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IL-6, IL-10 and TNF-α gene polymorphisms in preeclampsia: a case-control study in a Mexican population

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ABSTRACT

Objectives: We focused our study on examining the genotype and allele frequency of IL-6 (rs1800795), TNF-α (rs1800629) and IL-10 (rs1800872) single nucleotide polymorphisms (SNP) on preeclampsia (PE) diagnosed Mexican pregnant women.

Material and methods: A case-control study was designed including 86 preeclampsia patients and 100 normotensives pregnancies from Women's Hospital of Culiacan, Mexico. Genotyping of IL-6, TNF-α and IL-10 was performed using TaqMan SNP Genotyping.

Results: Not significant association was found between development of PE and genotypic ($p > 0.05$) and allelic ($p > 0.05$) frequencies of IL-6, TNF-α and IL-10 SNPs. Genotype distributions of IL-6 ($p = 0.599$), TNF-α ($p = 0.721$) and IL-10 ($p = 0.761$) polymorphisms in the two groups were in agreement with Hardy–Weinberg equilibrium.

Conclusions: According to the findings, the IL-6, TNF-α and IL-10 SNPs are not exponents of susceptibility to developing PE.
Keywords: IL-6; IL-10; TNF-α; preeclampsia; SNP

INTRODUCTION

Preeclampsia (PE) is a syndrome that affects women during pregnancy and delivery by increasing arterial pressure and, in some cases, multiorgan affectations. Daily, this complication is responsible for the death of approximately 830 women worldwide, [1] with 75% of maternal mortality being related to preeclampsia (PE). This syndrome affects between 2 and 8% of pregnancies and characterizes by a new-onset hypertension (> 140/90 mmHg) in the second half of pregnancy, coexisting with either proteinuria (> 300 mg/24 h) or multi-organ (kidney and liver failure) dysfunctions, neurological complications, thrombocytopenia and/or hemolysis [2]. For the newborn, PE has been associated with intrauterine growth restriction, neonatal hard breading syndrome, hypoxic-ischemic encephalopathy, bronchopulmonary dysplasia, and other complications [3].

The etiology of PE is still unknown. Predisposition to PE is likely multifactorial and polygenic. Preeclampsia had been previously associated with dysregulation of several gene and biological pathways in the basis of inflammation and remodeling activities, such as regulation of actin cytoskeleton [4]. Moreover, there are several studies that investigated the role of mutated genes, such as AGT, ACE, AGTR1, AGTR2, TNF, that exert implications in PE pathological processes, such as coagulation, vascular resistance, and metabolism, as well as inflammatory processes [5–7], of which 30–35% are attributed to maternal genotype, 20% fetal, 13% to the couple, and the rest to other effects [8].

It is believed that PE results from defective spiral artery remodeling, which leads to an imbalance between anti- and pro-angiogenic factors, resulting in a hypo-perfused placenta and the release of placental-derived factors causing maternal widespread endothelial dysfunction and organic failure. The main cause of the abnormal placentation remains unclear, but genetic, environmental, and immunological factors have been studied [9]. For instance, there is evidence supporting that both innate and adaptive immune responses participate in the pathogenesis of PE, suggesting that Th1 immunity is responsible for poor placentation and exacerbated inflammatory response and endothelial dysfunction seen in PE [10]. Preeclampsia has been associated with chronic immune activation that leads to an increased production of inflammatory cytokines by pro-inflammatory T cells, and a decrease

in regulatory and anti-inflammatory cytokines, which further promotes an inflammatory state during PE [11].

Th1/Th2 cytokine balance is important to maintain the success of normal pregnancy. In normal pregnancy, the production of Th1 cytokine is inhibited, and their overexpression predisposes to PE development. In this regard, Interleukin-6 (IL-6) is a Th2, multifunctional pleiotropic pro-inflammatory cytokine and plays a central role in the regulation of immune responses [12]. IL-6 represents one of the main mediators of the acute phase, stimulates T lymphocytes, induces the production of C reactive protein, and regulates the production of human chorionic gonadotropin. It also maintains the pregnancy through an immunosuppressive effect, although its expression is elevated during delivery. IL-6 plays an important role in trophoblastic proliferation, invasion and differentiation, and increased levels of this cytokine have been reported in PE pregnancy [13]. Tumor Necrosis Factor alpha (TNF-α) is a Th1-polarizing pro-inflammatory cytokine implicated in maternal endothelial dysfunction leading to PE, impairing placental formation, and deregulating the balance of vasodilatation and vasoconstriction present in maternal circulation [14]. Even more, both IL-6 and TNF-α have been found elevated in Latin-American preeclamptic patients [15]. In contrast, IL-10 is a potent immunosuppressive cytokine, which has been considered a key modulator of immune tolerance at maternal-fetal communication promoting successful placentation, controlling inflammation, and regulating vascular function. In this case, decreased levels of IL-10 in placenta tissues and serum samples have been associated to PE [16].

Previously, cytokine gene alterations, such as single nucleotide polymorphism (SNPs), had been related to physiopathology in some inflammatory and obstetric complications. In this regard, SNPs in the proximal and distal region of IL-10 promoter (-1082 A/G, -819 T/C y -592 A/C) [17] are known to work as transcriptional regulators. -1082 A/G has been related to lower IL-10 production [18], higher risk of PE [19] and early PE onset in an Indian population [20]. Additionally, -819 T/G has been associated with lower IL-10 production [20], higher risk of PE in a Chinese population [21] and early onset PE on Indians [20]. Other studies have associated -592A/C SNP with lower IL-10 production [22] and increased risk for PE in an Iranian population [23]. For TNF-α, evidence suggest an association between the transcriptional regulation of SNPs in its promoter region (-308 G/A, -238G/A) [24] and high levels of TNF-α in PE [25]. In contrast, IL-6 SNPs have yet to be associated with PE.

Considering this, we decided to explore the association between PE and some relevant cytokine SNPs for IL-6 (rs1800795), TNF-α (rs1800629) and IL-10 (rs1800872), in Mexican

population. In that respect, the relevance of this investigation relies on the necessity for understanding the roles of genetic variants and their participation on inflammatory processes of PE.

MATERIAL AND METHODS

Study participants

Written informed consent was obtained from all individual participants included in the study. To calculate the size of the samples of interest, the formula was used to estimate the proportion of cases and controls with a power of 80%, significance level of $p \leq 0.05$, and a loss value of the samples of 5% calculated a sample size of 86 individuals per group, for this reason, 186 pregnancies included in our case-control study, 100 normotensives with no complications and 86 PE patients. Biological samples and corresponding clinical data were collected from May 2018 to December 2019.

All procedures performed in a study involving people comply with the ethical standards of the institutional and/or national committee for research ethics and the 1964 Helsinki Declaration and its subsequent changes or comparable ethical standards. Our study was approved by Human Research Committee of Women's Hospital in Culiacan, Mexico (registry number #2018–032).

DNA isolation

Deoxyribonucleic acid (DNA) isolate was performed using whole blood sample with ethylenediaminetetraacetic acid (EDTA) anticoagulant following Gustincich protocol [26]. Deoxyribonucleic acid samples were quantified using NanoDrop One (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and their integrity was verified in 1.5 % agarose gels electrophoresis.

TaqMan genotyping assays

Real-time PCR was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) and TaqMan Universal PCR Master Mix (Thermo Fisher Scientific) for the following SNPs: IL-6 (rs1800795, -237C>G, assay ID C__1839697_20. Applied Biosystems, CA, USA), TNF-α (rs1800629, -488G>A, assay ID C__7514879_10. Applied Biosystems, CA, USA) and IL-10 (rs1800872, -627T>G, assay ID C__1747363_10. Applied Biosystems, CA, USA). The mixtures were incubated at 60°C for 30 s, then 95°C 10 min, followed by 40 cycles of 95° C for 15 s and 60° C for 1 min, and finally post read at 60°

C for 30 s. Automated genotype calling was done using the TaqMan Genotyper Software v1.3.

Database analysis

Different databases such as dbSNP by National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/snp/), the Population Architecture using Genomics and Epidemiology (PAGE) (https://www.pagestudy.org/) and SNPedia (https://www.snpedia.com/) were used to investigate variant details, clinical significance, and frequency of SNPs in the study.

Statistical analysis

Data was presented as mean ± standard deviation (SD) and frequencies. Differences in allelic and genotypic frequencies were evaluated using Pearson's Chi-squared test. SNPs associations were tested under dominant and recessive genetic models and odds ratios (OR) with 95% confidence intervals (CI) were used as the measure of association between specific alleles and genotypes with PE and its clinical subtypes. Hardy-Weinberg's equilibrium was calculated by Chi-squared test for all genotypic combinations of each SNP in patients and controls. PASW v20.0 (SPSS inc., Chicago, IL, USA) software package was used for analysis.

RESULTS

Table 1 summarizes the clinical characteristics of the study subjects. The mean of age of cases were similar to that of the control group (24.8 ± 6.4 vs 24.7 ± 7.2 years, respectively). Differences in the means of weight at last trimester of the groups studied were observed, 83.4 + 18.1 kg for cases, and 74.4 + 13.1 kg for the control group, with evident statistical differences ($p < 0.001$). These results impacted in body mass index (BMI) which were 27 + 6.4 vs 25.2 + 5.3 kg/m^2 for cases and control groups, respectively, presenting a statistical significance ($p = 0.041$). Cases showed higher blood pressure than the control group, in both systolic (144 + 16 vs 115 + 14 mmHg; $p < 0.001$) and diastolic (94 + 10 vs 74 + 9 mmHg; $p < 0.001$) measures. Interestingly, we found that 72.9% (62) of the total cases of newborns were at term and, there were no significant statistics between preterm birth and PE ($p = 0.659$). In the case of urinary protein, levels were higher in case groups than in the control group (1248.2 + 1863.1 vs 257.3 + 119.7 mg/24 h) observing statistical differences (p

< 0.001). Finally, the platelets were observed to have statistical differences as well (211.7 + 79.5 vs 237 + 69.7 103/mm^3 for cases and controls, respectively; p = 0.025).

Table 2 shows the distribution of genotypes and allele frequencies of IL-6 (-237C>G), TNF-α (-488G>A), and IL-10 (-627T>G) for the preeclamptic and normotensive patients. All frequencies for the three SNPs were similar between cases and controls (p > 0.05). Moreover, genotype distributions of IL-6 - (p = 0.599), TNF-α (p = 0.721) and IL-10 (p = 0.761) SNPs in the groups were in agreement with Hardy–Weinberg equilibrium. Interestingly, our allele frequencies results are similar to those reported in dbSNP by NCBI for Mexican (IL-6 and TNF-α) and Latin American population (IL-10), where IL-6 (-237C>G) shows allelic frequencies as follow A: 15% vs a: 85%, TNF-α (-488G>A) A: 93% vs a: 7% and IL-10 (-627T>G) A: 30% vs a: 70%.

DISCUSSION

As expected, in the case group, significant statistical differences were observed in the last trimester of pregnancy in the mother's weight, BMI, blood pressure, urine protein content, and platelets, in comparison to control group, as reported in most studies.

The role of inflammation in the pathogenesis of preeclampsia has been the object of recent studies. In this regard, a study described elevated levels of inflammatory markers such as TNF-α, IL-6, and C-reactive protein in preeclamptic patients and demonstrated that Latin-American women present a higher degree of inflammation than women from developed countries [15]. Interestingly, the induction of both IL-6 and TNF-α in these PE patients was attributed to chronic subclinical infections and insulin resistance [15]. The IL-6 (rs1800795) SNP has been associated with diseases such like chronic obstructive pulmonary disease, higher risk of death after an acute coronary syndrome and atherosclerosis [27]. As with our observations, IL-6 (-237C>G) SNP studied in other populations [28] have shown not association with PE (p = 0.969).

A study genotyped 503 tagSNPs in 40 genes related to inflammation on a cohort of pregnant African American mothers finding no association between IL-6 and PE; the same study found gene-level association of an upstream regulator of TNF-α with PE among European Americans. Their conclusion was that despite previous studies have suggested null associations, increased tagging and stratification by genetic ancestry suggests important association between TNF-α regulator and PE among European Americans [29]. TNF-α (rs1800629) SNP has been associated with some conditions such as acute kidney allograft rejection and acute myocardial infarction. Specifically, this SNP has been associated with PE

in Turkish, Iranian, and Finnish populations [23, 30]. In line with previous reports [31, 32], our study did not find an association between PE and TNF-α (488G>A) polymorphism (p = 0.649); perhaps not surprisingly considering the native-American genetic ancestry of our Mexican cohort.

The IL-10 (rs1800872) SNP has been associated with some diseases as squamous intraepithelial cervical lesions and PE, among others [33]. Our data did not find an association between PE and IL-10 (-627T>G) polymorphism (p = 0.573), agreeing with previous reports in Japanese and Iranian populations [34, 35]. Nevertheless, some studies show different results in the Chinese and Iranian population [13, 36]. Another study investigating association between PE and cytokine gene polymorphisms in Brazilian Mulatto women from the northeastern region of Brazil found no difference in genotype or allelic frequencies for TNF-α promoter (-308 G>A), IL6 promoter (-174 G>C), IFN-gamma intron 1 (+874 A>T), IL10 promoters (-1082 A>G), (-819 C>T) and (-592 C>A) and TGF-beta1 codon 10 (+869 T>C) and codon 25 (+915 G>C) [34].

It is well known that genetic variability between ethnic populations exists. While some SNPs may be more prevalent in some specific groups, contradictory reports on inflammatory cytokine SNPs associated to PE may also relate to unforeseen variables such as subclinical infections and chronic immune disorders. Of notice, while all pregnant women of our study were recruited in Culiacan city, a wide ethnic genetic diversity of our cohort was ensured by the fact that this hospital is the only concentrating Women Hospital in the public health systems across the entire state of Sinaloa, even receiving patients from neighboring states.

It is known that the up-regulation of pro-inflammatory cytokines such as IL-6, TNF-α, and downregulation of anti-inflammatory IL-10, may increase inflammatory response in pregnancies, causing an exacerbated immune system response, contributing to abnormal placentation and consequent hypoperfused placenta, conditions that have been widely recognized in preeclampsia.

CONCLUSIONS

There were no differences of IL-6 (rs1800795), TNF-α (rs1800629) and IL-10 (rs1800872) in genotypes and allele frequencies of the SNPs between preeclamptic and normotensives patients, ruling out their participation in the disease of this population. Moreover, genotype distributions for IL-6, TNF-α and IL-10 SNPs in both groups were in agreement with Hardy–Weinberg equilibrium.

Article information and declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Table *1*. Clinical characteristics of cases and controls groups *[m*ean ± *standard deviation (*SD)*]*

Variable	**Cases n = 86**	**Controls n = 100**	**p value**
Age *[*years*]*[a]	24.8 ± 6.4	24.7 ± 7.2	0.665
Weight last trimester *[*kg*]*[b]	83.4 ± 18.1	74.4 ± 13.1	< 0.001
BMI *[*kg/m^2*]*[b]	27.0 ± 6.4	25.2 ± 5.3	0.041
Systolic BP *[*mmHg*]*[a]	144 ± 16	115 ± 14	< 0.001
Diastolic BP *[*mmHg*]*[a]	94 ± 10	74 ± 9	< 0.001
Weeks at delivery[a]	37.6 ± 2.1	37.3 ± 3.5	0.731
Newborn birth weight *[*kg*]*[b]	3.0 ± 0.8	3.1 ± 0.6	0.804
Size of newborn *[*cm*]*[a]	49.6 ± 3.0	49.0 ± 3.9	0.367
24-h urinary protein *[*mg/24 h*]*[a]	1248.2 ± 1863.1	257.3 ± 119.7	< 0.001
Urea *[*mg/dL*]*[a]	17.3 ± 6.8	17.1 ± 7.7	0.829
Creatinine *[*mg/dL*]*[a]	0.7 ± 1.6	0.7 ± 0.4	0.785
Platelets *[*10ˆ3/mm^3*]*[b]	211.7 ± 79.5	237 ± 69.7	0.025
CRP *[*mg/dL*]*[a]	2.8 ± 5.0	5.0 ± 8.8	0.254
LDL cholesterol *[*mg/dL*]*[b]	134.5 ± 55.9	193.0	0.550
AST *[*U/L*]*[a]	25.1 ± 10.8	28.0 ± 10.4	0.284
ALT *[*U/L*]*[a]	25.7 ± 18.3	26.6 ± 14.4	0.831

[a]Mann-Whitney U statistical model*;* [b]t-student statistical model*;* BMI — *b*ody mass index*;* BP — *b*lood pressure*;* CRP — C-reactive protein*;* LDL cholesterol — low-density lipoprotein cholesterol*;* ALT — alanine aminotransferase*;* AST — aspartate aminotransferase

Table *2*. Gene and allele frequencies of IL-6, TNF-α and IL-10

	Genotype frequencies					**Allele frequencies**			
	NH	HT	MH	Total	**p value[a]**	A	a	Total	**p value[a]**

	# (%)	# (%)	# (%)	# (%)		# (%)	# (%)	# (%)	
IL-6 (-237C>G)									
Cases	2 (2.3)	26 (30.2)	58 (67.4)	86 (100)	0.969	30 (17.4)	142 (82.6)	172 (100)	0.890
Controls	2 (2.0)	29 (29.0)	69 (69.0)	100 (100)		33 (16.5)	167 (83.5)	200 (100)	
TNF-α (-488G>A)									
Cases	73 (84.9)	13 (15.1)	0 (0)	86 (100)	0.649	159 (92.4)	13 (7.6)	172 (100)	0.849
Controls	84 (84.0)	15 (15.0)	1 (1.0)	100 (100)		183 (91.5)	17 (8.5)	200 (100)	
IL-10 (-627T>G)									
Cases	16 (18.6)	37 (43.0)	33 (38.4)	86 (100)	0.573	69 (40.1)	103 (59.9)	172 (100)	0.347
Controls	21 (21.0)	48 (48.0)	31 (31.0)	100 (100)		90 (45.0)	110 (55.0)	200 (100)	

NH — normal homozygous; HT — heterozygous; MH — mutated homozygous; [a]Pearson's Chi-squared test

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