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Study of the Association of rs2228480 Polymorphism of the Estrogen Receptor Alpha Gene in Patients with Preeclampsia

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Abstract Preeclampsia (PE) is the one of the main cause of maternal death in the world. One of the genetic risk factors for the development of preeclampsia may be the rs2228480 polymorphism of the estrogen receptor alpha gene (ESR1). Purpose. The aim of the study was to study the rs2228480 polymorphism of the estrogen receptor alpha (ESR1) gene in patients with preeclampsia. Materials and methods. We investigated 140 people and divided it in two groups: the patient group included 72 pregnant women, in the third trimester of pregnancy, with preeclampsia of varying severity. The control group consisted of 68 healthy women with physiological pregnancy. The material for the study was DNA samples from a patient with a clinically diagnosed preeclampsia. Isolation of DNA from blood and PCR analysis were performed with kits of reagents and test systems from Ampli Prime Ribot-prep (LLC Next Bio, Russia). Testing of the ESR1 mutation was carried out on a PCR amplifier Corbett research (Corbett. Australia), using a test system of the company (OOO NPF "Sintol", Russia) Results. Comparison of the frequencies of genotypes and alleles of 2014G> A polymorphism of the ESR1 gene in the studied groups of patients and controls did not reveal statistically significant differences. In the patient group, the frequency of the G allele of polymorphism 2014G> A of the ESR1 gene was 88.3%, in the control group - 85.7%, which indicated an insignificant association with an increased risk of predisposition to preeclampsia ($\chi 2 = 0.31$; $P = 0.28$; $OR = 1.26$; 95% CI: 0.56-2.89). According to preliminary data, the presence of the G allele and the hetero- and homozygous G / A and GG genotypes increases, and the identification of the A allele and the AA genotype reduces the risk of preeclampsia.

Keywords Preeclampsia, rs2228480 polymorphism, ESR1, Genotype G / A and GG, PCR analysis

1. Introduction

Preeclampsia is the one of the main cause of maternal death in the world (2–5%), affecting women after 20 weeks of gestation, and is characterized by increased systemic vascular resistance, decreased blood volume, destruction of vascular endothelial cells and renal hemodynamic disorders. Preeclampsia is the result of the close interaction of genetic components with environmental factors; however, the etiology of preeclampsia remains to be seen.

One of the candidate genes that can serve as a risk factor for the development of preeclampsia is the gene for the estrogen receptor alpha (ESR1) [3]. The estrogen receptor α gene (ESR1) is located on the long arm of chromosome 6 (6q25.1) and contains eight exons [3] (Fig. 1, 2). ESR1 is a ligand-activated transcription factor that can be activated by growth factors in the absence of estrogen [4]. The N-terminus of ESR1 plays a critical role in the activation of ESR1-dependent genes, and any mutations at this site have been associated with high blood pressure [3]. Several polymorphisms of the ESR1 gene are associated with severe and mild preeclampsia, and the two most studied polymorphisms in intron 1 are ESR1 PvuII -397T / C (rs2234693) and XbaI -351A / G (rs9340799).

It has been established that the connection between the polymorphisms ESR1 PvuII -397T / C (rs2234693) and XbaI -351A / G (rs9340799) with the risk of severe and mild preeclampsia is controversial. Therefore, a meta-analysis of six articles (seven studies) was performed to investigate the association between ESR1 polymorphism and the risk of severe and mild preeclampsia.

The ESR1 and ESR2 genes are widely represented in various tissue types, but there are some differences in the pattern of their expression.

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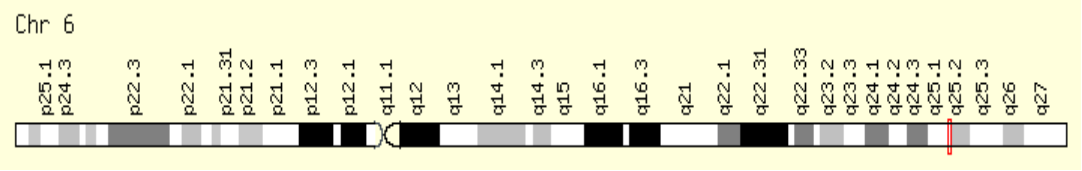

Figure 1. Genomic localization of gene ESR1 [4]

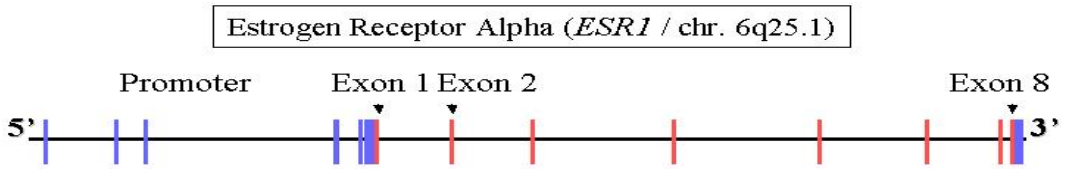

Figure 2. Gene of estrogen receptor-α ESR-α [5]

The estrogen receptor gene contains 8 exons (Fig. 1). Some of these exons are of particular importance in the hormone sensitivity of the cell or its absence. A different effect is observed depending on whether the receptor interacts with an agonist or antagonist (antiestrogen). This can explain the selectivity of the response of different tissues to the same estrogen receptor modulators.

This selectivity of estrogen receptors (ESR) is also determined by whether these changes cause activation and bringing them into a state of "ready to act" or, on the contrary, the inability of the receptor to bind to coactivators. The release of the estrogen receptor from the complex with the heat shock protein (hsp90) under the influence of the ligand activates its ability to interact with specific DNA sequences (steroid-sensitive elements). Binding of hsp90 to ESR is able to influence the processes of transcription regulation. ESR is a transcription factor induced by the emergence of estrogen in the environment.

Researchers have identified a point mutation in the ESR gene, leading to the loss of the functional properties of the estrogen receptor, which leads to development [6].

Estrogen receptors (ER) are a group of proteins classified as nuclear receptors and found in ligand-activated transcription factors. The effect of estrogen on target tissue is mediated by the ER [1]. Estrogen is the main endocrine hormone that plays a crucial role throughout pregnancy, including fetal development, uteroplacental blood flow, implantation, regulation of reproduction, and progesterone biosynthesis [2,3].

The purpose of the work is to study the rs2228480 polymorphism of the estrogen receptor alpha (ESR1) gene in patients with preeclampsia.

2. Materials and Methods

Materials: Research included several stages of clinical examination. The surveyed group consisted of 140 women, which divided in two groups. Patient group consist of 72 pregnant women, in the third trimester of pregnancy, with preeclampsia of varying severity, admitted to the Tashkent city maternity complexes No. 6 and No. 9. The age of women in the patient group was 20-45 years old. The control group consisted of 68 apparently healthy women with physiological pregnancy.

Methods: The material for the study was DNA samples from pregnant women with clinically diagnosed preeclampsia.

Isolation of DNA from blood and PCR analysis were performed with kits of reagents and test systems from Ampli Prime Ribo-prep (LLC Next Bio, Russia).

The concentration of the obtained preparation of nucleic acids in the samples was determined spectrophotometrically on a NanoDrop-2000 device (NanoDrop Technologies, USA).

Testing of the ESR1 mutation was carried out on a PCR amplifier Corbett research (Corbett. Australia), using the test system of the company (OOO NPF Sintol, Russia) according to the manufacturer's instructions.

Statistical processing of the results was carried out using the statistical software "EpiCalc 2000 Version 1.02". For statistical analyzing we use tests Pearson, Hardy-Weinberg and Fisher criteria.

3. Results

The prevalence of the frequencies of genotypes GG, GA and AA in preeclampsia was 79.2%, 18.0% and 2.8%, respectively, while in the control group - 72.1%, 26.4% and 1.5%, respectively (Table 1).

According to the results obtained, the frequency of the G and A allele is 85.3% and 14.7%, respectively (Table 2). The study of rs2228480 polymorphism of the ESR1 gene showed that in the group of apparently healthy people of Uzbek nationality, no significant deviations of the observed genotypes from those expected when checking for compliance with the Hardy-Weinberg equilibrium (HWE) ($\chi 2 = 0.207$; $p = 0.649$) were found. At the same time, the theoretically expected frequency of genotypes in the control group was: GG = 0.72; GA = 0.26; A / A = 0.015; actually observed: GG = 0.727; GA = 0.25; GG = 0.026 (Table 2).

Table 1. Frequency of distribution of alleles and genotypes of rs2228480 pol ymorphism of ESR1 gene in patient and control groups

№	Groups	n	Allele frequency				Genotype distribution frequency					
			G		A		GG		GA		AA	
			n	%	n	%	n	%	n	%	N	%
1	Control group (n = 68)	68	116	85,3	20	14,7	49	72,1	18	26,4	1	1,5
2	Patient group (n = 72)	72	127	88,2	17	11,8	57	79,2	13	18,0	2	2,8

Table 2. Distribution of genotypes of the polymorphic variant rs2228480 of the ESR1 gene to study groups

Groups	Allele frequencies		HWE	Genotype frequencies		
	G	A		G/G	G/A	A/A
Control group (n=68)	0,850	0,150	Observed	0,721	0,265	0,015
			Expected	0,727	0,251	0,022
			$\chi 2$	0,004	0,052	0,151
			$\sum$ p и $\sum \chi 2$	p =0,649; $\chi 2$=0,207		
Patient group (n=72)	0,88	0,12	Observed	0,792	0,181	0,028
			Expected	0,778	0,208	0,013
			$\chi 2$	0,018	0,265	0,990
			$\sum$ p и $\sum \chi 2$	p =0,25; $\chi 2$=1,27		

The data obtained from patients with preeclampsia are also consistent with the HWE law, where $\chi 2 = 1.27$ and p = 0.25. The expected frequency of genotypes in this group was: GG = 0.78; GA = 0.21; A / A = 0.013; observable: GG = 0.79; GA = 0.18; A / A = 0.028.

Thus, the rs2228480 polymorphism of the ESR1 gene in the population sample has the following characteristics: the frequency of the functionally unfavorable allele A is 15%, the distribution of the expected and observed genotypes of this polymorphism corresponds to HWE.

The study of the associative relationship between the rs2228480 polymorphism of the ESR1 gene and the risk of developing PE (Table 3):

Table 3. Association between the rs2228480 polymorphism of the ESR1 gene and the risk of developing Preeclampsia

Group	Alleles and genotypes	Statistical difference					
		Risk ratio		Odds ratio		$\chi 2$	p-value
		RR	95% CI:	OR	95% CI:		
Patient group (n=72)	G	1,13	0,78–1,64	1,29	0,64–2,57	0,51	0,23
	A	0,88	0,61–1,27	0,77	0,38–1,56		
	G/G	1,22	0,80–1,85	1,47	0,67–3,22	0,96	0,16
	GA	0,77	0,49–1,22	0,62	0,27–1,39	1,3	0,21
	AA	1,24	0,54–2,8	1,7	0,15–19,5	0,19	0,32

When comparing the frequencies of genotypes and alleles of the rs2228480 polymorphism of the ESR1 gene in the studied groups of patients and controls, we did not reveal statistically significant differences.

Thus, the presence of the unfavorable G allele and the homozygous G / G genotype significantly increases the risk of developing preeclampsia, while the presence of the A allele and the A / A genotype decreases it.

4. Discussion

When comparing the frequencies of genotypes and alleles of the rs2228480 polymorphism of the ESR1 gene in the studied groups of patients and controls, we did not reveal statistically significant differences. In the patient group, the frequency of the G allele of polymorphism 2014G> A of the ESR1 gene was 88.3%, in the control group - 85.7%, which also indicated an insignificant association with an increased risk of predisposition to preeclampsia ($\chi 2 = 0.31$; P = 0, 28; OR = 1.26; 95% CI: 0.56-2.89).

How we know, according to studies conducted in the Chinese population, the expression of estradiol is significantly reduced during preeclampsia, while we observed a significant increase in the expression of ESR α in patients with SPE [11].

Our data have demonstrated the insignificance of the observed changes in the frequency of detection of the studied genotypes in different groups.

As mentioned above, the data of our study showed a tendency for an increase in the detection of the G allele and the GG genotype in the groups of patients with preeclampsia, which could indicate the presence of a risk in identifying these genotypic and allelic variants of the rs2228480 polymorphism of the ESR1 gene.

At the same time, in the groups of conditionally healthy individuals in the control group, a tendency towards the prevalence of allele A and genotype AA and GA was established, which could characterize them as protective.

However, the insignificance of the revealed changes does not allow us to draw such conclusions. The lack of statistical reliability of the identified changes can be explained by the insufficient size of the sample under study.

At the same time, according to the meta-analysis conducted by Ge Zhao et al. (2019) that the GG genotype of the ESR1 Xbal polymorphism may be a genetic risk factor for a severe predisposition to preeclampsia [10, p. 1-10].

At the same time, the absence of statistically significant differences is confirmed by the studies of Hesham A. El-Beshbishy et al. (2015) ... in the population of Saudi patients with preeclampsia, no association was observed between preeclampsia and haplotypes C-G, T-G and C-A of ESR1 polymorphisms [12, p. S. 880-885]. Which also confirm the need for further studies using a large sample are needed to understand and study the mechanism of ESR 1 gene polymorphism in preeclampsia.

Consequently, the limited number of studies and small sample size may not provide sufficient statistical power to investigate the association between ESR1 polymorphism and the risk of severe or mild preeclampsia.

5. Conclusions

Thus, according to preliminary data, the presence of the G allele and homozygous GG genotypes increases, and the detection of the A allele of the heterozygote of the GA genotype and the AA genotype decreases the risk of developing preeclampsia.

Thus, we have obtained interesting data that require further research. It is recommended to conduct further studies of the association of ESR 1 gene polymorphism with the development of preeclampsia using a larger sample.

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