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STUDY OF GENOTYPE FREQUENCY DISTRIBUTION POLYMORPHIC MARKER OF T-786C OF ENOS3 GENE IN DIABETIC NEPHROPATHY IN PATIENTS WITH CD2 TYPE

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Abstract: This article presents the results of a study of 129 patients with 2-type diabetes and 110 healthy people to determine whether polymorphic markers T-786C of the ENOS3 gene are related to the development of diabetic nephropathy (DN). Patients in the main group: 65 patients with duration of disease up to 10 years, without diabetic nephropathy (33 patients) and with diabetic nephropathy (32 patients), 64 patients with diabetes lasting more than 10-20 years, without diabetic nephropathy (31 patients) and diabetic nephropathy (33 patients). Genotyping was performed by polymerase chain reaction method. The study showed that the association of Callele C and ENOS3 gene genotype play a role in the development of diabetic nephropathy in patients with type 2 diabetes mellitus in the studied Uzbek nation.

Keywords: diabetic nephropathy, diabetes mellitus, nitrogen oxide synthase, endothelin-1, gene, polymorphism, allele, genotype.

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Introduction. Today, chronic kidney disease or LDD is a pathology, with an increasing prevalence of an NCD epidemic, along with diseases such as diabetes mellitus and obesity. MGH develops in 13-15% of the general population and is much more common, up to 40-50%, in risk groups that include patients with diabetes mellitus type 2 [2]. The International Diabetes Federation predicts that the number of diabetes patients worldwide will increase to 587 million by 2035, of

whom 95% are patients with diabetes2 [15].

According to the data of the National Register of Diabetes Diabetes Surveys conducted in Uzbekistan in 2007, the cause of death in patients with diabetes was MI in 6.0%, CVD in 10.8%, and blindness was registered in 1.7%, i.e. the final stages of micro- and macroangiopathy, which lead to severe disability and mortality develop only in 2-22% of patients with diabetes type 2 [11]. It was found that in patients with the first-time diagnosis of microalbuminuria (MAH) type 2 diabetes was found in 15-40% of cases, proteinuria - in 7-10%, and CVD - in 1%, which reflects the difficulties of diagnosis of the disease. When determining the time of the first diabetes mellitus type 2 relatively accurately, the dependence of the frequency of DN development on the duration of the disease is the same as in the case of type 1 diabetes mellitus: 7-10% in the case of 5 years of diabetes, 20-35% in the case of 20-25 years of diabetes, and 50-57% in the case of a longer duration of the disease. [7]

In most developed countries of the world, diabetes mellitus occupies the 3rd-4th place in the mortality structure and is the leading cause of myocardial infarction, chronic renal failure (CNS), blindness, and amputations in adults [9,19]. Mortality rates of patients cause a lot of controversy and are far from being true, as these patients die not from diabetes itself, but from complications of diabetes and are among those who died from cardiovascular pathology and CVD, which significantly reduces the number of deaths from diabetes.

In recent years, vascular complications of type 2 diabetes have been detected not only in newly diagnosed diabetic patients, but even in people with intermediate hyperglycemia. By the time of clinical manifestation of type 2 diabetes, about 50% of patients already have various macrovascular complications. Therefore, in addition to metabolic, immunological, and hemodynamic factors, there are hereditary, molecular, and genetic factors that determine the development and progression, or vice versa, the protection of vascular complications in diabetes [16,18].

The risk of nephropathy development is definitely determined by genetic factors. Only approximately 40-50% of patients with both type 1 diabetes and type 2 diabetes subsequently develop DN. Genetic factors may directly influence the development of DM and/or act in conjunction with genes affecting cardiovascular diseases. The search for genetic markers of susceptibility or, conversely, disease resistance is one of the most important tasks of medical science. [5]

This is determined by the fact that the establishment of such markers allows clinicians to form risk groups for diseases and, in some pathologies, to establish an individual prognosis or diagnosis (including before the clinical

manifestation of disease). Evaluation of the role of a genetic marker in diabetes depends on racial and ethnic variations in the frequency of alleles and genotypes in the populations studied [11,20]. In recent years, the literature has been widely discussing the genetic risk of diabetes development and its complications depending on the genes of insulin resistance, genes that determine the reduced level of insulin, polymorphism of the gene Angiotensin-I-converting enzyme (ASE), and the gene of endothelial NO-synthase (NOS) in patients with both types of diabetes mellitus [1,3,4].

At present, endothelial dysfunction dominates in the pathogenesis of micro- and macrovascular complications of diabetes, accompanied by a deficit of vasodilators - nitrogen oxide (NO) - and activation of local secretion of vasoconstrictors, such as endothelin-1 (E-1). Therefore, the gene, endothelial nitric oxide synthase (eNOS3), is of interest as a candidate gene for diabetic nephropathy and CDP in type 2 diabetes.

It is known that endothelium regulates vascular tone through the release of vasodilating and vascular factors and modulates the contractile activity of smooth muscle cells.

Endothelial dilation factors include nitrogen oxide (NO). NO is the main vasodilator that prevents tonic contraction of vessels of neural, endocrine or local origin. In physiological conditions, NO is constantly involved in the adaptation of the vascular system to increased metabolic needs, physical activity. In diseases, excess NO is responsible for increasing peripheral vasodilation, and lack of NO can lead to severe diseases, including arterial hypertension, coronary heart disease and atherosclerosis (also renal glomerular system vessels). [22,24]

NO prevents platelet adhesion and aggregation, monocyte adhesion, affects the structure of the vessel, which protects the vascular wall and prevents remodeling of the vessels in various pathological conditions. Nitrogen oxide is formed under the action of the enzyme NO-synthase (NOS). NO-synthase exists in the form of three main isoforms, which are named after the type of cells in which they were first detected: neuronal NO-synthase (nNOS or NOS I), endothelial NO-synthase (eNOS or NOS III) and NO-synthase of macrophages or inducible NO-synthase (iNOS or NOSII). Neural and endothelial NO-synthases are enzymes with stable activity, while macrophage or inducible NO-synthase activity is more regulated by cytokines. Endothelial NO-synthase is stably expressed in endothelial cells. [23]

Inhibition of NO-synthase leads to all organic consequences of severe and prolonged arterial hypertension, including atherosclerosis and vascular organ lesions. [21]

The gene of endothelial nitric oxide synthase - eNOS3 is located on the long shoulder of 7

chromosomes (7q36.1) and consists of 26 exons [8]. It has three studied polymorphism variants: G894T, 4b/a, and T-786C. It was established in the experiment that the presence of the alleleC position of 786 promoter of the eNOS3 gene leads to a 52% decrease in its activity, and the resulting deficiency in eNOS3 causes a decrease in the synthesis and release of NO and endothelial dysfunction [12,14]. It has been shown that the presence of the C allele and CC genotype is an independent risk factor for the development of IBS and MI in the European and Japanese populations [13,17], as well as for the development of DM in patients with type 2 diabetes [10].

It is of interest to study and reveal the relationship between the polymorphism of the eNOS gene as a predictor of the development and progression of DM in patients with type 2 diabetes and to determine the genetic determinacy of their risk factors in the Uzbek nationality.

The polymorphism of the eNOS gene in case of type 2 diabetes and its macro- and microvascular complications in the Uzbek nationality has not been studied before.

Target. Evaluation of the contribution of polymorphic marker of eNOS3 gene to the risk of diabetic nephropathy development in persons of Uzbek nationality.

Material and methods: In the Republican Scientific and Practical Center of nephrology on the basis of TMA Clinic III the main group of 129 patients of Diagnostic Diabetes-2 type were examined and the control group consisted of 110 healthy persons of the Uzbek nation, included on the principle of "case-control". Patients in the main group were distributed as follows: 65 patients with duration of the disease up to 10 years, without diabetic nephropathy (33 patients) and with diabetic nephropathy (32 patients), 64 patients - with diabetes lasting more than 10-20 years, without diabetic nephropathy (31 patients) and diabetic nephropathy (33 patients). The results of general blood and urine tests, lipid spectrum, glycemic profile, glycosylated hemoglobin, microalbuminuria, glomerular filtration rate (GFR) by CKD-EPI formula, endotheline-1 plasma level, EchoKG, SMAD and Doppler examination of kidney vessels were studied.

T-786C polymorphism testing of ENOS3 gene was carried out on a programmable thermocycler by Applied Biosystems 2720 (USA) using test-systems of Litech (Russia) according to the manufacturer's instructions.

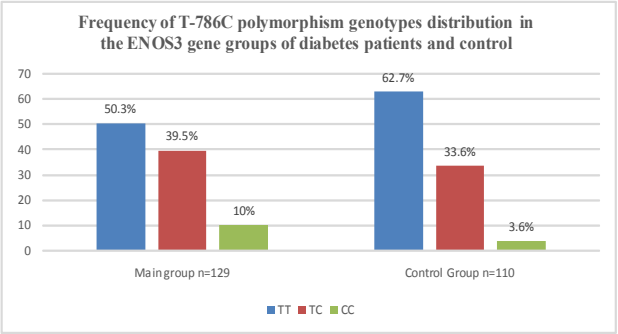
The STATISTICA 6 program was used for statistical processing of the material. The data are presented in the form of mean values with standard deviation (MëSD). Normal distribution was checked by Kolmogorov-Smirnov Criterion. The relative risk of disease in carriers of a certain alley and genotype was calculated as an indicator of the odds ratio (OR - oddsratio). The OR value was calculated using the online calculator of the Medical Statistics

program (http:// medstatistic.ru/ calculators.html).

Genotype distribution was checked for deviations from the Hardy-Weinberg equilibrium. The correlation coefficient r was calculated using the Spearman method. Differences at $p<0.05$ were considered statistically significant.

All patients signed an informed consent form before the examination.

Results and discussion: The frequency of alleles and genotypes of polymorphism T-786S of the ENOS3 gene in all patients (the main group) and the control sample is shown in Fig. 1.



The prevalence of allele T in the studied basic and control groups was 70.1% and 79.5%, respectively. The prevalence of the unfavourable C allele was 29.8% and 20.4% respectively. According to the statistical calculation, carriers of the C allele are 1.6 times more likely to develop the disease than carriers of the T allele ($X2 = 5.5$; $P = 0.02$; OR = 1.6; 95% CI 1.0844-2.524). Allel T ($X2 = 5.5$; $P = 0.02$; OR = 0.6; 95% CI 0.3962-0.9222) indicates that it has a protective effect on disease progression.

Table 1
The frequency of distribution of alleles and genotypes of polymorphismT-786C of the NOS3 gene in the main and control groups of 2-type diabetes patients.

Alleys and genotypes	Number of alleles and genotypes examined				χ^2	P	OR	95% DI
	Main group N	%	Control N	%				
T	81	0,1	75	79.5	5,5085	0,0189	0,6045	0,3962-0,9222
S	77	9,8	45	20.4	5,5085	0,0189	1,6544	1,0844-2,524
T/T	65	0,3	69	62.7	3,6702	0,0554	0,6035	0,3594-1,0132
T/S	51	9,5	37	33.6	0,888	0,346	1,29	0,7592-2,1919
S/S	13	0,0	4	3.6	3,7283	0,0535	2,9698	0,9392-9,3906

According to the results of the main and control groups, the frequency of TT, TS and CC genotypes distribution was 50.3%, 39.5%, 10% and 62.7%, 33.6% and 3.6%, respectively. According to the statistical calculation, CC genotype carriers are 2.9 times more likely to develop the disease than TT genotype carriers, and the difference between them has a reliable statistical significance ($X2$ = 3.7; P = 0.05; OR = 2.9; 95% CI 0.9392-9.3906).

The TT genotype was significantly lower in the main group than in the control group by 50.3%, 62.7%, and showed a protective function against disease progression ($X2$ = 3.7; P = 0.05; OR = 0.6; 95% CI 0.3594-1.0132). The TC genotype was also significantly lower in the main group than in the control group, at 39.5% and 33.6% respectively, and did not play a significant role in the development of the pathology ($X2$=0.9; P=0.3; OR=1.29; 95% CI=0.7592-2.1919) (Table 1.).

In our study, we demonstrated an association between the carriage of the C-allelele (CC genotype) of the ENOS3 gene and diabetic nephropathy in patients with type 2 diabetes. The obtained results are consistent with the data of domestic and foreign authors, who showed that the carriage of C-allelel is an independent risk factor for DM in patients with type 2 diabetes in different ethnic groups [6]. According to the meta-analysis of 2014, in which the results of 32 studies published before 2013 were analyzed, the association of three eNOS3 limorphisms with DN development was revealed: 4b/a, T-786C and G984T. Polymorphisms 4b/a and T-786C showed a reliable

association for all genetic models (OR=1,12-1,77 and 1,11-1,50, respectively). These data and the results of our study allow us to conclude that the eNOS3 gene plays an important role in the development of DN [13] in patients with type 2 diabetes mellitus in the studied Uzbek nation.

Conclusion

Thus, the study revealed a reliable association of the risk of diabetic nephropathy in patients with type 2 diabetes mellitus with endothelial encoding factor genes (NOS3), the expression products of which play a role in the pathogenesis of kidney damage in diabetes mellitus. The results of the present study indicate the importance of further study of molecular bases of development and progression of diabetes mellitus will lead to the development of new promising directions in the prevention of this pathology.

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