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FoxP3 gene promoter polymorphism affects susceptibility to preeclampsia

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ABSTRACT

Background: Preeclampsia (PE) is a multifactorial pregnancy disorder and is a major cause of maternal morbidity and mortality. Despite intense study, the pathophysiology of preeclampsia remains enigmatic. Recent studies have reported that regulatory T cells (Tregs) is linked with PE. It is well identified that FoxP3/Scurfin is involved in development and function of Tregs. However, the association between PE and the FoxP3 gene polymorphism has not been sufficiently investigated. In this study, we hypothesized that polymorphisms of the FoxP3 may be related to PE.

Methods: We assessed the relationship between four single-nucleotide polymorphisms (SNPs) in the FoxP3 genes with sequence-specific primers (PCR-SSP) in 81 PE patients and 90 age-matched controls.

Result: We identified significant difference of rs4824747 GG genotype frequency between the PE and control groups. Women with GG genotypes exhibited higher (OR = 6.25, 95% CI = 2.63–14.85; $P < 0.0001$) risk of developing PE. None of the other investigated SNPs (rs2232365, rs3761547 and rs3761548) showed significant association with PE.

Conclusion: We suggest that FoxP3 polymorphisms (rs4824747) could be a potential contributor for the development of PE in Iranian women.

1. Introduction

Preeclampsia (PE) is a severe pregnancy complication characterized with a worldwide incidence of 2–8% which plays a significant role in maternal and prenatal morbidity and mortality [45]. Development of preeclampsia has a multifactorial nature and is influenced by different factors such as fetal/paternal genetic and environmental risk factors [25]. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not completely understood.

Latest observations have led to the hypothesis that preeclampsia may be partially mediated by immune system. Normal pregnancy requires a maternal immunological tolerance to the semiallogeneic fetus [14].

Apparently an aberrant and persistent maternal systemic inflammatory response to pregnancy with cytokine-mediated endothelial damage plays an essential role in the pathogenesis of PE.

Emerging evidences suggest that homeostasis between Regulatory T (Treg) cells and proinflammatory CD4(+) T cells might be pivotal for the semiallogeneic fetus to be tolerated within the maternal environment [39]. Tregs play a crucial role in preventing destructive immunity in all the tissues via various mechanisms such as regulation of TH1/TH2 balance [1,56]. Tregs are characterized by expression of CD4, CD25, FoxP3 and their ability to produce inhibitory cytokines (TGFβ, IL-10, IL-35) and inhibitory receptors (CTLA4, LAG-3) [23,36,43,47,48,50]. Associations between PE and CTLA4 rs231775 variant [6,13,19,32] and IL-10 rs1800896 variant have been reported [10,11,12,24,46,49]. FoxP3, a member of the X chromosome-encoded fork head transcription factor family, is a master control gene, requisite for the development and functioning of Tregs [37]. Several studies have reported the associations between FoxP3 gene polymorphisms and autoimmune diseases [7,18], endometriosis and infertility [2,4,5]. The relations between

Abbreviations: PE, preeclampsia; SNPs, single-nucleotide polymorphisms; Treg cell, regulatory T cell.

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the genetic variants of FoxP3 and maternal susceptibility to PE gene have not been sufficiently explored. Few published studies on the FoxP3 variation from various geographic regions produced somewhat controversial conclusion [8,21,31]. In this study, we investigated the effects of polymorphisms of four loci in the promoter regions of the FoxP3 gene (rs2232365, rs3761548, rs3761547, rs4824747) on the pathogenesis of PE.

2. Materials and methods

2.1. Study population

Present investigation was conducted on a total of 171 individuals recruited from Shariaty Hospital, BandarAbbas, Hormozgan, Iran, which included 81 PE patients and 90 ethnically matched healthy pregnant women. Sample size estimation was based on existing information about prevalence of PE in Hormozgan Province [34]. Demographic data including age, gestational age and marriage were collected from the patient's medical records. All the subjects reside in the same geographic area (Hormozgan) and the sample exclusion criteria for cases and controls were the same. Informed consent was obtained from all patients and controls and documented. Written approval for the study obtained from the Ethics Committee of Hormozgan University of Medical Sciences. Patients were selected for the study based on their clinical symptoms and the diagnosis made by gynecologists.

2.2. Inclusion criteria

Patients were recruited based on International Society for the Study of Hypertension in Pregnancy (ISSHP) that defines PE as hypertension with systolic blood pressure $\geqslant$140 mmHg and diastolic blood pressure $\geqslant$90 mmHg after 20 weeks of gestation together with proteinuria $\geqslant$300 mg in a 24-h collection and/or, $\geqslant$1+ on dipstick testing not associated with urinary tract infection or ruptured membranes. Severe PE was defined as systolic blood pressure $\geqslant$160 mmHg and/or diastolic blood pressure $\geqslant$110 mmHg; severe proteinuria (urinary protein excretion $\geqslant$2.0 g per 24-h and/or $\geqslant$2+ on dipstick testing); evidence of pulmonary edema; seizures; oliguria (<500 mL/day); thrombocytopenia (platelet count, $<100 \times 10^9$/L); and severe central nervous system symptoms, such as altered mental status, headaches, blurred vision, or blindness. To analyze the differences according to disease severity, PE patients were divided into mild and severe subgroups. Controls are women with pregnancies uncomplicated by gestational hypertension and proteinuria.

2.3. Exclusion criteria

Subjects with chronic hypertension, diabetes, kidney diseases, chronic diseases (Autoimmune diseases, collagen vascular disease, ...), hemoglobinopathy, thrombophilia, chorioamnionitis, history of endometriosis, coagulation disorders, urinary tract infections, periodontal disease, history of cardiovascular disease, preterm delivery, premature rupture of membranes, multiple gestation, preterm delivery fetal infection, stillbirth, fetal anomalies, chronic aspirin use, history of PE in mother or sister excluded from the study.

2.4. Genotyping

DNA extraction was performed from peripheral blood samples of patients and healthy individuals with Diatom DNA Extraction kit. Four SNPs (rs2232365, rs4824747, rs3761548 and rs3761547) in FoxP3 gene were analyzed by SSP-PCR assay.

Table 1
Primer sequences used for SSP-PCR.

SNP	Primer sequences
rs2232365	F1 5′-CCAGCTCAAGAGACCCCG-3′
	F2 5′-CCAGCTCAAGAGACCCCA-3′
	R 5′-GCTATTGTAACAGTCCTGGCAAGTG-3′
rs4824747	F1 5′-AGCCACACCTACAGTTTCCTGG-3′
	F2 5′-AGCCACACCTACAGTTTCCTGT-3′
	R 5′-CGCTTTCTAGAGGACCAGTT-3′
rs3761548	F1 5′-CTGGCTCTCTCCCCAACTGA-3′
	F2 5′-TGGCTCTCTCCCCAACTGC-3′
	R 5′-ACAGAGCCCATCATCAGACTCTCTA-3′
rs3761547	F 5′-GCTTTCTATTCTGTTCTCTTCCC-3′
	R1 5′-TGCAGGGCTTCAAGTTGACAAC-3′
	R2 5′-TGCAGGGCTTCAAGTTGACAAT-3′
GAPDH	F 5′-GCAGCCCTGGAGCCTTCA-3′
	R 5′-TTACCATATACCCAAGGGAGCC-3′

Specific primers for the SNPs were designed using the software Primer Blast and Gene Runner (Table. 1). SSP-PCR reaction in a final volume of 20 μl contains 0.3 μl of *Taq*DNA polymerase buffer (5 unit/μl), 0.8 μl of MgCl2 (stock concentration 50 mM), 0.4 μl of each dNTP (stock concentration of 10 mM), 0.7 μl of each primer pair (stock concentration of 10 pM), 2 μl PCR buffer 10×, 3 μl of prepared DNA and sterile double distilled water. Then the PCR amplification reaction was carried out according to the following temporal temperature schedule: The first stage consisted of denaturation at temperature of 95 °C for 5 min. The second stage involved denaturation phase at 94 °C for 40 s, annealing phase at 57 °C for 40 s to specific primers (rs2232365), (rs4824747), (rs3761548) and a temperature of 60 °C for 40 s for the specific primer rs3761547 and the third phase was elongation at 72 °C for 1 min. The second stage involved the total of 35 repetitions. The third stage included the final elongation at 72 °C for 7 min. The amplified products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and photographed (Fig. 1).

2.5. Statistical analysis

The clinical and demographic data of the study groups were compared with the use of the Student *t*-test and Chi square test. Data were expressed as the mean ± standard deviation (SD) or number (%). The genotype, allele frequency in PE patients and control group were analyzed by standard Chi-square test. Genotype frequencies of the two Foxp3 polymorphisms were tested for deviation from the Hardy–Weinberg equilibrium by using Chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by a particular genotype or allele. Logistic regression was carried out with adjustment for potential confounding covariates (maternal age, BMI, and gestational age at delivery) to obtain the odds ratio (OR) for risk of PE at 95% confidence intervals (CI). $P < 0.05$ was considered as statistical significance. Statistical analysis was performed using the Statistical Package for Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Comparison of baseline characteristics between subjects with and without preeclampsia

A total of 81 patients with preeclampsia and 90 healthy controls were studied. Both nulliparous and multiparous women were enrolled. The characteristics of the participants were shown in Table 2. Thirty women (37%) had mild PE and 51 (63%) had severe PE. The median ages of healthy controls and PE women were