Investigation of rs1800469 and rs1800468 Polymorphisms of the *TGF- β1* Gene in Women with Pre-eclampsia

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Abstract

Objectives: to verify the contribution of polymorphisms rs1800469 and rs1800468 of the TGF-β1 gene and the risk factors for the pre-eclampsia development.

Methods: this is a case-control study with 257 women from the Uberaba region of Minas Gerais were selected, 88 of them were in the pre-eclampsia group and 169 in the control group. Genotyping was performed by allelic discrimination using the real-time PCR technique. The odds ratio and the 95% confidence interval were used to evaluate the probability of the polymorphisms studied contributing for the pre-eclampsia development. The logistic regression analysis was performed to evaluate the relation among family recurrence, smoking, primiparity and the presence of polymorphic alleles and susceptibility of pre-eclampsia.

Results: no association was found between polymorphisms rs1800469 and rs1800468 of the TGF-β1 gene and pre-eclampsia. The logistic regression analysis was statistically significant for family recurrence, showing that women with a family history of pre-eclampsia and primiparity are at an increased risk of developing the disease.

Conclusions: no association was found between polymorphisms rs1800469 and rs1800468 of the TGF-β1 and pre-eclampsia gene. Factors such as family history and primiparity were associated to the risk of developing pre-eclampsia.

Key words *Pre-eclampsia, Transforming Growth Factors, Genetic polymorphism*

Introduction

Pre-eclampsia (PE) is a hypertensive syndrome specific to human pregnancy whose clinical signs and symptoms occur after the twentieth week of pregnancy. It is characterized by the increased blood pressure levels (systolic blood pressure ≥ 140mmHg and diastolic blood pressure ≥ 90mmHg, measured on two occasions with intervals of at least 4 hours between them) and proteinuria > 300mg / 24 hours or ≥ 1+ protein detected in the urine analysis, type I).[1]

The risk factors the predispose for the onset of PE include the extreme of reproductive age, primiparity, extended time between pregnancies, increased body mass index, family history of PE, pre-existing hypertension, obesity, metabolic syndrome and *diabetes mellitus*.[2]

There is no consensus on what triggers PE, and it is considered to be the disease of theories. However, it is known that there is the participation of genetic, inflammatory and immunological factors. Among the genetic factors, the interaction between gene-environment occurs more in relatives of affected individuals than in the general population, since members of the same family share besides the genetic information, share the same environmental factors.[3]

In concern of the immunological factors, many cytokines appear as candidates in the development of PE, including the Transforming Growth Factor Beta-1 (*TGF-β1*).[4]

The *TGF-β1* is a multifunctional cytokine that has the potential to alter the balance between the syncytiotrophoblast and the invasive cytotrophoblast, in which the normal pregnancy development depends and contributes to the regulation of the maternal immune response against the fetal allograft.[5] The mean levels of *TGF-β1* seem to increase in patients with PE compared to normotensive women.[6]

Polymorphisms rs1800468 and rs1800469 are located in the promoter region of the *TGF-β1* gene and have the function of regulating expression levels of protein and are capable of triggering alterations in the splicing sites,[7] due to the exchange of the bases of these two variants in the beginning of the gene transcription.[8]

The presence of the polymorphic allele A of the polymorphism rs1800468 interrupts a binding consensus of the protein to the Camp Response Element (CRE) nuclear transcription factor, which causes a lower production of the total *TGF-β1* in the circulation.[9] Also, polymorphism rs1800469 appears

to be associated to an increased transcriptional activity,[10] raising circulating levels of *TGF-β1*.[8,9]

Considering that the *TGF-β1* gene exerts its influence in numerous of physiological and pathophysiological processes, such as fetal development, inflammatory processes, immune system suppression and the appearance of hypertension, it is interesting to evaluate the contribution of polymorphisms in this gene and the development of PE.[11]

Therefore, the objective of this work was to verify the contribution of polymorphisms rs1800469 and rs1800468 of the *TGF-β1* gene and the risk factors for the PE development.

Methods

It is a case-control study, where 257 women aged 18-45 years old were selected, with no history of hypertension, kidney or other chronic diseases. The study group was consisted of 88 women diagnosed with PE according to the classification criteria of the American College of Obstetricians and Gynecologists.[1] The mean age of the study group was 27.5 years old (± 6.6), the mean systolic blood pressure (SBP) was 155.4mmHg (± 22.4) and diastolic blood pressure (DBP) was 106.6mmHg (± 14.6). In relation to smoking, 12.5% of the PE group were smokers and 27.4% of the study group had a family history of PE. The control group was consisted of 169 women without any type of intercurrence. The mean age of the control group was 31.8 years old (±7.8), the mean SBP was 114.3mmHg (±11.6) and the DBP was 75.6mmHg (± 13.6). Regarding to smoking, 14.6% of the control group were smokers and the family history with PE was 5.3%.

All the participants were selected at the Maria da Glória Ambulatory and/or at the *Hospital de Clínicas da Universidade Federal do Triângulo Mineiro* (HC-UFTM) (Clinical Hospital of the Federal University in Triângulo Mineiro) and had to sign the Consent Form Term (TCLE). This project was approved by the Ethics and Research Committee at the UFTM under the document number: CEP/UFTM-CAAE 44460115.1.0000.5154.

10 mL of peripheral blood were collected from each study participant and the DNA extraction was performed by using the phenol-chloroform method.[12]

Genotyping of the samples for the polymorphisms rs1800469 and rs1800468 of the *TGF-β1* gene was done by real-time PCR allelic discrimination with TaqMan® hydrolysis probes (Applied Biosystems, USA), (Numbers of the assays:

C8708473_10; C8708474_20, respectively) . The equipment used for genotyping was Step One Plus (AB) and the conditions for each cycle were: 95ºC for 10 minutes and 40 amplification cycles of 95ºC for 15 seconds and 60ºC for 1 minute. At each cycle, the equipment analyzed the fluorescence emitted from the wild and mutant alleles, labeled as FAM and VIC.

The genetic models and haplotypes were analyzed by using SNPStat software. The Chi-square test (χ^2) was used to test whether the genotypic distributions followed the Hardy-Weinberg Equilibrium (HWE). The multiple logistic regression model was used to determine the effect of risk factors and the PE. The models included the following data: smoking habit (reference: non-smoker), family history of PE (reference: no), primiparity (reference: no), presence of the polymorphic allele T and A (reference: no). The results of the

logistic regression model were presented in *Odds Ratio* (OR) and 95% confidence intervals (95%CI). Statistical power presented 80% for detection association using G POWER 3.1 software and the level of significance considered was 5% ($p\leq0.05$).

Results

The analysis of the genetic models for polymorphisms rs1800469 and rs1800468 of the *TGF-β1* gene is shown in table 1.

For polymorphism rs1800469, no statistically significant difference was found in any of the genetic models analyzed (Codominance: p=0.93, Dominance: *p*=0.78, Recessive: *p*=0.87 and Over-dominance: *p*=0.70). Both groups are found in the Hardy-Weinberg Eequilibrium (PE: χ^2=0.24; *p*=0.62; C: χ^2=0.10; *p*=0.75).

Table 1

Analysis of the genetic models for the polymorphisms rs1800469 and rs1800468 of the *TGF- β1* gene and Pre-eclampsia.

Model	Genotype	Case		Control		OR (95%IC)	*p*
		n	%	n	%		
rs1800469							
Codominance	C/C	28	34.6	56	34.4	1.00	0.93
	C/T	40	49.4	72	46.8	0.90 (0.50-1.63)	
	T/T	13	16.1	26	16.9	1.00 (0.45-2.24)	
Dominance	C/C	28	34.6	56	36.4	1.00	0.78
	C/T-T/T	53	65.4	98	63.6	0.92 (0.53-1.62)	
Recessive	C/C-C/T	68	84	128	83.1	1.00	0.87
	T/T	13	16.1	26	16.9	1.06 (0.51-2.20)	
Over-dominance	C/C-T/T	41	50.6	82	53.2	1.00	0.70
	C/T	40	49.4	72	46.8	0.90 (0.53-1.54)	
rs1800468							
Codominance	G/G	84	95.5	158	93.5	1.00	0.59
	G/A	4	4.5	10	5.9	1.33 (0.40-4.37)	
	A/A	0	0.0	1	0.6	NA (0.00-NA)	
Dominance	G/G	84	95.5	158	93.5	1.00	0.52
	G/A-A/A	4	4.5	11	6.5	1.46 (0.45-4.73)	
Recessive	G/G-G/A	88	100.0	168	99.4	1.00	0.36
	A/A	0	0.0	1	0.6	NA (0.00-NA)	
Over-dominance	G/G-A/A	84	95.5	159	94.1	1.00	0.64
	G/A	4	4.5	10	5.9	1.32 (0.40-4.34)	

OR= *Odds ratio;*
Multiple Logistic Regression.

Table 2

Prevalence of the polymorphisms rs1800469 and rs1800468 haplotypes of the *TGF-β1* gene of women with pre-eclampsia and the control group.

Haplotype	PE group	C group	OR (-95%CI)	*p*
G-C	0.569	0.560	1.00	-
G-T	0.408	0.404	1.01 (0.68 – 1.50)	0.95
A-C	0.022	0.035	1.54 (0.50 – 4.73)	0.46
A-T	0.0	0.0	-	-

OR= *Odds ratio*; PE group= Pre-eclampsia group; C group= Control group; CI= confidence interval.
Algorithm EM

Table 3

Analysis of the risk factors contribution between Pre-eclampsia and the control group.

Variable analyzed	PE		C		OR	(95%) CI	*p*
	n	%	n	%			
Smoking					1.82	0.318-0.680	0.496
Yes	10	12.2	23	14.8			
No	72	87.8	132	85.2			
Family history					17.76	1.984-4.354	<0.001
Yes	22	26.8	9	5.8			
No	60	73.2	146	94.2			
Primiparity					19.32	2.163-5.311	<0.001
Yes	30	36.6	11	7.1			
No	52	63.4	144	92.9			
Polymorphisms							
rs1800469					2.25	0.147-0.432	0.665
CC	29	35.4	56	36.1			
CT-TT	53	64.6	99	63.9			
rs1800468					2.13	0.674-0.924	0.355
GG	78	95.1	144	92.9			
GA-AA	4	4.9	11	7.1			

OR= *Odds ratio*; CI: Confidence Interval; C= control; PE= Pre-eclampsia.
Multiple Logistic Regression

For the polymorphism rs1800468, also no statistically significant difference was found in any of the genetic models analyzed (Codominance: *p*=0.59, Dominance: *p*=0.52, Recessive: *p*=0.36 and Over-dominance: *p*=0.64). Both groups are found in the Hardy-Weinberg Equilibrium (PE: χ^2=3.12; *p*=0.07; C: χ^2=0.048; *p*=0.82).

The prevalence of the polymorphisms haplotypes rs1800469 and rs1800468 of the *TGF-β1* gene is shown in table 2 and no statistically significant difference was observed.

The logistic regression analysis among the known risk factors for the development of PE can be observed in table 3. A statistically significant difference was observed for family recurrence (*p*<0.001) and primiparity (*p*<0.001) of PE, indicating that these factors are involved in the development of pre-eclampsia.

Discussion

This present work investigated the polymorphisms rs1800469 and rs1800468 and the region promoter of the *TGF-β1* gene and the PE susceptibility.

The genetic models test (codominance, dominance, recessivity and over-dominance) did not find an association between the polymorphism rs1800469 of the *TGF-β1* gene and PE. Our results differ from those found by Deepthi *et al.*,[13] who analyzed the polymorphism rs1800469 of the *TGF-β1* gene with a sample of 469 Native Indian women, of those, 239 were in the group with PE and 230 in the control group. These authors demonstrated an association between the over-dominant model and observed that the TT genotype predisposes to PE, whereas the CT genotype seems to have a protective effect regarding the development of this disease. These results may have been found because the sample is larger in relation to the present study and due to the Indian population which is genetically homogeneous, but this is not the case of the Brazilian population.

For the polymorphism rs1800468 of the *TGF-β1* gene, the data regarding the analysis of genetic models that could be related to the results obtained in this present study were not found. Even this is a pioneer study, in the type of analysis, no statistically significant association was found between the inheritance model and the PE susceptibility.

In this current study, no statistically significant difference was observed between the haplotype prevalence of polymorphisms rs1800469 and rs1800468 of the *TGF-β1* gene and PE.

Aguilar-Duran *et al.*[14] analyzed a Mexican study, the haplotypes of three polymorphisms rs1800469, rs1800468 and rs1800470 of the *TGF-β1* gene and, in this case, a moderate imbalance binding was found. The most frequent haplotype was rs1800468G/rs1800469C/rs1800470C, however, this result was not associated to the development of PE.

The study done by Deepthi *et al.*[13] analyzed the polymorphism rs1800470 and rs1800469 of the haplotypes and the predisposition to develop PE and found that the C-C and T-T haplotypes are associated to the increased susceptibility of this disease.

For the logistic regression analysis, it was observed that women with a family history of PE had a risk of developing the disease by increasing 17 times higher when compared to women without family recurrence. These results are according to those performed in Norway and the United Kingdom where daughters of women who had PE were twice as likely to develop the disease compared to women with no family history. The data from the United Kingdom also showed that women who were not born from pregnancy with PE, but reported a family history of this disease were at greater risk than women without a family history.[15,16]

Mutze *et al.*[17] also observed this hereditary component, showing that 25 to 31% of daughters of women with PE develop this disease in their pregnancies. Cnattingius *et al.*[3] suggested that about 67% of the PE susceptibility is caused by genetic factors, while 32% are of several factors, reinforcing the multifactorial nature of PE.

In addition to the family history of PE, the logistic regression analysis showed that primiparous women present an increased risk of nineteen times to develop PE when compared to women with more than two pregnancies. These results are similar to those by Khader *et al.*,[18] observed that women in Jordanian had a twice the increased risk in developing PE in the first pregnancy than in later ones. Hernandez-Dias *et al.*[19] also showed that multiparous women with no history of PE had a 1% risk of developing the disease, while women with PE in one or two consecutive pregnancies presented a 15-30% risk of recurrence, respectively.

This increase in the risk of PE in primiparous women can be explained by the immaturity of the maternal immune system. The increase of the PE in primiparous can be justified by the reduction of exposed time of the paternal antigens; thus, the development of the necessary immunological tolerance to the fetal allograft is impaired, occurring rejection, and resulting to an increased susceptibility of PE. [20,21]

Thus, there are a few data in the world literature on the role of the polymorphisms rs1800469 and

rs1800468 of the *TGF-β1* gene and the susceptibility of PE. It is known that this disease is a complex disorder with clinical severity variable.[1,22] PE is a multigenic disease, so it is expected that an involvement of several genes associated to its developmental mechanisms will occur. Consequently, studies with different genes are necessary in an attempt to understand the real role of the genetic factors in the etiology of PE. It is noteworthy to mention that studies that evaluate genetic polymorphisms and disease susceptibility in the Brazilian population are complex due to the genetic heterogeneity of our population, what does not occur in European and Asian populations.[23,24]

Therefore, the present study found no association between polymorphisms of the *TGF-β1* gene, positions rs1800469 and rs1800468 and PE. However, factors such as family history and primiparity were associated with increased risk of developing PE. Although, further genetic studies are needed to understand the genesis of PE.

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