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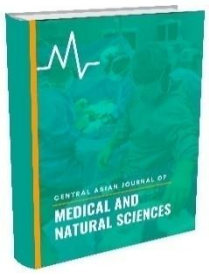

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THE ROLE OF ENDOTHELIAL DYSFUNCTION GENES IN THE DEVELOPMENT OF POSTPARTUM HEMORRHAGE

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Abstract: The vascular endothelium is a unique "endocrine tree" that lines absolutely all organs of the body's vascular system. Endothelial cells create a barrier between blood and tissues, perform a number of important regulatory functions, synthesizing and secreting a large number of different biologically active substances. The strategic location of the endothelium allows it to be sensitive to changes in the hemodynamic system, signals carried by the blood, and signals from the underlying tissues. In this article was given data about genes which leading to endothelial dysfunction and postpartum hemorrhage and were collected from women with uterine atony in postpartum period.

Key words: endothelial dysfunction, gene, hemodynamic system, postpartum hemorrhage, uterine atony.

Introduction. The vascular endothelium is a unique "endocrine tree" that lines absolutely all organs of the body's vascular system. Endothelial cells create a barrier between blood and tissues, perform a number of important regulatory functions, synthesizing and secreting a large number of different biologically active substances. The strategic location of the endothelium allows it to be sensitive to changes in the hemodynamic system, signals carried by the blood, and signals from the underlying tissues. A balanced release of biologically active substances helps maintain homeostasis. To date, data have been accumulated on the versatility of the mechanisms of endothelial participation in the occurrence and

development of various pathological conditions. This is due not only to its participation in the regulation of vascular tone, but also to its direct influence on the processes of atherogenesis, thrombus formation, and protection of the integrity of the vascular wall. Endothelial dysfunction is considered as a pathological condition of the endothelium, which is based on a violation of the synthesis of endothelial factors. As a result, the endothelium is unable to ensure the hemorheological balance of the blood, which leads to dysfunction of organs and systems. Endothelial dysfunction is a key link in the pathogenesis of many diseases and their complications. Currently, the role of endothelial dysfunction in the development of such chronic diseases as atherosclerosis, arterial hypertension, chronic heart failure, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease, inflammatory bowel disease, etc. has been proven.

Oxidative stress, the synthesis of powerful vasoconstrictors play the main role in the development of endothelial dysfunction, as well as cytokines and tumor necrosis factor, which suppress the production of nitric oxide (NO) [1]. Endothelial dysfunction may result from a decrease in the ability of the endothelium to synthesize, release or inactivate NO [2].

In humans and mammals, nitric oxide is mainly formed as a result of the oxidation of the guanidine group of the amino acid L-arginine with the simultaneous synthesis of another amino acid, citrulline, under the influence of the enzyme NO synthase (NOS). Three isoforms of this enzyme have been studied: neuronal (nNOS or NOS1), inducible (iNOS or NOS2) and endothelial (eNOS or NOS3) [3,4]. Two isoforms - NOS1 and NOS3 - are constitutive, that is, they are constantly expressed in neuronal and endothelial cells, respectively. The physiological effects of NOS are largely determined by polymorphisms in the structure of the NOS1 and NOS3 genes. Therefore, genetic polymorphisms of NOS1 and NOS3, which can partially/fully affect protein expression and/or biological activity, directly affect the level of NO in the body [5]. Currently, the number of works devoted to the search for candidate genes involved in the development of uterine atony is insignificant. In this regard, research devoted to identifying genetic mutations that lead to atonic uterine bleeding is relevant.

Aim of the work was to assess whether genetic polymorphism 786T>C of the NOS3 (rs2070744) and NOS1 (rs41279104) genes are associated with impaired myometrial contractility and the risk of developing atonic PPH in women of the Uzbek ethnic group.

Material and methods. We examined 101 women who were diagnosed with postpartum atonic bleeding of varying severity, and were included in the main group. The diagnosis of atonic PPH was made according to the criteria of the national clinical protocol of the Republic of Uzbekistan “Prevention and tactics of management of postpartum obstetric hemorrhage” approved on March 1, 2021. According to the protocol, the diagnosis of PPH was made when: blood loss ≥ 500 ml during vaginal delivery; for blood loss ≥1000 ml during cesarean section; and also, any clinically significant amount of blood loss (leading to hemodynamic instability) occurring within 12 weeks after birth [6]. Exclusion criteria included women who developed PPH due to retained placenta tissue or membranes, trauma to the birth canal, and a coagulation disorder not associated with bleeding. The control group consisted of 103 women without significant chronic somatic pathology, who had a history of natural childbirth without any complications or obstetric pathology. All women studied were of Uzbek nationality. All patients provided written informed consent to participate in the study.

All women underwent clinical, laboratory and instrumental studies, which also included standard methods of collecting anamnesis and physical examination.

The molecular genetic study was carried out on the basis of the Department of Molecular Medicine and Cell Technologies of the Republican Scientific and Practical Medical Center of Hematology of the Ministry of Health of the Republic of Uzbekistan. Whole venous blood was used as the test material for studying polymorphism. Genomic DNA preparations were used for molecular genetic detection of the 786T>C polymorphism in the NOS3 gene. Statistical analysis was performed using the OpenEpi v.9.2 application package. The distribution of genotypes was checked for compliance with the Hardy–Weinberg equilibrium using the computer program "GenePop" (http://wbiomed.curtin.edu.au/genepop) and assessed using the $\chi 2$ test.

Results. In table 1 were given the main clinical and anamnestic indicators of the patients we examined. The age of patients in all three subgroups did not differ significantly. When studying obstetric indicators in the main group of women who subsequently developed postpartum uterine atony, according to parity, multiparous women predominated in 61.4% of cases, rather than primiparous women in 38.6% of cases. A complicated course of pregnancy in the main group was noted in 27.7% of patients, and, apparently, complicated gestation does not have such a great influence on the development of bleeding after childbirth. As for the timing of delivery, premature birth was observed in 32.7% of postpartum women at 29-36 weeks of pregnancy.

Table 1. Main clinical and anamnestic characteristics of the examined patients

Index		Main group n=101	Control group n=103
Age, years (M±SD)		29,3±1,4	25±1,5
BMI, kg/m2 (M±SD)		28,5±1,1	30.7±3.1
Complicated pregnancy, n (%)		28 (27,7%)	-
Parity	Primiprava , n (%)	39 (38,6%)	34 (33%)
	Multiparous, n (%)	62 (61,4%)	69 (67%)
Gestation period	29-36 weeks, n (%)	33 (32,7%)	-
	37-42 weeks, n (%)	68 (67,3%)	103 (100%)

In the studied groups of patients and controls, the observed distribution of genotype frequencies for this polymorphism did not deviate from RCV, i.e. corresponded to the calculated expected value ($p>0.05$).

When analyzing the distribution of frequencies of alleles and genotypes of the 786T>C polymorphism in the NOS3 gene in women with uterine atony in the postpartum period in comparison with the control group, the following data were revealed (Table 2): there were no reliably significant differences in the frequency of occurrence of the functionally wild T allele and favorable T/T genotype in patients and women in the control group (69.8% versus 73.3%; 49.5% versus 52.4%, respectively). Results of the analysis of these odds ratio indicators, carriage of the T allele ($\chi 2=0.6$; $p=0.5$; OR=0.8; 95% CI:0.55-1.29) and homozygous genotype T/T ($\chi 2=0.2$; $p=0.7$; OR=0.9; 95% CI:0.51-1.54), did not reveal a significant protective effect on the risk of developing atonic postpartum hemorrhage (Table 2).

The frequency of distribution and the calculated odds ratio for the mutant allele C and the homozygous genotype C/C are noteworthy: the frequency of occurrence of these values in the group of patients and controls did not have significant differences (30.2% versus 26.7%; 9.9% versus 5.8%, respectively). The calculated odds ratio revealed an unreliable increase in risk by 1.2 times when carrying an unfavorable allele C

($\chi2$=0.6; p=0.5; OR=1.2; 95% CI:0.77-1.83) and 1.8 times, respectively , in the presence of a mutant homozygous genotype C/C ($\chi2$=1.2; p=0.3; OR=1.8; 95% CI:0.63-5.03). The above data indicate the absence of a clear association of the 786T>C polymorphism in the NOS3 gene in patients with postpartum atonic hemorrhage due to the lack of statistical significance of the results obtained (Table 2).

Table 2. Differences in the frequency of allelic and genotypic variants of the 786T>C polymorphism in the NOS3 gene in the main group of patients and controls

Alleles and genotypes	Number of examined alleles and genotypes				$\chi2$	p	OR	95%CI
	Main group		Control group					
	n	%	n	%				
C	61	30,2	55	26,7	0,6	0,5	1,2	0,77 - 1,83
T	141	69,8	151	73,3	0,6	0,5	0,8	0,55 - 1,29
C/C	10	9,9	6	5,8	1,2	0,3	1,8	0,63 - 5,03
C/T	41	40,6	43	41,7	0,0	0,9	1,0	0,55 - 1,67
T/T	50	49,5	54	52,4	0,2	0,7	0,9	0,51 - 1,54

The distribution of genotype and allele frequencies for the G84A polymorphism of the NOS1 gene is presented in Table. 3. Thus, it was found that in the sample under study, the dominant allele G occurred in 77.7% of cases, and the recessive allele A - in 22.3% of cases. The mutant genotype A/A in the patient group was recorded in 4.0% of cases, the heterozygous genotype G/A in 36.6% of cases, and the dominant genotype G/G in 59.4% of cases, in the control group 1.9%, 28 .2% and 69.9% of cases, respectively (Table 3).

Table 3. Frequency of distribution of alleles and genotypes of the G-84A polymorphism in the NOS1 gene in the patient and control groups

Num	Groups	Frequency of allels				Genotype distribution frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
1	Main group (n = 101)	157	77,7	45	22,3	60	59,4	37	36,6	4	4.0
2	Control group (n = 103)	173	84,0	33	16,0	72	69,9	29	28,2	2	1,9

To establish possible associations between allelic and genotypic variants of the G-84A polymorphism of the NOS1 gene with the development of atonic postpartum hemorrhage, a comparative analysis of the distribution of this marker in the groups of patients and controls was carried out. As can be seen from Table 4, in the main group and in the groups of patients with PPH, a tendency towards a significant difference in the frequency of occurrence of alleles and genotypes from the control sample was revealed for the G-84A polymorphism of the NOS1 gene (P<0.05).

Table 4. Differences in the frequency of allelic and genotypic variants of the G-84A polymorphism in the NOS1 gene in patient groups

Alleles and genotypes	Number of alleles and genotypes examined				χ2	p	OR	95%CI
	Main group		Control group					
	n	%	n	%				
G	157	77,7	173	84,0	2,6	0,2	0,7	0,41 - 1,09
A	45	22,3	33	16,0	2,6	0,2	1,5	0,91 - 2,47
G/G	60	59,4	72	69,9	2,5	0,2	0,6	0,35 - 1,12
G/A	37	36,6	29	28,2	1,7	0,2	1,5	0,82 - 2,66
A/A	4	4,0	2	1,9	0,7	0,4	2,1	0,39 - 11,23

The functionally unfavorable allele A did not statistically significantly prevail in women with PPH compared to the control sample (22.3% versus 16%, respectively; $\chi2=2.6$ and $p=0.2$), and allele G, on the contrary, was more common in the group of apparently healthy women compared with patients (84% versus 77.7%, respectively; $\chi2=2.6$ and $p=0.2$). According to the calculated odds ratio, carriers of the A allele tend to have a 1.5 times higher risk of developing postpartum atonic hemorrhage in pregnant women than in women who do not have it (OR=1.5; 95% CI: 0.91 - 2.47).

The wild G/G genotype was determined with a slightly higher frequency in the group of conditionally healthy women compared to the group of women giving birth with PPH (69.9% versus 59.4%, respectively). At the same time, the statistical difference in the studied groups did not reach the level of threshold significance ($\chi2=2.5$; $p=0.2$; OR=0.6; 95% CI:0.35-1.12). Perhaps this genotype has a protective effect against the development of PPH in pregnant women.

There is a higher frequency of occurrence of the homozygous mutant genotype A/A in women with PPH compared to controls - 4.0% and 1.9%, respectively ($\chi2=0.7$; $P=0.4$). The tendency to develop postpartum atonic bleeding in women carriers of this genotype was 2.1 times more likely than in carriers of other genotypes (OR=2.1; 95% CI 0.39-11.23).

The frequency of the unfavorable heterozygous G/A genotype of the G-84A polymorphism of the NOS1 gene was also significantly higher in the main group of women with PPH compared to the control sample (36.6% versus 28.2%, respectively, with $\chi2=1.7$; $p=0.2$). The risk of developing uterine atony in carriers of this genotype after childbirth is 1.5 times higher compared to other genotypic variants (OR=1.5; 95% 0.75-2.25).

The level and activity of NO depend on allelic variants of NO synthesis genes. In this regard, the study of genes involved in NO synthesis (primarily eNOS or NOS3) has become one of the frequently studied issues to determine genetic risk factors for various diseases, including obstetric and gynecological pathologies. It is also important to note that NOS3 inhibits platelet aggregation, prevents proliferation and migration of smooth muscle cells, and plays an important role in the processes of angiogenesis [7]. It is well known that there are significant differences in both hormonal levels and tissue-specific gene expression between men and women. An explanation for this effect may be the fact that sex hormones can have different effects on gene expression in somatic tissues, which leads to differences in susceptibility to diseases, characteristics of their course and severity [8].

Continuous exposure to NO promotes processes such as menstruation and implantation through prostaglandin synthesis and protein binding. Because three nitric oxide synthase genes are responsible for three separately expressed isoforms of NOS, the roles of NO in physiological processes are

different. Mutations of the NOS3 gene and their role in the development of such obstetric and gynecological pathologies as preeclampsia, gestational hypertension, recurrent miscarriage, fetal growth restriction syndrome, and disorders of uteroplacental and fetal blood flow were studied [8,9]. Clinical studies on the relationship between postpartum atonic hemorrhage and the G84A polymorphism of the NOS1 gene and 786T>C in the NOS3 gene have not been conducted to date, which explains the relevance of this study.

Conclusion. In women with postpartum atonic bleeding, according to the results of our studies, an association with the G-84A polymorphism of the NOS1 gene was revealed. Allelic variants A turned out to be risky in the development of this obstetric pathology. Our research data encourages further searches for genetic polymorphisms to predict the risk of postpartum hemorrhage and develop appropriate preventive measures.

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