SPSS Inc., Chicago, IL, USA). First, the quantitative data were assessed, using Kolmogorov-Smirnov test for normality. Data were expressed as mean \pm SD for parameters with a normal distribution or median and interquartile range for the data, which were not normally distributed.

One-way ANOVA was used for comparing the frequencies between the three genotype groups. Moreover, the frequencies of alleles and genotypes were compared, using a 2 \times 2 contingency table and Fisher's exact test. Group comparisons were performed, using sample *t*-test or Mann-Whitney U test for data without a normal distribution. Two-sided P-value less than 0.05 was considered statistically significant.

RESULTS

The subjects' clinical characteristics are presented in Table 1. Four cases were excluded from the control group due to technical issues. The mean age of the case and control groups was 28.2 \pm 6.1 and 27.1 \pm 6.3 years, respectively. The two groups were not significantly different in terms of mean age ($p=0.68$). Familial hypertension was more prevalent in the case group, compared to the control group ($p<0.001$).

Table 1. Subjects' clinical characteristics.

Variables	Pre-eclampsia (n=153)	No pre-eclampsia (n=150)	P-value
Age (mean \pm SD)	28.2 \pm 6.1	27.1 \pm 6.3	0.68
Gestational age (mean \pm SD)	35 \pm 2	36 \pm 3	0.048
Parity (No., %)	Nulliparous	79 (51.6)	0.18
	Multiparous	69 (46)	
Positive familial history of hypertension (No., %)	74 (48.4)	81 (54)	<0.001
Systolic blood pressure (mean \pm SD)	70 (45.8)	32 (22.9)	<0.001
Diastolic blood pressure (mean \pm SD)	160.4 \pm 5.1	105.4 \pm 4.7	<0.001
Body mass index (mean \pm SD)	105.4 \pm 4.7	74.5 \pm 4.1	0.785

Based on the findings, the expression of -238 TNF- α (AA) genotype was higher in the case group, compared to the control group ($p<0.001$) (Table 2). AA genotype was more frequent in the case group, while the frequency of GG genotype was lower in the case group, compared to the control group ($p<0.001$ for both genotypes).

Table 2: Genotypic and allelic frequencies of TNF- α (-238), TNF- α (-308), and IL-1 β (+3954) in the case (n=153) and control (n=150) groups.

Cytokine gene	Genotype	Case group N(%) n=153	Control group N (%) n=150	P-value
-238 TNF- α	AA	27(17.6)	3(2)	<0.001
	GA	104(67.9)	40(26.6)	
	GG	22(14.3)	93(62)	
-308 TNF- α	GG	80(52.2)	126(84)	0.001
	GA	73(47.8)	24(16)	
	AA	0(0)	0(0)	
+3954IL-1 β	CC	80(52.2)	84(56)	0.39
	CT	57(37.2)	60(40)	
	TT	16(10.4)	6(4)	

The frequency of A alleles of -238 TNF- α and G-308A polymorphisms was significantly higher in the case group, compared to the control group ($p < 0.001$) (Table 2). We also observed a significant difference in diastolic blood pressure among the three genotypes ($p < 0.001$).

In addition, the frequency of G-308A polymorphism was significantly higher in the case group, compared to the control group ($p = 0.001$). No significant relationship was found between IL-1 β (C+3954T) genotypes and pre-eclampsia (Table 2).

DISCUSSION

Pre-eclampsia is a disorder, characterized by augmented inflammatory response in maternal blood circulation due to the hypersensitivity of the immune system. Hypertension and dyslipidemia are other characteristics of this disorder (19,20). Although several etiologies and immune-related pathways have been suggested in the progression of pre-eclampsia, the exact etiology of this condition remains unknown (9). Several pro-inflammatory cytokines, including TNF- α , IL-1 β , and acute phase protein such as C-reactive protein, are speculated to play a key role in both the onset and progression of pre-eclampsia. These cytokines by inducing immune cells in tissues and stimulating the expression of other cytokines and enzymes may lead to oxidative stress, abnormal metabolic conditions, and probably pre-eclampsia (18,21). The over-expression of these cytokines may be due to the hypoxxygenation of placenta in patients with pre-eclampsia (22).

A number of studies have suggested an increase in the serum levels of some molecules such as P-selectin, E-selectin, VCAM-1, and ICAM-1 in patients with pre-eclampsia due to chronic inflammation. In this regard, based on a study by Farzadnia *et al.*, the serum levels of VCAM-1 and ICAM-1, which are released due to TNF- α secretion by the adaptive immune system, increased in patients with severe pre-eclampsia (21). On the other hand, no elevation has been reported in a few studies (23-26).

Based on several *in vitro* and human studies, incitation of TNF- α production by cells from -308 G/G homozygous and G/A heterozygous individuals can induce dissimilar effects on cytokine production. Also, according to several *in vivo* studies, this SNP and other polymorphisms within the TNF- α gene are associated with different inflammatory and metabolic states (13). Based on previous studies, IL-1 β polymorphisms are associated with inflammatory diseases such as psoriatic arthritis and coronary artery diseases (27,28). Also, production of pro-inflammatory mediators, including TNF- α , IFN- γ , IL-2, and IL-12, is stimulated by the generation of IL-1 β (13).

In a meta-analysis by Molvarec *et al.*, no general relationship was detected between G-308A polymorphism of TNF- α and pre-eclampsia; however, this SNP was considered to play a significant role in severe pre-eclampsia (29). Moreover, Schmid *et al.* by evaluating 100 women with preterm births and 100 control subjects showed that IL1 β +3953C>T polymorphism is associated with the reduced risk of preterm birth in Caucasian women, which is possibly due to the modulation of inflammatory responses during pregnancy (30).

In a meta-analysis on TNF- α polymorphisms, variations in the location of TNF- α promoter region were suggested to affect the expression level. Moreover, evaluation of SNPs at positions -308 and -238 has revealed a correlation between A alleles of TNF- α at position -308 and the onset of pre-eclampsia in the majority of conducted studies (31,32). Also, the association between polymorphisms at position -238 and development of pre-eclampsia has been confirmed in Iranian women, although the possible relationship between the presence of A and G alleles and pre-eclampsia is still controversial (11,32).

In this study, there was a significant association between TNF- α (G-308A) and (G-238A) genotype and pre-eclampsia, whereas no significant relationship was found between IL-1 β (C+3954T) genotype and pre-eclampsia. Also, in this study, the frequency of TNF- α polymorphism at position -238 was significantly different between preeclamptic patients and healthy individuals. The results also suggested that AA genotype and A allele may increase the risk of pre-eclampsia.

Based on the findings, there might be a relationship between inflammatory states and pre-eclampsia. Also, the onset and development of pre-eclampsia maybe influenced by various inflammatory mediators including TNF- α and IL-1 β . Further research is required to determine whether TNF- α and IL-1 β polymorphisms, which increase the expression of these cytokines, play a mechanistic role in pre-eclampsia.

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