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Original Article

Clinical and Genetic Characteristics of Preeclampsia

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Abstract

Preeclampsia (PE) is a severe complication of pregnancy accompanied by arterial hypertension, edema, or proteinuria with impaired functioning of various organs and systems. It is also an important medical and social problem, which has been one of the leading causes of maternal and perinatal mortality and morbidity worldwide. Despite the achievements of modern medicine, the etiology of this pathology is still unknown. Recently, many scientists have especially focused on the study of genetic factors underlying the etiopathogenesis of PE, namely, the contribution of individual polymorphic loci of various candidate genes. The current study aimed to investigate the clinical characteristics of PE and the contribution of the polymorphic loci rs1042838 of Progesterone Receptor (PGR) gene and rs8068318 of the T-Box Transcription Factor 2 (TBX2) gene to the development of PE. The study was conducted on 219 women with PE with the mean±SD age of 26.52±5.51 years and 329 women with the physiological course of pregnancy as the control group with the mean±SD age of 26.27±4.88 years. In total, 64.20%, 68.29%, 16.44%, 98.63%, and 35.48% of women with PE had increased systolic and normal diastolic blood pressure (SBP and DBP) values, proteinuria, edema, and overweight (BMI≥25), respectively. In the control group, 100%, 1.53%, 1.12%, and 35.48% of cases had normal SBP values with no proteinuria, DBP>90 mm Hg, edema, and overweight (BMI≥25), respectively. An association was observed between the CC genotype of the rs8068318 polymorphism of the *TBX2* gene with the risk of developing PE in women with PE (OR=2.12, 95%CI: 1.14-3.92, *P*=0.02). In addition, there was an association between the rs8068318 *TBX2* polymorphic locus with lower SBP (Me=140, Q25 – Q75 130 – 142.5, *P*=0.01) and PBP (Me=50, Q25 – Q75 40 – 55, *P*<0.01). According to the GeneCards database, the *TBX2* gene, a member of a phylogenetically conserved gene family, is located on the long arm of chromosome 17 and encodes the TBX2 T-box transcription factor protein, which is a regulator of the transcriptional activity of various genes (i.e., it suppresses the expression of *CDKN2A* (p19/ARF), inhibits cyclin-dependent kinase p21 Cip1 (*CDKN1A*), and affects the expression of *MYC*, *RAS*, *BRCA1*, and *BRCA2* genes).

Keywords: Edema, Preeclampsia, Polymorphic locus, Proteinuria, rs1042838 *PGR*, rs8068318, *TBX2*

1. Introduction

Preeclampsia (PE), as one of the main factors in maternal and perinatal mortality and morbidity, is among the most serious hypertensive complications of pregnancy worldwide, which complicates the course of pregnancy in 2-8% of all cases (1). The classic symptoms of PE include high blood pressure (systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg), proteinuria > 300 mg/24h, edema, and dysfunction of various organs and systems (2). The absence of high blood pressure, severe edema, or massive proteinuria are increasingly noted atypical (nonclassical) symptoms of PE, which are observed after the 20th week of pregnancy with proteinuria and indicate the need for more careful monitoring of vital signs in pregnant women (3). If left untreated, PE can lead to seizures, in which case it is known as eclampsia (4). In this case, the pregnancy

should be terminated, even by cesarean section or curettage.

The PE severity is classified as mild to severe. In mild disease, even slightly high blood pressure can be a sign of pregnancy poisoning, which may occur even without severe symptoms or the symptoms can go unnoticed. However, in severe cases, the disease is associated with high blood pressure and other symptoms may appear as well. Severe preeclampsia can lead to severe hospitalization and medical supervision. Although in very rare cases, there is a need for preterm delivery, the majority of women with pregnancy poisoning give birth to healthy babies (5, 6).

Despite the fact that eclampsia may not be certainly prevented, some precautions should be taken due to possible risk factors. In this regard, controlling weight, high blood pressure before pregnancy, and diabetes (if present) can help the patient. For some blood clotting disorders, practitioner may prescribe a small dose of aspirin to control the condition (7). The long-term side effects of aspirin include the risk of cardiovascular and kidney diseases, heart attack, stroke, brain injury, hypertension after childbirth, and a high chance of developing preeclampsia in the next pregnancy (8, 9).

The results of numerous studies performed on the relationship between the level of valuable markers measured in the quad marker test (alpha-fetoprotein [AFP], hCG, uE3, and Inhibin A) and pregnancy-related disorders (10) indicated a significant relationship between the level of these markers and the incidence of PE. In addition to the possibility of Down syndrome in the patient, these markers can also predict the possibility of preeclampsia, based on the corrected multiple of median (MoMc). It should be noted that the risk of EP increases with higher modified MoM of hCG, Inhibin A, AFP, and lower modified moles of free sterol (11). The highest potency of these markers is initially related to the increase of Inhibib A and hCG, and the lowest effect is related to AFP. In case the likelihood ratio of these markers are combined with the probability coefficient of the mother's clinical condition (e.g., weight, age, twin pregnancy, no history of birth, diabetes, and history of PE), up to about 60% of future mothers with Eclampsia will be considered to be at high risk of developing PE.

It is worth mentioning that PE is a multifactorial disease (12), which is highly subjected to both environmental and genetic factors the role of which is actively studied worldwide (13). Despite the large number of works devoted to the study of the PE etiology, the exact molecular mechanisms underlying the etiopathogenesis of this disease have remained unknown (14). Furthermore, a large number of established risk factors for PE include a family history of PE, arterial hypertension diagnosed before pregnancy, diabetes mellitus, high body mass index (BMI), infections, chronic kidney disease, and reproductive disorders. These were studied widely by Reshetnikov, Zarudskaya (15), Reshetnikov, Akulova (16). Recently, researchers have particularly focused on the study of genetic determinants as effective markers for the identification of high-risk groups for PE (17, 18). However, the obtained data were scattered, which dictated the need for further study of molecular genetic markers of candidate genes associated with the development of PE to assess the PE risk at the stage of periconceptional preparation and prevent this pregnancy complication timely.

This study was performed to evaluate the clinical features of preeclampsia and consider the role of polymorphic sites of the rs1042838 PGR gene and rs8068318 TBX2 gene in PE development.

2. Materials and Methods

The study participants included 219 PE patients with the mean±SD age of 26.52±5.51 years and the mean±SD BMI of 24.67±5.07 kg/m^2. The group of control in the current study included 329 women with physiological pregnancy with the mean±SD age of 26.27±4.88 years and the mean BMI±SD of 23.69±3.57 kg/m^2. All women were residents of the Central Black Earth Region of the Russian Federation without any kinship. Exclusion criteria included diseases of the uterus (i.e., fibroids of the uterus and abnormal

development of internal genital organs), other pregnancy abnormalities (e.g., abnormalities of placental attachment and location, placental insufficiency with fetal growth retardation syndrome, and Rh-conflict), fetal pathology (congenital malformations), and multiple pregnancies. All clinical and clinical-laboratory studies were conducted on the Perinatal Center of St. Joasaph Belgorod Regional Clinical Hospital during 2008-2015 after the informed written consent was obtained from the patients. The PE was diagnosed based on the clinical symptoms, and the participants underwent clinical and clinical-laboratory examination both before pregnancy and at the time of delivery. The studied polymorphic loci rs1042838 of the *PGR* gene (19) and rs8068318 of the *TBX2* gene (20) were selected for the study according to the available data on their involvement in the key pathogenetic mechanisms of PE. Genotyping of polymorphic loci was performed by PCR DNA synthesis on a CFX-96 Real-Time System (Bio-Rad, USA) using standard oligonucleotide primers and probes as previously described in the study conducted by Chambers, Zhang (21).

2.1. Statistical analysis

The statistical data were analyzed using STATISTICA software for Windows (Version 10.0; StatSoft, USA) as previously described in the study conducted by Ponomarenko, Reshetnikov (22).

3. Results and Discussion

In this study, 219 patients with PE were assessed for such parameters as blood pressure, proteinuria, and the presence of edema. In the study group, edema was absent in 1.37% of patients, and leg swelling, leg swelling in combination with pathological weight gain, edema of the legs and anterior abdominal wall in combination with pathological weight gain, and generalized edema were observed in 38.36%, 47.03%, 3.20%, and 9.59% of all cases, respectively.

Among the women with PE, 59.91%, 4.61%, 19.82%, 11.98%, 2.76%, and 0.92% had normal body weight (BMI=18.5-24.99), BMI<18.5 (underweight), BMI=25-30 (overweight-preobesity), BMI=30-35 (grade I obesity), BMI=25-40 (grade II obesity), and BMI>40 (grade III obesity), respectively.

Moreover, 83.56%, 4.57%, 6.85%, 0.91% of women with PE, had proteinuria≤300 mg/24h (the amount of protein in daily urine); between 301-1000 mg/24h, between 1001-3000 mg/24h, and >3000 mg/24h, respectively.

In total, 11.77% of women with PE were diagnosed with high SBP≥160 mm Hg (including 1.37% with SBP≥180 mm Hg) and 5.94% had high DBP≥110 mm Hg, corresponding to severe arterial hypertension in pregnant women (23). Moreover, SBP equal to 140-159 mm Hg was registered in 50.23%, and DBP equal to 90 mm Hg was observed in 63.65% of patients, corresponding to moderate arterial hypertension in pregnant women. In total, 37.80% and 31.71% of women with PE had SBP <140 mm Hg and DBP<90 mm Hg, respectively (Table 1).

The frequency of CC, CT, and TT genotypes of the polymorphic locus rs8068318 of the *TBX2* gene was estimated at 11.48% (n=24), 34.93% (n=73), and 52.59% (n=112) among women with PE, and obtained at 6.45% (n=20), 38.51% (n=119), and 55.34% (n=171) in the control group, respectively. In this study, the frequency of the C (minor allele) and T alleles in the control groups were 0.29 / 0.71 and 0.26 / 0.74, respectively. Based on the results, the CC genotype of the *TBX2* gene (rs8068318) was associated with the risk of developing PE (OR=2.12, 95% CI 1.14-3.92, *P*=0.02).

The frequency of TT, GT, and GG genotypes of the rs1042838 polymorphism of the PGR gene in patients with PE was estimated at 2.80% (n=6), 30.84% (n=66), and 66.36% (n=142), and the frequency of T (minor allele) and the G allele was obtained at 0.18 and 0.82, respectively. In the control group, the frequency of TT, GT, and GG genotypes was obtained at 2.15% (n=7), 24.62% (n=80), and 73.23% (n=238), and the frequency of T and G alleles was reported to

be 0.14 and 0.86, respectively. In addition, in the current study, no associations were observed between the PGR gene (rs1042838) and developing PE (P>0.05) (Table 2).

The distribution of genotypes did not differ from the expected distribution, according to the Hardy-Weinberg equilibrium, in terms of all the studied loci in the group of pregnant women with PE (n=219) and the group of control (n=329), (P>0.05).

The rs8068318 TBX2 polymorphic locus is associated with lower SBP (Me=140, Q25- Q75 130-142.5, P=0.01) and PBP (Me=50, Q25- Q75 40-55, P<0.01) in women with PE according to the results of Friedman ANOVA test (Table 3).

Table 1. Clinical characteristics of preeclampsia in women of Russian nationality, residing in the Central Black Earth Region (n=219)

Parameter	Value	Preeclampsia (n=219)	Control group (n=329)	*P*-value
SBP (mm Hg)	<140	37.80%	100%	<0.001
	140- 159	50.23%	0%	
	160-179	10.50%	0%	
	≥180	1.37%	0%	
DBP (mm Hg)	<90	32.42%	98.47%	0.001
	90 - 99	44.75%	1.53%	
	100 - 109	18.90%	0%	
	≥110	5.94%	0%	
Proteinuria (mg/24 h)	≤300	83.56%	100%	0.008
	300 - 1000	4.57%	0%	
	1001 - 3000	6.85%	0%	
	>3000	0.91%	0%	
Edema	Legs	38.36%	98.88%	0.015
	Legs+pathological weight gain	47.03%	1.12%	
	Legs+pathological weight gain+anterior abdominal wall	3.20%	0%	
	Generalized	9.59%	0%	
BMI (kg/m^2)	<18.5	4.61%	2.76%	0.001
	18.5 – 24.99	59.91%	61.54%	
	25 – 29.99	19.82%	29.85%	
	30 – 34.99	11.98%	5.54%	
	35 – 39.99	2.76%	0%	
	>40	0.92%	0.31%	

DBP: diastolic blood pressure; SBP: systolic blood pressure; mm Hg: millimeter of mercury; mg: milligrams; BMI: Body Mass Index; *P*-value: significance level; kg: kilograms; m: meter.

The distribution and frequencies of genotypes and alleles of the studied polymorphic loci rs8068318 of the *TBX2* gene and rs1042838 of the *PGR* gene among women with PE and pregnant women of the control group were assessed using the Hardy- Weinberg equilibrium. The frequencies of genotypes and minor alleles were calculated for the polymorphisms under consideration.

Table 2. Characteristics of the allele and genotype frequencies of the rs8068318 *TBX2* and rs1042838 *PGR* polymorphic locus in women with preeclampsia and the control group

Locus	Alleles, genotypes	Preeclampsia (n=219) No (%)	Control group (n=329) No (%)	OR (95%CI)	*P*-value
rs8068318 *TBX2*	C/C	24 (11.48%)	20 (6.45%)	2.12 (1.14-3.92)	0.02
	C/T	73 (34.93%)	119 (38.51%)	0.90 (0.62-1.30)	0.57
	T/T	112 (52.59%)	171 (55.34%)	0.96 (0.66-1.38)	0.82
	C	0.29	0.26	1.21 (0.91-1.61)	0.19
	T	0.71	0.74	0.85 (0.64-1.14)	0.29
rs1042838 *PGR*	T/T	6 (2.80%)	7 (2.15%)	1.31 (0.39-4.41)	0.85
	G/T	66 (30.84%)	80 (24.62%)	1.39 (0.93-2.07)	0.11
	G/G	142 (66.36%)	238 (73.23%)	0.73 (0.50-1.08)	0.12
	T	0.18	0.14	1.35 (0.97-1.89)	0.08
	G	0.82	0.86	0.76 (0.54-1.07)	0.12

Note: OR: odds ratio; CI: confidence interval; P-value: significance level; bold characters indicate a significant difference.

Table 3. Association of *TBX2* polymorphisms with blood pressure values in women with preeclampsia

Blood pressure values	rs8068318 *TBX2*			Friedman ANOVA test, P
	TT	CT	CC	
SBP, (mm Hg)	140 (130-150)	140 (130-150)	140 (130 -142.5)	0.01
DBP (mm Hg)	90 (80-90)	90 (80-95)	90 (85-95)	0.28
PBP (mm Hg)	50 (50-60)	50 (45-55)	50 (40-55)	<0.01
MAP(mm Hg)	105.8 (100-110)	103.3 (98.3-113.3)	105.0 (101.6-110.0)	0.60

Note DBP: diastolic blood pressure; MAP: mean arterial pressure; PBP: pulse blood pressure; SBP: systolic blood pressure; ANOVA: analysis of variance. The values of blood pressure level expressed a median (Me) and an interquartile range (Q25–Q 75).

According to the GeneCards database, the *TBX2* gene is located on the long arm of chromosome 17. This gene is a member of a phylogenetically conserved gene family and encodes the TBX2 T-box transcription factor protein, which is a regulator of the transcriptional activity of various genes (i.e. suppresses the expression of *CDKN2A* (p19/ARF), inhibits cyclin-dependent kinase p21 Cip1 (*CDKN1A*), and affects the expression of *MYC*, *RAS*, *BRCA1*, and *BRCA2* genes (24). Related biological pathways of the *TBX2* gene include the regulation of heart morphogenesis in the embryonic period, inhibition of ribosome biogenesis with p14/ARF, and cell differentiation, according to the Gene Ontology data. The *TBX2* gene is specifically expressed in the kidneys of adults, as well as the fetal lungs and the placenta, and plays a certain role in developing preeclampsia (25).

Therefore, the heterogeneity of the clinical signs of preeclampsia was established among women with PE in this study (n=219). In addition, 37.80% of women did not have an increase in SBP (<140 mm Hg), and 31.71%, 16.44%, 98.63%, and 59.91% of cases had DBP within normal limits (<90 mm Hg), significant proteinuria (>300 mg/24h), edema, and normal BMI (18.5-24.99), respectively. In the control group, 100% of women had normal SBP (<140 mm Hg) and 98.47% had DBP<90 mm Hg. Moreover, no woman had proteinuria (>300 mg/24h), 1.12% of women developed edema, and 61.54% had normal BMI (18.5-24.99).

5. Conclusion

Based on the obtained results, it can be concluded that the CC genotype of the polymorphic locus rs8068318 of the *TBX2* gene was significantly associated with the

risk of preeclampsia (OR=2.12, 95% CI: 1.14-3.92, *P*=0.02). Eventually, the rs8068318 *TBX2* polymorphism was also associated with lower SBP (Me=140, Q25-Q75 130- 142.5, P=0.01) and PBP (Me=50, Q25 – Q75 40 – 55, *P*<0.01) in women with PE.

Authors' Contribution

Study concept and design: O. V. G.
Acquisition of data: M. Y. A.
Analysis and interpretation of data: V. S. O.
Drafting of the manuscript: I. V. B.
Critical revision of the manuscript for important intellectual content: I. N. S.
Statistical analysis: M. Y. A.
Administrative, technical, and material support: M. Y. A.

Ethics

All procedures performed in this study involving human participants were in accordance with the Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia.

Conflict of Interest

The authors declare that they have no conflict of interest.

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