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GENETIC PREDISPOSITION TO ANTIPLATELET RESISTANCE IN UZBEK PATIENTS WITH CORONARY ARTERY DISEASE


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ЮРАК ИШЕМИК КАСАЛЛИГИ БЎЛГАН ЎЗБЕК МИЛЛАТИГА МАНСУБ БЕМОРЛАРДА АНТИАГРЕГАНТ РЕЗИСТЕНТЛИГИГА ГЕНЕТИК МОЙИЛЛИК

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ГЕНЕТИЧЕСКАЯ ПРЕДРАСПОЛОЖЕННОСТЬ К АНТИТРОМБОЦИТАРНОЙ РЕЗИСТЕНТНОСТИ У УЗБЕКСКИХ ПАЦИЕНТОВ С ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА

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Резюме. *Мақсад: юрак ишемик касаллиги билан оғриган ўзбек беморларида антиагрегантларга нисбатан резистентлик ривожланишига таъсир қилувчи генетик омилларнинг тарқалиши ва таркибини аниқлаш. Материаллар ва усуллар: тадқиқотда ўзбек миллатига мансуб 60 нафар соғлом ва юрак ишемик касаллиги билан касалланган 75 нафар беморлар иштирок этди. Тромбоцитлар функцияси икки каналли Алат-2 Биол лазерли анализаторида баҳоланди, сўнгра аггр AGGR компьютер дастури ёрдамида қайта ишланди. GP1BA ва тромбоцитлар АДФ рецепторлари P2RY12 мутациясининг полиморфизмини аниқлаш учун генетик тадқиқотлар ўтказилди. Тадқиқот натижалари: тромбоцитлар агрегацияси фаоллигини ўрганиш натижаларига кўра, аспиринга реакцияси бўлмаган 17 (22,6% ва 5,0 ммол АДФ да тромбоцитлар агрегациясининг ўртача даражаси 73,2% ни ташкил этди) беморлар аниқланди. Назорат гуруҳида ўрганилган полиморфизмлар учун мутант аллелнинг частотаси CP1BA генида (Thr145Met), H1/H2 генида (P2RY12) 2% ни ташкил етди, бу асосий гуруҳга қараганда анча паст (46.8% ва 62.6%, мос равишда p<0.05). Полиморфизм частоталарини таҳлил қилиш антиагрегант резистентлиги бўлган беморларда мутант CP1BA аллелларининг гомозигот шаклидаги (=1.94, мос равишда p<0.001) частоталарининг сезиларли даражада ошишини аниқлади. Хулоса: шундай қилиб, антиагрегант резистентлиги мавжуд ўзбек миллатига мансуб юрак ишемик касаллиги бўлган беморларда ADP P2Y12 рецепторлари генининг полиморфизмининг H2 генотипи билан ассоциациялари аниқланди.*

Калит сўзлар: *антиагрегант дориларга резистентлик, юрак ишемик касаллиги, тромбоцитлар агрегацияси, генлар.*

Abstract. *The purpose of the work: to ascertain the prevalence and composition of genetic variables that influence a person's susceptibility to developing antiplatelet resistance in Uzbek patients suffering from coronary heart disease. Materials and methods: There were 75 Uzbek participants with coronary heart disease and 60 healthy participants in the study. A two-channel laser analyzer called the Alat-2 Biol was used to assess platelet function. The AGGR program was then used for computer processing. To identify the mutation of the platelet ADP receptor P2RY12 and the polymorphism of the α-subunit GP1BA, genetic tests were conducted. Results of the study: According to the results of the study of platelet aggregation activity, 17 (22.6% and the average degree of platelet aggregation with 5.0 mmol of ADP was 73.2%) patients with no reaction to aspirin were identified.The control group had significantly lower mutant allele frequencies for the studied polymorphisms (17.4% in the CP1BA (Thr145Met) gene and 28.7% in the H1/H2 (P2RY12) gene) compared to*

the study group (46.8% and 62.6%, respectively, p<0.05). The analysis of the frequency of polymorphisms revealed significant excess frequencies of mutant CP1BA alleles in the homozygous form (=1.94, respectively, p<0.001) in patients with antiplatelet resistance. Conclusions: Therefore, correlations with the H2 genotype of the polymorphism of the ADP P2Y12 receptor gene were found in Uzbek nationals with coronary artery disease who also had antiplatelet resistance.
Key words: *resistance to antiplatelet agents, coronary heart disease, platelet aggregation, genes.*

Relevance. In developed nations, cardiovascular diseases (CVD) constitute the leading cause of morbidity and mortality. The cardiovascular pathology mortality structure indicates that 90–95% of deaths are caused by coronary heart disease (CHD). Antiplatelet medicines and medications that stop atherosclerosis from progressing are the primary ways to prevent cardiovascular disease (CVD) linked to acute ischemia. [1, 2, 7]. Achieving an optimal balance between the effectiveness and safety of drug treatment during prolonged antiplatelet therapy is not an easy clinical task, for which the doctor must take into account many factors related to the nature of the disease, the individual characteristics of the patient and the properties of the drugs used.

The most widely used medication for secondary prevention of atherothrombotic events in cardiovascular disorders is acetylsalicylic acid, also known as aspirin. Thus, the issue of aspirin therapy's efficacy need to be regarded as one of the main ones in cardiology [8,15,17]. Since there is no solid evidence to support the use of aspirin for primary prevention in the general population, the question of personalizing antiplatelet medication is still pertinent. The antiplatelet effect of antiplatelet agents in humans is not the same. There is documented variability among both patients and healthy volunteers in laboratory evaluation of platelet aggregation during antiplatelet therapy. In some patients, the blocking properties of antiplatelet agents in relation to platelet aggregation may be minimal or may be lost over time.

Despite the standard antiplatelet therapy, thrombotic complications often occur. One of the reasons for this condition is resistance to antiplatelet agents [3,5,6]. There is a correlation between antiplatelet resistance and clinical outcomes. Therefore, the identification of patients with "potentially high risk" is important, as it allows timely correction of antiplatelet therapy and reduces the likelihood of developing cardiovascular events [10, 11]. Depending on the approach and patient type, the prevalence of clopidogrel and acetylsalicylic acid (ASA) resistance varies from 20% to 45% and 5% to 45%, respectively; resistance to dual antiplatelet therapy ranges from 6 to 8% [16,18,20].

In such variability of response to antiplatelet agents, great importance is attached to genetic, metabolic, and epigenetic factors [4,9,13]. In the future, it will be feasible to identify the highest risk group, forecast the possibility of thrombotic complications, and promptly modify therapy in patients with coronary heart disease by searching for and studying genetic markers that determine each patient's unique sensitivity to antiplatelet agents [14,19,21]. At the same time, to assess the importance of introducing genetic testing into clinical practice, it is important to take into account the ethnoterritorial specificity in the prevalence of allelic variants of candidate genes.

The purpose of the work: to ascertain the prevalence and composition of genetic variables that influence a person's susceptibility to developing antiplatelet resistance in Uzbek patients suffering from coronary heart disease.

Materials and methods: The initiative was based on the Cardiology Department of the Republican Specialized Scientific and Practical Medical Center for Therapy and Medical Rehabilitation. A cross-sectional genetic and clinical examination is the study's design. The study comprised 75 respondents with coronary heart disease (39 men and 36 women; average age 59.5±8.4 years, main group) and 60 healthy Uzbek respondents (control group), who were similar in age and gender. The following metrics were used to evaluate each patient: total protein, glucose, triglycerides, total cholesterol, hemoglobin concentration, platelet count, and erythrocyte sedimentation rate (ESR). Coagulological parameters were also determined: activated partial thromboplastin time, prothrombin time with calculation of the international normalized ratio, fibrinogen level and D-dimer concentration using latex particles with highly specific monoclonal antibodies to D-dimer. All patients received the enteric-soluble form of ASA 100 mg/day (THROMBOASS). If patients had not previously received acetylsalicylic acid preparations, they were prescribed upon admission.

Platelet aggregation was studied by the Born turbidimetric method based on recording changes in light transmission of platelet-rich plasma. Platelet aggregation was determined several times: against the background of ASA therapy — upon admission, after 10 days, after 1 month of taking the drug. The method is based on the analysis of fluctuations in light transmission of a platelet- and plasma-enriched sample with the addition of an adenosine diphosphate (ADP) inducer at final concentrations of 0.1, 1.0, and 5.0 micrograms/ml, followed by computer processing using the AGGR program. The normal activity limits of the platelet aggregation process with the addition of 5.0 mmol of ADP are 25-72%. The level of platelet aggregation with 5.0 mmol of ADP >72% was used as a criterion of resistance.

Table 1. Clinical features of coronary artery disease patients

The risk factor	abs (n=75)	%
Women	43	57
Men	32	43
Age, years	59,5±8,4	
Body mass index	31,3±2,4	
Smoking	16	21
Fatness	26	34
Arterial hypertension	69	92
postinfarction cardiosclerosis	20	26
Hyperlipidemia	42	56
Type 2 diabetes mellitus	14	18

Table 2. The frequency of alleles in the analysis of Thr145Met polymorphisms in the GP1BA gene and H1/H2 in the P2RY12 gene in the studied groups

Polymorphism	Alleles	Frequency of alleles, %		X^2	p
		The main group	The control group		
Thr145Met in the gene GP1BA	0	55,4	81,2	42,02	<0,001
	1	43,5	17,4		
H1/H2 in the geneP2RY12	0	56,5	72,3	8,09	0,021
	1	42,0	28,1		

Molecular genetic study included primer creation, polymerase chain reaction (PCR), electrophoresis in a 1–1.5% agarose gel for qualitative DNA analysis, DNA separation, and purity and concentration evaluation. The outcome was assessed as a heterozygote (1), mutant homozygote (3), and normal homozygote (0). Genetic studies were performed to determine the polymorphism of the alpha subunit GP1BA and mutation of the platelet ADP receptor P2RY12.

The static analysis of the obtained results was carried out using the SPSS19.0 program and Microsoft Excel 2007. The differences at $p<0.05$ were considered statistically significant. The odds ratio (OR) was calculated to describe the relative risk of developing the disease. The $\chi 2$ criterion was used to compare qualitative data. The gene counting method was used to determine the frequency of alleles, and $\chi 2$ was employed to identify deviations from the Hardy-Weinberg equilibrium. Each polymorphism's genotype distribution was examined using $\chi 2$ (3x2) between people with coronary artery disease and controls.

The results of the study: Analysis was done on coronary heart disease patients. Of these, 15 had myocardial revascularization and 20 suffered postinfarction cardiosclerosis. The primary risk factors for coronary heart disease include: 69 patients had arterial hypertension of I-II-III degree, 14 patients had type 2 diabetes mellitus, and 42 patients had hyperlipidemia. Every patient gave their informed consent to take part in the research.

Table 1 displays the clinical features of the patients under study.

According to the results of the study of platelet aggregation activity, 17 (22.6% and the average degree of platelet aggregation with 5.0 mmol of ADP was 73.2%) patients with no reaction to aspirin were identified.

The control group had significantly lower mutant allele frequencies for the studied polymorphisms (17.4% in the CP1BA (Thr145Met) gene and 28.7% in the H1/H2 (P2RY12) gene) compared to the study group (46.8% and 62.6%, respectively, $p<0.05$). In patients with antiplatelet resistance, the study of polymorphism frequency showed significant excess frequencies of mutant CP1BA alleles in the homozygous form (=1.94, respectively, $p<0.001$). There was less variation in the frequencies of the H1/H2 gene's polymorphisms (OR=1.17, p=0.01). There was a significant dependence of fibrinogen content in the blood on (and GP1BA_Thr145Met genotype (p=0.030).

Table 2 displays the frequencies of Thr145Met polymorphisms of the GP1BA gene and H1/H2 of the P2RY12 gene based on the findings of a genetic analysis of 135 coronary artery disease patients who took and did not take aspirin.

When the significance of the presence of the Thr145Met mutant allele was investigated, 12 heterozygotes were discovered in 60 healthy individuals and 29 in 75 patients with coronary heart disease (18 non-mutant homozygotes and 11 mutant homozygotes). The groups' differences were statistically significant according to the Pearson criterion ($\chi 2=24.5$; $p<0.001$). Based on the presence of the mutant H1/H2 allele, there was a significant difference ($\chi 2=14.3$, $p<0.01$) between the groups of patients with coronary heart disease and healthy individuals. The extremely high statistical significance indicators that were discovered using the selected methodology demonstrate the prevailing clinical importance of the mutant gene

in the homozygous form. The genotypes of the Thr145Met polymorphism in the GP1BA gene were distributed according to the Hardy-Weinberg equilibrium (p=0.84 and p=0.34 in the patient and control groups, respectively). Nevertheless, compared to the control group (17% and 2%, respectively), the frequency of the mutant allele was eight times higher in the group of patients with coronary heart disease.

According to different models, there was no correlation between the genotype distribution in the patient and control groups and the disease. The dominant model (OR=2.78 95% CI (0.11-70.93), p=0.52) and the codominational model (OR=3.75, 95% CI (0.14-99.88), p=0.55) both showed a correlation between the H2 genotypes of this polymorphism and the risk of coronary heart disease.

When analyzing the correlations of the parameters under investigation among the group of patients with coronary heart disease who also had antiplatelet resistance, strong statistically significant correlations were found between blood cholesterol and triglyceride levels (r=0.727; p=0.001) and between cholesterol and fibrinogen levels (r=0.283; p=0.004). There were significant associations between the diastolic and systolic blood pressure levels (r=0.803; p=0.001). Additionally, correlations were larger in the group of patients with coronary heart disease than in the control group.

Conclusions: P2RY12_H1H2 and GP1BA_Thr145Met mutations are associated with the emergence of antiplatelet resistance in coronary heart disease patients in the Uzbek population. It was discovered that the GP1BA gene's Thr145Met mutation had the most fluctuations in polymorphism frequency.

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ГЕНЕТИЧЕСКАЯ ПРЕДРАСПОЛОЖЕННОСТЬ К АНТИТРОМБОЦИТАРНОЙ РЕЗИСТЕНТНОСТИ У УЗБЕКСКИХ ПАЦИЕНТОВ С ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА

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Резюме. *Цель работы: установить распространенность и состав генетических факторов, влияющих на предрасположенность человека к развитию антитромбоцитарной резистентности у узбекских пациентов, страдающих ишемической болезнью сердца. Материалы и методы: В исследовании приняли участие 60 здоровых узбекских респондентов и 75 респондентов с ишемической болезнью сердца. Функцию тромбоцитов оценивали на двухканальном лазерном анализаторе Alat - 2 Biol с последующей компьютерной обработкой с использованием программы AGGR. Были проведены генетические исследования для определения полиморфизма α-субъединицы GP1BA и мутации АДФ-рецептора тромбоцитов P2RY12. Результаты исследования: По результатам изучения агрегационной активности тромбоцитов было выявлено 17 (22,6%, а средняя степень агрегации тромбоцитов при 5,0 ммоль АДФ составила 73,2%) пациентов, у которых отсутствовала реакция на аспирин. В контрольной группе частота мутантного аллеля для изучаемых полиморфизмов составила 17,4% в гене CP1BA (Thr145Met), 28,7% в гене H1/H2 (P2RY12), что достоверно ниже, чем в основной группе (46,8% и 62,6% соответственно, p<0,05). Анализ частот полиморфизмов выявил значительное превышение частот мутантных аллелей CP1BA в гомозиготной форме (=1,94 соответственно, p<0,001) у пациентов с антитромбоцитарной резистентностью. Выводы: Таким образом, у пациентов с ишемической болезнью сердца узбекской национальности с наличием антитромбоцитарной резистентности были выявлены ассоциации с генотипом H2 полиморфизма гена рецептора ADP P2Y12.*

Ключевые слова: *резистентность к антиагрегантным препаратам, ишемическая болезнь сердца, агрегация тромбоцитов, гены.*