

Polymorphisms of the Apoptotic genes TP53 and MDM2 and Preeclampsia Development

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Abstract

Preeclampsia (PE) is a multisystemic disorder responsible by high levels of maternal/fetal mortality and morbidity. Although its exact physiology is not well established, apoptosis has a pivotal role in PE pathophysiology, and an elevated rate of cell death is related to PE development. Here it was performed a case-control study, where the frequencies of two polymorphisms in major genes of the apoptosis pathway (TP53 rs1042522 and MDM2 rs2279744) were genotyped in 119 women suffering PE and 99 without preeclampsia. The genotyping was performed by allelic discrimination using a Taqman SNP genotype analyzer. Considering clinical features, the mean of birth weight was lower among children delivered by PE women ($p=0.004$). Also, a higher number of children from PE women was classified as having Low Birth Weight ($p<0.001$). PE women had Cesarean delivery more frequently than controls ($p=0.004$) and also presented overweight and hypertension ($p<0.001$). Despite the clinical features, the distribution of the genotypic variants was similar between case and controls, suggesting that the genetic variants evaluated did not directly influence the risk of PE among our subjects.

Keywords: Apoptosis; Pregnancy; Preeclampsia; p53; Polymorphisms

Introduction

Preeclampsia (PE) is one of the most impactful hypertensive diseases during pregnancy, being responsible for almost 25% of maternal deaths in Latin America [1]. Its etiology and pathogenesis involve a combination of maternal-fetal genetic and immunological factors [2]. This disease is often related to predisposing disorders, such as chronic hypertension, diabetes, and overweight. Moreover, PE presents a complex pathogenesis that involves abnormal placentation as well as implantation impairment. PE is also considered as an inflammatory disorder since immune cells secrete inflammatory cytokines capable to induce apoptosis of cells in the extravillous cytotrophoblast [3]. Nevertheless, the exact cause of PE remains poor understood. Thus, the components of immune system, the apoptosis pathways and angiogenesis process have been a focus of interest as potential predictors of PE development.

Several studies have suggested the importance of the apoptosis pathways and its related genes in the regulation of PE [4,5]. A dysfunction or imbalance in apoptotic pathways can trigger pathological processes in an organism, broadly affecting its homeostasis [6]. Interestingly, apoptosis has been shown to be elevated during complicated pregnancies [7]. The p53 protein, encoded by *TP53* gene, plays an important role in maintenance of several biological processes, being pivotal in the prevention of tumors and keeping the genomic stability of somatic cells. This protein acts as a transcription factor that regulates a large number of genes in response to cell damage [8]. Under cell stress conditions, p53 is activated, initiating a process of cell cycle arrest, DNA repair, senescence and apoptosis [9,10], acting in tissue remodeling and elimination of unwanted cells [11]. High p53 levels are seen in placentas during complicated pregnancies, highlighting the role of P53 in trophoblast apoptosis [12].

The p53 protein can be negatively modulated by its inhibitor, the Mdm2 protein, that down regulates p53 through ubiquitination, leading to its degradation. Single nucleotide polymorphisms (SNPs) on *TP53* and *MDM2* genes have already been related to different aspects of

human reproduction such as recurrent pregnancy and/or implantation failure, endometriosis, *in vitro* fertilization success or even twinning rate and trisomy 21 [13-20]. A well-recognized polymorphism in *TP53* gene (P72R, rs1042522) is characterized by a G>C substitution at codon 72, leading to a less efficient P53 protein and consequently to less efficient apoptosis [21-23]. In *MDM2* gene, the c.14+309T>G polymorphism (SNP309, rs2279744) is an important SNP in the promoter region of the gene. The *MDM2* G allele is associated with high Mdm2 protein expression. The high Mdm2 levels are correlated to the attenuation of p53 function [16,24,25].

Although several studies have addressed the role and effects of p53 protein levels on apoptosis, there are no studies evaluating polymorphisms in *TP53* and *MDM2* genes and correlation with PE development. Taking into account that a deregulated pathway of apoptosis could be related to PE [11], we analyzed the frequencies of *TP53* c.215G>C (P72R, rs1042522) and *MDM2* c.14+309T>G (SNP309, rs2279744) genes polymorphisms and their role as risk factors for PE development.

Material and Methods

Subjects

Subjects were recruited at the Maternity Unit of the hospital Nossa

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Senhora da Conceição in Rio Grande do Sul, the southernmost state of Brazil. We recruited 119 preeclamptic women and 99 healthy pregnant women. Preeclampsia was defined as the presence of hypertension and proteinuria. Hypertension and proteinuria were accessed accordingly to previous recommendations [26,27]. The PE was classified as severe according the following criteria: blood pressure was $\geq 160/110$ mmHg; urinary protein excretion ≥ 5 g per 24h; platelet counts of <100000 mm^{-3} in at least two samples; the combination of haemolysis, abnormal liver enzymes associated with persistent epigastric or upper right quadrant pain; persistent and severe symptoms as altered mental status, headaches, blurred vision or blindness; presence of multiorgan involvement such as pulmonary edema, oliguria (<500 mL per day) [28,29]. The inclusion criteria for the control group comprised: no rise in blood pressure, no hypertension or proteinuria, similar age and no biological relationship with PE patients. Controls were followed up for at least three months after delivery. Women that presented chronic hypertension, autoimmune diseases, renal diseases, collagen vascular diseases, cancer or thrombosis were not included in the study.

All subjects gave their written informed consent, and all procedures were approved by the local ethics committee of the Hospital Nossa Senhora da Conceição and by CONEP (National Committee of Ethics on Research, Brazil).

Genotypic analysis

Genomic DNA was extracted from blood samples as described by Lahiri and Nurnberger [30]. The TP53 c.215G>C (P72R) and MDM2 c.14+309T>G (SNP309) SNPs were genotyped by allelic discrimination by Taqman probes (Applied Biosystems) accordingly to the manufacturer directions. The reaction products were analyzed on a StepOne V2.2.2 Software.

Statistical analysis

Statistical analyses were performed using SPSS software, version 20.0. Chi-square was used to test agreement with the Hardy-Weinberg equilibrium, to compare allelic and genotypic frequencies and to characterize and compare clinical parameters between PE and control individuals. To analyze the gene-gene additive effect on the risk of PE, chi-square test and binary logistic regression models were used. In this analysis, potential confounders (such as maternal age at delivery, maternal body mass index, maternal smoking, ethnicity, gestational age, primiparity and number of previous miscarriages) were included as covariates when associated with the studied groups (or at least one of

them) and with the outcome for a $p < 0.20$. Statistical significance was considered when $P < 0.05$.

Results

The clinical and obstetric data of the sample are shown in Table 1. PE women and controls presented similar age (mean age 30.25 yrs \pm 7.94 vs 28.84 yrs \pm 7.03, respectively). PE women presented higher weight as compared to the control group ($p = 0.004$). In addition, PE women delivered more children with low birth weight when compared to the healthy subjects ($p = 0.004$). Moreover, in PE group, the frequency of low birth weight was higher than in control group ($p < 0.001$). PE women presented high index of cesarean ($p < 0.001$) and chronic hypertension ($p < 0.001$) when compared to the control group. Considering ethnicity, no difference was found comparing the studied groups ($p = 0.371$).

The genotypic and allelic frequencies distribution between PE and control groups were in Hardy-Weinberg equilibrium (*data not shown*) and did not differ when analyzed separately or when controlled for ethnicity and previous occurrence of spontaneous abortion. The allelic and genotypic frequencies of the studied polymorphisms in our population, as well as another european and euro-descendant populations are shown in Table 2. No statistical significance difference were observed in genotypic and allelic frequencies of TP53 and MDM2 genes between the studied groups (TP53; Chi-square Test, $p=0.396$ and MDM2; Chi-square Test, $p=0.195$). We also analyzed the effects of the genotypes on the risk of PE using binary logistic regression model. The preeclampsia sample were coded as 1 and the control group as 0 (reference category). No statistical significance was observed [TP53 ; CG : $p=0.772$, OR 0.917 (0.511-1.646). GG : $p=0.198$, OR 1.808 (0.733-4.459) ; MDM2 ; TG : $p=0.085$, OR 1.693 (0.931-3.044). GG : $p=0.861$, OR 0.927 (0.399-2.156)].

Discussion

In the present work, we evaluated clinical features and the frequency of polymorphic variants in genes of the apoptotic pathway in preeclamptic women. Women with PE presented a higher mean of weight when compared to the control group. It is well established that overweight is a risk factor for PE development, therefore our data are in accordance with this [3]. In addition, children delivered by PE women presented a lower mean of birth weight and a higher frequency of child with low birth weight was observed among preeclamptic mothers as compared to the control group. This finding corroborates previous

	Case	Control	P
Age during gestation [mean (\pm sd)]*	35.09 (\pm 4.01)	38.43 (\pm 3.78)	0.597 ^a
Ethnicity**			
European ancestry [N (%)]	82 (68.3)	74 (74.8)	
African ancestry [N (%)]	38 (31.7)	25 (25.2)	0.371 ^b
Weight [mean (\pm sd)]*	187.1 lbs(\pm 17.32)	160.9 lbs(\pm 23.64)	0.004 ^b
Birth weight [mean (\pm sd)]*	5.9 lbs(\pm 0.939)	6.9 lbs(\pm 1.679)	0.004 ^a
Low birth weight [N (%)]	44 (36.1)	16 (16.2)	<0.001 ^b
Primiparity	34 (26.3)	18 (18.2)	0.102 ^b
Previous miscarriage [N (%)]	27 (23.1)	28 (29.2)	0.396 ^b
Cesarean delivery [N (%)]	27 (49.1)	12 (20)	<0.001 ^b
Hypertension	37 (32.5)	4 (4.1)	<0.001 ^b
Alcohol consumption	3 (2.4)	4 (4.1)	0.703 ^b
Smoking	23 (18.8)	21 (21.2)	0.767 ^b

^aStudent t-test; ^bChi-square test

Table 1: Clinical and Obstetric Data of Case and Control Groups.

Gene	Genotype / Allele	Case n (%)	Control n (%)	P*	Expected allele frequency (%)**	
					Euro-descendant	Afro-descendant
TP53 (rs1042522)	GG	51 (41.8)	45 (45.5)			
	GC	51 (41.8)	44 (44.4)			
	CC	20 (16.4)	10 (10.1)	0.396		
	G	153 (62.7)	134 (67.7)	0.322	72.0	33.0
MDM2 (rs2279744)	TT	48 (39.3)	46 (46.5)			
	TG	60 (49.2)	37 (37.4)			
	GG	14 (11.5)	16 (16.1)	0.195		
	T	156 (63.9)	129 (65.2)	0.868	64.0	90.0
Risk allele combination	TP53 C + MDM2 G	64 (52.5)	47 (47.5)	0.547	NA	NA

*Chi-square Test; **Data from the 1000 Genomes Project Consortium; NA = Not available

Table 2: Allelic and genotypic frequencies of SNPs in the TP53 pathway.

studies associating PE and restriction of fetal growth [31]. Our data also revealed that the number of cesarean deliveries were elevated in PE women. This delivery mode is common in women presenting PE due to the indication of emergency situation as well as the high rate of labor induction [27,32]. In this way, our results assert that our sample is well characterized in accordance with the clinical criterious for PE. The prevalence of preeclampsia can vary between populations and ethnic groups [33]. However, in our population, ethnicity was not related to PE development. Our data differs from those from Dempsey and Trostad that described that African-descendant women have a higher risk of preeclampsia as compared to European-descendant women [34,35].

In this study, we evaluated a possible role of TP53 and MDM2 gene polymorphisms in the development of preeclampsia. No statistically significant associations were observed between the genetic variants studied and PE development (Table 2). Although these polymorphisms have never been investigated as possible risk factors for preeclampsia, some studies have already described the importance of polymorphic variants in genes related to apoptotic pathways during pregnancy. Regarding the MDM2 gene, the MDM2 GG genotype has been already associated with missed abortion [25]. Other studies evaluating the TP53 gene, showed an association between SNPs of p53 gene and fertility, suggesting a specific role of p53 in the regulation of human reproduction. For instance, a statistically significant association between carriage of the Pro allele and the occurrence of idiopathic recurrent miscarriages [19]. Also, a higher frequency of homozygous for this allele was observed among women with recurrent pregnancy loss [14], recurrent implantation failure or low implantation rates after *in vitro* fertilization [15,17] and endometriosis-associated infertility [18] were already described. Nevertheless, it is also true that the real involvement of such variant in gestational success is still controversial, since recently Fraga et al. [36] associated homozygosity for the Arg variant with recurrent pregnancy loss.

Our data were analyzed using actual statistical tools in order to evaluate the role of gene-gene interaction and its effects on PE development. A recent study also tested the gene-gene additive effect of TP53 P72R and MDM2 SNP309 polymorphisms and maternal susceptibility to aneuploidy, showing an increased risk of offspring with Down Syndrome in presence of the combined alleles (TP53 P72 and MDM2 G) [13].

In conclusion, our results suggested that the genetic variants here accessed did not influence the risk for PE. Additional studies focusing on other genes involved in apoptosis could help in understanding the molecular events involved in PE. Moreover, post-transcriptional process

could also affect the mRNA viability/stability as well as the protein formation, revealing a new scenario of investigation on PE pathology. Thus, studies in populations with different ethnic background and/or a larger sample size should be conducted to clarify the molecular events that could link apoptosis and PE development.

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Author Disclosure Statement

The authors declare no competing financial interests.

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