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ORIGINAL ARTICLE

Cytokine gene polymorphisms in preeclampsia and eclampsia

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The clinical spectrum of preeclampsia (PE) ranges from mild hypertension to severe vasospasm associated with convulsions and multiple organ damage. The biological factors that determine the progression of PE to eclampsia (E) are unknown. Endothelial cell activation seems related to an impaired maternal immune response. The production of cytokines, *IL-10* and *TGF-β1,* is apparently suppressed, and altered *IL-2/IL-10* and *TNF-α/IL-10* ratios have been reported in preeclamptic cases. The relationship between PE and cytokine gene polymorphism has been studied, but there are few studies that include eclamptic patients. This study aimed at investigating whether polymorphisms in genes, *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoters (−1082 A>G), (−819 C>T) and (−592 C>A) and *TGF-β1* codon 10 (+869 T>C) and codon 25 (+915 G>C) are associated with E and/or PE. Genotyping was carried out in 266 Mulatto women from the northeastern region of Brazil who were referred to a single maternity hospital: 92 with PE, 73 with E and 101 normotensive controls. The χ^2 or Fisher's exact tests were used to compare genotype frequencies. Among the six single-nucleotide polymorphisms (SNPs) studied, we found no difference in genotype frequencies between the groups. There was a higher frequency of *IFN-γ* (+874 A) in eclamptic patients in comparison with that in controls. (70.3 *vs.* 57.8%, respectively; *P*=0.02). There were no other significant differences in allelic frequencies between eclamptic, preeclamptic and control groups We found no independent association between any single SNP and PE or E risk in this population of Mulatto women from the northeastern region of Brazil.
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INTRODUCTION

Preeclampsia (PE) is a common systemic obstetric disorder and a major cause of maternal and neonatal morbidity and mortality. The clinical spectrum of PE is large, ranging from mild hypertension with minimal proteinuria to severe hypertension associated with convulsions and multiple end-organ damage that can lead to maternal demise.[1] Current knowledge suggests that the underlying physiopathology for eclampsia (E) is a posterior reversible encephalopathy syndrome with vasogenic edema because of hyperfusion rather than vasospasms.[2] The biological factors that determine the progression of PE to E are unknown. General endothelial cell activation is present in both conditions, and seems to be related to an impaired maternal immune response.[3]

Excessive innate activity and a shift toward an inflammatory cytokine profile have been reported in PE.[4,5] Elevated levels of Th-1 cytokines, such as tumor necrosis factor *TNF-α*, *IFN-γ* and low *IL-4* production by phytohemagglutinin-stimulated peripheral blood mononuclear cells have been identified in preeclamptic patients. The production of immunoregulatory cytokines, *IL-10* and *TGF-β1,* is apparently suppressed, and altered *IL-2/IL-10* and *TNF-α/IL-10* ratios have been reported in placental tissue from preeclamptic cases.[6]

Cytokine gene polymorphisms have been associated with certain inflammatory and infectious diseases, including some obstetric disorders.[7–10] The relationship between PE and single-nucleotide polymorphisms (SNPs) in cytokine genes has been investigated, but is still unclear.[11–15]

The controversial results reported by different investigators may in part be because of selection criteria. Genotype frequencies of SNPs and linkage disequilibrium patterns can differ among ethnic groups, leading to different results.[16] Moreover, distinct clinical patterns may involve different pathological mechanisms.[17,18] Few studies have evaluated patients with E.

Considering that cytokine gene polymorphism may influence disease susceptibility, severity and outcome,[7,19,20] our purpose was to investigate whether polymorphisms in genes, *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoters (−1082 A>G), (−819 C>T) and (−592 C>A) and *TGF-β1* codon 10 (+869 T>C) and codon 25 (+915 G>C) are associated with E and/or PE in a northeastern Brazilian population.

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METHODS

All patients were Mulatto (of mixed white and black ancestry) and lived in the same geographical location (Maceio, Alagoas, Brazil). All women with a single live pregnancy and who were referred to a single hospital (Santa Monica Obstetric Clinic) were eligible.

This case–control study included 92 women with PE, 73 patients with E and 101 healthy women (control). The control women had to be normotensive in the index pregnancy, with a history of at least one previous normal pregnancy, without any maternal or fetal disorder.

The diagnoses of PE and E were based on the criteria of the Report of the 'National High Blood Pressure Education Program.'[21]

Exclusion criteria were multiple gestation, fetal death, autoimmune disease, diabetes, uterine malformation, *in vitro* fertilization treatment, placental abruption, any infection, cancer or any other systemic disease, including preexisting hypertension.

On admission, 5 ml of venous blood was drawn from each participant. The samples were collected in tubes containing ethylenediamine tetraacetic acid (Sigma-Aldrich Corp., St Louis, MO, USA), immediately centrifuged to separate the buffy coat containing polymorphonuclear cells and frozen at −20°C for later DNA extraction.

The study protocol was approved by the ethics committee of Alagoas State University. Written informed consent was obtained from each participant. Experimental procedures on humans followed the ethical standards for human experimentation that were established by the Declaration of Helsinki of 1975, revised in 1983.

Genotyping

DNA was extracted from whole blood by the dodecyl trimethyl-ammonium bromide/cetyl trimethyl-ammonium bromide (DTAB/CTAB, Sigma-Aldrich Corp.) technique.[22] Cytokine genotyping was carried out by the PCR sequence-specific primer method, using the 'Cytokine Genotyping Tray' (One Lambda Inc., Canoga Park, CA, USA), according to the manufacturer's instructions. The polymorphisms analyzed in this study were *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoters (−1082 A>G), (−819 C>T) and (−592 C>A) and *TGF-β1* codon 10 (+869 T>C) and codon 25 (+915 G>C).

Following the kit's manufacturer's suggestion and literature recommendations,[23–25] cytokine genotype and phenotype were grouped as follows: for the *TNF-α* gene, the alleles were distributed as A/A and A/G (high) and G/G (low); for the *IL-6* gene, the groups were G/G and G/C (high) and C/C (low); for the *IL-10* gene, the alleles for each polymorphism were grouped as GCC/GCC (high), GCC/ATA and GCC/ACC (intermediate) and ACC/ACC and ACC/ATA (low); for the *IFN-γ* gene, the alleles were distributed as T/T (high), T/A (intermediate) and A/A (low); and for the *TGF-*β1 gene, the groups were T/T G/G and T/C G/G (high), T/C G/C and C/C G/G (intermediate) and C/C G/C, C/C C/C, T/T C/C and T/C C/C (low).

Statistical analysis

Sample size estimates were based on a reported 67% frequency of *TNF-α* (308G) in the general population and a 43% frequency of *TNF-α* (−308G) in patients with increased susceptibility to E.[26] Assuming a similar difference in *TNF-α* (−308G) frequencies between patients and controls, a two-tailed-α of 0.05 and 80% power, a sample size of 67 per group would be sufficient to detect an association between this allele and E/PE. We selected this specific *TNF-α* SNP for sample size calculation as it is the most frequently assessed in PE studies.[12–14,26,27]

Hardy–Weinberg equilibrium tests were carried out by calculating the expected frequencies of each genotype and comparing them with the observed values. Arlequin software (Geneva, Switzerland) was used for these analyses.[28] Single allelic and single genotype frequencies (obtained by direct count) were analyzed by Fisher's exact or χ^2 tests, with the level of significance set at $P \leqslant 0.05$. Odds ratios and 95% confidence intervals were calculated.

RESULTS

The clinical characteristics of preeclamptic, eclamptic and control women are presented in Table 1.

Linkage disequilibrium was detected between the three alleles of the *IL10* promoter, (−1082 A>G), (−819 C>T) and (−592 C>A), and between the two alleles of *TGF-β1,* codon 10 (+869 T>C) and codon 25 (+915 G>C) in all patient and control groups, confirming the earlier findings.[29]

All SNPs in PE, E and in control patients were in Hardy–Weinberg equilibrium. Genotyping data are presented in Table 2. Among the six SNPs studied, we found no difference in genotype frequencies between the groups.

We detected a higher frequency of *IFN-γ* (+874 A) in eclamptic patients in comparison with that in controls. (Fisher's exact test: 70.3 vs. 57.8%, respectively; P=0.02). There were no other significant differences in allelic frequencies between eclamptic, preeclamptic and control groups (Table 3).

DISCUSSION

We did not find an association between polymorphisms in genes, *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoters (−1082 A>G), (−819 C>T) and (−592 C>A) and *TGF-β1* codon 10 (+869 T>C) and codon 25 (+915 G>C) and E or PE.

These results are in line with other publications,[12,13,30–33] including our earlier investigation.[11] In that study, we evaluated cytokine gene polymorphisms in preeclamptic and control women of three different ethnic groups, and there was a significant association between PE and *IL-10* (−1082-G/G) in white women.

The significantly higher frequency of *IFN-γ* (+874 A) in eclamptic patients in comparison with that in controls was unexpected and could be because of chance. We did not detect a corresponding expression in genotype frequency. This finding should be interpreted with caution as it is the first time that this SNP is being investigated in patients with E. This finding needs to be further investigated.

Table 1 Clinical characteristics of women with preeclampsia, eclampsia and controls[a]

Variable	*Preeclampsia* (n=*92*)	*Eclampsia* (n=*73*)	*Controls* (n=*101*)	(*PE* vs. *C*)[b]	(*E* vs. *C*)[b]
Age (years)	23 (19–28)	18 (17–21)	26 (21–31)	<0.0065	<0.0001
Prepregnancy BMI	23.43 (22.90–25.91)	24.27 (22.59–27.30)	23.02 (22.36–23.38)	0.14	0.32
SBP (mm Hg)	160 (150–180)	160 (140–180)	110 (110–120)	<0.0001	<0.0001
DBP (mm Hg)	110 (100–120)	110 (100–120)	70 (60–80)	<0.0001	<0.0001
GA delivery (weeks)	37 (34–39)	37 (34–39)	39 (39–40)	<0.0001	<0.0001
Birth weight (g)	2450 (1760–3100)	2500 (1890–2995)	3200 (2950–3970)	<0.0001	<0.0001

Abbreviations: BMI, body mass index (kg m^{-2}); DBP, diastolic blood pressure; E, eclampsia; GA, gestational age; PE, preeclampsia; SBP, systolic blood pressure.
[a]Data are expressed as median and interquartile ranges (25th–75th percentiles).
[b]Mann–Whitney test.

Table 2 Genotype frequencies of the *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoter (−1082 A>G), (−819 C>T), (−592 C>A) and *TGF-β1* codon 10 (+869 T>C), codon 25 (+915 G>C) polymorphisms in women with preeclampsia, eclampsia and controls[a]

	Eclampsia			*Preeclampsia*			*Controls*	
Gene (position) genotype	N	%	P	N	%	P	N	%
TNF-α (−308 G>A)	64			88			97	
GG	54	84.3		69	78.4		71	73.1
GA	9	14.2	0.24	16	18.2	0.49	24	24.7
AA	1	1.5		3	3.4		2	2.2
IFN-γ (+874 T>A)	64			85			96	
TT	6	9.3		7	8.2		18	18.8
TA	26	40.6	0.08	46	54.2	0.12	45	46.8
AA	32	50.1		32	37.6		33	34.4
IL-6 (−174 G>)	60			82			93	
GG	31	51.7		54	65.8		56	60.3
GC	21	35.0	0.30	24	29.3	0.72	31	33.3
CC	8	13.3		4	4.9		6	6.4
TGF-β (+10 T>C)	52			78			97	
TT	13	25.0		22	28.3		25	25.8
TC	31	50.6	0.97	42	53.8	0.87	56	57.7
CC	8	15.4		14	17.9		16	16.5
TGF-β (+25 C>G)	52			78			97	
GG	42	80.8		68	87.2		82	84.5
GC	10	19.2	0.72	10	12.8	0.77	15	15.5
CC	00			00			00	
IL10 (−1082 G>A)	62			88			95	
GG	3	4.8		5	5.6		8	8.4
GA	30	48.4	0.67	36	40.9	0.35	46	48.4
AA	29	46.8		47	53.5		41	43.2
IL10 (−819 C>T)	62			88			95	
CC	21	33.8		39	44.3		40	42.1
CT	32	51.6	0.19	36	40.9	0.11	49	51.6
TT	9	14.6		13	14.8		6	6.3
IL10 (−592 C>A)	62			88			95	
CC	21	33.8		39	44.3		40	42.1
CA	32	51.6	0.19	36	40.9	0.11	49	51.6
AA	9	14.6		13	14.8		6	6.3

[a]χ^2 test, Fisher's exact test.

We selected SNPs in cytokine genes that are known to influence cytokine production.[23,24] However, the protocol used to measure cytokine, the interactions between genes and environmental factors involved in the activation of these genes may also affect cytokine production. Therefore, the relationship between genotype and phenotype cannot always be clearly demonstrated.[25]

PE and E are considered as expressions of the same syndrome, with E being the most severe form of PE. Although other studies have investigated the relationship between PE and cytokine gene polymorphism, few have evaluated eclamptic patients. The PE syndrome is a state of excessive inflammatory response. However, both excessive Th1 (pro-inflammatory) and Th2 (anti-inflammatory) cytokine productions have been associated with PE.[3–5,34,35] Hence, although we selected only patients with PE, we may have evaluated a heterogeneous population, including women with different cytokine and genetic profiles.

Polymorphisms in *IL6*, *IL10*, *IFN-γ* and *TGF-β1* genes in preeclamptic patients have been investigated earlier, but most of these studies involved the analysis of SNPs in the *TNF-α* gene. Several polymorphisms in the promoter region of the *TNF-α* gene have been described. Specifically, the *TNF-α* −308 G>A polymorphism has been extensively evaluated because of functional significance, and discordant results have been reported.[11–14,27,36–38]

Similarly, there is no consensus regarding cytokine production and PE/E. There are studies indicating a consistent increase in *TNF-α* production,[39] others reporting that a part of these patients exhibit

Table 3 Allele frequencies of the *TNF-α* promoter (−308 G>A), *IL6* promoter (−174 G>C), *IFN-γ* intron 1 (+874 A>T), *IL10* promoter (−1082 A>G), (−819 C>T), (−592 C>A) and *TGF-β1* codon 10 (+869 T>C), codon 25 (+915 G>C) polymorphisms in women with preeclampsia, eclampsia and controls[a]

	Eclampsia				*Preeclampsia*				*Controls*	
Gene (position) allele	N	*%*	P	*OR (IC 95%)*	N	*%*	P	*OR (IC 95%)*	N	*%*
TNF-α (−308 G>A)	128				176				194	
G	117	91.4			154	87.5			166	85.6
A	11	8.6	0.16	1.7 (0.8–3.7)	22	12.5	0.64	1.1 (0.6–2.1)	28	14.4
IFN-γ (+874 T>A)	128				170				192	
T	38	29.7			60	35.3			81	42.2
A	90	70.3	0.02[a]	0.5 (0.3–0.9)	110	64.7	0.19	0.7 (0.4–1.1)	111	57.8
IL-6 (−174 G>C)	120				164				186	
G	83	69.1			132	80.5			143	76.9
C	37	30.9	0.14	0.6 (0.4–1.1)	32	19.5	0.43	1.2 (0.7–2.0)	43	23.1
TGF-β (+10 T>C)	104				156				194	
T	57	54.8			86	55.1			106	54.6
C	47	45.2	1.00	1.0 (0.6–1.6)	70	44.9	1.00	1.0 (0.6–1.5)	88	45.4
TGF-β (+25 C>G)	104				156				194	
G	94	90.4			146	93.6			179	92.3
C	10	9.6	0.66	0.7 (0.3–1.8)	10	6.4	0.68	1.2 (0.5–2.8)	15	7.7
IL10 (−1082 G>A)	124				176				190	
G	36	29.1			46	26.2			62	32.6
A	88	70.9	0.53	0.8 (0.5–1.3)	130	73.8	0.20	0.7 (0.4–1.1)	128	67.4
IL10 (−819 C>T)	124				176				190	
C	74	59.7			114	64.7			129	67.9
T	50	40.3	0.14	0.6 (0.4–1.1)	62	35.3	0.58	0.8 (0.5–1.3)	61	32.1
IL10 (−592 C>A)	124				176				190	
C	74	59.7			114	64.7			129	67.9
T	50	40.3	0.14	0.6 (0.4–1.1)	62	35.3	0.58	0.8 (0.5–1.3)	61	32.1

Abbreviations: CI, confidence interval; IL, interleukin; IFN, interferon; OR, odds ratio; TNF, tumor necrosis factor.
[a]Fisher's exact test, χ^2-test.

high TNF levels,[40] whereas some investigators did not detect any change in cytokine levels.[41] These discrepancies have been reported for almost all cytokines.[42]

These several conflicting findings could be because of heterogeneity in study designs, definition of phenotype, SNP selection, population diversity and sample size. These factors interfere with the interpretation of the results, especially in a complex disease such as PE.

Multiple mechanisms and mediators are involved in the development of PE; therefore, this disorder can be associated with more than one cytokine and with other non-cytokine genes.[43,44] Moreover, gene–gene interaction and environmental factors are likely to play a role in determining an individual's risk for a disease.[29,45–47] These interactions can be so important that they can obscure eventual associations between gene polymorphisms and the disease and lead to discordant results in different studies involving the same disorder.

To circumvent possible ethnic interference, we included only Mulatto women from a single geographical location. This is the first study that evaluates the relationship between six different cytokine polymorphisms in preeclamptic and eclamptic patients and healthy women without any obstetric or systemic disease. One of the limitations of this study is the small sample size. However, the size was sufficient to detect (with an 80% power) a possible association between cytokine gene polymorphism and E/PE, in accordance with earlier studies.[26,27,48]

These findings need to be replicated in future studies. The role of these cytokine gene SNPs in PE/E has to be further investigated, because they may be involved with etiology and not with susceptibility to disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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