Cytokine 71 (2015) 232–237

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

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

Melina B. Pinheiro [a,b], Karina B. Gomes [a,*], Carla R.S.C. Ronda [c], Gabrielle G. Guimarães [c], Letícia G. Freitas [a], Andréa Teixeira-Carvalho [d], Olindo Assis Martins-Filho [d], Luci M. Dusse [a]

[a] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Faculdade de Medicina, Universidade Federal de São João Del Rei, Divinópolis, Minas Gerais, Brazil
[c] Simile Instituto de Imunologia Aplicada, Belo Horizonte, Minas Gerais, Brazil
[d] Laboratório de Biomarcadores de Diagnóstico e Monitoração – Centro de Pesquisas René Rachou-Fundação Osvaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form 12 August 2014
Accepted 28 October 2014
Available online 21 November 2014

Keywords:
Preeclampsia
Inflammation
Cytokine gene polymorphism
Cytokine levels

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.

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

* Corresponding author at: Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av Antonio Carlos, 6627, Pampulha, CEP: 31270-901, Belo Horizonte, MG, Brazil. Tel.: +55 (31) 3409 6895; fax: +55 (31) 3409 6985.
E-mail address: karinabgb@gmail.com (K.B. Gomes).

http://dx.doi.org/10.1016/j.cyto.2014.10.021
1043-4666/

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.

and non-pregnant women showed similar ages ($P = 0.207$) and body mass index (BMI) ($P = 0.128$). Normotensive pregnant and PE women did not show differences regarding gestational age ($P = 0.799$). As expected, systolic and diastolic blood pressures were significantly higher in PE women, comparing to the other two groups ($P < 0.001$, in both of cases), as well as the gestational weight gain, when compared to normotensive pregnant women ($P = 0.002$).

The case (PE) and control group (normotensive pregnant and non-pregnant women) were under Hardy–Weinberg equilibrium ($P = 0.289$ and $P = 0.364$, respectively.)

Phenotyping data are presented in Table 2. A higher frequency of the IFN-γ (+874) T/T genotype in PE women compared to normotensive pregnant group was observed (PE women: T/T 28% and A/A 28%; normotensive pregnant: T/T 5% and A/A 58%, $P < 0.001$). However, no differences between cases and controls were found in phenotypes distribution for TNF-α (−308), IL-10 (−1082) and IL-6 (−174) polymorphisms.

Cytokine plasma levels were analyzed as mean fluorescent intensity (MFI) provided by CBA immunoassay (Fig. 1). To assess whether pregnancy is able to induce different levels of cytokines, this analysis was performed separately in each studied group. TNF-α, IL-6 and IFN-γ plasma levels were higher in PE women compared to non-pregnant women ($P < 0.001$; $P < 0.001$; $P = 0.004$, respectively). Furthermore, IL-6 and IFN-γ levels were also higher in PE women compared to normotensive pregnant women ($P < 0.001$; $P = 0.010$, respectively). However, IL-10 levels were higher in normotensive pregnant compared to PE women ($P < 0.001$) and non-pregnant women ($P < 0.001$). Aiming to evaluate whether the polymorphisms in TNF-α, IL-10, IL-6 and IFN-γ genes influence the genic expression, plasma levels of these cytokines were compared to phenotypes determined by genotypes (Table 3). Increased levels of IL-6 in "high" phenotype compared to "low" phenotype ($P = 0.05$) were observed in normotensive pregnant women. Furthermore, increased levels of IFN-γ in "high" compared to "intermediate" ($P = 0.012$) and "low" phenotypes ($P < 0.001$) were found in PE women.

In order to investigate the correlation between genotypes and cytokines plasma levels, the three groups were analyzed together. A significant positive correlation between IFN-γ plasma levels and the presence of +874T allele was observed ($P < 0.001$, $r = 0.302$). When the three groups were evaluated separately, a significant positive correlation between IL-6 levels and −174C allele ($P = 0.05$, $r = 0.236$) in normotensive pregnant women was evidenced. Moreover, in PE women, it was found a significant positive correlation between IFN-γ plasma levels and +874T allele ($P = 0.004$, $r = 0.372$). The other polymorphisms did not show correlation with cytokines levels.

4. Discussion

In the present study, the +874 T/T genotype in IFN-γ gene was more frequent in PE women than in the control group (normotensive pregnant and non-pregnant women). Therefore, given the decisive role of IFN-γ in pregnancy (and the presence of functional polymorphisms in the first intron of the IFN-γ gene), our data suggest that this gene might plausibly be a candidate for susceptibility gene in PE. In contrast, a study involving Brazilian preeclamptic and eclamptic women showed higher frequency of IFN-γ + 874 A in eclamptic women compared to controls [21]. The authors admitted that these results were unexpected and could have occurred by chance, since they did not detect a corresponding expression in genotype frequency. However, other studies have investigated this polymorphism in PE women and did not find association between genotypes or allele frequencies of IFN-γ gene and this disease [19,24]. These conflicting findings could have resulted from the heterogeneity in study designs, definition of phenotype, population diversity and sample size. Our study evaluated a huge sample size in a clinical homogeneous group, since only severe PE cases were investigated. Therefore, this result suggests that +874 T/T genotype in IFN-γ gene is associated with severe PE in the studied population.

No association between TNF-α (−308 G → A), IL-6 (−174 G → C), or IL-10 (−1082 G → A) polymorphisms and PE was observed. These results are in line with other publications [18,19,21,22,24,28,29,31,36–43] and in disagreement with others [19,23–27,31]. A reason for discrepant results among different studies might be the selection bias and small sample size in retrospective studies, or ethnic differences in the studied populations.

There are several lines of evidences suggesting that IL-10 has an important role in pregnancy. IL-10 has a critical function in different obstetric pathologies associated to down regulation of inflammatory responses in the placenta [44]. PE has also been associated with a deficiency of placental IL-10, which induces T lymphocytes to differentiate along the Th2 pathway and block IFN-γ production. It is known that IFN-γ is the major Th1 lymphocyte product that induces pro-inflammatory cytokine synthesis [44]. Mirhamadian et al. [26] found significantly higher C/C genotype frequency of

Table 2
Phenotype frequencies of TNF-α, IL-10, IL-6 and IFN-γ polymorphisms in women with preeclampsia (PE) and the control groups.

Polymorphisms genotypes (phenotype[a])	Non-pregnant ($N = 58$)	Normotensive pregnant ($N = 107$)	PE ($N = 116$)	P
TNF-α (−308 G → A)				0.781
A/A; A/G (high)	16 (0.28)	30 (0.28)	28 (0.24)	
G/G (low)	42 (0.72)	77 (0.72)	88 (0.76)	
IL-10 (−1082 G → A)				0.785
G/G (high)	7 (0.12)	9 (0.08)	11 (0.09)	
G/A (intermediate)	25 (0.43)	53 (0.50)	61 (0.53)	
A/A (low)	26 (0.45)	45 (0.42)	44 (0.38)	
IL-6 (−174 G → C)				0.258
G/G; G/C (high)	55 (0.95)	104 (0.97)	107 (0.92)	
C/C (low)	3 (0.05)	3 (0.03)	9 (0.08)	
IFN-γ (+874 A → T)				<0.001*
T/T (high)	6 (0.10)	5 (0.05)**	33 (0.28)***	
T/A (intermediate)	19 (0.33)	40 (0.37)	51 (0.44)	
A/A (low)	33 (0.57)	62 (0.58)***	32 (0.28)**	

Values in parentheses are frequency.
[a] Cytokine production phenotype according to the Hoffmann et al. [46], Pravica et al. [47], Turner et al. [48], and Wilson et al. [49]. Pearson chi-square test.
* $P < 0.05$.
** <−1.96, less frequent.
*** >1.96, more frequent (analysis of adjusted residual).

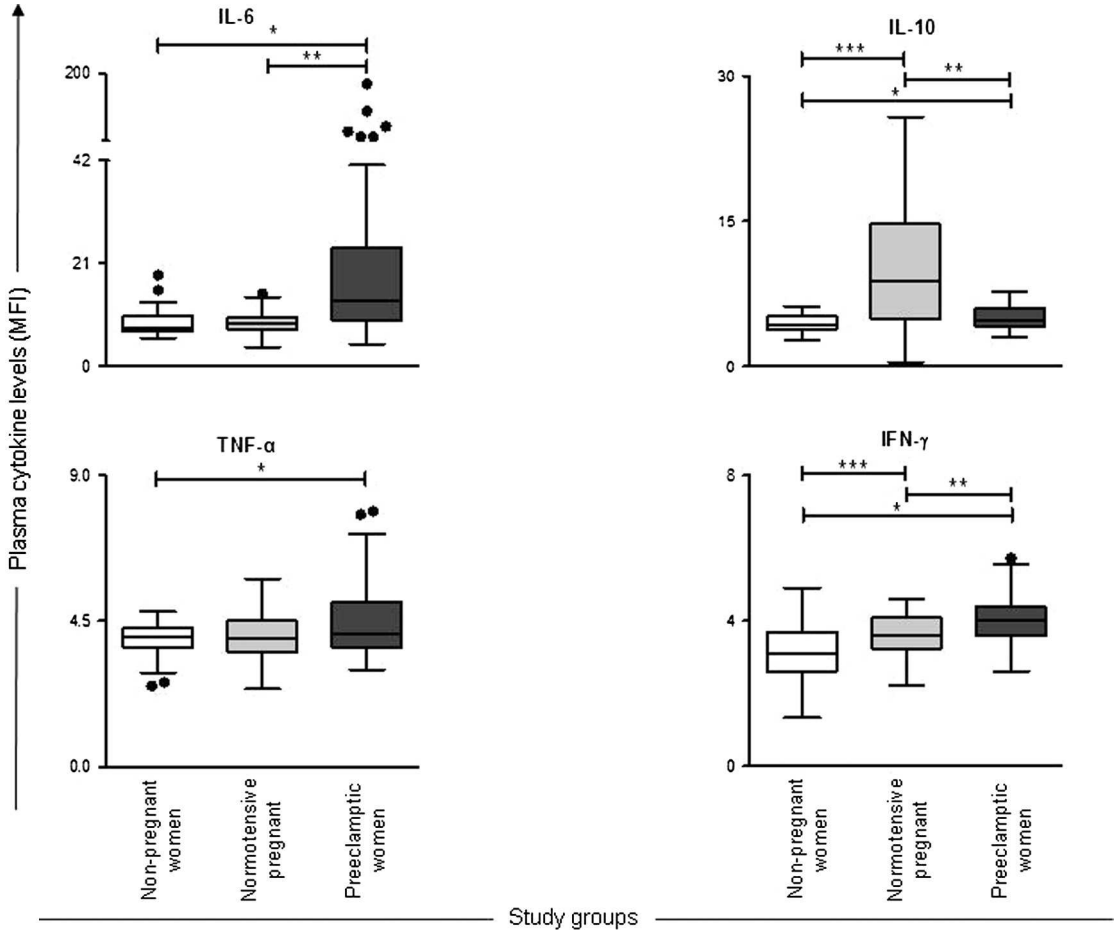

Fig. 1. Cytokine plasma levels in preeclamptic women (■) as compared to normotensive pregnant women (▭) and non-pregnant women (□). Plasma levels of cytokines were determined by cytometric beads array. Results are expressed in mean fluorescence intensity (MIF) data are presented in a box plot format. The lines stretch from the 10th percentile to the upper 90th percentile, highlighting the outliers (○). The median is shown as a line across the box. Statistical analysis was performed by non-parametric Mann–Whitney test. Significant differences at $P < 0.05$ are highlighted by connecting lines. *non-pregnant X PE; **normotensive X PE; ***non-pregnant X normotensive.

IL-10 (−819 C → T) and (−592 C → A) in PE women. However, in agreement with other studies [20–22,30] our data did not show association between PE and IL-10 gene polymorphism. Accordingly, a recent meta-analysis showed no association between PE and IL-10 polymorphisms (−1082 G → A) [43].

IL-6 is a critical cytokine in the cascade of host response to infection. IL-6 activates the acute phase response, stimulates T lymphocytes, induces the terminal differentiation of B-lymphocytes, and induces C reactive protein production [45]. It has recently been reported that in PE, endothelial cells phagocytes kill trophoblasts shedding from placenta to maternal blood. Phagocytosis of necrotic trophoblasts cause endothelial cells activation and subsequent IL-6 release [46,47]. Several studies have been reporting increased IL-6 levels in PE [43,48–52].

It is known that the IL-6 production is under genetic regulation. A polymorphism in the promoter region of IL-6 (−174 G → C) gene, on chromosome 7 [53] is associated with the production of IL-6 [32]. The C/C genotype of this polymorphism is related to reduced IL-6 production, whereas homozygous G/G or heterozygous G/C displays normal production. In agreement with our results, other studies did not find an association between polymorphism in IL-6 gene promoter (−174 G → C) and PE occurrence [19,21,28,29,42], which was confirmed by a recent metanalysis [43].

TNF-α is a potent and multi-functional cytokine produced by macrophages, lymphocytes and trophoblast. It contributes to the abnormal placental invasion [54], endothelial cell damage [55] and oxidative stress [56]. An excessive inflammatory response to pregnancy seems to characterize PE, and TNF-α represents a major mediator of this reaction [38]. Our data did not show any association between polymorphism in TNF-α gene and PE occurrence or TNF-α plasma levels. In agreement with our data, two metanalysis [36,43] revealed no association between polymorphism −308 G → A and PE. Nonetheless, in one of these metanalysis [43] it was found an association between high TNF-α plasma levels and PE. In accordance, our data showed high TNF-α plasma levels in PE comparing to non-pregnant women, which could suggest that this cytokine may have a role in PE.

Our data showed increased inflammatory cytokines levels, IL-6 and IFN-γ, in PE women comparing to normotensive pregnant women. Supporting our findings, some studies have demonstrated an increase in IFN-γ [48–51,57] and IL-6 in PE [43,48–52], which was confirmed by a recent metanalysis [43]. However, we found decreased levels of the regulatory cytokine IL-10. It has been suggested that decreased IL-10 production in PE may cause a pro-inflammatory cytokine response.

As IL-10 has a very short half-life, it is not consistently present in the circulation. Thus, a single blood sample may fail to detect a sporadic elevation or reduction in this cytokine level. Different factors, such as the effect of gestational age at the time of blood sample collection, the influence of body mass index and the assay sensitivity to measure IL-10 may also explain the divergences in results found in the studies [19].

Table 3
Evaluation of polymorphism influence in circulating levels of cytokines.

Population	Cytokines	Polymorphism Genotype (phenotype[a])			*P* value
		High	Intermediate	Low	
Non-pregnant	TNF-α	4.00 (3.80–4.20)	NA	3.90 (3.20–4.10)	0.812
	IL-10	4.75 (3.80–5.70)	4.00 (3.70–4.90)	4.55 (3.80–5.70)	0.515
	IL-6	7.71 (5.73–18.60)	NA	–	–
	IFN- γ	3.32 (2.00–4.91)	3.53 (2.09–4.01)	2.83 (1.33–3.96)	0.183
Normotensive pregnant	TNF-α	4.00 (3.80–4.70)	NA	3.90 (3.50–4.70)	0.585
	IL-10	8.20 (6.00–9.60)	8.65 (4.50–13.20)	8.25 (6.50–11.10)	0.456
	IL-6	8.82 (3.90–14.86)	NA	6.66 (6.21–7.10)	0.050[c]
	IFN-γ	4.2 (3.79–4.61)	3.54 (2.76–4.33)	3.53 (2.23–4.61)	0.390
Preeclamptic women	TNF-α	4.10 (4.20–6.00)	NA	4.10 (4.50–6.80)	0.637
	IL-10	4.55 (3.70–5.30)	5.20 (4.20–6.80)	4.15 (3.30–5.90)	0.155
	IL-6	12.92 (4.57–176.24)	NA	19.99 (9.73–75.67)	0.187
	IFN- γ	4.45 (3.18–5.73)	3.79 (2.67–4.41)	3.89 (2.62–4.83)	0.012[b] <0.001[c] 0.494[d]

Levels of plasma cytokine measured by median fluorescence intensities (MFI); NA, Not applicable; (–) no woman had the phenotype "low" (C/C). Data were compared by the Kruskal–Wallis and Mann–Whitney test. Values are presented as median (25th–75th centiles).
[a] Cytokine production phenotype according to the Hoffmann et al. [46], Pravica et al. [47] Turner et al. [48], and Wilson et al. [49]. b. High × Intermediate; c. High × low; d. Intermediate × low.

Our data revealed higher levels of IL-6 in pregnant women with "high" phenotype compared to "intermediate" and "low" phenotypes. This finding suggests that pregnancy is able to increase IL-6 levels and this cytokine may be important to physiologic gestational development. However, it is known that IL-6 levels depend on the genotype that determines the "high" phenotype. Some cytokines such as IL-4, IL-6 and IL-10 seem to favor pregnancy success whereas others such as TNF-α and IFN-γ are harmful. In pregnancy, there is a greater increase in IL-6 production compared to the non-pregnant state [58]. IL-6 may induce prostaglandin synthesis by intrauterine tissues, suggesting its physiological role in labor. Moreover, IL-6 is considered a Th2 cytokine, but it may perform "Th1-type" or "Th2-type" functions depending on the biological condition [7]. However, several studies showed that IL-6 plasma levels are higher in women presenting pregnancy complications, when compared to healthy pregnant women, which suggests a role for this cytokine in these disturbances [43,48–52,59].

To the best of our knowledge, this is the first study investigating the relationship between IFN-γ levels and gene polymorphism in PE. Our data revealed increased IFN-γ plasma levels in preeclamptic women with "high" phenotype compared to "intermediate" and "low" phenotypes. Besides, a positive correlation between IL-6 levels and "high" phenotype in normotensive pregnant women was revealed. Moreover, this association was also observed evaluating the three groups together. These results point to the importance of IL-6 production in healthy pregnancy. On the other hand, IFN-γ seems to play an essential role in PE occurrence (Fig. 2).

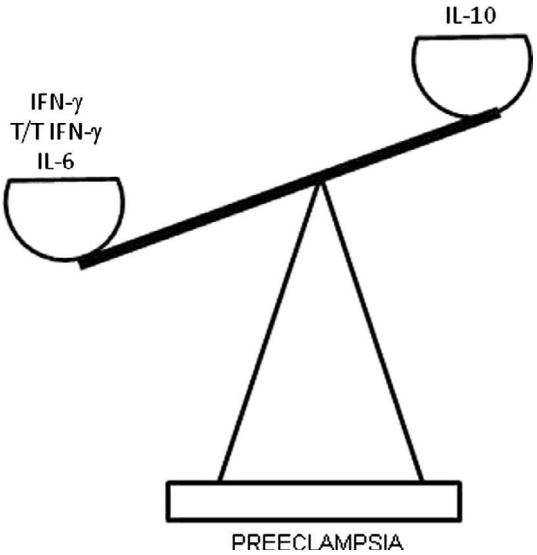

Fig. 2. Cytokine levels and genotype associated to favorable and unfavorable PE occurrence.

5. Conclusion

Our results suggest that IFN-γ seems to play a role in PE occurrence.

The inflammatory markers results will certainly reflect such differences, leading to controversial research conclusions. Moreover, maternal and fetal genes interacting with each other (and a variety of environmental stimuli) interfere on the PE severity and outcome. Based on these considerations, further studies are undoubtedly needed in order to clarify the association of genes polymorphisms and maternal cytokines production. In this sense, reproducing our findings in other populations will help defining the influence of genes polymorphisms and cytokine production in PE pathophysiology.

Acknowledgements

The authors thank FAPEMIG and CNPq/Brazil. DUSSE, Luci M, MARTINS-FILHO Olindo Assis, TEIXEIRA-CARVALHO Andrea, GOMES, Karina are grateful to CNPq for Research Fellowship (PQ).

References

[1] ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet 2002;2002(77):67–75.
[2] Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. Am J Reprod Immunol 2011;65:377–94.
[3] Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol 2010;63:534–43.
[4] Redman CWG, Sargent IL. Pre-eclampsia, the Placenta and the maternal systemic inflammatory response—a review. Placenta 2003;24:S21–7.
[5] Laresgoiti-Servitje E, Gomez-Lopez N, Olson DM. An immunological insight into the origins of pre-eclampsia. Hum Reprod Update 2010;16:510–24.

[6] Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinski J, Oleszczuk J. T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. Eur J Obstet Gynecol Reprod Biol 1999;86:165–70.
[7] Daher S, de Arruda Geraldes Denardi K, Blotta MH, Mamoni RL, Reck AP, Camano L, et al. Cytokines in recurrent pregnancy loss. J Reprod Immunol 2004;62:151–7.
[8] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683–765.
[9] Hennessy A, Pilmore HL, Simmons LA, Painter DM. A deficiency of placental IL-10 in preeclampsia. J Immunol 1999;163:3491–5.
[10] Wilczynski JR, Tchorzewski H, Banasik M, Glowacka E, Wieczorek A, Lewkowicz P, et al. Lymphocyte subset distribution and cytokine secretion in third trimester decidua in normal pregnancy and preeclampsia. Eur J Obstet Gynecol Reprod Biol 2003;109:8–15.
[11] Azizieh F, Raghupathy R, Makhseed M. Maternal cytokine production patterns in women with pre-eclampsia. Am J Reprod Immunol 2005;54:30–7.
[12] Omu AE, Al-Azemi MK, Al-Qattan F, Al-Yatama M. Connection between human leucocyte antigens D region and T helper cytokines in preeclampsia. Arch Gynecol Obstet 2004;269:79–84.
[13] Saito S, Umekage H, Sakamoto Y, Sakai M, Tanebe K, Sasaki Y, et al. Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with preeclampsia. Am J Reprod Immunol 1999;41:297–306.
[14] Yoneyama Y, Suzuki S, Sawa R, Yoneyama K, Power GG, Araki T. Relation between adenosine and T-helper 1/T-helper 2 imbalance in women with preeclampsia. Obstet Gynecol 2002;99:641–6.
[15] Haimovici F, Hill JA, Anderson DJ. The effects of soluble products of activated lymphocytes and macrophages on blastocyst implantation events in vitro. Biol Reprod 1991;44:69–75.
[16] Clark DA, Chaouat G, Arck PC, Mittruecker HW, Levy GA. Cytokine-dependent abortion in CBA x DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase [correction of prothombinase]. J Immunol 1998;160:545–9.
[17] Yui J, Garcia-Lloret M, Wegmann TG, Guilbert LJ. Cytotoxicity of tumour necrosis factor-alpha and gamma-interferon against primary human placental trophoblasts. Placenta 1994;15:819–35.
[18] Canto-Cetina T, Canizales-Quinteros S, de la Chesnaye E, Coral-Vazquez R, Mendez JP, Canto P. Analysis of C-850T and G-308A polymorphisms of the tumor necrosis factor-alpha gene in Maya-Mestizo women with preeclampsia. Hypertens Pregnancy 2007;26:283–91.
[19] Daher S, Sass N, Oliveira LG, Mattar R. Cytokine genotyping in preeclampsia. Am J Reprod Immunol 2006;55:130–5.
[20] de Groot CJ, Jansen MW, Bertina RM, Schonkeren JJ, Helmerhorst FM, Huizinga TW. Interleukin 10-2849AA genotype protects against pre-eclampsia. Genes Immun 2004;5:313–4.
[21] de Lima TH, Sass N, Mattar R, Moron AF, Torloni MR, Franchim CS, et al. Cytokine gene polymorphisms in preeclampsia and eclampsia. Hypertens Res 2009;32:565–9.
[22] Haggerty CL, Ferrell RE, Hubel CA, Markovic N, Harger G, Ness RB. Association between allelic variants in cytokine genes and preeclampsia. Am J Obstet Gynecol 2005;193:209–15.
[23] Kaiser T, Grehan M, Brennecke SP, Moses EK. Association of the TNF2 allele with eclampsia. Gynecol Obstet Invest 2004;57:204–9.
[24] Kamali-Sarvestani E, Kiany S, Gharesi-Fard B, Robati M. Association study of IL-10 and IFN-gamma gene polymorphisms in Iranian women with preeclampsia. J Reprod Immunol 2006;72:118–26.
[25] Kim SY, Lim JH, Park SY, Yang JH, Kim MY, Kim MH, et al. Transforming growth factor-beta 1 gene polymorphisms in Korean patients with pre-eclampsia. Am J Reprod Immunol 2010;63:291–8.
[26] Mirahmadian M, Kalantar F, Heidari G, Safdarian L, Mansouri R, Amirzargar AA. Association of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in Iranian patients with pre-eclampsia. Am J Reprod Immunol 2008;60:179–85.
[27] Pazarbas A, Kasap MI, Güzel AI, Kasapa H, Özbakır B, et al. Polymorphisms in the tumor necrosis factor-alpha gene in Turkish women with pre-eclampsia and eclampsia. Acta Med Okayama 2007;61:153–60.
[28] Saarela T, Hiltunen M, Helisalmi S, Heinonen S, Laakso M. Polymorphisms of interleukin-6, hepatic lipase and calpain-10 genes, and preeclampsia. Eur J Obstet Gynecol Reprod Biol 2006;128:175–9.
[29] Stonek F, Metzenbauer M, Hafner E, Philipp K, Tempfer C. Interleukin 6-174 G/C promoter polymorphism and pregnancy complications: results of a prospective cohort study in 1626 pregnant women. Am J Reprod Immunol 2008;59:347–51.
[30] Stonek F, Metzenbauer M, Hafner E, Philipp K, Tempfer C. Interleukin-10-1082 G/A promoter polymorphism and pregnancy complications: results of a prospective cohort study in 1616 pregnant women. Acta Obstet Gynecol Scand 2008;87:430–3.
[31] Vural P, Degirmencioglu S, Saral NY, Demirkan A, Akgul C, Yildirim G, et al. Tumor necrosis factor alpha, interleukin-6 and interleukin-10 polymorphisms in preeclampsia. J Obstet Gynaecol Res 2010;36:64–71.
[32] Hoffmann SC, Stanley EM, Cox ED, Craighead N, Dimercurio BS, Koziol DE, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28 stimulated peripheral blood lymphocytes. Transplantation 2001;72:1444–50.
[33] Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet 1999;26:1–3.
[34] Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An Investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1–8.
[35] Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J Exp Med 1993;177:557–60.
[36] Bombell S, McGuire W. Tumour necrosis factor (-308A) polymorphism in pre-eclampsia: meta-analysis of 16 case-control studies. Aust N Z J Obstet Gynaecol 2008;48:547–51.
[37] Dizon-Townson DS, Major H, Ward K. A promoter mutation in the tumor necrosis factor-alpha gene is not associated with preeclampsia. J Reprod Immunol 1998;38:55–61.
[38] Freeman DJ, McManus F, Brown EA, Cherry L, Norrie J, Ramsay JE, et al. Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. Hypertension 2004;44:708–14.
[39] Livingston JC, Park V, Barton JR, Elfering S, Haddad B, Mabie WC, et al. Lack of association of severe preeclampsia with maternal and fetal mutant alleles for tumor necrosis factor alpha and lymphotoxin alpha genes and plasma tumor necrosis factor alpha levels. Am J Obstet Gynecol 2001;184:1273–7.
[40] Stanczuk GA, McCoy MJ, Hutchinson IV, Sibanda EN. The genetic predisposition to produce high levels of TGF-beta1 impacts on the severity of eclampsia/pre-eclampsia. Acta Obstet Gynecol Scand 2007;86:903–8.
[41] Stonek F, Bentz EK, Hafner E, Metzenbauer M, Philipp K, Hefler LA, et al. A tumor necrosis factor-alpha promoter polymorphism and pregnancy complications: results of a prospective cohort study in 1652 pregnant women. Reprod Sci 2007;14:425–9.
[42] Stonek F, Hafner E, Metzenbauer M, Katharina S, Stumpflen I, Schneeberger C, et al. Absence of an association of tumor necrosis factor (TNF)-alpha G308A, interleukin-6 (IL-6) G174C and interleukin-10 (IL-10) G1082A polymorphism in women with preeclampsia. J Reprod Immunol 2008;77:85–90.
[43] Xie C, Yao MZ, Liu JB, Xiong LK. A meta-analysis of tumor necrosis factor-alpha, interleukin-6, and interleukin-10 in preeclampsia. Cytokine 2011;56:550–9.
[44] Rein DT, Breidenbach M, Honscheid B, Friebe-Hoffmann U, Engel H, Gohring UJ, et al. Preeclamptic women are deficient of interleukin-10 as assessed by cytokine release of trophoblast cells in vitro. Cytokine 2003;23:119–25.
[45] Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998;128:127–37.
[46] Chen Q, Stone P, Ching LM, Chamley L. A role for interleukin-6 in spreading endothelial cell activation after phagocytosis of necrotic trophoblastic material: implications for the pathogenesis of pre-eclampsia. J Pathol 2009;217:122–30.
[47] Chen Q, Stone PR, McCowan LM, Chamley LW. Phagocytosis of necrotic but not apoptotic trophoblasts induces endothelial cell activation. Hypertension 2006;47:116–21.
[48] Molvarec A, Szarka A, Walentin S, Beko G, Karadi I, Prohaszka Z, et al. Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia. Reprod Biol Endocrinol 2011;9:124.
[49] Sharma D, Singh A, Trivedi SS, Bhattacharjee J. Intergenotypic variation of nitric oxide and inflammatory markers in preeclampsia: a pilot study in a North Indian population. Hum Immunol 2011;72:436–9.
[50] Sharma D, Singh A, Trivedi SS, Bhattacharjee J. Role of endothelin and inflammatory cytokines in pre-eclampsia – a pilot North Indian study. Am J Reprod Immunol 2011;65:428–32.
[51] Szarka A, Rigo Jr J, Lazar L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol 2010;11:59.
[52] Jonsson Y, Ruber M, Matthiesen L, Berg G, Nieminen K, Sharma S, et al. Cytokine mapping of sera from women with preeclampsia and normal pregnancies. J Reprod Immunol 2006;70:83–91.
[53] Sutherland GR, Baker E, Callen DF, Hyland VJ, Wong G, Clark S, et al. Interleukin 4 is at 5q31 and interleukin 6 is at 7p15. Hum Genet 1988;79:335–7.
[54] Hunt JS, Soares MJ, Lei MG, Smith RN, Wheaton D, Atherton RA, et al. Products of lipopolysaccharide-activated macrophages (tumor necrosis factor-alpha, transforming growth factor-beta) but not lipopolysaccharide modify DNA synthesis by rat trophoblast cells exhibiting the 80-kDa lipopolysaccharide-binding protein. J Immunol 1989;143:1606–13.
[55] Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986;163:740–5.
[56] Stark JM. Pre-eclampsia and cytokine induced oxidative stress. Br J Obstet Gynaecol 1993;100:105–9.
[57] Mansouri R, Akbari F, Vodjgani M, Mahboudi F, Kalantar F, Mirahmadian M. Serum cytokines profiles in Iranian patients with preeclampsia. Iran J Immunol 2007;4:179–85.
[58] Krasnow JS, Tollerud DJ, Naus G, DeLoia JA. Endometrial Th2 cytokine expression throughout the menstrual cycle and early pregnancy. Hum Reprod 1996;11:1747–54.
[59] Dudley DJ, Hunter C, Mitchell MD, Varner MW. Clinical value of amniotic fluid interleukin-6 determinations in the management of preterm labour. Br J Obstet Gynaecol 1994;101:592–7.