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FEATURES OF CHANGES IN PLATELET FUNCTIONAL ACTIVITY IN PATIENTS WITH CORONARY ARTERY DISEASE AND HYPERTENSION DEPENDING ON SENSITIVITY TO ANTIPLATELET DRUGS

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Hypertension is an urgent problem of cardiology, due to its prevalence and adverse complications. The purpose of the study was to optimize the diagnosis and treatment of patients with coronary artery disease and hypertension based on the researching of functional activity of platelets and the effectiveness of antiplatelet therapy. A total of 147 patients were included, the control group consisted of 30 people. It was determined that 54% of patients treated with acetylsalicylic acid had impaired sensitivity to treatment and 27% – to thienopyridine drugs. The most noticeable changes were observed in the degree of induced platelet aggregation with arachidonic acid and collagen among patients receiving acetylsalicylic acid, and thienopyridines as for adenosine diphosphate – and adrenaline-induced platelet aggregation. Given the heterogeneity of indices in groups of patients with hypertension and various forms of coronary artery disease, the study of vascular-platelet hemostasis in the whole blood by express method makes it possible to more accurately determine the degree of sensitivity to antiplatelet therapy.

Key words: hypertension, coronary artery disease, vascular-platelet hemostasis, sensitivity to antiplatelet therapy

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ОСОБЛИВОСТІ ЗМІН ФУНКЦІОНАЛЬНОЇ АКТИВНОСТІ ТРОМБОЦИТІВ У ПАЦІЄНТІВ З ІШЕМІЧНОЮ ХВОРОБОЮ СЕРЦЯ ТА ГІПЕРТОНІЧНОЮ ХВОРОБОЮ В ЗАЛЕЖНОСТІ ВІД ЧУТЛИВОСТІ ДО АНТИТРОМБОЦИТАРНИХ ПРЕПАРАТІВ

Гіпертонічна хвороба є актуальною проблемою кардіології, що пов'язано з її поширеністю і несприятливими ускладненнями. Метою роботи було оптимізувати діагностику та лікування хворих на ішемічну хворобу серця з гіпертонічною хворобою на підставі вивчення особливостей функціональної активності тромбоцитів та ефективності антитромбоцитарного лікування. Було включено 147 пацієнтів, група контролю – 30 осіб. Визначено, що 54 % хворих, які отримували у складі терапії ацетилсаліцилову кислоту мали знижену чутливість до проведеного лікування та 27 % – до тієнопіридинів. Найбільш помітні зміни спостерігались у ступені індукованої агрегації тромбоцитів арахідоновою кислотою та колагеном серед пацієнтів, які отримували ацетилсаліцилову кислоту, та у ступені аденозиндифосфат- та адреналін-індукованої агрегації тромбоцитів – тієнопіридини. Враховуючи гетерогенність показників, у групах пацієнтів з гіпертонічною хворобою та різними формами ішемічної хвороби серця, дослідження тромбоцитарної ланки гемостазу експрес-методом у цільній крові дає можливість більш точно визначити ступінь чутливості до антитромбоцитарної терапії.

Ключові слова: гіпертонічна хвороба, ішемічна хвороба серця, тромбоцитарний гемостаз, чутливість до антитромбоцитарного лікування

Hypertension is an urgent problem of cardiology, due to its prevalence and adverse complications [7, 15]. In the absence of complete treatment, hypertension leads to coronary artery disease (CAD), heart failure (HF), stroke, renal failure, and early mortality [13]. Increased attention of physicians is explained by the fact that these diseases often develop in able-bodied active people, significantly limiting their social and labor activity, exacerbating socio-economic problems in society [8, 11].

According to the official statistics of the Ministry of Health of Ukraine in 2017, 7,751,199 people with coronary artery disease and 10,388,376 patients with hypertension were registered, most of whom were women – 6,310,243 people [5]. Atherothrombotic complications of hypertension – myocardial infarction and stroke, occupy a special place. Platelets play a leading role in the pathogenesis of these diseases: changes in their number and functional properties are accompanied by the release of vasoactive mediators that provoke local vasospasm and increase platelet aggregation, which increases the risk of thrombotic complications [2, 3]. According to modern concepts, one of the main mechanisms of development and progression of CAD is also

the destabilization of vascular hemostasis [6]. Analysis of platelet aggregation properties allows identifying the risk, to predict dangerous complications of the disease, to perform timely preventive therapy, which determines the relevance of studying the various mechanisms of their adhesion and aggregation [4, 9].

Thus, timely diagnosis and correction of platelet disorders and atherothrombosis can be a guarantee of improving the prevention and treatment of life-threatening conditions and, as a consequence, reducing cardiovascular mortality [1].

The purpose of the study was to optimize the diagnosis and treatment of patients with coronary artery disease and concomitant hypertension based on the study of the functional activity of platelets and the effectiveness of antiplatelet therapy.

Materials and methods. The study included 147 patients with CAD and concomitant hypertension. Inclusion criteria in the study were hypertension diagnoses in combination with various forms of CAD confirmed on the basis of laboratory, clinical and instrumental data: stable angina pectoris (II, III FC) and acute coronary syndrome (ACS); written informed consent to participate in the study. Exclusion criteria were: age less than 18 and older than 85 years, class III heart failure, liver, renal failure), long-term anticoagulant treatment (mechanical heart valve prostheses), pregnancy, history of stroke and intracranial bleeding, history of severe comorbidities: history of cancer ≤ 5 years, pulmonary tuberculosis, AIDS, HIV, alcohol and/or drug dependence, diseases that cause hemolysis or red blood cells instability, as well as acute infectious disease at the time of examination.

Group 1 included 75 patients with CAD with concomitant hypertension (stable forms), group 2 consisted of 72 patients with hypertension and ACS. Patients in both groups received antiplatelet therapy in the form of acetylsalicylic acid (ASA) at a dose of 75-100 mg/day, thienopyridines (clopidogrel, ticagrelor) and their combinations. To determine the functional activity of platelets, aggregometry was used to assess the degree of spontaneous aggregation and aggregation potentiated by arachidonic acid (ARA), adenosine diphosphate (ADP), collagen and adrenaline. To determine the sensitivity to antiplatelet drugs, aggregometry by AggreGuide A-100, laser-light scattering platelet aggregometer when adding whole blood to the test cartridge – AA/ADP. The result was recorded by platelet activity index (PAI). Different degrees of treatment resistance with the method using indicated the value of the platelet index above 5.

Statistical analysis was performed on a personal computer using the program SPSS-23 (Sublicense Agreement no. 138 of 04.08.2016, licensee LLC "Prognostic Solutions"). The type of index distribution was assessed during evaluation of each group of indices – Gaussian distribution or one that differs from the normal one. The normality of distribution was assessed using the Shapiro-Wilk test. For descriptive statistics, the mean index values, standard deviation (SD), standard error (m), 95% confidence interval for the mean were determined. Comparison of the two independent groups was performed using Student's t-test. The Mann-Whitney U test was used to compare the two independent groups. The one-way analysis of variance (ANOVA) with Fisher's test was used to analyze the differences between the mean values of several groups. The results are presented as mean±standard deviation (M±SD), for data with a distribution similar to normal. The difference between the groups was considered significant at $p<0.05$.

Results of the study and their discussion. The sensitivity to antiplatelet therapy was studied in two study groups: ASA monotherapy was prescribed to 55 patients (45 people of group 1 and 10 people of group 2), thienopyridines – 32 patients (20 – group 1, 12 – group 2) and a combination of both drugs were received by 60 people (10 patients of the first group and 50 persons of the second group). 15 people from the total number of patients received ASA, both in the form of mono- and as part of combination therapy. 54% (62 patients) had a impaired sensitivity (PAI> 5) to this antiplatelet drug. 73% (67 people) were sensitive (PAI 1-5) to thienopyridine drugs that were part of combination therapy. It was noteworthy that in groups 1 and 2 there was a decrease in sensitivity to antiplatelet therapy, which included ASA. In the group of patients with hypertension+stable forms of CAD (1) in 29 patients out of 55 people (53%), the activity of vascular-platelet hemostasis indicated an insufficient treatment response. In the group of patients with hypertension+ACS (2), 33 patients out of 60 people (55%) had varying degrees of impaired sensitivity. In patients receiving thienopyridines, in both study groups, the degree of platelet activity impairement indicated the treatment efficacy ("sensitive"). Among patients of group 1, in 77% (23 persons) out of 30 patients the value of platelet index below 5 was registered, similar results were determined in patients of group 2 – 71% (44 patients).

During the study of spontaneous aggregation degree in the "sensitive" subgroup of group 1, the degree of spontaneous platelet aggregation exceeded the control values by 53% (1.35±0.47), however, no significant difference from the control was determined (0.88±0.30) (table 1).

Table 1

Functional platelet activity in patients with stable CAD and ACS in response to antiplatelet therapy with ASA

Index, units of measurement	Group	Subgroup	M	SD	m	95% confidence interval		p
						lower limit	upper limit	
Degree of spontaneous aggregation, %		Control (n=30)	0.88	0.30	0.05	0.77	1.00	p=0.14
	1 n=55	sensitive (n=26)	1.35	0.47	0.29	0.75	1.95	
		impaired sensitivity (n=29)	2.03*	1.86	0.34	1.32	2.74	
	2 n=60	sensitive (n=27)	1.89*	1.02	0.19	1.49	2.29	p=0.44
		impaired sensitivity (n=33)	2.09*	0.93	0.16	1.76	2.41	
Degree of ARA-induced aggregation, %		Control (n=30)	28.82	4.87	0.88	27.00	30.64	
	1 n=55	sensitive (n=26)	15.10*	9.37	1.83	11.32	18.89	p=0.0001
		impaired sensitivity (n=29)	28.12	4.19	0.77	26.53	29.72	
	2 n=60	sensitive (n=27)	18.67*	10.07	1.93	14.68	22.65	p=0.0001
		impaired sensitivity (n=33)	32.35*	4.65	0.81	30.70	34.00	
Degree of collagen-induced aggregation, %		Control (n=30)	20.94	4.68	0.85	19.19	22.69	
	1 n=55	sensitive (n=26)	20.73	12.21	2.39	15.8	25.66	p=0.02
		impaired sensitivity (n=29)	28.12*	10.33	1.95	24.11	32.12	
	2 n=60	sensitive (n=27)	24.54	7.39	1.42	21.62	27.47	p=0.03
		impaired sensitivity (n=33)	29.80*	0.79	1.87	25.99	33.64	
Degree of adrenalin-induced aggregation, %		Control (n=30)	18.41	9.10	1.66	15.01	21.81	
	1 n=55	sensitive (n=26)	16.08	8.9	1.74	12.49	19.68	p=0.05
		impaired sensitivity (n=29)	21.02	9.28	1.72	17.49	24.56	
	2 n=60	sensitive (n=27)	18.07	5.93	1.14	15.72	20.41	p=0.07
		impaired sensitivity (n=33)	21.09	6.89	1.2	18.65	23.54	
Note. * – probability of differences with the control group (p<0.05).								

However, in the analysis of spontaneous aggregation among patients of group 1 with impaired sensitivity, it was shown that this degree was 2.3 times higher than in the control group (2.03±1.86). Therefore, it was further decided to perform a study of the functional activity of platelets separately in groups of patients according to the degree of sensitivity.
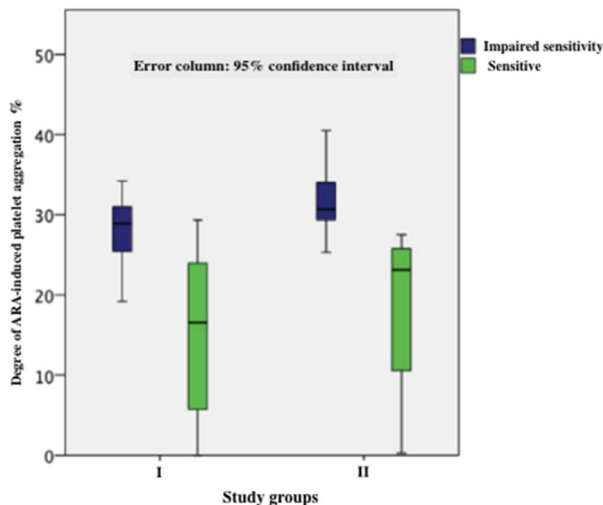

Fig. 2 The degree of ARA-induced platelet aggregation among groups of patients depending on their sensitivity to antiplatelet therapy

Analyzing the spontaneous aggregation in patients of group 2, there was noted an increase in the level of spontaneous aggregation among patients with impaired sensitivity (2.09±0.93). In both subgroups, the degree of spontaneous aggregation was probably higher than in the control group, without significant differences between them (1.5 times higher in the group of patients with impaired sensitivity).

We found more significant differences during the analysis of data of ARA-induced platelet aggregation. Thus, this degree in patients of group 1 who had a positive response to antiplatelet therapy was 52% lower than the value of the control group (15.10±9.37 and 28.82±4.87, respectively), while among patients with varying degrees of insensitivity, it did not differ from control values, despite the treatment with ASA (28.12±4.19). It should be noted that a significant difference was registered between the group of sensitive patients and people with impaired sensitivity – 86% (F=45.78; p <0.0001). Similar values were registered in persons of group 2 (fig. 2).

It should be noted that there were also significant differences in collagen-induced platelet aggregation among "sensitive" patients and people with impaired sensitivity. Thus, in patients of group 1 the difference between sensitive and impaired sensitive individuals (20.73±12.21 and 28.12±10.33, respectively) was 35% and reached a significant difference (F = 5.78; p = 0.02). A similar dependence was observed in patients of group 2 – by 21% (24.54±7.39 and 29.8±10.79, respectively; p = 0.03).

Analyzing adrenaline-induced platelet aggregation, we noted differences, which reached a significant difference in patients of group 1 (F = 4.03; p = 0.05). Such dependence was not found in patients of group 2.

As part of antiplatelet therapy, 61% of patients out of 147 received thienopyridines (clopidogrel, ticagrelor), both as monotherapy and in combination with ASA. Since we found some features in the functional activity of platelets against ASA, it was further decided to perform a similar study in patients receiving thienopyridines.

Among this cohort of patients, significant differences were observed already in the degree of spontaneous platelet aggregation. In contrast to the data on the background of ASA, in patients with impaired sensitivity of group 1, spontaneous aggregation was 92% higher than the control values and 2.8 times higher than the obtained data in "sensitive" patients, which reached a significant difference (1.69±0.74 and 0.59±0.33; p = 0.03). In patients of group 2, this trend remained, and the obtained data on the degree of spontaneous aggregation probably differed from the control group among both subgroups. However, there was no statistically significant difference between the groups (table 2).

Table 2

Functional platelet activity in patients with hypertension, stable CAD and ACS in response to antiplatelet therapy with thienopyridines

Index, units of measurement	Group		M	SD	m	95% confidence interval		p
						lower limit	upper limit	
Degree of spontaneous aggregation, %	Control (n=30)		0.88	0.30	0.05	0.77	1.00	
	1 n=30	sensitive (n=23)	0.59	0.33	0.19	0.18	0.99	p=0.03
		impaired sensitivity (n=7)	1.69	0.74	0.66	0.07	3.3	
	2 n=62	sensitive (n=44)	1.79**	0.8	0.12	1.54	2.03	p=0.09
		impaired sensitivity (n=18)	2.22**	1.15	0.27	1.65	2.8	
Degree of ADP-induced aggregation, %	Control (n=30)		34.75	5.23	0.95	32.80	36.71	
	1 n=30	sensitive (n=23)	27.24*	7.84	1.63	23.85	30.63	p=0.0001
		impaired sensitivity (n=7)	40.43	4.83	1.82	35.95	44.9	
	2 n=62	sensitive (n=44)	28.59*	10.62	1.6	25.36	31.8	p=0.0001
		impaired sensitivity (n=18)	40.38*	6.14	1.44	37.32	43.43	
Degree of collagen-induced aggregation, %	Control (n=30)		20.94	4.68	0.85	19.19	22.69	
	1 n=30	sensitive (n=23)	23.99	8.01	1.67	20.5	27.45	p=0.68
		impaired sensitivity (n=7)	25.56	11.14	4.21	15.25	35.87	
	2 n=62	sensitive (n=44)	26.66*	10.16	1.53	23.57	29.75	p=0.9
		impaired sensitivity (n=18)	26.51	10.56	2.48	21.26	31.76	
Degree of adrenaline-induced aggregation, %	Control (n=30)		18.41	9.10	1.66	15.01	21.81	
	1 n=30	sensitive (n=23)	17.0	8.65	1.80	13.26	20.74	p=0.007
		impaired sensitivity (n=7)	29.02	12.19	4.60	17.74	40.30	
	2 n=62	sensitive (n=44)	19.37	6.36	0.95	17.43	21.30	p=0.5
		impaired sensitivity (n=18)	18.26	5.17	1.21	15.69	20.83	

Note. * – probability of differences with the control group (p<0.05).

Among group 2 patients who were sensitive to thienopyridines, the degree of ADP-induced platelet aggregation was 22% lower than the control (27.24±7.84 and 34.75±5.23, respectively), in contrast to persons with varying degrees of impaired sensitivity, in whom this level was higher by 48% (40.43±4.83) and exceeded the control values by 1.2 times. The difference between the subgroups was statistically significant (F = 17.5; p = 0.0001).

Similar changes were also reported among sensitive patients of group 2: ADP-induced platelet aggregation was 18% lower than control values (28.59±10.62); among patients with varying degrees of impaired sensitivity, it was 16% higher than the control values (40.38±6.14). The difference between the groups was 41% and reached a significant difference (F = 19.38; p = 0.0001). We did not find a similar trend in the analysis of collagen-induced platelet aggregation. The values of collagen-induced aggregation did not differ among the subgroups of sensitivity in the studied groups.

In patients of group 1, among patients with impaired sensitivity, the degree of adrenaline-induced platelet aggregation was 1.7 times higher than among the group of "sensitive" patients, which reached a significant difference (17.00±8.65 and 29.02±12.19; F = 8.54; p = 0.007). Such pattern was not found in patients of group 2.

Thus, among patients treated with thienopyridines, we found some differences among the subgroup of "sensitive" patients and patients with impaired sensitivity, the most pronounced changes were registered from spontaneous, ADP-induced and adrenaline-induced platelet aggregation (the latter among patients of group 1).

Despite the constant long-term receiving of antiplatelet drugs, in some patients with CAD thrombotic events continue to develop. In recent years, the literature has described a possible relationship between residual platelet activity determined by various laboratory tests and clinical outcome, which increases the possibility that "resistance" to antiplatelet drugs, may underlie many clinically adverse effects [12]. The most significant differences were found by us in the analysis of the degree of ARA-induced platelet aggregation, which in sensitive patients with stable CAD, was 52% lower than the control values, while among people with

impaired sensitivity it did not differ from the control group despite the treatment with ASA. It should be noted that a significant difference of 86% was found between subgroups (p <0.0001, F = 45.78). Collagen-induced platelet aggregation also had significant differences among sensitive patients and less sensitive ones. Thus, in patients of group 2, the difference between sensitive and less sensitive patients was 35% and reached reliability (p = 0.02, F = 5.78). We observed a similar dependence (21%) in patients with hypertension and ACS. Thus, collagen-induced platelet aggregation also had a response to ASA. In some previous studies, ASA affects only ARA-induced platelet aggregation, and also has some effect on collagen-induced aggregation, which was confirmed by our studies [10].

Among patients of group 2, who according to the express method of laser-light scattering platelet aggregometry in whole blood responded to therapy with thienopyridines, the degree of ADP-induced platelet aggregation was 22% lower than the control in contrast to patients with impaired sensitivity, in whom this level was higher by 48% and exceeded the value of the control group by 1.2 times. Similar changes were also found in sensitive patients of group 2: ADP-induced platelet aggregation was 18% lower than the control, among patients with impaired sensitivity it was 16% higher than the control values. The difference between the groups was 41% and was statistically significant [14].

Conclusion

Therefore, in our study it was shown that it's necessary to take into account the degree of patient's response to a particular group of antiplatelet drugs and to perform a detailed analysis, taking into account the data of the express method of laser-light scattering platelet aggregometry in whole blood, when analyzing the functional activity of platelets on the background of antiplatelet therapy. Given the heterogeneity of indices in groups of patients with hypertension and various forms of CAD, this study makes it possible to more accurately determine the degree of sensitivity to antiplatelet therapy.

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