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Research Article

On the Results of the Detection of Allelic Variants and the Association of Polymorphism IIe 105Val of the GSTP1 Gene in the Mechanism of the Development of Asherman Syndrome in the Uzbek Population

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Summary

Abstract: Asherman syndrome or intrauterine synechia is one of the urgent problems of obstetric practice, characterized by the formation of adhesions and outgrowths of the endometrium with its sclerosis and fibrosis. The search for pathogenetic aspects of morbidity is a priority area of medical science.

Aim: The Aim of the Study was to study allelic variants and the association of polymorphism IIe 105Val of the GSTP1 gene of the xenobiotic biotransformation enzyme in the mechanism of the development of Asherman syndrome.

Material and Methods of Research: We examined 26 patients with Asherman syndrome aged 19 to 42 years, who were observed at the clinic of RSSPMC O&G of the Ministry of Health of the Republic of Uzbekistan. All patients underwent clinical, functional, molecular genetics and statistical studies. The control group consisted of 22 women of healthy reproductive age of the corresponding age.

Research Results: Molecular genetic studies of the association of IIe 105 Val genotype polymorphism of the GSTP1 gene revealed the presence of favorable A/A genotypes - 7.7% (2/26), which was 11.8 times lower than in the control group. ($\chi 2=33.5$ $p<0.0008$; OR=0.01; 95%CI 0.0-0.06). Whereas the heterozygous genotype A/G of the GSTP1 gene in the main group of patients was 65.4% (17/26), which was 7.2 times higher than in the control healthy individuals. ($\chi 2=33.5$ $p<0.0008$; OR=18.9; 95%CI 3.58-99.64). And the unfavorable homozygous genotype G/G of the GSTP1 gene was not determined in the control group, while in the main group it was 26.9% (7/26). ($\chi 2=33.47$ $p<0.0008$; OR=17.31; 95% CI 0.93-322.9).

Conclusion: The analysis of molecular genetic studies showed that the carriage of the heterozygous genotype of the GSTP1 gene polymorphism can be a predisposition factor to the development of Asherman syndrome in women of the Uzbek population, increasing its risk by 18.9 times (OR = 19.9). ($\chi 2=33.5$ $p<0.0008$; OR=18.9; 95%CI 3.58-99.64).

Keywords: Asherman syndrome, Genetics, GSTP1 detoxification enzyme genes, Prediction

Introduction

In global healthcare, infertility is still one of the major challenges of reproductive health. According to WHO, the frequency of infertile marriages is 11 to 16.4%, and at the present stage does not tend to decrease [1-3].

According to studies, infertility and hypomenorrhea are the most common clinical manifestations of Asherman syndrome, accounting for 54.9% and 32.1% of cases, respectively [1,3,4]. Asherman syndrome is an intrauterine pathology, which is characterized by the formation of numerous synechiae, because of which the endometrium atrophies and becomes sclerosed. More than 50% of cases of female infertility are accompanied by pathological changes in the uterus, and intrauterine pathology is detected in 39.7% of women [1,3]. It should be noted that there are several difficulties in diagnosing Asherman syndrome, and in most cases the main leads to the re-development of the disease, which is an aggravating factor for reproduction.

Due to this, the search for genetic markers responsible for the development of this pathology is currently one of the priority areas of obstetric science and practice [2,5-7]. In medical science, special attention is paid to genomic and proteomic research aimed at studying genetic and biochemical polymorphic systems and the relationship of individual allelic variants of genes with various pathological processes, as well as with the intensity of biochemical reactions in the pathogenesis of many diseases [5,6]. In the pathogenesis of obstetric pathology, along with the main trigger factors of an endo- and exogenous nature, the so-called modifier genes are involved, the effect of which is largely determined by environmental factors. Among these genes, the Glutathione-S-Transferase (GST) genes encoding enzymes of the second phase of xenobiotic biotransformation are of particular interest [6-11]. These enzymes are responsible for the biotransformation of chemicals, biological agents, pharmaceutical products, etc. that enter the body. Particular attention is paid to the enzyme of the second phase of detoxification, i.e., glutathione transferase, a product of the GSTP1 gene.

Studies of the determinability of the xenobiotic enzyme genes of the Uzbek population were carried out in patients with FGRS (Fetal Growth Restriction Syndrome) *Mavlyanova NN and Boboyev K.T* (2018) [3]. However, no patients with Asherman syndrome have been studied in Uzbekistan. In this regard, studying the genes of xenobiotic enzymes of the second phase in this category of female patients of the Uzbek population was of interest to us.

The Aim of Our Research

was to study allelic variants and the association of IIe 105Val polymorphism of the GSTP1 gene of the xenobiotic biotransformation enzyme in the mechanism of development of Asherman syndrome.

Material and Methods of Research

The object and subject of the research were patients with Ash

erman syndrome, DNA samples from patients and healthy donors, the glutathione transferase gene GSTP1 (IIe 105 Val). The study included 26 patients with Asherman syndrome aged 19 to 42 years, who were observed at the clinic of the RSSPMC O&G of the Ministry of Public Health of the Republic of Uzbekistan. 15 women aged 19 to 25, 7 women aged 26 to 30, 2 ones aged 31 to 36, and 2 ones aged over 36. The control group consisted of 22 women of healthy reproductive age. Molecular genetic examination of biomaterials (DNA) was performed on the basis of the SI RSNPMC of Hematology and Blood Transfusion of the Republic of Uzbekistan under a scientific agreement. The object and subject of the study were DNA samples of patients and healthy people, the GSTP1 (A/G) gene.

DNA samples were isolated from peripheral blood lymphocytes in accordance with a modified method. The concentration and purity of the isolated DNA were evaluated by measuring the optical density of DNA-containing solutions at a wavelength of 260 and 280 nm against TE on a NanoDrop 2000 spectrophotometer (USA). Genotyping of A/G polymorphism, GSTP1 gene was carried out on a Rotor Gene 6000 Model 65H0-100 (Australia) PCR amplifier in real-time mode, using the test system of "Synthol" (Russia), Cat. No.-NP_555_100_RG, in accordance with the manufacturer's instructions. Statistical analysis of the results was carried out using the statistical software package "OpenEpi 2009, Version 2.3." The frequency of variants of alleles and genotypes (f) was calculated by the formula: f=n/2N and f=n/N, where n is the occurrence of the (allele and genotype) variant, and N is the sample size.

Research Results

Table 1: The sequence of oligonucleotide primers used for PCR.

No.	Gene, Localization	Polymorphism	Structure of Oligoprimers
1	GSTP1 (11 (11.g13))	detection	5'-ACCAGGGCTCTATGGCCAA- 5'-TGACCCGAGAAGAACGGGT-3', '

In terms of age, 15 women aged 19 to 25, 7 women aged 26 to 30, 2ones aged 31 to 36, and2 ones aged over36. Molecular genetic studies of the IIe 105 Val polymorphism of the GSTP1 gene were carried out with the informed consent of the patients. Information

about gene sequences and the structure of primers was obtained taking into account the original literature source and from GeneBank. The characteristics of the genetic marker and the sequence of the synthesized oligoprimers are shown in (Table 1).

The results of molecular genetic studies of the GSTP1 gene had the following definitions, which are given in (Table 2).

Table 2: Frequency distribution of alleles and genotypes of IIe 105 Val polymorphism of the GSTP1 gene in groups of patients with Asherman syndrome and the control group of healthy women.

No.	Group	Frequency of Alleles				Frequency of Genotype Distribution					
		A		G		A/A		A/G		G/G	
		n	%	n	%	n	%	n	%	n	%
1	Main group n= 26 (52)	21	40.4	31	59.6	2	7.7	17	65.4	7	26.9
2	Control group n=22 (44)	42	95.5	2	4.5	20	90.9	2	9.1		

The results of the study of allelic variants of the GSTP1 gene showed that in the control group of healthy subjects, the favourable allele A was detected in 95.5% of cases (42/44), and in the main group of patients with Asherman syndrome, it was found in 40.4% of cases (21/52), which was 2.4 times lower than in the control healthy group. ($\chi2$=32.04 p<0.00008; OR=0.03; 95%CI 0.01-0.15). Then the detection of the unfavorable allele G in the control group was 4.5% of cases (2/44), and in the main group, it was 59.6% (31/52), which was 13.2 times higher than in healthy subjects. ($\chi2$=32.04 p<0.00008; OR=31.0; 95% CI 6.76-142.15).

Analysis of the data obtained indicates the presence of an association between the mutant allele "G" IIe 105 Val of the GSTP1 gene and Asherman syndrome, with a high odds ratio (OR= 31.0). The study of the association of polymorphism of IIe 105 Val genotypes of the GSTP1 gene revealed the presence of favorable A/A genotypes in 90.9% (20/22) in the control group of healthy subjects, while in the main group this genotype was 7.7% (2/26), which was 11.8 times lower than in the control group. ($\chi2$=33.5 p<0.0008; OR=0.01; 95%CI 0.0-0.06). The heterozygous genotype A/G of the GSTP1 gene in the control group was determined in 9.1% of cases (2/22), and in the main group, it was determined in 65.4% of cases (17/26), which was 7.2 times higher than in the control group of healthy subjects. ($\chi2$=33.5 p<0.0008; OR=18.9; 95%CI 3.58-99.64). The unfavorable homozygous genotype G/G of the GSTP1 gene was not determined in the control group, while in the main group it was 26.9% (7/26). ($\chi2$=33.47 p<0.0008; OR=17.31; 95% CI 0.93-322.9).

Taking into account the fact that in the main group there was a significant detectability of the unfavourable allele G and the association of polymorphism of unfavorable genotypes – 13.2 and 7.2 times more than in the control group, the data obtained may indicate that the carriage of the unfavourable allele G and the heterozygous genotype A/ G polymorphism of the GSTP1 gene may be a factor of predisposition to the development of this syndrome, increasing its risk by 18.9 times (OR=18.9) (Table 3).

Table 3: Differences in the frequency of occurrence of alleles and genotypes of the IIe 105 Val polymorphism of the GSTP1 gene in the main and control groups.

Alleles and Genotypes	Number of Examined Alleles and Genotypes		Statistical Difference
	Main group	Control	
Allele A	21	42	$\chi2$=32,04 p<0,00008; OR=31,0; 95%CI 6,76-142,15
Allele G	31	2	
A/A genotype	2	20	$\chi2$=33,5 p<0,0008; OR=0,01; 95%CI 0,0-0,06
A/G genotype	17	2	$\chi2$=33,5 p<0,0008; OR=18,9; 95%CI 3,58-99,64
G/G genotype	7	0	$\chi2$=33,47 p<0,0008; OR=17,31; 95%CI 0,93-322,9

Thus, the results of molecular genetic studies have shown that the unfavorable variant allele "G" of the IIe 105 Val polymorphism of the GSTP1 gene, which leads to the replacement of A with G at position 105 of the amino acid sequence, may be associated with the development of Asherman syndrome. It was found that the risk of developing Asherman syndrome in women in the Uzbek population in the presence of the variant allele G polymorphism in the genome increased by 31.0 times (OR=31.0).

The result also indicates that the heterozygous A/G genotype of the IIe 105 Val polymorphism of the GSTP1 gene is a genetic determinant that is a predisposition factor to the risk of developing this pathology, increasing its risk by 31.0 times (OR=31.0). In the analysis of the results of molecular genetic studies, it is important to assess the expected and observed frequency of the genotypes of the studied polymorphic genes, potentially associated with the development and pathogenesis of diseases, which can be determined in accordance with the distribution of frequencies according to the *Hardy-Weinberg Equilibrium* (HWE) (Table 4).

Table 4: Expected and observed frequency of distribution of genotypes according to HWE of the IIe 105 Val polymorphism of the GSTP1 gene in the main group of patients with Asherman syndrome.

Genotypes	Frequency of Genotypes		$\chi 2$	P
	Observed	Expected		
A/A	7.7	53.01	0.163	0.23
A/G	65.4	39.6	0.482	
G/G	26.9	7.4	0.355	
Total	100	100	1.45	

As follows from Table 4, the frequency indicators of genotype distribution according to HWE of the IIe 105 Val polymorphism of the GSTP1 gene in the main group of patients, the observed frequency of A/A genotypes occurred in 7.7%, and the expected frequency occurred in 53.01% of cases. Whereas the expected heterozygous genotype was 39.6% of cases, and the observed heterozygous A/G genotype was 65.4%, which was 1.6 times higher than expected. The homozygous unfavourable variant of G/G genotypes in the observed frequencies was 26.9%, and in the expected frequencies it was 7.4%, which was 3.6 times lower compared to the observed ones, respectively. The results obtained are of great importance in predicting the risk of developing morbidity. In the control group, the observed and expected frequency of favourable genotypes was 90.9 and 60.6%, respectively, while the heterozygous variant of the observed frequency was 9.1%, and the expected frequency was 34.5%, which is 3.8 times exceeded the observed frequency ($P<0.05$). The homozygotic variant of the favourable G/G genotypes of the observed frequency was not detected, while in the expected one it was 4.9% (Table 5).

Table 5: Expected and observed frequency of distribution of genotypes according to HWE of the IIe 105 Val polymorphism of the GSTP1 gene in the control group.

Genotypes	Frequency of Genotypes		$\chi 2$	P
	Observed	Expected		
A/A	90.9	60.6	0.911	1
A/G	9.1	34.5	0.087	
G/G	0	4.9	0.002	
Total	100	100	0	

A comparative analysis of the expected and observed frequencies of the genotypes of this polymorphism showed a statistically significant deviation of indicators ($P<0.05$) in all the studied groups and subgroups (Tables 4,5). This fact indicates that the observed proportion of genotypes in the studied samples corresponds to the Hardy-Weinberg equilibrium.

The analysis showed that both in the control group and in the main group with Asherman syndrome, the values of the expected and observed heterozygosity of the studied polymorphism were as follows. Thus, in patients of the main group, with the carriage of favourable A/A genotypes of the GSTP1 gene, there is an increase in the frequency of expected genotypes by 6.8 times, while in the control group of healthy subjects, there is an increase in the frequency of heterozygous variants of the A/G gene IIe 105 Val of the GSTP1 gene by 3.4 times, which is important in predicting the risk of developing Asherman syndrome.

The analysis of the obtained results shows that the distribution of all genotypes of the IIe 105 Val polymorphism of the GSTP1 gene in the main group and in the control group corresponds to HWE, indicating the absence of the influence of systematic or random factors that can change the genetic structure of populations. The study of the genetic structure of this marker revealed a relatively high level of the expected A/G IIe 105 Val heterozygosity of the GSTP1 gene in the main group of patients with Asherman syndrome in relation to the control group (39.6% and 34.5%, respectively). In both groups, the indicator D is to the left of 0, that is, it is negative ($D<0$). The revealed fact testifies to higher frequencies of expected heterozygotes, but not actually calculated heterozygotes. When an-

alyzing the distribution of frequencies of occurrence of alleles and genotypes of this polymorphism in the main group of patients, significant differences were found compared to the control group. The functionally unfavorable allele G in the main group was 13.2 times higher than in healthy subjects. ($\chi2$=32.04 p<0.00008; OR=31.0; 95% CI 6.76-142.15).

The distribution of frequencies of the A/G IIe 105 Val genotypes of the GSTP1 gene also revealed significant differences between the main group and the control group in the total sample (P<0.05). Associations of "functionally unfavorable" A/G genotypes of the GSTP1 gene ($\chi2$=33.5 p<0.0008; OR=18.9; 95% CI 3.58-99.64) and the unfavorable homozygous G/G genotype of the GSTP1 gene ($\chi2$=33.47 p<0.0008; OR=17.31; 95%CI 0.93-322.9) with the risk of developing Asherman syndrome, responsible for the detoxification functions of the body, were found.

Thus, the allele G and the association of polymorphism of the heterozygous genotype IIe 105 Val of the GSTP1 gene are markers of an increased risk of developing Asherman syndrome in women of the Uzbek population. (P<0.05). Allele A and functionally favourable A/A genotype are reliable functional markers for the development of pathology ($\chi2$=32.04 p<0.00008; OR=0.03; 95%CI 0.01-0.15).

Considering the fact that in the main group there was a significant detectability of the association of polymorphism of unfavorable heterozygous genotypes, namely 7.2 times more compared to the control group, the data obtained may indicate that the carriage of the heterozygous genotype of polymorphism of the GSTP1 gene polymorphism may be a predisposition factor to the development of this pathology, increasing its risk by 18.9 times (OR=19.9). ($\chi2$=33.5 p<0.0008; OR=18.9; 95%CI 3.58-99.64)

Conclusions

a) Allele G and the association of polymorphism of the heterozygous genotype IIe 105 Val of the GSTP1 gene are markers of an increased risk of developing Asherman syndrome in women of the Uzbek population. (P<0.05). Allele A and the functionally favourable A/A genotype are reliable functional markers of the development of pathology ($\chi2$=32.04 p<0.00008; OR=0.03; 95%CI 0.01-0.15).

b) Analysis of molecular genetic studies showed that the carriage of the heterozygous genotype of polymorphism of the GSTP1 gene may be a predisposition factor to the development of Asherman syndrome in women of the Uzbek population, increasing its risk by 18.9 times (OR = 19.9). ($\chi2$=33.5 p<0.0008; OR=18.9; 95%CI 3.58-99.64).

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None.

Conflict of Interest

No conflict of interest.

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