METHODS OF DIAGNOSIS AND TREATMENT

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USE OF PHARMACOGENETIC ANALYSIS AT PATIENTS WITH ACUTE CORONARY SYNDROME ASSOCIATED WITH IN-STENT RESTENOSIS

ABSTRACT

We studied the influence of CYP2C19 gene polymorphism on the development of in-stent restenosis after transdermal coronary intervention in patients with acute coronary syndrome. The obtained data have revealed a gene almost in the half of patients with restenosis in coronary artery, it being responsible for the reduction of clopidogrel metabolism.

Keywords: restenosis, clopidogrel, pharmacogenetics, acute coronary syndrome.

INTRODUCTION

Acute coronary syndrome (ACS) is the term characterizing any group of clinical signs or symptoms, allowing suspecting acute myocardial infarction (AMI) or unstable angina (UA) [3].

For the purpose of visualization and treatment of coronary artery atherosclerosis (CA) a method of transdermal coronary intervention (TCI) with the use of stents associated with active antiaggregant, antithrombotic and lipid-lowering therapies [1] is conducted.

At present the inhibitor P2Y12 of clopidogrel receptor is considered one of most common antiaggregants. Research results by CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events), CURE (Clopidogrel in Unstable angina Recurrent Events), CREDO (Clopidogrel for the Reduction of Events During Observation) have brought to wide application of clopidogrel when treating atherosclerosis [9,10,12]. However, for most patients treated with antithrombotic preparations ischemic complications have been noted. Due to such manifestations some research works on genetic factors associated with clopidogrel metabolism is being carried out at present among them cytochrome CYP2C19 allele variants being the most important [15,6,11,2]. For patients carrying allele variants CYP2C19*2 and CYP2C19*3 slight antiaggregant effect of clopidogrel has been noted due to dyspoiesis of its active metabolite in a liver that causes genetically determined resistance to the given preparation [7,13]. And the patients with ACS as a group of high cardiovascular risk are of great concern in the course of studying the resistance to clopidogrel.

Aim. To analyse effect of polymorphism of CYP2C19 gene in the development of in-stent restenosis after coronary artery angioplasty at patients with acute coronary syndrome.

MATERIALS AND METHODS

The research was carried out on the basis of urgent cardiology department with a group of intensive therapy of the regional vascular Centre of Republican hospital №2-Centre of emergency medical care for the period 2013 and 2015. Patients' eligibility for the research was carried out after signing of their informed consent. The research report was approved by the local Ethical committee. 35 cases were included in the research of coronary artery in-stent restenosis (ISR) at patients with acute coronary syndrome. The given diagnosis was stated on the basis of clinic, typical changes of electrocardiogram, dynamics of myocardium necrosis markers (troponin I) of the research according to recommendations of the All-Russia national society of cardiologists about diagnostics and treatment of patients with acute myocardium infarction with ST segment rise on ECG (ARNSC, 2007), national recommendations about treatment of acute coronary syndrome without resistant ST segment rise on electrocardiogram (ARNSC, 2006). All the patients had a pharmacogenetic survey, blood genetic analysis on polymorphism of gene CYP2C19*2 and CYP2C19*3 by allel-specific polymerase chain reaction (PCR). Initial dose calculation of clopidogrel was conducted by means of the program PharmSuite. DNA extraction was carried out with 0.2 ml of blood using a DNA extraction kit GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA). Amplification of gene polymorphisms CYP2C19 * 2 and * 3 SYP2C19 performed using TaqMan kits (Applied Biosystems, USA) in a system of detection of PCR products in real time.

The patients were divided into 3 groups based on results of the pharmacogenetic survey in cooperation with Teaching and research laboratory 'Genome medicine' of Medical institute in the Northeast federal university.

Group 1 - gene CYP2C19*1 carriers, n - 19, including 15 men and 4 women. Middle age is 57,8 ±12,7 years;

Group 2 - gene CYP2C19*2 carriers, n - 12, men-9 and women-3. Middle age has made 66,7±7,6 years;

Group 3 - gene CYP2C19*3 carriers, n - 4, male patients only. Middle age - 60±6,6 years.

When admitted to the department of Emergency Cardiology all the patients underwent the antiaggregant therapy in a loading dose: aspirin 250-325 mg and clopidogrel 600 mg.

The analysis of laboratory data included lipid profile indices. Due to higher risk of cardiovascular complications, high parameters were considered as follows: TC ≥ 4,0 mmol/l, LDL-C ≥ 1,8 mmol/l, HDL-C ≥ 1,0 mmol/l at men and 1,2 mmol/l at women, TG ≥ 1,7 mmol/l (recommendations of the Russian cardiologic society (RCS 2012), National society on Atherosclerosis (NSA) and Russian society of cardiosomatic rehabilitation and secondary prophylaxis (RosCSR). Body mass index (BMI) was estimated on Ketle II index calculated as followed: weight (kg) / growth (m ²). Normal BMI within 18,5-24,9 kg/m ²; 25 29,9 kg/m ² - overweight (pre-obese); ≥ 30 kg/m ² obesity (RCS, 2012).

Coronary angiography was conducted by the standard technique on 'INNOVA 3100'. At visual analysis of the coronary angiography main coronary arteries (left coronary artery trunk, anterior interventricular artery, circumflex artery, diagonal artery, right coronary artery, and obtuse

marginal branch) were studied. A type of coronary blood supply to the heart and the number of diseased arteries was estimated as well, and a function of previously implanted stent (DES stent – with drug-induced covering, BMS stent – bare metal) was defined. In-stent restenosis was classified based on extension and localization restenosis area in relation to stent (R.Mehran and coauthors, 1999): I type - local (length less than 10 mm); II - diffuse (length more than 10 mm); III– proliferative (beyond the stent, length more than 10 mm); IV type – stent occlusion.

The restenosis angiographic criterion was characterized by lumen reduction in a zone of previously implanted stent on 50 % and less that demanded repeated revascularization. The ACS standard treatment including stenting of infarct-connected coronary artery was performed.

All patients after the primary stenting were recommended to take two-componental disaggregant therapies (acetylsalicylic acid, clopidogrel or ticagrelor) and IHD basic therapy (β-adrenoblokatory, ACE inhibitors, statins).

Procedures of the statistical analysis were carried out by means of statistical package SPSS-19. Results are presented in the form of MyoSd, where M - average value, SD - standard deviation, CI - 95 % (confidence interval). Check for normal distribution of the studied quantity indicators in two surveyed groups was conducted by Kolmogorov-Smirnov's test. Variable distribution differed from the normal one, as a consequence it was presented in the form of median and interquartile ranges (25 and 75 percentiles). The authenticity of the average quantitative differences between two groups were checked by means of the nonparametric Mann-Whitney test, the qualitative indicators were checked by means of the contingency table based on the confidence analysis of χ2-Pearson criterion for independent samples. Authentic differences were noted when p <0,05.

RESULTS AND DISCUSSION

In I group examined (patients with normal genotype CYP2C19*1) the coronary artery in-stent restenosis was manifested clinically on the average in 18,7 (3,7; 21,3) months. In II group, who were slow gene carriers (CYP2C19*2), manifestations were revealed on the average in 16,8 (1,25; 19,3) months. In III group of patients with genotype CYP2C19*3 repeated cardiovascular events were noted in 5,5 (2,0; 9,5) months that corresponded to the research results of other authors to identify the causes of coronary artery in-stent restenosis [2, 4]. Despite early manifestation of the disease after the previous coronary intervention in III group, no authentic differences in temporal indicators of in-stent restenosis depending on genotype were revealed (p<0,05).

In table 1 average indices of lipid profile at the surveyed are presented. The analysis has revealed high indicators TC and LDL-C. According to Iliodromitis E. [14] dyslipidemia as well as absence of cholesterol lowering preparations increase risk stent restenosis. TC average level at I group patients has made 4,2 mmol/l [3,2; 5,0], at II group the indicator of total cholesterol has appeared a bit higher as compared with I group - 4,6 mmol/l [3,7; 5,4]. In III group the TC average index is significantly higher than at 1 group patients - 5,4 mmol/l [4,6; 6,1], p I - III <0,05. The LDL-C average value in III group has appeared higher in comparison with other groups' parameters - 3,7 mmol/l (2,8; 4,5), but authentic differences are not obtained. The HDL-C and TC in all groups were within the values recommended by RCS, with exception of HDL-C slightly lower parameters in II group (tab. 1). The atherogenicity index (AI) has been higher at the gene carriers CYP2C19*2 and CYP2C19*3 and has made 3,7 in comparison with the indices in the 1 group - 2,5.

The analysis of the coronary angiography data has revealed differences in frequency of coronary artery in-stent restenosis at the patients investigated (fig. 1). The greatest percent of damage is revealed in anterior descending arteriy (ADA), on the second place is in the right coronary artery (RCA), then in the circumflex artery.

Of the total number of the patients who had PCI with stenting CA (n=35), in I group BMS stents had been implanted at 2 patients earlier, DES stents had been at 9 patients (tab. 2). In II group investigated, of the patients with genotype responsible for resistance to clopidrogrel (CYP2C19*2), BMS stents had been installed at 4 persons (33,3 %), DES stents at 3 people (25 %), unknown - 5 people (41,7 %), of the patients with genotype CYP2C19*3 3 people (75 %) had BMS stents, 1 person had DES stent (25 %). The highest percent of BMS stents were noted in III group.

The analysis of the remote results after stenting has revealed high frequency of two restenosis types in all groups: proliferative and occlusive damages. At the same time, stent occlusion (IV type) is significantly often noted in group CYP2C19*3. Lumen loss in stent > 90 % at PC is much higher in groups with genotypes CYP2C19*2 GA (83,3 %) and CYP2C19*3 AA (75 %).

The patients with smaller diameter of revascularized vessel are considered to have the greater risk of an adverse outcome after coronary stenting [5]. In our research we have also obtained the similar data (fig. 2). The average index of coronary artery diameter in the investigated groups was not bigger than 3,5 mm. So in I group the CA average diameter has been only 2,6 mm, it probably causing restenosis development. In II group of patients the similar indicator was equal to 2,98 mm, in III group - 3,17 mm. Significant distinctions are noted between I and II groups, p <0,05.

In our work we have analyzed ACS clinical diagnoses among the surveyed. We have found out that the patients with no gene on resistance to clopidogrel had a small proportion of unstable angina (Figure 3).

To all patients with in-stent restenosis

Table 1

Average indices of lipid profile at patients with CA in-stent restenosis

Index	I group, gene carriers CYP2C19*1, n – 19	II group , gene carriers CYP2C19*2, n - 12	III group , gene carriers CYP2C19*3, n - 4	P I-II	P I-III	P II-III
Total cholesterine, mmol/l	4,2 [3,2;5,0]	4,6 [3,7;5,4]	5,4 [4,6;6,1]	>0,05	<0,05	>0,05
Low density lipoproteins, mmol/l	2,7 [1,9;3,1]	2,8 [2,2;3,4]	3,7 [2,8;4,5]	>0,05	>0,05	>0,05
High density lipoproteins, mmol/l	1,3 [1,0;1,6]	0,9 [0,7;1,2]	1,3 [0,8;1,7]	>0,05	>0,05	>0,05
Triglycerides	1,1 [0,86-1,3]	1,5 [1,2;1,9]	1,0 [0,6;1,4]	<0,05	>0,05	>0,05
Atherogenicity index	2,5 [1,8;3,0]	3,7 [2,5;4,9]	3,7 [2,2;5,1]	<0,05	>0,05	>0,05

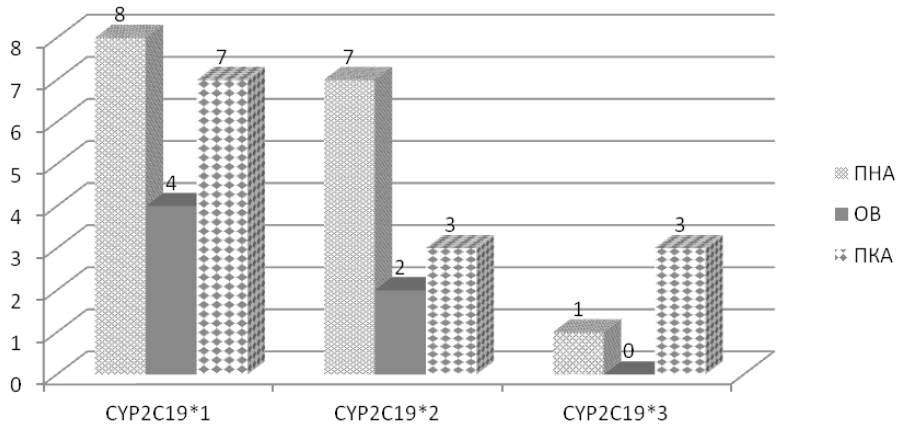

Fig. 1. Stent restenosis localization in coronary artery depending on genotype

Table 2

Characteristics of Angiographic Indices

Indices	I group , gene carriers CYP2C19*1, n - 19		II group , gene carriers CYP2C19*2, n - 12		III group , gene carriers CYP2C19*3, n - 4		P I-II	P I-III	P II-III
	n	%	n	%	N	%			
Character of previously implanted stent:									
BMS	2	10,5	4	33,3	3	75,0	>0,05	<0,01	>0,05
DES	9	47,4	3	25,0	1	25,0	>0,05	>0,05	>0,05
Unknown	8	42,1	5	41,7	-	-	>0,05	-	-
Restenosis Type:									
I	3	15,8	1	8,3	-	-	>0,05	-	-
II	6	31,6	1	8,3	-	-	>0,05	-	-
III	6	31,6	5	41,7	1	25,0	>0,05	>0,05	>0,05
IV	4	21,1	5	41,7	3	75,0	>0,05	<0,05	>0,05
Degree of Contraction:									
Lower than 50%	4	21,1	-	-	1	25,0	-	>0,05	-
50-75%	3	15,8	1	8,3	-	-	>0,05	-	-
75-90%	4	21,1	1	8,3	-	-	>0,05	-	-
Higher than 90%	8	42,1	10	83,3	3	75,0	<0,05	>0,05	>0,05

the antiaggregant Tikagrelor (Brilinta) was appointed in a dose of 180 mg/days to decrease in risk of repeated restenosis.

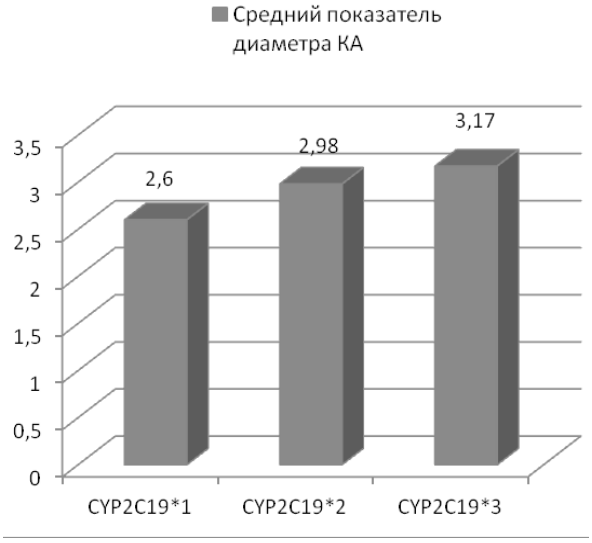

Fig. 2. The average diameter of the coronary artery at patients with in-stent restenosis

CONCLUSION

1. Genotypes CYP2C19*2 and CYP2C19*3 were revealed at patients with coronary artery in-stent restenosis in 45.7 % of cases.

2. Lipid metabolism is considered to be one of the unfavorable factors of the development of in-stent restenosis that demands more careful supervision and treatment of the given categories of patients.

3. The higher prevalence of coronary artery restenosis is noted in ADA, on the second place in RCA and then CA, with a smaller diameter of the vessel (no more than 3,0 mm on the average) and with a higher attack rate of bare metal stent (BMS) that corresponds to the literary data [8,5].

4. According to the above-stated results of our research, all the patients undergone PCI (coronary intervention procedure) by means of stenting are recommended to be tested on thrombocyte aggregation. If no decrease of the aggregation properties against the background of two-componental antiaggregant therapy is noted it is necessary to carry out pharmacogenetic assays due to the high risk of in-stent restenosis.

5. If CYP2C19*2 or CYP2C19*3 are carried by patients, another antiaggregant, for example, prasugrelor or tikagrelor is recommended. When revealing the genotype CYP2C19*1/*1 clopidogrel is appointed in the doses regulated in the instructions for medical use: a loading dose - of 300 mg, further on 75 mg/days [7].

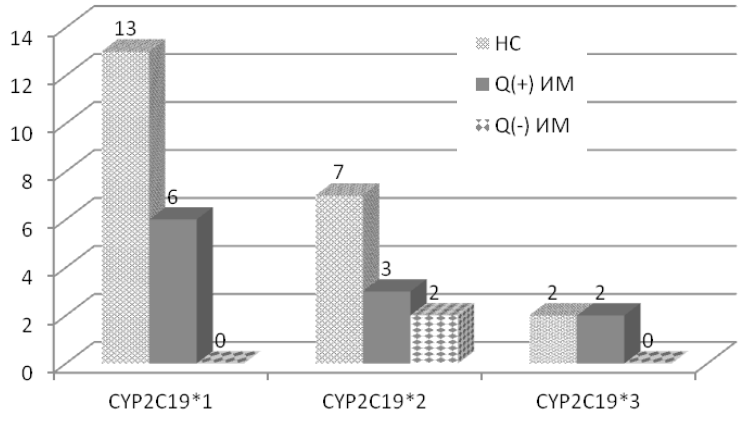

Fig. 3. Vascular complications at patients with in-stent restenosis
UA– unstable angina, Q (+) MI – Q positive myocardial infarction, Q (-) – Q negative myocardial infarction.

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