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ANTIPHOSPHOLIPID AND ANTINEUTROPHIL ANTIBODIES LEVELS IN MEN WITH STABLE CORONARY HEART DISEASE AND POSTINFARCTION CARDIOSCLEROSIS AND ITS RELATIONSHIP WITH THE DISEASE MANIFESTATION

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ABSTRACT

The aim is to study the levels of antiphospholipid (aPL) and antineutrophil antibodies in men with stable coronary heart disease (CHD) with postinfarction cardiosclerosis and to evaluate its relationship with the disease manifestation.

Materials and methods: 164 men with stable CHD and postinfarction cardiosclerosis (53.0 ± 9.14 (M ± σ) years) and 48 age-matched men without CHD were examined. The total aPL IgG and IgM, beta-2 glycoprotein 1 antibodies (anti-β2-GP 1) IgG and IgM, and antibodies for neutrophil proteinase-3 / myeloperoxidase (anti-PR3 / MPO) IgG were determined by ELISA.

Results: Positive levels of aPL and anti-β2-GP 1 of IgG were identified in 56.7% (33.5% double positivity of aPL + anti-β2-GP 1) and 29.2% of control group ($p < 0.001$), while the IgM was lower (11.6% vs. 6.2%, $p = 0.55$, respectively). Significantly higher (1.5-1.7 times) levels of aPL and anti-β2-GP 1 were identified in patients who underwent myocardial infarction (MI) aged less than 44 years, after Q-MI, recurrent MI, in the presence of ischemic stroke, livedo reticularis. In 6.7% of patients with positive levels of aPL and anti-β2-GP 1 low IgG anti-PR3 / MPO levels were detected.

Conclusions: In men with postinfarction cardiosclerosis, IgG positivity according to total aPL and anti-β2-GP 1 is associated with a higher incidence of Q-MI and with recurrent MI. Men with postinfarction cardiosclerosis have a tendency to increase anti-PR3 / MPO levels of IgG under conditions of double aPL positivity and anti-β2-GP1 of IgG.

KEY WORDS: coronary heart disease, antiphospholipid syndrome, antineutrophil antibodies

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INTRODUCTION

Antiphospholipid syndrome (APS) as an independent factor of myocardial infarction (MI) and other forms of coronary heart disease (CHD) is attracting increasing attention [1; 2; 3]. The prevalence of primary APS is 40-50 cases per 100,000 population and annually approximately 5 new cases per 100,000 population are detected [4]. Among patients with acute coronary syndrome, the frequency of APS ranges from 6.1% to 43.3% [5]. The development of APS is due to the formation of antibodies to the own phospholipids (cardiolipin, phosphatidylserine, phosphatidylinositol), cofactor proteins and their complexes with phospholipids (antibodies to β2-glycoprotein 1 (anti-β2-GP1), annexin, thrombomodulin etc). Antiphospholipid antibodies (aPL) cause immune inflammatory activation of the endothelium, initiate the development of thrombophilia, thrombosis and atherothrombosis of various vessels, including coronary arteries, leading to MI [2]. On the other hand, ischemic-reperfusion lesions of the myocardium can act as a trigger for antigen-dependent aPL synthesis and the development of APS, as cardiolipin, a phospholipid with the most potent immunogenic properties enter the circulatory channel [6]. There is evidence in one of the studies that elevated aPL levels of IgG and IgM were detected in 100% of patients in the acute period of MI [7].

Another factor influencing inflammatory activation of the coronary artery endothelium may be antibodies to neutrophil proteinase-3 and myeloperoxidase (anti-PR3 / MPO) [8; 9]. Anti-PR3 / MPO cause leukocyte degranulation and endothelial lining of small vessels (arterioles, venules, capillaries) and cause microcirculatory disorders. Microcirculatory disorders significantly impair myocardial status and increase the risk of CHD progression [10; 11]. It has been recently demonstrated that anti-PR3 / MPOs can be produced concurrently with aPL, which aggravates systemic vasculitis [12]. The question of possible associations of APS components with anti-PR3 / MPO in CHD patients with postinfarction cardiosclerosis remains open.

THE AIM

The aim is to study the levels of aPL and antineutrophil antibodies in men with stable CHD with postinfarction cardiosclerosis and to evaluate its relationship with the disease manifestation.

MATERIALS AND METHODS

An open cross-sectional study was conducted on a case-control basis. 164 patients with stable CHD with

Table I. Clinical and demographic features of CHD group and control group

	Patients with CHD, n=164	Control, n=48	p value
Age, years (M±σ)	53.0±9.14	52.1±8.69	0.860
Body mass index, kg / m2 (M±σ)	29.9±3.74	29.2±2.68	0.141
Body mass index ≥ 30 kg / m2, n (%)	83 (50.6 %)	18 (37.5 %)	0.139
Waist circumference, sm (M±σ)	102.9±9.0	100.5±9.9	0.110
Waist circumference ≥ 94 sm, n (%)	133 (81.1%)	34 (70.8 %)	0.159
AH, n (%)	140 (85.4%)	36 (75.0 %)	0.124
SBP, mm Hg (M±σ)	138.4±15.5	134.7±12.5	0.261
DBP, mm Hg. (M±σ)	86.9±10.1	85.6±10.2	0.592
Smoking, n (%)	67 (40.9 %)	15 (31.3 %)	0.243

Table II. Frequency of detection of phospholipid antibodies IgG class in men with stable CHD with postinfarction cardiosclerosis

Group	Frequency of IgG class antibody levels, n (%)						
	Normal	Low-positive			Medium-positive		
		aPL	anti-β2-GP 1	aPL + anti-β2-GP 1	aPL	anti-β2-GP 1	aPL + anti-β2-GP 1
Control, n=48	34 (70.8%)	6 (12.5 %)	4 (8.3%)	2 (4.2%)	1 (2.1%)	1 (2.1%)	0 (0.0%)
Patients with CHD, n=164	71 (43.3%)	7 (4.3 %)	9 (5.5%)	42 (25.6 %)	11* (6.7%)	13* (7.9%)	11 (6.7%)
p	<0.001	0.078	0.496	<0.001	0.305	0.199	0.073

Note. * - medium-positive levels of one type of antibodies were combined with low-positive levels of another type of antibodies.

Table III. Phospholipid antibody levels in men with stable CHD disease with postinfarction cardiosclerosis

Group	Total aPL, U / ml (M±σ)		Anti-β2-GP 1, U / ml (M±σ)	
	IgG	IgM	IgG	IgM
Control, n=48	6.56±3.67	3.38±2.26	6.70±3.67	3.80±2.91
Patients with CHD, n=164	11.5±5.89	6.14±4.07	12.7±6.74	6.72±3.93
p	<0.001	<0.001	<0.001	<0.001

postinfarction cardiosclerosis, at the age of 53.0 ± 9.14 years and total disease duration 42.0 [14; 99] months were examined. All patients were treated at the cardiology and polyclinic departments of Vinnytsia Regional Clinical Hospital named after Pirogov within 2013-2018. The study was conducted in compliance with bioethic norms according to the Helsinki Declaration "Ethical Principles for Medical Research Involving Human Subjects" with subsequent revisions, European Convention of Human Rights and Biomedicine.

The diagnosis of stable CHD was established according to the recommendations of the AHA / ACC (2014) and ESC (2013). Postinfarction cardiosclerosis was verified on the basis of examination of relevant medical records and the results of instrumental studies. The criteria for inclusion of patients in the study were as follows: male; age > 25 years; verified postinfarction cardiosclerosis; duration of the disease from 3 months or more after the last MI; patient's consent to participate in the study.

The study involved men with postinfarction cardiosclerosis. Exclusion criteria were the following: female, acute coronary syndrome, uncontrolled arterial hypertension (CBP ≥ 180 mm Hg, DBP ≥ 100 mm Hg), hemodynamically unstable arrhythmias, type 1 and 2 diabetes mellitus with decompensated conditions. The study included 123 (75%) patients who underwent Q-MI and 41 (25%) patients who underwent non-Q-MI, including 17 (10.4%) patients who had recurrent MI. Comorbid conditions were found in 143 (87.2%) patients, the most common being arterial hypertension (AH) (85.4%) and abdominal obesity (50.6%). The control group consisted of 48 men aged 52.1 ± 8.69 years and corresponded to the main group by clinical and demographic parameters (Table I).

Blood for the studies was obtained under standard conditions, in the morning on an empty stomach after a night of fasting. The serum was stored in eppendorf microtubes at – 20°C until the study. The determination of total aPL (to cardiolipin, phosphatidylserine, phosphatidylinositol) IgG and IgM was performed by ELISA using the Anti-Phospholipid Screen IgG / IgM kit (Orgentec Diagnostika GmbH, Germany). The results were interpreted as follows: negative result (normal level) – aPL < 10 U / ml, positive result – ≥ 10 U / ml. aPL levels above 40 U / ml were considered high-positive, 20 – 40 U / ml – medium-positive, 10 – 20 U / ml – low-posi-

Table IV. Levels of antibodies to neutrophilic proteinase-3 / myeloperoxidase (anti-PR3 / MPO) IgG class in men with stable CHD with postinfarction cardiosclerosis

Group	Anti-PR3 / MPO IgG, U / ml (M±σ)	Frequency level anti-PR3/MPO, n (%)	
		Negative	Low-positive
Control, n=48	1.81±1.51	48 (100 %)	0 (0.0%)
Patients with CHD, n=164	3.43±2.84***	153 (93.3 %)	11 (6.7 %)
Including those depending on antibodies levels to phospholipid IgG class			
Group 1, n=71	2.78±1.80 *	70 (98.6 %)	1 (1.4 %)
Group 2, n=58	3.39±3.06 **	54 (93.1 %)	4 (6.9 %)
Group 3, n=24	4.76±3.56*** #	20 (83.3 %)	4 (16.7 %)* #
Group 4, n=11	4.91±4.05*** #	9 (81.8 %)	2 (18.2 %)*#

Notes: group 1 – normal levels of aPL and anti-β2-GP 1; group 2 – low-positive levels of aPL and/or anti-β2-GP 1; group 3 – medium-positive levels of aPL or anti-β2-GP 1; group 4 – medium-positive levels of aPL + anti-β2-GP 1.
* – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ compared to control group;
# – $p < 0.05$ – compared to group 1.

Table V. Levels of IgG autoantibodies in patients with CHD with postinfarction cardiosclerosis depending on clinical and demographic features

Feature		Levels IgG, U/ml (M±σ)		
		aPL	anti-β2-GP 1	anti-PR3/MPO
Age at which the 1st MI occurred	< 44 years (n = 41)	16.6±6.10	18.0±7.10	3.46±2.48
	≥ 44 years (n = 123)	9.86±4.77**	11.0±5.65**	3.42±2.96
Duration of the disease	≤ 5 years (n = 107)	11.7±5.93	13.4±7.02	3.34±2.71
	> 5 years (n = 57)	12.6±7.15	13.1±7.84	3.99±3.47
Variant of the 1st MI	Q-IM (n = 123)	12.0±5.51	13.6±6.46	3.39±2.52
	Not Q-IM (n = 41)	9.75±5.81*	10.2±6.85*	3.48±3.40
Number of MI	Single IM (n = 147)	10.9±5.22	12.0±5.68	3.35±2.65
	Recurrent IM (n = 17)	17.1±8.32*	19.2±10.9*	4.31±4.17
Comorbid hypertension	CHD without hypertension (n = 24)	11.8±5.84	12.1±5.07	2.99±1.73
	CHD with hypertension (n = 140)	11.6±6.16	12.9±7.05	3.51±2.98
Vascular comorbidity	without CVD and livedo reticularis (n = 147)	10.8±5.43	12.0±6.31	2.81±1.70
	with CVD + livedo reticularis (n = 17)	18.6±7.40***	19.9±6.96***	8.78±4.64**
Body mass index	<30 kg / m2 (n = 81)	11.4±5.95	12.2±5.86	3.18±2.57
	≥ 30 kg / m2 (n = 83)	11.9±5.84	13.3±7.51	3.67±3.07

Notes. * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ compared to control group

Table VI. Odds ratio for MI in men with CHD with postinfarction cardiosclerosis depending on the laboratory components of APS

Levels of aPL IgG and anti-β2-GP 1 IgG	Odds ratio, OR (95% CI)		p1	p2
	Q-MI	Recurrent MI		
Negative	1	1	-	-
Positive	2.58 (1.26 – 5.28)	2.52 (0.83-7,67)	0.010	0.067
Including:				
Medium-positive	2.63 (0.98 – 7.18)	4.64 (1.36-15.8)	0.059	0.018
Low-positive	2.53 (1.11-5.77)	1.54 (0.66-5.64)	0.028	0.730

Notes. p1 – authenticity about «1st Q-MI »; p2 – «recurrent MI».

tive. The levels of antibodies to anti-β2-GP 1 of IgG and IgM were determined by the Aeskulisa β2-Glyco-GM kit (AESKU diagnostics, Germany, lot 18200). The results were interpreted as follows: negative result (normal level) – < 12 U / ml, low-positive – 12 – 18 U / ml, positive – > 18 U / ml, including medium-positive – 18 – 40 U / ml, high-positive – above 40 U / ml. The verification of clinical and laboratory components of APS was performed according to the diagnostic

criteria of Sapporo (2006) [13] and the latest EULAR-2019 guidelines [14]. Statistical processing was performed using SPSS Statistics 22.0. Student's t-test was used to estimate the difference between groups in the normal distribution and Mann-Whitney U-test was used for the distribution which is different from normal. The normality of the distribution was checked by Kolmogorov-Smirnov and Shapiro-Wilk criteria. Pearson correlation analysis was used to determine relationships between indicators, using Fisher's exact method when comparing the frequency of changes. The odds ratio (OR), confidence intervals (95% CI) were evaluated. The difference at $p < 0.05$ was considered significant.

RESULTS

It was found (Table II) that negative results on both IgG antibodies (aPL and anti-β2-GP 1) in the control group were found to be 1.63 times more frequent than in patients with CHD ($p < 0.001$). Low positive levels of one type of antibody were found in 20.8% of the control group and in 9.8% of patients with CHD ($p < 0.05$). The combination of low-positive levels of aPL + anti-β2-GP 1 was found in 25.6% of patients with CHD, which was 6.1 times higher than in the control group. In patients with CHD, the presence of medium-positive levels of one of the antibodies (aPL or anti-β2-GP 1) was combined with the presence of low-positive levels of another type of anti-β2-GP 1 antibody, and in 6.7% of patients the medium-positive levels of both antibodies were detected simultaneously. In the control group, low-positive aPL and anti-β2-GP 1 IgM levels were detected in 3 (6.2%) and 4 (8.3%) individuals. Among patients with CHD, low-positive aPL and anti-β2-GP 1 IgM levels were detected in 12 (7.3%) and 15 (9.1%) individuals, with medium-positive levels in 7 (4.3%) and 5 (3, 0%) of individuals, respectively, and differences in control group were not statistically significant. Analysis of the absolute values of aPL and anti-β2-GP 1 IgG and IgM showed (Table III) that in patients with CHD these indicators are significantly higher (1.7-1.8 times, $p < 0.001$) than in the control group. 17 (10.4%) patients with CHD, who were detected with positive levels of aPL and anti-β2-GP 1 IgG and IgM, had had a history of non-coronary vascular manifestations of probable APS, 13 (7.9%) had experienced an ischemic stroke or transient stroke attack, and 7 (4.3%) people had had livedo reticularis including 3 of them with the previous stroke.

Among patients with CHD, 11 (6.7%) individuals with low-positive levels of IgG (Table IV) anti-PR3 / MPO were detected, while in the control group 100% of individuals showed negative levels of these antibodies ($p = 0.073$). Positive anti-PR3 / MPO levels were more commonly found in patients with medium-positive aPL and anti-β2-GP1 IgG levels than in patients with negative and low antibody phospholipid levels. On average, the level of anti-PR3 / MPO IgG in patients with CHD significantly exceeded the control group value (1.8 times), and these differences were amplified with increasing levels of antibodies to phospholipids.

The analysis of the levels of aPL, anti-β2-GP 1 and anti-PR3 / MPO IgG class in patients with CHD, depending on clinical and demographic parameters revealed certain features (Table V). So, significantly higher levels of aPL and anti-β2-GP 1 IgG were found in patients with CHD who underwent their first MI under 44 years (1.68 and 1.63 times respectively), in patients with Q-MI (1,24 and 1.33 times respectively), recurrent MI (1.56 and 1.60 times respectively), in the presence of non-coronary vascular manifestations (1.72 and 1.66 times respectively). Differences in the level of aPL and anti-β2-GP 1 in patients with CHD, depending on the duration of the disease, hypertension and body mass index have not been established. Patients with cerebrovascular disease (CVD), such as stroke or transient ischemic attacks and livedo reticularis, were found to have significantly higher levels of anti-PR3 / MPO IgG (3.12 times) than patients without vascular manifestations. There were no other clinical manifestations for differences in anti-PR3 / MPO IgG levels in patients with CHD.

Thus, in men with postinfarction cardiosclerosis, IgG positivity according to total aPL and anti-β2-GP 1 is associated with a higher incidence of Q-MI (OR 2.58, 95% CI 1.26 – 5.28, $p = 0.01$) and in the presence of medium-positive levels of these autoantibodies with recurrent MI (OR 4.64, 95% CI 1.36-15.8, $p = 0.018$). (Table VI).

DISCUSSION

According to the results of this study, in the primary testing of 164 men with postinfarction cardiosclerosis, the overall frequency of aPL positivity was 56.7%, including two antibodies (aPL + anti-β2-GP 1) – 33.5 %. There is evidence that in individuals with triple aPL positivity during primary testing (lupus anticoagulant + cardiolipin antibodies (aCL) + anti-β2-GP 1), this aPL profile is confirmed after 12 weeks in 98% of cases, in individuals with double aPL- positivity in 88% of cases, and in individuals with isolated aPL positivity in only 40% of cases [15]. The results of long-term prospective studies on asymptomatic carriers of laboratory markers of APS indicate a high risk of thrombotic events in the case of double or triple positivity for aPL, while positivity for one type of antibodies is not associated with an increased risk of thrombosis [16]. A meta-analysis of 11 studies with a total of 2425 patients with CHD (283 individuals with IgG positive aCL) showed an increase in the relative risk of major cardiac events in 12 and 24 months (RR 2.17 and 2,11 respectively). [5]. In IgG aCL-positive patients with "juvenile" CHD (younger than 50 years), the relative risk of recurrent major cardiac events after 12 and 24 months increased more significantly (3.21 and 3.24, respectively) [5].

CONCLUSIONS

Thus, in men with postinfarction cardiosclerosis, double positivity for total aPL and IgG anti-β2-GP 1 is closely associated with the early manifestation of CHD (at the age of 44 years), Q-MI and recurrent MI. Besides, in the case

of double positivity to aPL and anti-β2-GP 1 IgG, there is a tendency to increase the levels of anti-PR3 / MPO IgG class, which is associated with vascular pathology (strokes, livedo reticularis). Therefore, the persistence of the production of aPL and antineutrophil antibodies forms an unfavorable pathogenetic pattern that can significantly modify the course of CHD in men.

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Topic: "Metabolic risk factors, cardiovascular remodeling and functional status of kidneys in patients with cardiovascular pathology. Possibilities of pharmacological correction"

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