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Interleukin-1 alpha variation is associated with the risk of developing preeclampsia

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ABSTRACT

Objective: Preeclampsia is a syndrome that affects 5% of all pregnancies, producing substantial maternal and prenatal morbidity and mortality. Several studies have reported that cytokine genes are associated with the persistence of preeclampsia or the severity of the disease. The aim of this study is to investigate the relationships between the polymorphisms of interleukin-1 alpha-889 (IL-1A) gene and preeclampsia.

Method: Genomic DNA was extracted from the peripheral blood of 305 patients with preeclampsia and 325 normal controls from Sayyad Shirazi Hospital of Golestan University. Then subjected to SSP-PCR amplification. STATA software and the chi square test were used for statistic calculations.

Results: The frequencies of IL-1A -889 genotypes C/C, T/T and C/T in preeclampsia cases were 34.8%, 8.2%, 57% and in controls were 20.9%, 7.6% and 71.3% respectively. There was a significant 1.5 fold excess frequency in genotype C/C in cases (CI = 1.44–3.07, OR = 2.1, $P = 0.0001$). There was a significant difference in the frequencies of alleles or genotypes in IL-1A promoter regions between patients with preeclampsia and the control group. Turkomans showed the highest frequency of the C allele and Sistanies had the lowest frequency of the C allele in preeclampsia compared to control groups (CI = 1.5–3.9, OR = 2.48, $P = 0.0001$).

Conclusion: Our findings suggest that the IL-1A-899C/C genotype and C allele are associated with susceptibility to preeclampsia.

Introduction

Preeclampsia (PE) is a placenta-dependent disease that causes mother and fetus systemic disorders [1,2]. It is characterized by endothelial dysfunction throughout the maternal circulation, resulting in hypertension and proteinuria which typically occurs after 20 week gestation to after delivery during the postpartum period [3–6]. PE[2] is a major cause of maternal and fetal mortality and morbidity worldwide [4]. The prevalence of this disease is 5–7.5% in the USA and 4–4.5% in Iran [4,7]. Several studies on PE indicated that ethnic and genetic factors may contribute to the increased risk of PE [1,8,9]. Also, the role of immune system variations in the pathophysiology of preeclampsia has been reported in many studies [10].

Cytokines are a major group of extracellular signal proteins that regulate, proliferate and make cell-to-cell connections in the immune system. Cytokine signals have an important role in the different stages of health and diseases and increases in inflammatory cytokines such as IL-1, IL-10, IL-6 and INF-gamma that are produced in response to local hypoxia and ischemia of the placenta which might trigger endothelial and result in preeclampsia [11–13]. Therefore, the gene encoding pre-inflammatory cytokines participating in the regulation of the immune response are good candidates for further studying maternal genetic susceptibility to preeclampsia.

IL-1 is one of cytokines that plays a central role in the inflammation and endothelial function. All family of these genes

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(IL-1A, IL-1B, IL-1RN) on chromosome (2q13:14) are polymorphic. These polymorphisms are in regulatory regions; they have an important role in regulating the production proteins of IL-1, and are associated with several diseases [14,15]. IL-1A is a subtype of pre-inflammatory cytokines which induce the expression of 40 several inflammatory factors [16]. Haggeri et al. (2005) found an association between this position and preeclampsia; but there are not more studies in the position of IL-1A apparently [17].

In this study, the polymorphism in IL-1A gene was investigated in preeclamptic women to elucidate the association between IL-1A gene polymorphism (-889) and susceptibility to preeclampsia.

Materials and methods

In this study, the blood samples were collected from 305 preeclampsia women and 325 control women with no history of preeclampsia who referred to Sayyad Shirazi Hospital of Golestan University between March 2010 and May 2013. Women with inflammatory, autoimmune and chronic diseases or infections such as HBV were excluded. Controls were selected from among healthy pregnant women who were admitted to the labor ward. Preeclampsia was defined as the development of hypertension of above 140 and 90 mm Hg of systolic and diastolic pressure with proteinuria of +1 or >300 mg in 24 h without renal diseases and infection (according to the report of National High Blood Pressure Education Program Working Group, 2000). Women with chronic hypertension or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) without preeclampsia were also excluded from the study. Ethical approval for data collection and the project was approved by the ethics committee of Golestan University of Medical Sciences (Registration Number: 351599012254 and date of issue: 2/16/2012).

DNA was extracted from the whole blood using a standard phenol-chloroform extraction method and diluted to 50 ng/μl. IL-1A gene polymorphism (-889C/T) was genotyped by specific sequence primer polymerase chain reaction (SSP-PCR). Details of primer sequences and fragment size for determining of IL-1A gene polymorphism and human growth hormone (HGH) gene as an internal control are provided in Table 1. The PCR (polymerase chain reaction) amplification was followed by a 15 μl reaction mixture containing 1 μl of genomic DNA, 0/9 μl of 25 mm mgcl2 (Qiagen, USA), 1/5 μl of each 10x buffer (Qiagen, USA), 1/5 μl of 10 mm dNTP, 2/2 μl of sucrose 60%, 0/5 VL of each 10pm specific primer, 0/5 μl of each 10pm HGH primers and 0/2 of taq polymerase (Qiagen, USA). The cycling protocol was included in an initial denaturation at 96°C for 1 min, 10 cycles for 15 s at 96 °C, 50 s at 63 °C, 40 s at 72 °C (loop 1) and was followed by 20 cycles for 1 min at 96 °C, 50 s at 58 °C, 40 s at 72 °C (loop 2). A total of 30 cycles was carried out, with a final extension at 72 °C for 5 min. The PCR products were subjected to electrophoresis on 1/5% agarose gels and photographed under a UV trans-illuminator (2006 bio-red gel doc) after staining with ethidium bromide. Allele frequencies were tested for hardy-Weinberg equilibrium using Chi-squared test and/or Fisher exact test. These tests were used to compare the genotype frequency between preeclamptic and control groups. SPSS 16 software (IBM, Armonk, NY, USA) was also applied, the level of statistical significance was defined as $P < 0.05$, odds ratios (ORs) were calculated on a 95% confidence interval (CI).

Table 1
PCR primer sequences for IL-1A (-889).

Gene	Annealing temperature	Fragment size (bp)	Primers
IL-1A	63	221	FW: 5′-CTTTAATAATAGTAACCAGGCAACAT-3′ FM: 5′-CTTTAATAATAGTAACCAGGCAACAC-3′ R: 5′-AAGTAGCCCTCTACCAAGGA-3′
HGH	64	429	HGH1: 5′-GCC TTC CCA ACC ATT CCC TTA-3′ HGH2: 5′-TCA CGG ATT TCT GTT GTG TTT C-3′

Results

Using SSP-PCR, the fragments containing the positions where the IL-1/_889 SNP occurred in 305 patients with preeclampsia and 325 control subjects were amplified. Statistical analysis showed a significant difference between preeclampsia and control groups in terms of allelic and genotype frequency (Table 2) so that the C/C genotype frequency was significantly higher in the patients (CI = 1.44–3.07, OR = 2.1, $P = 0.0001$). Also, the C allele frequency was significantly higher in the patients than control group (CI = 1.04–1.66, OR = 1.32, $P = 0.016$), while T allele did not show any significant difference between the two groups. However the present study demonstrated a significant relationship between of the IL-1A/-889 gene polymorphism and preeclampsia. Case and control groups of this work were separated into three groups based on reproductive age that included: <18, between 18 and 40 and >40 years old. In terms of ethnicity, they were divided into four subgroups of Persian, Turkoman, Sistani and others[3] based on the participant's biography. Statistical observations are summarized in Table 3.

Investigation in the mentioned age groups showed no statistically significant differences between the case and control groups. But, studying the ethnicity provided the following results: Although genotyping of the following ethnic groups was slightly different, these changes were not statistically significant and needed to be studied in a larger population. However, studies demonstrated significant allelic differences so that the C allele significantly increased in the sub-groups of Turkomans, Persians, and Sistanies respectively. Turkomans showed the highest frequency of the C allele and Sistanies had the lowest frequency of the C allele in preeclampsia compared to control groups. However, T allele was significantly increased in the sub-groups of Sistanies, Persians, and Turkomans respectively. The obtained results are separately shown in Table 4.

Comments

In this study, the region of the IL1a gene position -889 was analyzed using SSP-PCR and a T to C polymorphism was identified in this position relative to preeclampsia. Highly significant correlation was found between homozygosity for the IL1a -889*C allele and development of PE. Also, the investigation of sub-ethnic groups in this study showed that Turkomans had the highest percentage of the C allele and Sistani ethnicity had the lowest percentage in PE compared with control groups. These data suggested that the C allele might be as a susceptibility factor for preeclampsia in Turkoman ethnicity and T allele can have a protective role against PE in Sistani ethnicity.

Both genetics and inflammatory factors play a vital role in the pathogenesis of preeclampsia [8,18]. Numerous studies have demonstrated the role of inheritance in preeclampsia [19,20]; but, the way genetics and molecular mechanisms work in preeclampsia is still unknown.

An aggressive inflammatory response may result in numerous adverse reproductive outcomes including preeclampsia [21–24]. An abnormal genetic predisposition, for example, exaggerates cytokine and may promote an inflammatory response which leads

[3] Other groups included Baluchistan and Afghanistan ethnicities.