ORIGINAL ARTICLE

CONTENTS

Biomarker diagnostics of endothelial dysfunction in patients with acute coronary syndrome and non-alcoholic fatty liver disease

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ABSTRACT

Aim: To determine the severity of endothelial dysfunction (ED) in patients with acute coronary syndromes (ACS) in non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: The study included 124 patients with ACS. Group 1 included 74 patients after ACS and NAFLD, and group 2 consisted of 50 patients after ACS without liver damage. The patients' ED was determined in a test with reactive hyperaemia, and the levels of endothelin-1 (ET-1), P-selectin, and von Willebrand factor (wWF) were determined.

Results: The reactive hyperaemia test revealed ED in patients with ACS regardless of liver damage. More pronounced changes in EDV and EIVD were found in patients with ACS in combination with NAFLD. Laboratory markers of ED, namely ET-1, P-selectin and WwF, were also significantly higher in patients with ACS and confirm the pronounced vasoconstrictor effect of endothelial dysregulation in these patients. A significant difference was found between the levels of ET-1, P-selectin and WwF in patients of groups 1 and 2.

Conclusions: ED is established in patients with ACS, which is more pronounced in NAFLD. Biomarkers such as ET-1, WwF, P-selectin in the blood serum are highly specific substances for determining the severity of ED in patients with ACS, especially when it is combined with NAFLD.

KEY WORDS: Acute coronary syndrome; nonalcoholic fatty liver disease; diagnostics; endothelial dysfunction, endothelin-1, P-selectin, von Willebrand factor

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) - the most common chronic liver disease in worldwide, affecting up to ~30% of adults in the general population, up to ~70% of patients with type 2 diabetes and almost all patients with severe obesity [1]. The pathogenesis of NAFLD includes a profound disturbance of metabolic homeostasis; reprogramming of the interaction between hepatocytes, sinusoidal endothelial cells and hepatic stellate cells; restructuring of the liver immune landscape; remodelling of the hepatic microvasculature and stromal microenvironment. The key effect of constant metabolic stress on the liver is the activation of hepatic stellate cells and the development of liver fibrosis, which usually dictates the natural course of NAFLD [2, 3].

Patients with NAFLD often suffer from obesity and/or insulin resistance and type 2 diabetes mellitus, dyslipidaemia, which are metabolic factors that complicate its course [4, 5, 6]. Namely, obesity is a key link in the chain of future metabolic disorders, in particular NAFLD, and hyperinsulinaemia leads to an increase in adipose tissue in the liver and contributes to the development of NAFLD [7].

When common diseases coexist and share common risk factors, it can be difficult to disentangle cause and effect relationships and understand the role of potential complicating factors. Metabolic syndrome (MS) is common in patients with NAFLD; however, components of MS, including obesity and hypertension, also increase the risk of developing NAFLD [8]. Non-alcoholic fatty liver disease and coronary heart disease share common pathogenetic links. Evidence of the association of NAFLD with acute coronary syndromes (ACS), complex multivessel coronary artery disease and increased mortality risk in patients with ACS is still under investigation [9]. Therefore, the study of pathogenetic links, including biomarkers of endothelial dysfunction (ED) in ACS and NAFLD, is an urgent medical issue.

AIM

The aim of the stady to determine the severity of endothelial dysfunction in patients with acute coronary syndrome in non-alcoholic fatty liver disease.

MATERIALS AND METHODS

124 patients with ACS with ST-segment elevation were examined and treated at the clinical base of Hospital therapy of the Medical Faculty of the State Higher Educational Institution «Uzhhorod National University» (Municipal Non-Profit Enterprise «Transcarpathian Regional Clinical Centre of Cardiology and Cardiac Surgery» of the Transcarpathian Regional Council) in 2019-2024. The study was conducted at the stage of outpatient observation of patients after ACS (on average up to 4.4±0.7 months). All patients who survived ST-segment elevation STEMI and were included in this study underwent stenting or coronary artery bypass grafting during inpatient treatment. After discharge, patients were prescribed individually tailored drug therapy aimed at normalising blood pressure, rhythm and conduction disorders, treating/preventing the progression of chronic heart failure, and including anticoagulants and statins.

The diagnosis of ST-segment elevation ACS was made in accordance with the unified clinical protocol for emergency, primary, secondary (specialised), tertiary (highly specialised) medical care and cardiac rehabilitation, as well as the evidence-based clinical guideline «Acute coronary syndrome with ST-segment elevation» (Order of the Ministry of Health of Ukraine No. 1936 of 14.09.2021). Patients underwent electrocardiographic examination (ECG), echocardiography and CT-coronary angiography, as well as biomarkers of myocardial infarction (troponin levels in the blood).

The average age of patients after ACS was 53.1±7.2 years. Men prevailed among the examined patients, namely 94 (75.8%), and women were 30 (24.2%). The control group consisted of 20 practically healthy individuals (16 (80.0%) men and 4 (20.0%) women). The average age was 51.1±7.4 years.

All studies were conducted in compliance with the basic provisions of the «Rules for Ethical Principles for Scientific Medical Research Involving Human Subjects» with the consent of the patients (all patients gave written consent to the relevant diagnostic and treatment measures), and the methodology was in line with the Helsinki Declaration of Human Rights (1964-2013), the Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation.

Patients after ACS were divided into two groups. Group 1 included 74 patients after ACS and NAFLD, and group 2 consisted of 50 patients after ACS without liver damage (no history of NAFLD). All examined patients with ACS and NAFLD were subjected to general clinical, anthropometric, instrumental and laboratory methods. All examined patients was carried out an ultrasound examination of the abdominal cavity according to the generally accepted method. The anthropometric examination included height, weight and body mass index (BMI).

Standard general and biochemical tests were performed in the blood serum to determine the functional state of the liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT)), lipid metabolism (total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL)), carbohydrate metabolism (glucose, insulin, glycated haemoglobin (HbA1c, %)).

NAFLD diagnosed in according with the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine of 06.11.2014, No. 826) and the EASL-EASD-EASO guidelines for the diagnosis and treatment of NAFLD [10]. The degree of liver damage was calculated using surrogate markers of fibrosis using online calculators, namely NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), Fibrotest. The patients also underwent liver elastometry.

The levels of von Willebrand factor (WwF), apolipoprotein, insulin, C-peptide, glycosylated haemoglobin (HbA1c) were determined by chromogenic analysis (Sysmex 500 and 560, Japan) using Siemens reagents. In the blood serum, endothelin-1 (ET-1) was measured by ELISA using test kits from Biomedica (Austria), P-selectin using a test kit from eBioscience (Austria).

The state of vascular endothelium and its dysfunction were determined by the method of D.Celermajer, assessing endothelium-dependent vasolilatation (EDV) in the brachial artery (BA). Endothelium-independent vasodilation (EIVD) was determined after 0.5 mg of nitroglycerin sublingually at rest, causing dilatation of peripheral vessels. The study was repeated 2 and 5 minutes after nitroglycerin administration. The difference between the diameter of the PA in the background of reactive hyperaemia and the initial diameter was calculated to assess the response to increased blood flow and the difference between the diameter of the PA 2 min after nitroglycerin administration and its initial diameter. An increase in the diameter of the PA against the background of reactive hyperaemia by 10.0% or more was considered normal.

The criteria for exclusion of patients from the study were: Type 1 diabetes mellitus, type 2 diabetes mellitus (severe - with severe manifestations of diabetic angioneuropathy), chronic hepatitis of alcoholic, viral (hepatitis B, C, D virus) etiologies, autoimmune hepatitis, Wilson-Conovalov disease, haemochromatosis.

Table 1. Indicators of the functional state of the liver in the blood serum of the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
ALT, U/l	20.4±0.8	128.7±1.8 **,++	42.3±2.6 *
AST, U/	18.6±1.1	109.2±1.6 **, ++	38.7±1.7 *
TB, mmol/l	12.5±0.7	30.1±1.0 *	20.1±0.6
ГГТ, U/l	38.9±2.4	82.4±2.1 **, +	42.7±1.2

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 2. Indicators of lipid metabolism in blood serum in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
TG, mmol/l	1.12±0.07	3.48±0.12 **,++	1.92±0,27 *
TC, mmol/l	4.56±0.44	7.21±0.24 **,+	5.96±0,26 *
LDL, mmol/l	1.70±.,21	3.28±0.26 **,+	2.30±0,24 *
VLDL, mmol/l	0.56±0.09	1.84±0.07 **,++	0.98±0,14 *
HDL, mmol/l	1.84±0.09	1.04±0.08 **,+	1.50±0,11 *

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 3. Indicators of carbohydrate metabolism in blood serum in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
Glucose, mmol/l	4.88±0.17	6.92±0.16 *	6.42±0,16 *
HbA1c, %	4.32±0.36	6.76±0.28 *	6.07±0,38 *
Insulin, U/l	8.44±0.21	22.38±0.77 **,+	14.23±0,77 *
C-peptide, ng/ml	4.12±0.18	10.92±0.51 **,+	7.12±0,21 *
HOMA-IR	1.69±0.27	8.84±0.24 ***,++	3.95±0,26 **

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - p<0.05; ** - p<0.01; between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - p<0.05; ++ - p<0.01.

The analysis and processing of the results of examining the patients was performed using the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the received results.

RESULTS

In patients after ACS, at the stage of outpatient follow-up, indicators of the functional state of the liver were determined (Table 1).

In patients of group I, a significant increase in ALT and AST activity was detected compared with patients of group 2 (3.0 and 2.8 times, respectively - p<0.01). In group 1 of patients after ACS in combination with NAFLD, a significant increase in serum levels of TB and GGT was diagnosed.

The lipid metabolism in the blood serum in both groups of patients was evaluated - Table 2.

There was a statistically significant increase in all lipid metabolism parameters in the blood serum in patients after ACS of both groups. However, it should be noted that in patients of group I (ACS in combination with NAFLD), the levels of TG, VLDL and LDL were 1.8 (p<0.01), 1.2 (p<0.05), 1.4 (p<0.05) and 1.9 (p<0.01) times higher than in patients of group 2.

Indicators of carbohydrate metabolism in patients after ACS were evaluated (Table 3). A statistically significant increase in the levels of glucose, insulin, C-peptide, and HOMA-IR index in patients of group I was found compared with those in patients of group 2.

Patients of group I (combination of ACS and NAFLD) had metabolic disorders in the body, which was manifested by a significant increase in carbohydrate and lipid metabolism in these patients.

In our patients with ACS and NAFLD, we determined the indicators of endothelial dysfunction (ED), which are presented in Table 4.

Table 4. Laboratory and instrumental parameters of endothelial dysfunction in the subjects

Indicator	Control group (n=20)	Examined patients	
		Group 1 (n=74)	Group 2 (n=50)
Diameter of BA at the beginning of the study, mm	4.38±0.06	3.52±0.07 *+	3.87±0.05
Diameter of BA for 30 sec. of reactive hyperaemia, mm	5.52±0.08	4.26±0.04 **+	4.50±0,06 *
Diameter of BA for 60 sec. of reactive hyperaemia, mm	4.78±0.05	3.81±0.05 **+	4.03±0,06 *
Blood flow rate through the BA, sm/sec.	102.26±2.14	65.12.±1.49 **+	84.12±1,71 *
EDV,%	14.59±0.65	8.06±0.21 *+	9.26±0,63
EIVD,%	25.12±0.73	14.23±0.29 *+	16.21±0,55
ET-1, fmol/ml	0.41±0.06	1.45±0.09 ***+	1.07±0,08 **,+
P-selectin, ng/ml	109.32±3.11	467.70±4.23 **+	341.12±3.91 **
wWF, %	53.7±2.5	193.7±3.2 *+	123.8±1,7**+

Note: the difference of indicators between of the control group and the examined patients of groups 1 and 2 is statistically significant: * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$;
between the indicators of patients of groups 1 and 2 the difference is statistically significant: + - $p<0.05$.

The reactive hyperaemia test revealed endothelial dysfunction in patients with ACS regardless of liver damage. However, more pronounced changes in EDV and EIVD were found in patients with ACS in combination with NAFLD. Laboratory markers of ED, namely ET-1, P-selectin and wWF, were also significantly higher in patients with ACS and confirm the pronounced vasoconstrictor effect of endothelial dysregulation in these patients. A significant difference was found between the levels of ET-1, P-selectin and wWF in patients of groups 1 and 2.

DISCUSSION

Cardiovascular disease is the leading cause of death worldwide. Coronary artery disease (CAD) is the most common and is characterized by the accumulation of lipids and immune cells in the subendothelial space of the coronary arteries or atherosclerosis. This process involves the inflammatory response of the vascular endothelium. Endothelial cells (EC) form a semipermeable monolayer that separates the wall of the arteries from the components of intravascular flow. This barrier regulates vascular tone, prevents platelet aggregation, and maintains fluid homeostasis. The endothelium produces vasodilator and vasoconstrictor molecules such as nitric oxide and endothelin, respectively; the imbalance in production of these vasoactive substances results in the loss of its function, which is defined as endothelial dysfunction. Endothelial dysfunction plays an essential role in the development of atherosclerosis and can be triggered and exacerbated by different cardiovascular and cardiometabolic risk factors. Currently, there is a wealth of data on endothelial dysfunction and the risk of developing atherosclerosis and CAD [11].

In resistance arteries, the endothelium plays a fundamental role in the regulation of vascular tone, local blood flow and systemic blood pressure via the generation of various vasoactive stimuli. This monolayer operates to sense, integrate and transduce signals present in the blood and local tissue environment, which then initiate dynamic modulation of contractile activity of the surrounding vascular smooth muscle. In response to mechanical (e.g., shear stress due to blood flow) and chemical (e.g., acetylcholine, bradykinin, ATP) stimuli, EC release vasodilatory factors that regulate the vascular tone. The main vasoconstrictors produced by the endothelium are thromboxane A2 and ET-1, while the main endothelial vasodilator factors are NO, prostacyclin, and endothelium-derived hyperpolarization factor (EDHF) [12].

There is growing evidence of a direct link between the vascular system and NAFLD. Changes in endocannabinoids and adhesion molecules, such as P-selectin, derived from the endothelium and platelets, in children and adolescents with obesity and NAFLD were investigated. It was found that childhood obesity leads to vascular inflammation and, therefore, may contribute to the development of atherosclerosis at an early age. [13]. P-selectin is an adhesion molecule translocated to the surface of endothelial cells and platelets under inflammatory stimuli [14].

Thus, the study of changes in the levels of ED biomarkers may reveal new potentiated links between patients with cardiovascular disease and NAFLD.

CONCLUSIONS

1. Endothelial dysfunction is established in patients with ACS, which is more pronounced in NAFLD.
2. Biomarkers such as ET-1, WwF, P-selectin in the blood serum are highly specific substances for determining the severity of ED in patients with ACS, especially when it is combined with NAFLD.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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