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Genetic Factors Of Pregnancy Pathology.

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ABSTRACT

To study associations of genetic polymorphisms of cytokines with the risk of preeclampsia development. The study involved 250 pregnant women with preeclampsia and 245 women with a physiological pregnancy. All pregnant women with preeclampsia and women of the control group were typed four genetic polymorphisms of cytokines: tumor necrosis factor α (-308G/A *TNFα,* rs1800629), lymphotoxin α (+250A/G *Ltα*, rs909253), tumor necrosis factor receptor 1 (+36A/G *TNFR1*, rs767455), interferon-inducible T-cell alpha chemoattractant (A/G *I-TAC*, rs 4512021). The combination of alleles +250A *Ltα* and G *I-TAC* (rs 4512021) with genotype +36GG *TNFR1* was observed in 13.38% of pregnant women with PE, whereas the frequency in the control group was 20.00% (OR=0.62, 95% CI 0.38-1.00, p=0.03). The results indicate the connection of genetic cytokine polymorphisms with the risk of preeclampsia development in the population of the Central Black Earth Region of Russia: the combination of genetic variants +250A *Ltα*, G *I-TAC* (rs4512021), +36GG *TNFR1* has a protective effect on the development of preeclampsia (OR=0.62).
Keywords: pregnancy, preeclampsia, gene polymorphism, cytokine

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INTRODUCTION

Preeclampsia (PE) is a complication of pregnancy, characterized by the development of endothelial dysfunction, multiple organ failure, disruption of coagulation and anticoagulation systems, microcirculation, metabolic processes, immune response (Eiland, Nzerue, 2012).

According to the world literature and WHO, the incidence of preeclampsia is 2-8% (Duley, 2009; Abalos et al., 2014). Preeclampsia remains an important cause of maternal, perinatal and neonatal morbidity and mortality (Abalos et al., 2014; Vogel et al., 2014).

Severe preeclampsia and eclampsia causes the risk of complications such as hemorrhages and cerebral edema, placental abruption, disseminated intravascular coagulation syndrome, massive obstetric hemorrhages, HELLP syndrome, hemorrhage and rupture of the liver capsule, pulmonary edema, adult respiratory distress syndrome, acute renal and hepatic insufficiency (Abalos et al., 2014).

According to a number of studies, this complication of pregnancy has a multifactorial nature (Pinheiro, 2015; Zhou et al., 2016; Reshetnikov et al., 2017). Local gene networks of preeclampsia include endothelial dysfunction genes, vascular reaction genes, growth factor and cytokine genes, major histocompatibility genes, etc. (Goddard et al., 2007; Johnson et al., 2012; Bell et al., 2013; Zhao et al., 2013; Alpoim et al., 2014; Mistry et al., 2015; Reshetnikov et al., 2017).

It should be noted that the results of molecular genetic studies of preeclampsia obtained by authors in different populations are often contradictory (Johnson et al., 2012; Ozkan et al., 2015; Wan et al., 2016; Zhao et al., 2016; Reshetnikov et al., 2017; Reshetnikov et al., 2018).

MATERIALS AND METHODS

Object of study

To conduct the study, a sample of 495 women was formed, which included 250 pregnant women with preeclampsia and 245 women with a physiological pregnancy. Samples of pregnant women with preeclampsia and control women included individuals of Russian nationality, born in the Central Black Earth Region of the Russian Federation and not related to each other. The average age of patients with preeclampsia is 31.2±7.5 years, control - 30.2±6.3 years (p>0.05). The group of patients with preeclampsia included individuals diagnosed with preeclampsia confirmed by clinical and clinical-laboratory examination. Preeclampsia was determined by the presence of hypertension, accompanied by proteinuria, as defined by a 24 h urine protein excretion more than 300 mg (ACOG Committee on Practice Bulletins-Obstetrics, 2002).

The control group included women with a physiological pregnancy. Examination of pregnant women was conducted in the Perinatal Center of St. Joasaph Belgorod Regional Clinical Hospital.

All patients with preeclampsia and individuals of the control group were typed four genetic polymorphisms of cytokines: tumor necrosis factor α (-308G/A *TNFα*, rs1800629), lymphotoxin α (+250A/G *Ltα*, rs909253), tumor necrosis factor receptor 1 (+36A/G TNFR1, rs767455), interferon-inducible T-cell alpha chemoattractant (A/G *I-TAC*, rs 4512021).

Molecular and genetic methods

A genomic DNA was isolated from peripheral blood by the method of standard phenol-chloroform extraction from frozen venous blood.

DNA markers genotyping was produced by the method of TaqMan probes detection according to RFU values (relative fluorescence unit) of each probe on the thermocycler IQ5 with detecting system in real time. Bio-Rad IQ5-Standart Edition program was used for the alleles discrimination.

Statistical methods

Statistical processing of data was carried out using STATISTICA for Windows 6.0 and Microsoft Excel 2007 software packages. To verify the compliance of the observed distribution of genotypes with the expected one, based on Hardy-Weinberg equilibrium, we used χ^2 test. Associations of alleles and genotypes of the studied DNA markers with the formation of preeclampsia were evaluated by analyzing the 2×2 contingency tables with the calculation of the χ^2 criterion with the Yates correction for continuity and the odds ratio (OR) with a 95% confidence interval. Analysis of the role of combinations of genetic variants of cytokines in the occurrence of preeclampsia was carried out with the APSampler software, using the Markov chain Monte Carlo method and Bayesian nonparametric statistics (Favorov et al., 2005).

RESULTS AND DISCUSSION

The study of the concentrations of allele frequencies and genotypes of polymorphic markers -308G/A *TNFα* (rs1800629), +250A/G *Ltα* (rs909253), +36A/G *TNFR1* (rs767455) in the group of pregnant women with preeclampsia and in the control group showed no statistically significant differences (Table 1).

Table 1: Frequency of genetic variants of cytokines in pregnant women with preeclampsia and without preeclampsia, (%)

Locus	Alleles, Genotypes	Pregnant women without preeclampsia (n=245)		Pregnant women with preeclampsia (n=250)		OR (95% CI) χ^2, p
		n_i	%	n_i	%	
-308G/A *TNFα* (rs1800629)	-308G	436	89.02	438	88.66	1.03 (0.69-1.57) χ^2=0.01. p=0.93
	-308A	54	10.98	56	11.34	
	-308GG	192	78.37	195	78.95	1.03 (0.65-1.63) χ2=0.01. p=0.96
	-308GA	52	21.14	48	19.43	0.90(0.56-1.42) χ2=0.15. p=0.70
	-308AA	1	0.40	4	1.62	4.02 (0.42-96.74) χ2=0.79. p=0.37
+250A/G *Ltα* (rs909253)	+250A	364	74.59	371	74.80	1.00 (0.74-1.35) χ^2=0.01. p=1.00
	+250G	124	25.41	125	25.20	
	+250AA	132	54.10	138	56.65	1.06 (0.73-1.54) χ2=0.07. p=0.78
	+250AG	100	41.00	94	37.90	0.88 (0.60-1.28) χ2=0.37. p=0.54
	+250GG	12	4.90	16	6.45	1.33 (0.58-3.08) χ2=0.29. p=0.59
+36A/G *TNFR1* (rs767455)	+36A	242	48.40	246	50.83	1.10 (0.85-1.43) χ2=0.49.p=0.49
	+36G	258	51.60	238	49.17	
	+36AA	59	23.60	63	26.03	0.87 (0.57-1.35) χ2=0.27.p=0.60
	+36AG	124	49.60	120	49.59	1.00 (0.69-1.45) χ2=0.01. p=1.00
	+36GG	67	26.80	59	24.38	0.90 (0.59-1.38) χ2=0.16. p=0.69

	G	213	44.01	208	41.60	1.10 (0.85-1.43) χ^2=0.49. p=0.49
	A	271	55.99	292	58.40	
A/G *I-TAC* (rs4512021)	AA	78	32.23	81	32.40	1.01(0.68-1.50) χ^2 = 0.01. p=1.00
	GA	115	47.52	130	52.00	1.20 (0.83-1.73) χ^2 = 0.82. p=0.37
	GG	49	20.25	39	16.00	0.73 (0.45-1.19) χ^2 = 1.51. p=0.22

The role of combinations of the cytokine genes in the formation of preeclampsia was studied with the help of bioinformational methods. As a result of the integrated analysis of the carriage of the combinations of alleles and genotypes of the cytokine genes studied, significant differences were found between pregnant women with preeclampsia and the control group. Thus, the combination of alleles +250A *Ltα* and G *I-TAC* (rs 4512021) with +36GG *TNFR1* genotype is observed in 13.38% of pregnant women with preeclampsia, whereas in the control group it was diagnosed in 20.00% (p=0.03). This combination protects from the development of preeclampsia (OR=0.62 .95% CI 0.38-1.00).

The results of the study indicate the association of combinations of alleles and genotypes of polymorphic loci +250A/G *Ltα* (rs909253), +36A/G *TNFR1* (rs767455), A/G *I-TAC* (rs4512021) with a reduced risk of preeclampsia.

Our data agree with the literature on the medical and biological significance of cytokines in the body. Interferon-inducible T-cell alpha chemoattractant plays a fundamental role in maintaining homeostasis, the functioning of the immune system, affecting central nervous system cells, as well as endothelial cells involved in angiogenesis (Antoshina et al, 2005, Lockwood et al, 2013). According to Hirota et al. (2006), I-TAC has a positive proliferative effect on endothelial endometrial cells, which underlies the formation of preeclampsia (Lockwood et al, 2013). The tumor necrosis factor receptor 1 controls an acute inflammatory response, accumulates in most cells, and stabilizes circulating TNF, increasing the half-life of this cytokine, consistent with literature data on the pathogenetic mechanisms of development of preeclampsia (Chan et al., 2000; Paludan et al., 2001). Lymphotoxin α influences the activity of fibroblasts, takes part in the metabolism of glucose, initiates the production of stress hormones, plays an important role in apoptosis processes (Ketlinskiy et al., 2008; Gallitano et al., 2016). Lymphotoxin α is produced in the early stages of vascular inflammatory processes and activates adhesion molecules and cytokines (RANTES, IP-10, MCP-1, BLC, SLC, ELC) secreted by endothelial cells (Asselbergs et al., 2007). These pathogenetic mechanisms are important in the development of preeclampsia (Steegers et al., 2010; Hartgill et al, 2013; Kartik, 2010).

The results of other studies on the search for associations of genetic polymorphisms of cytokines with a risk of preeclampsia development turned out to be conflicting.

A number of studies in the Slovak, Iranian and Chinese, North American populations have identified associations of α-308G/A *TNFα* polymorphism with the risk of developing preeclampsia (Harmon et al., 2014, Zubor et al., 2014; Tavakkol Afshari et al., 2016; Zhou et al., 2016).

Other researchers have not revealed such associations (Lachmeijer et al., 2001; Pissetti et al., 2015).

The discrepancy between the results obtained in various studies can be related to differences in the ethnic and, respectively, the genetic background of the populations under study.

CONCLUSION

Thus, the results obtained indicate the involvement of the genetic polymorphisms +250A/G *Ltα* (rs909253), +36A/G *TNFR1* (rs767455), A/G *I-TAC* (rs 4512021) in susceptibility to the development of preeclampsia in the population of the Central Black Earth Region of Russia: a combination of genetic variants

+250A *Ltα*, G *I-TAC* (rs 4512021), +36GG *TNFR1* has a protective effect on the development of preeclampsia (OR=0.62).

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