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Severe preeclampsia: Association of genes polymorphisms and maternal cytokines production in Brazilian population

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ABSTRACT

Introduction: Preeclampsia (PE) is a multi-system disorder of pregnancy characterized by hypertension and proteinuria. Healthy pregnancy is associated with a controlled inflammatory process, which is exacerbated in PE in response to excessive placental stimuli. Gene expression levels can affect inflammation and immune regulation. It is known that differences in cytokine allele frequencies amongst populations may contribute to difference in the incidence of several diseases.
Objective: The aim of this study was to investigate the frequency of TNF-α, IL-6, IFN-γ and IL-10 genes polymorphisms and their relationship with the cytokines plasma levels in PE.
Methods: A total of 281 women were included in this study; 116 with severe PE, 107 normotensive pregnant and 58 non-pregnant women. Cytokine genotyping was carried out by the polymerase chain reaction. The analyzed polymorphisms were: TNF-α (−308 G → A), IL-10 (−1082 G → A), IL-6 (−174 G → C), and IFN-γ (+874 A → T). Cytokine plasma levels were measured by Cytometric Bead Array method.
Results: A higher frequency of the IFN-γ (+874) T/T genotype in severe PE comparing to normotensive pregnant women was found ($P < 0.001$). TNF-α, IL-6 and IFN-γ plasma levels were higher in PE women compared to non-pregnant women ($P < 0.001$; $P < 0.001$; $P = 0.004$). IL-6 and IFN-γ levels were also higher in PE women compared to normotensive pregnant ($P < 0.001$; $P = 0.010$). IL-10 levels were higher in normotensive pregnant women compared to PE ($P < 0.001$). IFN-γ and IL-6 genes polymorphisms influenced the genic expression in PE and normotensive pregnant women, respectively.
Conclusions: These results suggest that IFN-γ seems to play a role in PE occurrence.
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1. Introduction

Preeclampsia (PE) is a multifactorial disease characterized by systolic blood pressure ⩾140 mmHg or diastolic ⩾90 mmHg at bed rest, on at least two occasions, six hours apart, and proteinuria ⩾0.3 g/24 h, measured after the 20th week of pregnancy [1]. Symptoms frequently observed in PE include headache, blurred vision, and abdominal pain. The etiology of PE is unknown and the delivery of placenta remains the only known treatment. Clinically, it is important to diagnose the severe form of PE when hypertension and proteinuria are even higher. This disease can progress to eclampsia (characterized by seizures as a sign of affection of the cerebral vessels), syndrome HELLP (hemolysis, elevated liver enzyme, low platelets) or disseminated intravascular coagulation [2]. PE is associated with placental disorder, endothelial cell dysfunction and systemic vasospasm. The events leading to these alterations remain unclear, but it seems that abnormal immune system activation plays a relevant role in PE development [2,3].

Healthy pregnancy is associated with a controlled inflammatory process, which is exacerbated in PE in response to excessive placental stimuli [4]. Previous studies suggested that cytokines might be involved in the PE pathogenesis. High levels of interleukin (IL) IL-1, IL-6 and tumor necrosis factor alpha (TNF-α), as well as IL-2 and interferon gamma (IFN-γ), have been detected in plasma and amniotic fluid of PE women. All these inflammatory cytokines seem to have deleterious effects on pregnancy development [5–7]. IL-10 has been identified as an important cytokine in successful

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pregnancy [8]. It has been suggested that decreased IL-10 production in PE may cause a pro-inflammatory cytokine maternal response, resulting in pregnancy complications [6,9,10].

It has been reported that phytohemagglutinin (PHA)-stimulated IFN-γ production in peripheral blood mononuclear cells (PBMC) in PE women is significantly higher compared to normotensive pregnant women [6,11–14]. Elevated IFN-γ levels in pregnancy can be potentially harmful to the fetus. It is known that IFN-γ inhibits the outgrowth of trophoblast cells *in vitro* [15] and synergistically stimulates the programmed death of primary villous trophoblast cells [16,17].

Point mutations and single nucleotide substitutions (SNPs) in the regulatory regions of cytokine genes may affect cytokine transcription and influence its production.

The relationship between PE and SNPs in cytokine genes has been investigated, but remains unclear [18–31]. Therefore, the aim of this study was to investigate whether the TNF-α (−308 G → A), IL-6 (−174 G → C), IFN-γ intron 1 (+874 A → T) and IL-10 (−1082 G → A) genes polymorphisms are associated with severe PE occurrence.

2. Subjects and methods

2.1. Ethical aspects

This study was approved by the Ethics Committee of Federal University of Minas Gerais-Brazil and informed consent was obtained from all participants. The research protocol did not interfere with any medical recommendations or prescriptions.

2.2. Study design

The present case-control study included 281 women; 116 with severe PE, 107 normotensive pregnant and 58 non-pregnant women, selected from Odete Valadares Maternity-Belo Horizonte, Brazil, Regional Public Hospital of Betim, Brazil and Healthy Center Guanabara, Betim, Brazil from 2008 to 2011.

2.3. Inclusion criteria

Severe PE was defined by systolic blood pressure ⩾160 mmHg or diastolic blood pressure ⩾110 mmHg, presented in two consecutive occasions at bed rest at least four hours apart; and proteinuria >2 gL–1/24 h or at least 2+ protein by dipstick. Normotensive pregnant women had systolic/diastolic blood pressure below 120/80 mmHg and no history of hypertension or proteinuria. All pregnant women showed gestational age ⩾20 weeks. Non-pregnant women had no clinical and laboratory alterations, including hypertension.

2.4. Exclusion criteria

Exclusion criteria common for the three groups were chronic hypertension, haemostatic abnormalities, cancer, diabetes mellitus, cardiovascular, autoimmune, renal and hepatic diseases, and anticoagulant therapy.

2.5. Cytokine gene polymorphism analysis

DNA was extracted and purified from whole blood, collected in EDTA using Biopur Mini Spin Kit (Biometrix, Brazil).

Cytokine genotyping was carried out by the polymerase chain reaction (PCR) sequence-specific primer method, using the 'Cytokine Genotyping Tray' (One Lambda Inc., Canoga Park, CA, USA). The kit accuracy was checked by our laboratory using known DNA samples. The PCR products were then visualized by electrophoresis in 2% agarose gel stained with ethidium bromide and documented with a Polaroid camera. The polymorphisms analyzed in the present study were: TNF-α (−308 G → A), IL-10 (−1082 G → A), IL-6 (−174 G → C), and IFN-γ (+874 A → T).

The cytokine genotypes were grouped according to the final phenotype on gene expression. For the TNF-α gene, the genotypes were distributed as A/A and A/G (high) and G/G (low); for the IL-10 gene, the genotypes were distributed as G/G (high), G/A (intermediate) and A/A (low); for the IL-6 gene, the genotypes were distributed as G/G and G/C (high) and C/C (low); and for the IFN-γ gene, the genotypes were distributed as T/T (high), T/A (intermediate) and A/A (low) [32–35].

2.6. Determination of cytokine plasma levels

Samples collected in EDTA were centrifuged at 2500*g* for 20 min at 4 °C to obtain plasma, which was stored at −80 °C until analysis. Data acquisition and analysis were performed in dual-laser FACScalibur™ flow cytometer (BD Biosciences Pharmingen, San Jose, CA, USA), using the BD Bioscience CBA software. IFN-γ was determined using the Human Th1/Th2 Cytometric Bead Array method (BD Biosciences Pharmingen, USA). IL-6, IL-10 and TNF-α were determined using Human Inflammation Kit (BD Biosciences Pharmingen, USA), according to the manufacturers' instructions. Results were expressed as mean fluorescence intensity (MFI) for each cytokine.

2.7. Statistical analysis

Statistical analysis was carried out using SPSS (version 13.0) and GENEPOP software. Hardy–Weinberg equilibrium was investigated through probability test. Data normality was tested by Shapiro–Wilk test. Comparisons between two groups were made by Student *t* test for parametric variables and Mann–Whitney for non-parametric variables. A comparison of non-parametric variables was done by Kruskal–Wallis test amongst three groups. When differences were detected among groups, these were compared in pairs by Mann–Whitney method, followed by Bonferroni test. The comparison of categorical variables was performed using the chi-square test (χ^2). When $P < 0.05$, residue adjusted analysis was made to identify where was the difference. Spearman's correlations were computed to assess correlations with cytokine plasma levels and cytokine genotype. *P* values <0.05 were considered statistically significant.

3. Results

Table 1 summarizes the clinical characteristics of the 281 women enrolled in this study. PE women, normotensive pregnant

Table 1
Clinical characteristics of participants.

Characteristics	Control group	Preeclamptic women	*P* value
Age (years)	25.8 (6.22)	26.8 (7.16)	0.207
GA (weeks)	32.9 (4.68)[a]	33.0 (4.04)	0.799
GWG (kg)	10.0 (6.75–13.55)[a]	12.7 (8.50–16.50)	0.002*
BMI (kg/m^2)	23.25 (20.53–26.90)	23.98 (21.63–28.13)	0.128
SBP (mmHg)	110 (100.0–120.0)	170 (160.0–180.0)	<0.001*
DBP (mmHg)	70 (70.0–80.0)	110 (100.0–120.0)	<0.001*

GA: gestational age; GWG: gestational weight gain; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index.
Age and GA are presented as mean (standard deviation). Student *t* test.
GWG, BMI, SBP and DBP are presented as median (25th–75th centiles). Mann–Whitney test.
[a] Only normotensive pregnant.
* $p < 0.05$ – Statistic significant.

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