ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
September 2012; 11(3): 224-229.

Association of Single Nucleotide Polymorphisms in the Human Tumor Necrosis Factor-α and Interleukin 1-β Genes in Patients with Pre-eclampsia

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Received: 24 August 2011; Received in revised form: 9 October 2011; Accepted: 25 January 2012

ABSTRACT

Pre-eclampsia is a pregnancy-specific syndrome that may be dangerous especially to the fetus. Different cytokines have been found to be elevated in women with pre-eclampsia and may have possible roles in the development of this disorder. Alleles of the interleukin-l-beta (IL-lβ) and tumor necrosis factor alpha (TNF-α) genes are associated with pr-eeclampsia in several studies in different populations. The aim of the present study was to investigate the relationship between IL-lβ (C+3954T) and TNF-α (G-308A) gene polymorphisms with pre-eclampsia in north east of Iran (Khorasan province).

This study included 54 diagnosed patients with pre-eclampsia and 50 normal pregnant women as control group. DNA was extracted from peripheral blood and the polymorphisms were determined by PCR-RFLP method. Data was analyzed using chi-square and Fisher's exact tests.

There was significant association between TNF-α (G-308A) genotype and pre-eclampsia (p=0.001) but we did not find any significant association between IL-lβ (C+3954T) genotype and pre-eclampsia (p=0.39).

The present study might suggest a role for TNF-α in the development of pre-eclampsia; however, IL-lβ (C+3954T) polymorphism could not be considered as a marker of susceptibility to preeclampsia in our population.

Keywords: Gene Polymorphism; Interleukine-1β; PCR-RFLP; Pre-eclampsia; Tumor Necrosis Factor-α

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Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Pre-eclampsia is a known complication of pregnancy which reduces organ perfusion secondary to endothelial activation and vasospasm.[1,2]

Pre-eclampsia belongs to a group of hypertensive disorders in pregnancy that can be divided into gestational hypertension, chronic hypertension, pre-eclampsia, and pre-eclampsia superimposed on chronic hypertension. Hypertension is usually defined as systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg. Women with mild pre-eclampsia generally have no symptoms. However, women with severe pre-eclampsia (usually BP ≥ 160/110 mmHg and/or proteinuria ≥ 2–5 g/24 hours) may have signs and symptoms such as renal insufficiency, liver disease, neurological disturbances, and haematological disturbances.[3,4]

Pre-eclampsia is considered a syndrome due to hyperactivation of leukocytes in the maternal circulation.[1,2] Different cytokines during pregnancy are released by immune cells and lymphocytes at the interface of trophoblast and decidua. Several of these cytokines have been found to be elevated in women with pre-eclampsia and are of interest as possible markers for the development of this disorder.[5]

Plasma concentration of placental proinflammatory cytokines such as tumor necrosis factor–α (TNF-α) and interleukin (IL)-1β are higher in patients with pre-eclampsia than in normotensive pregnant women.[6-11]

The TNF-α gene is positioned within the highly polymorphic major histocompatibility complex (MHC) region on chromosome 6p21.3. It embodies many polymorphisms including microsatellites and single nucleotide polymorphisms (SNPs). There are many SNPs within the TNF-α gene promoter. The -308 G/A SNP has been the most studied polymorphism.

In vitro stimulation of TNF-α production by cells from −308 G/G homozygous individuals and G/A heterozygote individuals has produced contradictory results. Also many human studies have shown that this SNP and others within the TNF-α gene associate with different inflammatory and metabolic conditions.[12]

Secretion of IL-1β leads to a proinflammatory cascade, including production of TNF-α, interferon gamma (IFN-γ), IL-2, and IL-12.

The IL-1 gene family is located on the chromosome 2q13-14. The IL-1β gene is highly polymorphic, and several diallelic polymorphisms have been reported.[13]

In pre-eclampsia, pro-inflammatory cytokines IL-1β and TNF-α mediate inflammatory response by attracting and activating leukocytes in tissues, and stimulating the secretion of other lymphocytotropic cytokines and catabolic enzymes that may contribute to the oxidative stress associated with preeclampsia.[14]

In this study we investigated the distribution of the polymorphisms of IL-1β (C+3954T) in exon 5 and TNF-α (G-308A) in the promoter region of their genes in patients with pre-eclampsia and compared them with healthy controls.

MATERIALS AND METHODS

Pre-eclampsia is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks' gestation and can present as late as 4-6 weeks postpartum. It is clinically defined by hypertension, with or without pathologic edema.[15-17]

Fifty four patients with pre-eclampsia from Khorasan Province, north-east of Iran took part in this study. Fifty normal pregnant women also randomly were selected. All of women in control group were from the same region (Khorasan Province) without evidence of hypertension or proteinuria and gave birth to healthy neonates of appropriate size for gestational age. Patient and control groups were matched for gestational age.

Informed consents were obtained from all patients and controls who took part in this study.

Genomic DNA was extracted from peripheral white blood cells using a standard salting out method procedure[18]. The polymorphisms of IL-1β gene (C+3954T) in exon 5 and TNF-α gene (G-308A) in the promoter region were screened by polymerase chain reaction (PCR)-based methods. We used primer sequences, as below:

TNF-α:

Forward: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′;

Reverse: 5′-ACACTCCCCATCCTCCCGGCT-3′.

IL-1β:

Forward: 5′-GTTGTCATCAGACTTTGACC-3′;

Reverse: 5′- TTCAGTTCATATGGACCAGA-3′.

Amplification of fragments from the genomic region containing the SNP was performed in PCR assay. Each 25µl of PCR mixture contained 100ng of genomic DNA, 0.5U of Taq DNA polymerase (Qiagen), 1x PCR reaction buffer (Qiagen), 0.2 mM each dNTP (Sigma), 0.5µM each primer.

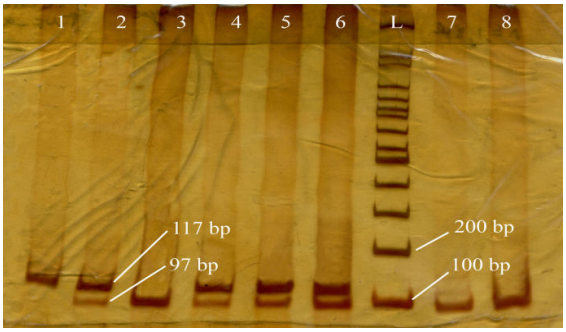

Figure 1. Analysis of the TNF-α -308 (G/A) Promoter polymorphism in patients with pre-eclampsia (1, 2, 3, 4, 5, 6, 7 and 8). Lane 1: homozygote for allele G (G/G); Lane 2, 4, 5 and 6: heterozygote (G/A); Lane 3, 7 and 8: homozygote for allele A (A/A); L: Ladder 50 bp.

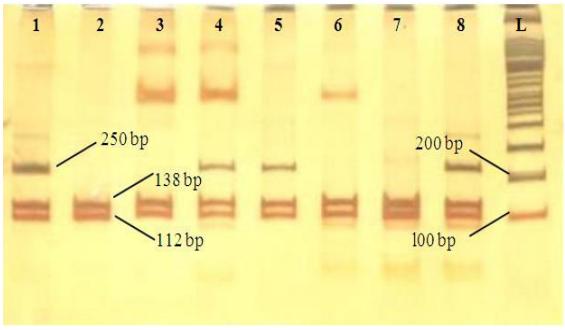

Figure 2. Analysis of the IL-1β+3954 (C/T) polymorphism in patients with pre-eclampsia (1, 2, 3, 4, 5, 6, 7, and 8). Lane 1, 4, 5 and 8: heterozygote C/T ; Lane 2, 3, 6 and 7: homozygote for allele C (C/C); L: Ladder 100 bp.

Reactions were carried out in a thermal cycler (CORBETT RESEARCH) consistent with the following scheme: 95°C for 2 min, 35 cycles at 95 °C for 1 min, 62 °C for 1 min, 72°C for 1 min and 72 °C for 5 min.

Aliquots of the PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide before digestion to verify the proper amplification of the fragments. The PCR products of the IL-1β and the TNF-α gene were digested with TaqI endonuclease and NcoI *e*ndonuclease subsequently [19]. TaqI digestion of the 250bp PCR product resulted in two fragments of 138 and 112 bp (allele C), or remained intact (allele T). The PCR product of TNF-α (117 bp) was mixed with 2 units of NcoI according to the manufacturer's instructions. Homozygote A showed two fragments of 97 and 20 bp. Homozygote G was undigested and resulted in a single band of 117 bp.[20] Heterozygote A/G was detected by the presence of all three fragments. Cleaved DNA fragments were subjected to electrophoresis on 17% polyacrylamide gel and stained with silver nitrate (Figure 1, 2).

Statistical analyses were performed using Fisher's exact and chi square tests. The results were considered to be significant when the *p*-value was less than 0.05. Deviation from Hardy-Weinberg equilibrium was controlled. This study was approved by the ethics committee of Mashhad University of Medical Science.

RESULTS

In this study, 54 patients with pre-eclampsia and 50 normal pregnant women were included. Average age of patients and controls was 24.9±3.2 and 25.2±2.4 years, respectively (p=0.676). Mean of systolic blood pressure in patient group was 160.4±5.1 mmHg and in normal controls was 114.7±5.5 mmHg (p<0.001). Mean of diastolic blood pressure in patients was 105.4±4.7 mmHg and in control group was 74.5±4.1 mmHg (p<0.001). Mean prepregnancy body mass index (BMI) in patients and control group was 24.4±2.9 and 24.2±2.6 Kg/m^2, respectively (p=0.758).The gestational age of pre-eclamptic group was 35±2 and control group was 36±3 weeks.

Table 1. TNF-α and IL-1β genotype in healthy controls and in patients with Pre-eclampsia

Gene	Genotype	Pre-eclampsia group N=54 (%)	Control group N=50 (%)	*p*-value
TNF-α (G-308A)	G/G	28 (51.9 %)	42 (84.0 %)	0.001
	G/A	26 (48.1%)	8 (16.0 %)	
	A/A	0 (0 %)	0 (0 %)	
IL1-β (C+3954T)	C/C	28 (51.9 %)	28 (56.0 %)	0.39
	C/T	20 (37.0 %)	20 (40.0 %)	
	T/T	6 (11.1 %)	2 (4.0 %)	

Table 2. TNF-α and IL-1β allele frequencies in healthy controls and in patients with pre-eclampsia

Gene	Allele frequencies	Pre-eclampsia group N=54 (%)	Control group N=50 (%)	*p*-value
TNF-α (G-308A)	Allele G	82(75.9 %)	92(92.0 %)	0.002
	Allele A	26 (24.1%)	8 (8.0 %)	
IL-1β (C+3954T)	Allele C	76 (70.4 %)	76 (76.0 %)	0.36
	Allele T	32 (29.6%)	24 (24.0%)	

Polymorphism of G-308A, in the promoter region of the TNF-α gene, was significantly associated with pre-eclampsia ($p<0.001$), but there was no significant association between IL-1β (C+3954T) genotype and pre-eclampsia in Iranian population from north-east province of Iran ($p=0.397$) (Table 1).

Allele A of the G-308A polymorphism was found to be associated with an increased risk of pre-eclampsia but there is not any significant association between allele C and allele T of IL-1β Gene according to table 2.

We found a significant correlation between systolic and diastolic blood pressure and polymorphism of TNF-α (G308A) ($p=0.001$) but did not show any correlation between systolic and diastolic blood pressure and polymorphism of IL-1β (C+3954T) gene ($p=0.55$).

DISCUSSION

Several genetic polymorphisms in cytokines have already been shown by Rinehart et al in pre-eclampsia. Increased expression of TNF-α, IL-1β and IL-10 was demonstrated in pre-eclamptic patients' placentas. This event may be due to decreased oxygenation of placenta and may be the result of endothelial dysfunction observed in preeclampsia.[21]

IL-1β is a proinflammatory cytokine produced by monocytes, macrophages, and epithelial cells. IL-1 creates a high variety of biologic activities that initiate and perpetuate an inflammatory response. Different IL-1β polymorphisms have been found in association with a number of disorders like psoriatic arthritis, periodontitis and cardiovascular diseases.[22-24] Hefler LA et al. showed that in 69 women with pre-eclampsia and 47 controls, there was not any role for polymorphisms of the interleukin-1β (promoter region and exon 5) and IL-1 receptor antagonist genes in the pathogenesis of pre-eclampsia among Hispanic women; however; polymorphisms within the IL-1β gene cluster might have influenced the severity of preeclampsia.[23]

Tosun M et al showed in 26 women with pre-eclampsia and 21 normal pregnant women that maternal serum levels of IL-8 and TNF-α were significantly related to preeclampsia.[25]

The results of study of Mirahmadian M et al also showed that TNF-α (G308A) A allele and-238 G allele frequencies are significantly elevated in preeclamptic patients compared to those of the control group.[26]

In another study, Vural P et al showed no notable differences were observed in allele or genotype frequencies for TNF-α (G308A) and IL-6 (-174) genes between preeclamptic patients and controls.[27]

In the present study our data do not support a role for polymorphism of IL-1β gene at position +3954 in exon 5 for the risk of pre-eclampsia and severity of it.

The polymorphism of TNF-α gene at the position of G308A in the promoter region was reported to be associated with elevated TNF-α levels and a number of infectious and metabolic diseases.[28,29] TNF-α is elevated in plasma and amniotic fluid of patients with severe pre-eclampsia [30]. One of the distinguished features of pre-eclampsia is over expression of the TNF-α gene.[31,32] Chen YP et al showed that maternal A allele of TNF-α promoter region at position -308 could play a role in the alteration of blood pressure and might augment urinary protein excretion during pregnancy. They concluded that TNF-α promoter region at position-308 might play an important role in the development of both gestational hypertension and preeclampsia.[33] Galbraith et al demonstrated an association between the two polymorphisms in the promoter region of the TNF-α gene and preeclampsia.[34] Haggert CL et al examined cytokine genotypes among 150 primiparous preeclamptic women. They showed that preeclamptic white women had upregulated TNF-α –308 A/A genotype.[35]

Our findings were in agreement with mentioned study. We showed a significant association between polymorphism of promoter region of TNF-α gene at position-308 with pre-eclampsia.

Our results do not support the hypothesis that IL-1β exon 5 (C+3954T) allele affects the risk of pre-eclampsia. The inconsistent findings for this allele might also be due to differences in linkage disequilibrium with other relevant loci in the IL-1 gene cluster.

We recommend further studies on polymorphism of IL-1 gene cluster except +3954 location in IL-1β, in different ethnic samples for more accurate results.

These results confirm that different genetic factors may be responsible for development of pre-eclampsia. The differences between populations suggest that ethnicity plays an important role in susceptibility to pre-eclampsia. In spite of some limitations in our study, including small sample size that influences the precision of this study, it can be cautiously concluded that: TNF- α (G-308A) polymorphism can be considered as a marker of susceptibility to pre-eclampsia in our population.

ACKNOWLEDGMENTS

This study was supported by the Vice Chancellor for Research, Mashhad University of Medical Sciences, Mashhad, Iran (Research Grant Number: 85149).

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