GENETIC MARKERS OF ENDOTHELIAL DYSFUNCTION AND THE STRUCTURE OF DYSLIPIDEMIA IN HYPERTENSIVE PATIENTS WITH METABOLIC SYNDROME

M.D., Ph.D. Associate Professor Bondar Vadym

Ukraine, Odessa, Odessa national medical university, department of internal medicine

Abstract. *The purpose of the research were analysis of the distribution of polymorphisms of eNOS gene and assessment of the features of dyslipidemia depending on the distribution of the isolated and combined polymorphisms of eNOS gene in patients with arterial hypertension (AH) and metabolic syndrome (MS) in Ukrainian population.*

128 patients with AH and MS were examined. In the surveyed group polymorphisms T(-786)C and G894T of eNOS gene were determined by PCR. Patients were divided into subgroups with isolated polymorphism T(-786)C, with isolated polymorphism G894T, with combination of two polymorphisms and with "normal genotypes" of eNOS gene. In each subgroup we additionally studied the blood lipid fractions and type of dyslipidemia.

The obtained results shows that the features of dyslipidemia in patients with AH and MS is associated with polymorphism of eNOS gene. This association indicates the relationship of endothelial dysfunction with impaired lipid metabolism and shows the possible primacy of endothelial dysfunction. The most unfavorable polymorphism, which is associated with dyslipidemia of very high atherogenic and cardiovascular risks, is a combination of polymorphisms T(-786)C and G894T of eNOS gene.

Keywords: *arterial hypertension, metabolic syndrome, endothelial dysfunction, dyslipidemia, gene polymorphism*

Introduction. The cardiovascular risk in patients with arterial hypertension (AH) and metabolic syndrome (MS) is largely determined by the severity and features of dyslipidemia, which is associated with endothelial dysfunction [1]. The secondary nature of dyslipidemia and atherosclerotic process in relation to endothelial dysfunction has been widely discussed recently. Nowdays we have a reasonable confirmation that endothelial dysfunction forms an internal need for changing of the structure of blood lipid spectrum, which is necessary for further plastic changes in the vascular wall that are aimed at leveling of initiating factor [2, 3].

Low-density lipoproteins (LDL) are the universal lipid-protein particle and main cholesterol transporter in cells of various organs and tissues, including endothelial cells. When the rabbits are kept on an atherogenic diet, the level of endothelial proliferation after 3 weeks of the experiment is increased by 10 times, and after 4-5 weeks - by 17 times. Endothelium of arteries is the only cell barrier in the way of transport of lipoproteins (LP) to the vascular wall, so it is natural that its functional state and integrity largely determine the speed and pathways of LP transport to the intima of arteries. The endothelium performs a double load: provides the intake of cholesterol in composition of LDL for own energy needs of cells with the participation of apoB-receptors and controls the intake of cholesterol in the composition of LP in the intima of the arteries. Both these processes are interrelated [4, 5].

Control of endothelial dysfunction is currently carried out in several ways: instrumental methods, the checking of biochemical markers. The study of polymorphisms of endothelial nitric oxid (NO)-synthetase (eNOS) gene is perspective. Presently, a direct correlation between the severity of endothelial dysfunction and polymorphisms T (-786) C and G894T of eNOS gene is shown [6].

The polymorphism T (-786) C of eNOS gene promoter, according to modern data, is more closely associated with spastic states of vessels and the development of coronary heart disease (CHD) [7, 8]. Replacing of nitrogen base thymine (T) for cytosine (C) at the 5'-end of eNOS gene leads to significant inhibition of the gene promoter activity and to decrease in the synthesis of endothelial NO. In modern literature it has been shown that in patients with acute coronary syndrome (ACS) the homozygotes with pathological genotype CC of NOS3 gene promoter have been revealed 3 times more often than in health people, which indicates the role of T(-786)C polymorphism in the pathogenesis of ACS, especially in men with premature development of atherosclerosis [7].

The G894T polymorphism of the exon 7 of eNOS gene is structural and consists of transversion of G/T at position 894 of nucleotide sequence of eNOS gene, which leads to the replacement of glutamine with asparagine at 298 position [9, 10]. According to meta-analysis of 26 researches of dependence of various eNOS gene polymorphisms with the presence of hypertension and coronary heart disease, homozygous TT were associated with an increased risk of development of

these diseases [11]. However, the results of other studies are very controversial, depending on ethnicity, sex, characteristics of the course of hypertension [12].

The presence of MS, which includes insulinresistance, abdominal obesity, hypertension and dyslipidemia, significantly aggravates the course of hypertension, endothelial dusfunction and worsens the prognosis. The role of T(-786)C and G894T polymorphisms of eNOS gene in concomitant MS can be significant and used for diagnostic and prognostic purposes.

The purpose of the research were analysis of the distribution of polymorphisms of eNOS gene and assessment of the structure of dyslipidemia depending on the distribution of the isolated and combined polymorphisms of eNOS gene in hypertensive patients with MS in Ukrainian population.

Materials and methods. 128 patients with arterial hypertension and metabolic syndrome were examined. Diagnosis of arterial hypertension was established based on the guidelines of the European Society of Cardiologists 2013 [13]. In order to detect the metabolic syndrome, IDF (International Diabetes Federation) 2005 criteria with modifications of 2009 were used [14].

In the surveyed group polymorphisms T(-786)C and G894T of eNOS gene were determined by PCR in international medical laboratory. For determination of T(-786)C polymorphism of eNOS gene we used pair of primers: the sense pimer 5'-CAGATGACACAGAACTACAA-3' and the antisense pimer 5'-GAGTCTGACATTAGGGTATCC-3. We obtained the following genotypes of T(-786)C polymorphism: TT, TC, CC. Genotype TT we considered as "normal", genotypes TC and CC – "pathological". The determination of G894T polymorphism of eNOS gene was made using the sense pimer 5'-GGCTGGACCCCAGGAAAC-3' and the antisense pimer 5'-CCACCCAGTCAATCCCTTTG-3'. We obtained the following genotypes of G894T polymorphism: GG, GT, TT. Genotype GG we considered as "normal", genotypes GT and TT – "pathological".

The significance of differences and correlation level were determined using Fisher's exact test (φemp) and non-parametric correlation index.

Subsequently, the patients were divided into subgroups with isolated polymorphism T(-786)C (subgroup 1), with isolated polymorphism G894T (subgroup 2), with combination of two polymorphisms (subgroup 3) and with "normal genotypes" of eNOS gene (subgroup 4). In each subgroup we additionally studied the blood lipid fractions and type of dyslipidemia (Fredrickson's classification).

According to Fredrickson's classification dyslipidemias are divided on I, IIa, IIb, III, IV and V types. The I type is characterized by elevated level of chylomicrons, normal or little elevated level of cholesterol (CH) concentration and very high level of triglyceride (TG) concentration, has low degree of atherogenesis. The IIa type is characterized by elevated level of low-density lipoproteins (LDL), moderate elevated level of CH and normal level of TG, has very high degree of atherogenesis. The IIb type is characterized by elevated level of LDL and very low-density lipoproteins (VLDL), moderate elevated level of CH and moderate elevated level of TG, has very high degree of atherogenesis. The III type is characterized by elevated level of intermediate-density lipoproteins (IDL), moderate elevated level of CH and high elevated level of TG, has very high degree of atherogenesis. The IV type is characterized by elevated level of VLDL, normal or little elevated level of CH, moderate elevated level of TG, has low degree of atherogenesis. The V type is characterized by elevated level of VLDL and chylomicrons, little or moderate elevated level of CH concentration and very high level of TG, has low degree of atherogenesis [1].

Results and its discussion. Clinical and demographic parameters of examined patients are shown in Table 1.

Table 1. Clinical and demographic parameters of examined patients, (M ± m)

Parameters	AH with MS (n=128)
Average age (years old)	50,6±2,9
Male/female	60/68
Duration of hypertension (years)	6,3±1,3
SBP (mmHg)	157,8 ± 2,5
DBP (mmHg)	93,0 ± 1,4
BMI, kg/m^2	33,6±1,2
Waist circumference, cm	102,2±3,5

The examined group of patients (n=128) with arterial hypertension and metabolic syndrome was enough homogeneous: the average age was (50,6±2,9) years, male/female - 60/68 respectively, the duration of arterial hypertension - (6,3±1,3) years. In the examined group, an increasing of waist circumference (102,2±3,5 cm) and an increasing of BMI (33,6±1,2 kg/m^2) were observed.

Analyzing the prevalence of polymorphisms of NO-synthase gene, the following results were obtained (Table 2).

Table 2. The frequency of polymorphisms of eNOS gene in patients with AH and MS, n/ %

	AH with MS (n=128)	
	n	%
Polymorphism T(-786)C of eNOS gene		
TT ("normal" genotype)	54	42
TC + CC ("pathological" genotypes)	74	58
p=0,18		
Polymorphism G894T of eNOS gene		
GG ("normal" genotype)	19	15
GT + TT ("pathological" genotypes)	109	85
p=0,008		

Note: p - significance of differences in the distribution of "normal" and "pathological" genotypes.

In the surveyed group, the frequency of isolated polymorphism T(-786)C was (17,9 %/23), isolated polymorphism G894T - (29,6 %/38), combination of polymorphisms T(-786)C and G894T - (35,9 %/46), "normal genotypes" of eNOS gene - (16,4 %/21). There is a high frequency of occurrence of isolated polymorphism G894T and combination of polymorphisms T(-786)C and G894T compared to isolated polymorphism T(-786)C and "normal genotypes" of eNOS gene.

Analyzing the relationship of eNOS gene polymorphisms with the structure of dyslipidemia in the examined group of patients with AH and MS, the following patterns were revealed (Table 3).

Table 3. Association of polymorphisms T(-786)C and G894T of eNOS gene (abs/ %; r, p)

Type of dyslipidemia	Subgroup 1 (isolated polymorphism T-786C) n=23	Subgroup 2 (isolated polymorphism G894T) n=38	Subgroup 3 (combination of two polymorphism) n=46	Subgroup 4 ("normal genotypes" of eNOS gene) n=21
Type I	0	1(3 %)	0	0
Type IIa	13(58 %), r=0,64, p=0,04	4(9 %), r=0,14, p=0,32	10(22 %), r=0,24, p=0,1	5(23 %), r=0,22, p=0,1
Type IIb	4(17 %), r=0,12, p=0,24	8(21 %), r=0,27, p=0,08	27(58 %), r=0,66, p=0,03	5(23 %), r=0,17, p=0,24
Type III	5(21 %), r=0,24, p=0,14	24(64 %), r=0,72 p=0,01	6(13 %), r=0,12, p=0,08	9(42 %), r=0,56, p=0,04
Type IV	1(4 %)	0	1(2 %)	1(6 %)
Type V	0	1(3 %)	2(5 %), r=0,11, p=0,3	1(6 %)

Type IIa dyslipidemia (with elevation of LDL and total cholesterol levels) was predominant in subgroup (1) (58 %, r = 0,64, p=0,04); in subgroup (2) - type III dyslipidemia (64 %, r=0,72, p=0,01) with elevation of IDL, total cholesterol and triglycerides levels; in the subgroup (3) - type IIb dyslipidemia (58 %, r=0,66, p=0,03) with elevation of LDL and VLDL, total cholesterol and triglycerides levels; in the subgroup (4) - type III dyslipidemia (42 %, r = 0,56, p=0,04).

Conclusions. Thus, the features of dyslipidemia in patients with AH and MS is closely associated with polymorphism of eNOS gene. This association indicates the relationship of endothelial dysfunction with impaired lipid metabolism, which can confirm the primacy of endothelial dysfunction in relation to the structure of dyslipidemia. Dyslipidemia is formed "on demand" by the impaired function of endothelium.

In addition, it was revealed that the most unfavorable polymorphisms of eNOS gene, which are associated with dyslipidemias of very high atherogenic risk and, respectively, cardiovascular risk (type IIa, IIb, III), is a combination of polymorphisms T(-786)C and G894T.

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