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MARKERS OF ENDOTHELIAL DYSFUNCTION AS CRITERIA FOR DESTABILIZATION OF THE DISEASE COURSE IN PATIENTS WITH CORONARY ARTERY DISEASE

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To determine the presence and severity of vascular endothelial dysfunction in patients with coronary artery disease and the possibility of diagnosing exacerbation of the atherosclerotic process in such patients by determining markers of endothelial dysfunction, 173 patients with different variants of the disease were examined. It was found that patients with unstable coronary artery disease were characterized by a more pronounced impairment of vascular endothelial function, as evidenced by a significant increase in biochemical markers of endothelial dysfunction (ET-1, sVCAM, and PAPP-A) not only compared to the control group but also to the patients with stable coronary artery disease. Therefore, such markers can be considered criteria for destabilizing the atherosclerotic process. Based on determining the threshold values of endothelial dysfunction in patients with coronary artery disease, groups with definite and possible ("grey zone") destabilization and without destabilization were identified. Values of ET- 1 ≥10.43 ng/mL, sVCAM ≥1320.0 ng/mL, and PAPP-A ≥10.10 mIU/L were the basis for including patients in the group of patients with a destabilization of the disease even in the absence of clinical manifestations.

Key words: coronary artery disease, destabilization of the atherosclerotic process, endothelial dysfunction, endothelin-1, soluble vascular cell adhesion molecules, vasodilation function of the endothelium.

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МАРКЕРИ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКІЇ В ЯКОСТІ КРИТЕРІЇВ ДЕСТАБІЛІЗАЦІЇ ПЕРЕБІГУ ЗАХВОРЮВАННЯ У ХВОРИХ НА ІШЕМІЧНУ ХВОРОБУ СЕРЦЯ

З метою визначення наявності і виразності порушення функцій судинного ендотелію у хворих на ішемічну хворобу серця та можливості діагностики загострення атеросклеротичного процесу у таких пацієнтів шляхом визначення маркерів ендотеліальної дисфункції, обстежено 173 пацієнти з різними варіантами перебігу захворювання. Встановлено, що пацієнти з нестабільним перебігом ішемічної хвороби серця характеризувалися більш виразним порушенням функцій судинного ендотелію, свідченням чого було достовірне зростання біохімічних маркерів ендотеліальної дисфункції (ЕТ-1, sVCAM і PAPP-A) не лише відносно контрольної групи, а й відносно хворих зі стабільною ішемічною хворобою серця. Тому такі маркери можна розцінювати в якості критеріїв дестабілізації атеросклеротичного процесу. На підставі визначення граничних величин показників ендотеліальної дисфункції у хворих на ІХС виділені групи з достовірною і можливою («сіра зона») дестабілізацією та без дестабілізації. Величини показників ЕТ-1 ≥10,43 нг/мл, sVCAM ≥1320,0 нг/мл і PAPP-A ≥10,10 мМО/л стали підставою для включення пацієнтів в групу хворих з дестабілізацією захворювання навіть за відсутності клінічних проявів.

Ключові слова: ішемічна хвороба серця, дестабілізація атеросклеротичного процесу, ендотеліальна дисфункція, ендотелін-1, розчинні судинні молекули адгезії, судинно-рухлива функція ендотелію.

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According to world statistics, coronary artery disease (CAD) has been the leading cause of death and disability worldwide, including in Ukraine, for several decades [2; 12; 13]. Cardiovascular diseases cause about 4 million deaths in Europe each year and cost countries about 192 billion euros in direct and indirect economic losses [13].

The prognosis of a patient with coronary artery disease is largely determined by the destabilization of the process, which is clinically manifested by acute coronary syndrome (ACS), which is occurred due to the activation of atherothrombogenesis. The methods used to diagnose ACS are based on the detection of focus of necrosis or transient myocardial dysfunction. However, it is equally important to search for markers that would allow predicting the development of ACS or diagnosing it in a patient before the onset of irreversible changes in the myocardium [9].

The main pathophysiological mechanism of coronary artery disease is the discrepancy between the myocardial oxygen demand and insufficient oxygen supply, which is caused in the vast majority of cases by coronary atherosclerosis.

One of the important links in the pathogenesis of atherosclerosis is endothelial dysfunction, an important initial link in the cardiovascular continuum that determines the course and prognosis of cardiovascular diseases. To date, the vascular endothelium is considered a powerful paracrine organ that performs barrier, secretory, hemostatic, and vasoregulatory functions and plays an important role in the processes of inflammation and vascular wall remodeling.

The purpose of the study was to identify markers of endothelial dysfunction in patients with different variants of coronary artery disease and to evaluate the possibility of their use as criteria for exacerbation of the atherosclerotic process.

Materials and methods. The study included 173 patients with coronary artery disease (mean age: 57.24±5.12 years) who were treated in the cardiology department of the Vinnytsia M.I.Pirogov Memorial Regional Clinical Hospital and the department for patients with myocardial infarction of the Vinnytsia Regional Clinical Treatment and Diagnostic Center for Cardiovascular Pathology. After the examination, the patients were divided into 2 main clinical groups – 92 patients with stable CAD (45 with II and 47 with III functional classes, respectively) and 81 patients admitted to the hospital with ACS (subsequently, 43 patients were diagnosed with unstable (progressive) angina (UA), and 38 patients with acute myocardial infarction (AMI)).

The control group consisted of 30 practically healthy individuals of comparable age and gender.

Blood sampling from the cubital vein for clinical and biochemical examination was performed on the first day of admission to the hospital (in the case of ACS – in the first 2 hours). In 82 patients (47.4%), CAD was combined with hypertension.

The diagnosis of stable coronary artery disease and variants of the acute coronary syndrome was established following 2012, 2013, and 2015 European Society of Cardiology Guidelines and Orders of the Ministry of Health of Ukraine No. 455 of July 02, 2014, No. 164 of March 03, 2016, and No. 152 of March 02, 2016.

The study did not include people over 75 years of age with congestive heart failure of III-IV functional classes (NYHA), malignant tumors, secondary arterial hypertension, acute inflammatory or exacerbation of chronic diseases at the time of the examination, obesity of II-III degrees, liver and kidney diseases with impaired function, diseases causing secondary dyslipidemia (diabetes mellitus, hypothyroidism, nephrotic syndrome, cholestasis).

All patients underwent lipid profile, glucose level, electrolyte composition (K+, Na+), urea and creatinine levels, total protein, fibrinogen, prothrombin index or INR, total bilirubin and its fraction, alanine and aspartate aminotransferase activity before the start of the study, in addition to a complete blood count and urinalysis.

Markers of endothelial dysfunction in the blood serum of patients with coronary artery disease were determined by enzyme-linked immunosorbent assay using special reagent kits (ELISA kits "Endotelin-1" manufactured by DRG, USA; "sVCAM" by Bender Medsystems, Austria; highly sensitive "PAPP-A" by Diagnostics Systems Laboratories, USA).

Excel-2010 spreadsheets and StatSoft "Statistica" v.10.0 software were used to create the database and analyze the results. The significance of the differences was determined using Student's and Mann-Whitney's t-test. Limit values were calculated using the formula of Antomonov M.Yu. [1].

Results of the study and their discussion. A comparative assessment of biomarkers of endothelial dysfunction in groups of patients with stable and unstable coronary artery disease (Table 1) showed significant differences of parameters with a high degree of reliability for sVCAM and PAPP-A levels ($p<0.001$), which gives reason to associate their increase with the destabilization of the process. The degree of differences in ET-1 levels was also significant ($p<0.01$), although somewhat smaller. Levels of the blood lipid spectrum did not differ significantly in patients with different disease courses, except HDL cholesterol, which was slightly but significantly higher in patients with stable CAD than in patients with ACS ($p<0.05$).

The degree of severity of changes in endothelial vasoconstrictor function depends on the nature of the disease and the severity of the process. The lowest degree of changes was observed in patients with FC II of angina. With increasing severity of angina FC, the level of ET-1, increased significantly ($p<0.05$), although moderately (by 11.8%). The highest ET-1 values were in patients with ACS without any significant difference within the groups with AMI and UA ($p>0.05$) (fig. 1). The presence of a significant difference in the levels of ET-1 in patients with ACS compared with patients with stable angina of FC III gives grounds to consider this indicator as a criterion for destabilization of the atherosclerotic process.

Table 1

Biochemical parameters in patients with different courses of coronary artery disease

Index	Stable course (n=92)	Unstable course (n=81)	p
ET-1, ng/mL	8.84±0.28	11.08±0.37	<0.01
sVCAM, ng/mL	1195.3±31.44	1724.3±41.20	<0.0001
PAPP-A, mIU/L	4.44±0.27	16.15±0.24	<0.0001
Total cholesterol, mmol/L	5.99±0.13	6.01±0.17	ns
TG, mmol/L	1.80±0.05	1.90±0.07	ns
LDL cholesterol, mmol/L	4.05±0.12	4.07±0.14	ns
LDL cholesterol, mmol/L	0.80±0.03	0.85±0.04	ns
HDL cholesterol, mmol/L	1.14±0.02	1.07±0.02	<0.05
Atherogenicity index, units	4.25±0.21	4.62±0.19	ns

Notes: p – the significance of the difference between the groups with stable and unstable courses; ns – the difference is not significant (p>0.05)

An increase in the levels of sVCAM and PAPP-A above the reference values was found in all groups of patients, with the greatest degree in patients with an unstable course of coronary artery disease (see fig. 1). The highest values of sVCAM and PAPP-A were found in patients with AMI, which significantly differed not only from patients with stable forms of CAD but also from patients with UA (p<0.001).

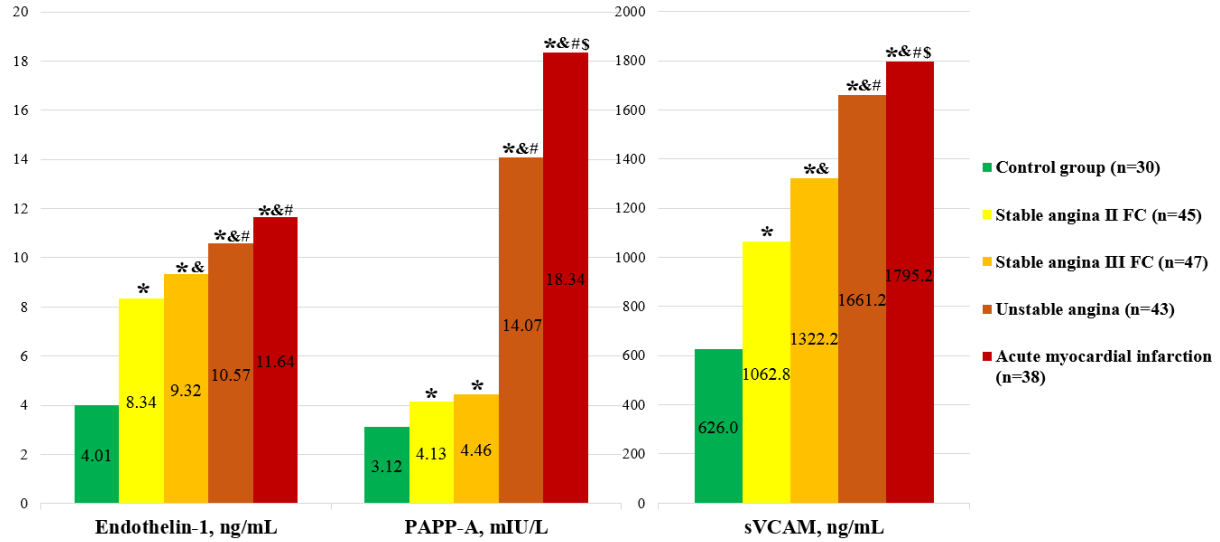

Fig. 1 Levels of biomarkers of endothelial dysfunction in patients with different clinical variants of coronary artery disease.

Notes: * – significantly compared to the control group (p<0.05); & – significantly compared to the stable angina II FC group (p<0.05); # – significantly compared to the stable angina III FC group (p<0.01); $ – significantly compared to the unstable angina group (p<0.001)

Since the mean values of ET-1, sVCAM, and PAPP-A were highest in patients with ACS and significantly differed from patients with stable angina, they can be attributed to the criteria for destabilization of the atherosclerotic process.

Limit values were established for the indicators characterizing the destabilization of the process (Table 2), based on which 3 main groups of patients were identified: without criteria for destabilization (group A), with definite destabilization (group C), and with possible destabilization (group B). Further analysis of the indicators in the group with possible destabilization ("gray zone") led to the division of the group B into 2 subgroups: with moderate (B-1) and significant (B-2) possibility of destabilization.

As can be seen from Table 2, ET-1 ≤6.37 ng/mL, sVCAM ≤912.0 ng/mL, and PARP-A ≤4.10 mIU/L are typical values for patients with a stable course of the disease.

The values of ET-1 ≥10.43 ng/mL, sVCAM ≥1320.0 ng/mL and PAPP-A ≥10.10 mIU/L was the basis for including these patients in the group of patients with a destabilization of the disease even in the absence of clinical manifestations, which, in turn, determines the need to activate therapy to reduce the destabilization process.

The values of ET-1 (6.38-10.42 ng/mL), sVCAM (913.0-1319.0 ng/mL), and PAPP-A (4.11-10.09 mIU/L) corresponded to the "gray zone", i.e. patients with possible destabilization of the process. In patients of subgroup B-2, the values of biomarkers were close to those of the cohort of patients with definite destabilization of the process, so it can be assumed that activation of therapy is desirable for such patients.

Table 2

Limit values of indices associated with destabilization of the process in patients with coronary artery disease

Indices	No criteria for destabilization	Possible destabilization of coronary artery disease			Definite destabilization of coronary artery disease
		The whole group	Moderate possibility of destabilization	Significant possibility of destabilization	
ET-1, ng/mL	≤6.37	6.38-10.42	6.38-7.87	7.88-10.42	≥10.43
sVCAM, ng/mL	≤912.0	913.0-1319.0	913.0-1169.0	1170.0-1319.0	≥1320.0
PAPP-A, mIU/L	≤4.10	4.11-10.09	4.11-7.14	7.15-10.09	≥10.10

It should be noted that the increase in the levels of various biomarkers, of course, destabilization does not always occur in parallel and is manifested unequally often in different course variants. The level of PAPP-A reached the typical threshold values of destabilization of the process mainly in patients with unstable angina and AMI and less often increased in clinically stable disease.

It was found that the criteria for probable destabilization and definite destabilization were more common in patients with ACS with not significant differences between patients with unstable angina and AMI. This may indicate that these criteria do not reflect the degree of myocardial damage but are predictors of such damage. The presence of criteria for definite and probable destabilization in some patients with angina of III FC requires more careful monitoring of such patients.

Our data on the diagnostic significance of markers of endothelial dysfunction in patients with coronary artery disease agree with the results of other authors. Thus, according to Davenport AP et al, the level of ET- 1 in unstable atherosclerotic plaques is significantly higher than in stable ones, which may indicate its effect on plaque destabilization and progression of the atherosclerotic process [4; 9].

Jankowich M et al. [7] observed a significant increase of ET-1 in acute myocardial ischemia, and its level correlated with the severity of the pathological process and prognosis in patients with acute MI and the severity of symptoms in angina pectoris.

According to the results of the ROC analysis of soluble adhesion molecules in 63 patients with ACS completed by Hulok A. et al. [6], a significantly higher risk of ACS is present at a level of sVCAM-1 over 700.15 ng/mL and sICAM-1 over 407.8 ng/mL. The authors believe that the level of sVCAM-1 is an independent risk factor for Non-STEMI (OR – 1.003 (95% CI: 1.0007-1.004); p=0.007), but not STEMI (p>0.05).

According to the results of the study by C. Heeschen [5], an increase in plasma PAPP-A levels above 12.6 mIU/L (4.4 ng/mL) signals an approximately twofold increase in the risk of ACS. It is believed that PAPP-A contributes to plaque instability by causing degradation of the extracellular matrix of the fibrous capsule [3; 14].

When comparing the level of biomarkers of endothelial dysfunction with the content of troponins I and T, determined in the first hours of MI, in our study, no significant relationship between them was found, this gives reason to consider these indicators as criteria for destabilization of coronary artery disease, but not as markers of myocardial damage or necrosis. Thus, an increase in ET-1, sVCAM, and PAPP-A levels may indicate destabilization of the atherosclerotic process and the possibility of developing ACS, as pointed out by other authors [4; 7; 8; 10; 11].

Based on the determination of the threshold values of endothelial dysfunction markers in patients with coronary artery disease, groups with definite and possible ("grey zone") destabilization and without destabilization were identified, which makes it possible to diagnose exacerbation of coronary artery disease not only in the presence but also in the absence of clinical manifestation of destabilization of the process.

Conclusions

1. Patients with coronary artery disease have impaired vascular endothelial function, as evidenced by increased plasma levels of ET-1, sVCAM, and PAPP-A. The degree of changes in these markers is

associated with the severity of the disease and its progression, reaching the highest values in patients with acute myocardial infarction.

2. Increased levels of ET-1 ≥10.43 ng/mL, sVCAM ≥1320.0 ng/mL, and PAPP-A ≥10.10 mIU/L in patients with coronary artery disease indicate destabilization of the atherosclerotic process even in the absence of clinical manifestations, which determines the need to activate therapy in such patients.

Further research in this area will improve the early diagnosis of coronary artery disease exacerbation, help form risk groups, and organize preventive measures in the early stages of the disease before irreversible changes in the myocardium, thereby improving the prognosis of patients with coronary artery disease.

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