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Interleukin-10 rs1800871 (−819C/T) and ATA haplotype are associated with preeclampsia in a Tunisian population

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ABSTRACT

Objectives: Interleukin-10 (IL-10) is implicated in several aspects of pregnancy. As genetic predisposition can be involved in the development of preeclampsia, the association between IL-10's promoter region polymorphisms and this pathology has been investigated, although with conflicting results. To date, only a small cohort study (total n = 40) has evaluated this association in the African continent, and none have been conducted in Tunisia. Hence, we evaluated the association between these polymorphisms and the risk of preeclampsia in a large Tunisian cohort.

Study design: 345 preeclampsia patients and 300 controls were genotyped for the IL-10 promoter region variants −1082A/G, −819C/T and −592A/C using real-time PCR.

Main outcome measures: Differences in means were determined by Student's *t*-test, while intergroup significance was assessed by Pearson χ^2 or 2-way ANOVA. Genotypes were tested for Hardy–Weinberg equilibrium (HWE) in the control and cases. Logistic regression analysis was performed in order to determine the odds ratios and 95% confidence intervals associated with the linkage disequilibrium risk.

Results: An increased frequency of the −819 T (minor) allele and the −819 T/T genotype was seen in preeclampsia cases. Also, three-locus haplotype (−1082AG/−819CT/−592AC) analysis identified the ATA haplotype as having a higher incidence in women with preeclampsia (OR = 1.48, 95% CI: 1.03–2.11) and this was confirmed by multivariate regression analysis (OR = 1.65, 95% CI: 1.13–2.43) after controlling for covariates.

Conclusions: We suggest that the IL-10 −819 T/T variant and the ATA haplotype, which are associated with low production of IL 10, represent genetic risk factors for preeclampsia in Tunisian women.

1. Introduction

Preeclampsia (PE) is a pregnancy complication characterized by hypertension and proteinuria after 20 weeks of gestation [1]. It is a major cause of perinatal and maternal mortality and morbidity, affecting about 2–10% of pregnant women worldwide [2]. Risks factors associated with this pathology include nulliparity, multifetal gestations, previous history of PE, obesity, diabetes mellitus, maternal age (more than 40 or less than 20 years old), pre-existing hypertension, chronic renal disease and thrombotic vascular disease [3]. However, the etiology of PE is still unknown. It has been hypothesized that PE may be related to uteroplacental ischemia, endothelial dysfunction and an exaggerated maternal inflammatory response to the trophoblast invasion deficiency [4]. Increasing evidence suggests that an abnormal maternal immune response may also play an important role in the development of PE [5]. As such, PE is considered a complex and multifactorial

Abbreviations: BMI, Body Mass Index; CI, confidence intervals; DBP, diastolic blood pressure; DNA, deoxyribonucleic acid; EDTA, Ethylenediaminetetraacetic acid; H, hour; HELLP, hemolysis, elevated liver enzymes and low platelets; HLA-G, human leukocyte antigen-G; HWE, Hardy–Weinberg equilibrium; IL-10, interleukin 10; LD, Linkage disequilibrium; MAF, minor allele frequency; NK, natural killer; OR, odds ratios; PCR, polymerase chain reaction; PE, preeclampsia; Rpm, Rotation per minute; SBP, systolic blood pressure; SEng, soluble endoglin; SFlt-1, soluble fms-like tyrosine kinase 1; SNP, single nucleotide polymorphisms; Th1, helper T cells type 1; Tregs, regulatory T cells

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disease. In addition, it is thought that its development is influenced by genetic predisposition and environmental factors. Numerous investigations have focused on the role of genetic factors on the incidence of PE [6–8]. For instance, heritability studies have shown that daughters and sisters of previously preeclamptic women have an increased risk of PE during their pregnancy [9].

In addition, many studies have reported that an imbalance between pro- and anti-inflammatory cytokines at the maternal-fetal interface plays a major role in the etiology of this pathology by contributing to poor placentation, persistent leukocyte and platelet activation, endothelial dysfunction, induction of vasoactive anti-angiogenic factors, oxidative stress and vasoconstriction [5,10,11]. Several studies have focused on the cytokine interleukin-10 (IL-10) as it is thought to be important because of its pleiotropic activities and since it is a potent regulator of the inflammatory process during pregnancy. More specifically, IL-10 is an immunosuppressor cytokine which is secreted by antigen presenting cells, activated T and B lymphocytes, regulatory T cells (Tregs), monocytes and trophoblast cells (the main cell type within the placenta) [12,13]. Moreover, this anti-inflammatory cytokine is known to inhibit the production of various pro-inflammatory cytokines and chemokines [14]. Alternatively, the expression of human leukocyte antigen-G (HLA-G) is stimulated by IL-10, which may trigger fetal allograft tolerance by inhibiting lysis of the embryo by the maternal natural killer (NK) cells. Given that IL-10 expression in human placenta is higher during the first and second trimesters compared to the third trimester, this suggests an interesting regulator role for IL-10 over the course of human pregnancy [15,16]. As a result, it has been suggested that IL-10 deficiency may contribute to the development of PE through the production of defective placental invasion, excessive maternal inflammatory response with associated endothelial dysfunction resulting in hypertension and proteinuria [15,17].

The gene encoding IL-10 is located on human chromosome 1 (1q31-1q32) [18]. Several single nucleotide polymorphisms (SNPs) have been identified in the IL-10 promoter region which appear to be correlated with variations in transcription and have been shown to regulate the levels of circulating IL-10 [19]. More specifically, it is well known that the mutant IL-10 (−1082) G allele is associated with an increased production of IL-10, while the IL-10 promoter (−819) T and (−592) carrying allele A are linked with low production of IL-10 [20–24]. Interestingly, associations between the IL-10 promoter region polymorphisms and the susceptibility to PE have been studied in different populations, specifically from Europe, Asia and America but with inconsistent results due to heterogeneity in study population ethnicity, and genotypes [7]. Indeed, it has been demonstrated by Liu et al. that the IL-10 −819C/T polymorphism was associated with an increased risk of PE in a Chinese population, while the −1082A/G was not [25]. Similar results were obtained by Sowmya et al. in an Indian population which revealed that the distribution of genotype and allele frequencies of IL-10 −1082 were not statistically different between the PE and controls women, while the other two polymorphisms (−819 and −592) showed a significant difference between the two groups [26]. In contrast, a PE case-control study in a population from the Netherlands demonstrated that there was no association between the distribution of the genotypes of the three IL-10 polymorphisms and the development of PE [27]. This is in line with data demonstrating that stratification by ethnicity must be taken into consideration to explain the potential source of heterogeneity and gene-environment interactions among the population [28]. Moreover, results from other ethnicities such as Africans must be investigated because, to date, only one group has evaluated this association in the African continent, in a small Egyptian population (n = 40, 20 cases and 20 controls) and reported that the IL-10 variant (−1082A/G) was associated with a higher risk of developing PE [29].

As such, the present study aimed to evaluate the association between polymorphisms in the promoter region of IL-10 and the susceptibility to PE in a large Tunisian (North Africa) cohort (300 controls and 345 cases). The IL-10 gene promoter being highly polymorphic, we focused on the most important three sites which are −1082 (A/G), −819 (C/T), and −592 (A/C).

2. Materials and methods

2.1. Human cohort

This is a retrospective case-control study involving 645 unrelated Tunisian women who were recruited between May 2012 and June 2013 from the gynecology services of Farhat Hached University Hospital (Sousse, Central Tunisia), Fattouma Bourguiba University Hospital (Monastir, Central Tunisia), Taher Sfar University Hospital (Mahdia, Eastern Tunisia), and Gafsa Hospital (Southern Tunisia). Out of those 645 patients, 345 were diagnosed with PE. Diagnostic criteria were the presence of systolic blood pressure (SBP) ≥ 140 and diastolic blood pressure (DBP) ≥ 90 mmHg on 2 separate occasions, at least 4 h apart, and the new onset proteinuria of ≥2+ by dipstick or ≥300 mg/24 h after 20 weeks of gestation. Severe PE was defined as SBP ≥ 160 or DBP ≥ 110 mmHg and proteinuria of ≥3+ by dipstick or ≥500 mg/24 h. Patients who met the criteria for PE but not for severe PE were defined as moderate PE (1). Women with previously diagnosed chronic hypertension and a history of PE were included in the study.

As controls were age-matched with PE cases, 300 unrelated women with normal pregnancies, no known personal or family history of hypertension and PE and from the same geographical area were recruited. The ethics committee of the Farhat Hached University Hospital approved the study protocol (project number: PI-15-91) and all subjects gave written informed consent for their participation in the study. Demographic and clinical data of the study participants were collected from a designed questionnaire (see Online Supplement) and medical record.

2.2. Sample collection and preparation

5 ml of venous blood was collected in EDTA-anticoagulant tubes from each participant. Blood was centrifuged at 1500 rpm for 10 min. Plasma was isolated, genomic DNA was extracted from peripheral blood leukocytes using the salting-out method [30], and all samples were stored at −20 °C for further analysis.

2.3. IL-10 genotyping

IL-10 genotyping was performed on the leukocyte DNA using the allelic (VIC- and FAM-labeled) discrimination method, using TaqMan assays as assay-on-demand which were ordered from Applied Biosystems: C_1747363_10 (rs1800872; −592C > A), C_1747362_10 (rs1800871; −819C > T), and C_1747360_10 (rs1800896; −1082A > G) and a TaqMan master mix (4371355, Applied Biosystems). The reaction was performed in a 6 µl volume using a Step One Plus real-time PCR system, according to manufacturer's instructions (Applied Biosystems). Duplicate blinded quality control samples were included to assess the reproducibility of the genotyping procedure; concordance was > 99%.

2.4. Statistical Analysis

Statistical analysis was performed on SPSS v21.0 (SPSS Inc., Chicago, IL). Categorical and continuous data were expressed as percentages of total or as mean ± SD, respectively. Differences in means were determined by Student's *t*-test, while intergroup significance was assessed by Pearson χ^2 or 2-way ANOVA. Genotypes were tested for Hardy–Weinberg equilibrium (HWE) in the control and cases using Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview). All analyses were conducted under additive genetic effect as it is the conservative model, using SNPStats software (bioinfo.iconcologia.net/