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EVALUATION OF THE SIGNIFICANCE OF THE G/A POLYMORPHISM OF THEF7 GENE IN THE DEVELOPMENT OF AN UNFAVORABLE IVF OUTCOME IN WOMEN WITH THROMBOPHILIA

Hafizova Dilnoza Bakhodirovna

Abstract: The article presents the results of molecular genetic studies полиморфизма of the G/A polymorphism F7 of the F7 90 gene in patients with unsuccessful IVF in women with thrombophilia. The results of the study showed that the A and hetero - G/A allele and homozygous non-functional A / A genotypes полиморфизма of theF7 polymorphism are one of the markers of an increased risk of thrombophilia in women of the Uzbek population. And the functional allele G and the functionally favorable genotype G/G are functional genotypes for the development of pathology (χ^2=15.48; p<0.0004; OR=0.03; 95%CI0.00-0.46).

Keywords: thrombophilia, genetics, полиморфизм G/A polymorphism of theF7 gene

The study of the mechanisms of development of obstetric complications (SPD, SORP, AFN, non-developing pregnancies), taking into account the genetic factors of the body, may provide some understanding in the pathogenesis, clinical course and development of preventive measures of the disease. [1,2,4,5,9,16,17]

Studies conducted by scientists Makatsaria A.D., Bitsadze V. O. (2006) on the role of thrombophilia, in particular, APS, mutations of factor V Leiden, prothrombin G202 10A and MTHFR C677T, showed an extremely high frequency of them not only in patients with various thromboembolic complications, but also typically obstetric complications, such as habitual miscarriages, severe gestosis, premature detachment of the normally located placenta (PONRP), intrauterine growth retardation syndrome (SVRP), antenatal fetal death (AHP), etc.

At the same time, thrombophilia as a complex integral factor in the pathogenesis of gestosis was found in 80% of patients with gestosis. It was notedthat among patients with mild forms of gestosis , thrombophilia was detected in 54%, while in the control group of pregnant women with a physiological course of pregnancy-in 16% (p<0.05). The results of the study showed that the mutation of MTHFR C677T was found in 56.8% of cases, while in the group of mild forms of gestosis and in patients with a physiological course of pregnancy - 44 and 12%. While the PAI-1 gene polymorphism was detected in 49.1% . In the group of pregnant women with mild forms of gestosis, this polymorphism was found in 40%, and in the control group of pregnant women with a physiological course of pregnancy-only16%(p<0.05).[6,7,8,10,12-15]

Also multigenic forms of thrombophilia, which accounted for 72.5%, while in the group of mild forms of gestosis only 14in the group of physiological pregnancy - 4%, and it should be noted that in the two control groups, multigenic thrombophilia was represented only by a gene polymorphism or heterozygous MTHFR C677T, but not by a mutation of FV Leiden or prothrombin G20210A.

Antiphospholipid antibodies were detected in 17.3% in the retrospective group, 16.25% in the prospective group, 10% in the group of mild gestosis, and 4% in the control group with a physiological course of pregnancy. [6,7,9,13,17,18]

The aim of our research was to study the detectable polymorphisms of the G/A genotypes of theF7 gene in women of the Uzbek population.

Material and methods of research. A total y of 118 women aged 20 to 39 years were examined. Among 118 women, the main group consisted of 90 (76.3%) with unsuccessful IVF in a woman with thrombophilia and 28 - a control group of healthy women without thrombophilia.

By informed consent, moleculargenetic analysis of the G/A gene of theF7 gene was

performed by real-time polymerase chain reaction. All pregnant women underwent general clinical, instrumental, functional (ultrasound), and ELISA studies. Pregnant women were consulted by related specialists. (general practitioner, neurologist, infectious diseases specialist, dermatologist, endocrinologist, etc.) Among 118 patients, the main group consisted of 90 women with an established diagnosis of thrombophilia and 28 women were the control group of the corresponding age. Molecular genetic examination of biomaterials (DNA) was performed at the clinical laboratoryof Genotechnologies LLC. DNA extraction from all biological blood samples was performed usingthe Ribot-prep kit(Interlabservis, Russia).

To detect polymorphism of the genotype consisting of alleles G>A Fof the F7gene, alleles-specific primers from the manufacturer were selected from DNA samples. For genotyping of DNA samples by polymerase chain reaction (PCR), 200 DNA samples were studied. To do this, the 96-cell automated amplifier "Applied Biosystems Veriti" was optimized according to the following program: initial denaturation once at 180 seconds 94°C, 94°C-10 seconds, 64°C-10 seconds, 72°C-20 seconds in the program, we performed these specified actions 40 times to make the operation occur polymerase chain reaction. Statistical analysis of the results was performed using the statistical software package "OpenEpi 2009, Version 2.3".**Research results. The results of molecular genetic studies are presented in the following table. (table 1) Table 1.**

№	Group	Allele				frequency Genotype distribution					
		frequency G		A		G/G		G/A		A / A	
		n	%	n	%	n	%	n	%	n	%
1.	Main group n=90(180)	138	76,6	42	23,3	55	61,1,1	28	31,1*	7	7,7,7
2	Counter. group n=28 (56)	56	100			28	100				

N -the number of patients examined; *n - the number of alleles studied; * - a confidence indicator in relation to the control group ($P<0.05$)

As can be seen from the table, a comparative analysis of the distribution frequencies of alleles and genotypes Fof the F7 (G/A) polymorphism of the homeostasis system gene among 180 DNA samples in 90 patients with unsuccessful IVF in women with thrombophilia , the presence of the functional аллеляG allele was 76.6% (138/180) of cases, and in the control group this allele was detected-in 100% (56/56), which was 1.3 times higher than in the main group.

(χ^2=15.90; p<0.0001; OR=0.03; 95%CI 0.00-0.48). While the mutant allele A was detected in the main group in 23.3% of cases (42/180), and in the control group this allele was not detected in the studied isolated DNA of healthy women. (χ^2=15.90; p<0.0001; OR=34.68; 95%CI2.10 – 573.21) General model наследования генаof F7 gene inheritance F(chi-square test, df = 2) is presented in the following table (table 2.) **Table 2.**

Alleles and genotypes	**Frequency of alleles and genotypes**		**Statistical difference**
	Main group	***Control***	
Allele G	138	138	$\chi^2$56/2=15.90; p<0.0001; OR=34.68; 95%CICI 2.10-573.21
Allele A	42	0	
Genotype **G/G**	55	55	$\chi^2$28/2=15.48; p<0.0004; OR=0.03; 95%CI0.00-0.46
Genotype G / A	28	0/2	$\chi^=$=15.48; p<0.0004; OR=25.99; 95%CI 1.53 – 440.86
Genotype **A/A**	7	7	$\chi^2$0/2=15.48; p<0.0004; OR=5.12; 95%CI 0.28 – 92.51

The results of molecular genetic studies of genotypes of theF7 gene polymorphism showed in the examined patients that the homozygous variant of functional G/G genotypes in the control group of women without thrombophilia was detected in 100% of cases (28/28), and in the main group – 61.1% (55/90), which was 1.6 times lower than in the control group. (χ^2=15.48; p<0.0004; OR=0.03; 95%CI0.00 – 0.46) . Whereas the heterozygous G/A variant of the F7 gene was not detected in the control group. And in the main group of patients, the heterozygous вариант G/A variant of the F7 gene was detected in 31.1% of cases (55/90) (χ^2=15.48; p<0.0004; OR=25.99; 95%CI 1.53-440.86) and the mutant homozygous A / A variant was detected in 7 patients, which was 7.7%. (χ^2=15.48; p<0.0004; OR=5.12; 95%CI 0.28 – 92.51). In the control group of patients, this genotype was not determined.

Analysis of the obtained results indicates that in patients with unsuccessful IVF with thrombophilia, the carrier of the heterozygous and mutant homozygous variants of theF7 gene genotypes was statistically significantly higher . (P <0.001).

According to literature data, the allele variant c. 1238A (heterozygote c. 1238G/A and homozygote c. 1238A/A) of the F7 gene leads to a decrease in gene expression and a decrease in the level of factor 7 in the blood, and is considered as a protective marker for the development of thrombosis and myocardial infarction.

Taking into account the peculiarities of the physiological adaptation of the hemostatic system to pregnancy, the absolute majority of genetic forms of thrombophilia are clinically manifested during the gestational process and, as it turned out, not only in the form of thrombosis, but also in the form of typical obstetric complications.

In the studies of biklo, it was found that during this period, the mother's body

undergoes a restructuring of the coagulation, anticoagulation and fibrinolytic system, which leads to an increase in blood clotting factors by 200%. Moreover, in the third trimesterснижается , the blood flow rate in the veins of the lower extremities decreases by half due to partial mechanical obstruction of the venous outflow by the pregnant uterus. The tendency to blood stasis in combination with hypercoagulation during physiological pregnancy predisposes to the development of thrombosis and thromboembolism. And with pre-existing (genetic) TF, the risk of thrombotic and obstetric complications increases tens and hundreds of times, which corresponds to our own researchя.

To assess the frequency of occurrence of various genotypes of theF7 polymorphic gene and the potential influence of a number of dynamic factors, that determine the genetic structure of the population, as well as to assess the population risk of developing dysfunctional IVF , we analyzed the expected and observed frequency of genotypes of the studied polymorphism and the correspondence of the frequency distribution равновесию to the Hardy-Weinbergequilibrium (XB).

Table 3.

Expected and observed frequency of distribution of genotypes by RCB of the F7 polymorphism7 in the main group of patients.

Genotypes	genotype frequency		χ^2	P
	Observed	expected		
G/G	61.1,1	53.01,01	0.588	
G /A	31.1	39.6	0.358	
A/A	7.8	7.4	0.054	
Total	100.00	100.00	0.64	**0.42**

Based on the XB equation, the frequency of observed favorable G/G genotypes in the main group was 1.2 times higher than the expected frequencies-61.1% and 53.01%, respectively. The heterozygous G/A variant of the observed frequency гена of theF7 gene was 31.1%, and the theoretically expected frequency was 39.6%, respectively, which indicates a 1.3-fold increase in this indicator. (P <0.05). The frequencyof the observed mutant homozygous variant A/A генаof the F7 gene was 7.8% , and the expected one-7.4 %, which was 1.05 times higher than the expected values (P> 0.05).

See Table 4.

Expected and observed frequency of distribution of genotypes by RCB of the F7 polymorphism7 in the control group of patients .

Genotypes	genotype frequency		χ^2	P
	Observed	expected		
G/G	100.0	60.66	1.000	
G /A	0	34.51	0.000	
A/A	0	4.9	0.000	
Total	100.00	100.00	0	**1**

The results of the analysis of the expected frequencies of genotypes of theF7 gene in the control group showed that the observed frequency of functional genotypes G/G was 100%, while the expected frequency was 60.6%, which was 1.4 times lower than the observed values. While the observed frequency of the heterozygous G/T variant and mutant homozygous variants of the F7 gene was 0, the expected frequency was 34.5 and 4.9%, respectively, which indicates an increase in the definability of the carrier polymorphism of the mutant genotype association.

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