THE ROLE OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 IN THE DEVELOPMENT OF ENDOTHELIAL DISFUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND TYPE 2 DIABETES MELLITUS*

D. V. Minukhina¹, V. D. Babadzhan¹, P. G. Kravchun¹, I. P. Dunaeva², D. V. Minukhin¹, A. G. Krasnoyarskiy¹, D. A. Yevtushenko¹

¹ Kharkiv National Medical University, Kharkiv, Ukraine;
² Kharkiv Postgraduate Medical Academy, Kharkiv, Ukraine
minukhinadv@ukr.net

According to the World Health Organization report published in 2013, cardiovascular diseases are the main cause of population disability worldwide [1]. Currently, about 422 million people suffer from diabetes mellitus, by 2040 this number will increase to 642 million [2].

Coexistence of cardiovascular diseases and metabolic disorders, namely type 2 diabetes mellitus (type 2 DM), is one of the most commonly occurring comorbidities and is the main cause of death at an earlier stage of the disease [3, 4]. Hemostatic impairments [5], along with metabolic disorders, occupy key positions in the development of cardiovascular pathology in type 2 DM. Assessment of mortality structure provides the most objective illustration of this connection: thrombosis is the cause of death in 80 % of patients with type 2 DM, 75 % of which are of coronary localization, 25 % is cerebrovascular and peripheral thrombosis [4].

Plasminogen activator inhibitor type 1 (PAI-1) is a member of the superfamily of serine protease inhibitors and the major inhibitor of fibrinolysis in the plasminogen activator system [6, 7]. High PAI-1 concentration plays a key role in the pathogenesis of arterial and venous thrombosis and, therefore, contributes to the development of thrombotic events [8, 9].

In addition, a higher PAI-1 level is associated with insulin resistance, diabetes, hyperlipidemia and premature aging [10, 11].

Patients with type 2 DM are commonly found to have an increase in the content of proinflammatory agents (C-reactive protein (CRP),...
TNF-alpha, interleukin-6), which are considered to be predictors of cardiovascular risk [12, 13].

In recent years, a new mechanism has been discussed in the literature, which explains the variety of aspects of endothelial properties alteration in type 2 DM by the development of endothelial dysfunction [14, 15].

The high risk of vascular catastrophes is a motive for the study of pathogenic mechanisms of thrombotic formation in type 2 DM. Meanwhile, changes in hemostasis associated with metabolic changes revealing the nature of these relationships are of indisputable practical interest.

The study implied the assessment of serum PAI-1 levels in patients with AMI with and without type 2 DM to identify the clinical value of PAI-1 as a potential biomarker in type 2 DM.

**MATERIALS AND METHODS**

The study involved examination of 130 patients, among them 44 (42.6 %) women and 86 (57.4 %) men who underwent inpatient treatment at the myocardial infarction department of Kharkiv City Clinical Hospital № 27 (clinical center of the Department of Internal Medicine № 2 and Clinical Immunology and Allergology of Kharkiv National Medical University of the Ministry of Health of Ukraine) and at the cardiology department of Kharkiv Regional Clinical Hospital «Center for Emergency and Catastrophe Medicine».

All the patients were divided into groups: the main one included 73 patients with AMI with accompanying 2 type DM (among them 43 men and 30 women, average age 62.73 ± 1.39 years); group of comparison comprised 57 patients with AMI without type 2 DM (43 men and 14 women, average age 63.98 ± 1.47); control group with 20 practically healthy individuals (among them 10 men and 10 women, mean age 60.85 ± 1.37).

AMI was diagnosed in accordance with the Order of the Ministry of Health of Ukraine No. 455 as of 02.07.2014 «Unified clinical protocol of emergency, primary, secondary (specialized) and tertiary (highly specialized) medical care and medical rehabilitation of patients with acute coronary syndrome with segment ST elevation, based on clinical, electrocardiographic and biochemical criteria» [16].

Duration of type 2 DM in patients with AMI was from one to 30 years. Type 2 DM was diagnosed according to the Order of the Ministry of Health of Ukraine № 1118 as of 21.12.2012. The exclusion criteria were rheumatologic, oncological diseases, diffuse connective tissue disorders, pituitary and hypothalamic diseases, thyroid gland diseases, and symptomatic hypertension.

Plasminogen activator inhibitor type 1 (PAI-1, IAP-1) was determined by the immunoassay method using a commercial test system manufactured by Technoclone PAI-1 ELISA Kit (Austria) on the first day of myocardial infarction. The level of NO synthase was determined by the immune enzyme method using a commercial test system manufactured by Bender MedSystem (Austria) on the first day of myocardial infarction. Biochemical study included determination of the level of total cholesterol (TC) and high-density lipoprotein (HDL), low (LDL) and very low density lipoprotein (VLDL), and was carried out by peroxidase method using «Cholesterol Liquicolor» reagents manufactured by «Human» (Germany) in blood serum stabilized by heparin. The level of triglycerides (TG) was determined by enzymatic colorimetric method using «Triglycerides GPO» reagent kit manufactured by «Human» (Germany). Determination of the level of glycosylated hemoglobin was carried out by high-speed chromatography using the automatic analyzer Adams A1c according to the generally accepted method. Determination of blood insulin levels was performed using a commercial test system manufactured by DRG Instruments GmbH (Germany).

The design of the study was agreed with the Ethics Committee of Kharkiv National Medical University. All the patients involved in the research signed a voluntary informed consent to participate in the study.

Statistical computer processing of the results was performed using the Microsoft Office Excel 2003 and «Statistica 10.0» (StatSoft Inc., USA) software. Comparative analysis of the samples implied calculation of the arithmetic mean and statistical error of the mean arithmetic (M ± m). Differences between groups at
distribution close to normal were estimated using the Student’s t-criterion. Differences were considered statistically significant at \( p < 0.05 \).

The difference in frequencies in two independent samples was analyzed using the Pearson criterion.

RESULTS AND THEIR DISCUSSION

The group of patients with AMI and type 2 DM was found to have an increase in the average PAI-1 level as compared to the group of patients with AMI without type 2 DM (68.85 [72.23; 12.19] and 53.1 [69.47; 21.61] ng/ml, respectively, \( p < 0.05 \)) and the control group (17.51 [27.05; 12.81] ng/ml, \( p < 0.05 \)) (Fig. 1).

Patients with AMI and type 2 DM were shown to have a significant (\( p < 0.05 \)) increase in lipid metabolism rates due to elevated levels of total cholesterol (TC), low density lipoprotein cholesterol (LDLC), very low density lipoprotein cholesterol (VLDLC) and triglycerides (TG), which was accompanied by a decrease in high density lipoprotein cholesterol (HDLC) as compared to control group patients (\( p < 0.05 \)). Moreover, there was a statistically significant increase in carbohydrate metabolism indices due to an increase in fasting glucose, glycated hemoglobin and insulin as compared to the control group (\( p < 0.05 \)) (Table).

Concerning hemostasis, namely, fibrinogen and Quick’s prothrombin, endothelial dysfunction indices, particularly PAI-1 and NOS, and proinflammatory factor, C-reactive protein, there was a statistically significant increase in these parameters in patients with acute myocardial infarction and type 2 diabetes mellitus as compared to the control group (\( p < 0.05 \)).

Comparison of carbohydrate, lipid metabolism, hemostasis and endothelial dysfunction indices in patients with AMI with type 2 DM showed a statistically significant increase in glucose, glycated hemoglobin, insulin, atherogenic lipid fractions, PAI-1 and CRP, and decreased LDLC and NOS as compared to the group without type 2 DM (\( p < 0.05 \)).

Assessment of PAI-1 content on the first day in patients with AMI in the presence or absence of type 2 DM showed that PAI-1 levels in patients with AMI and type 2 DM increased by 70.5 % and by 36.5 % in patients without type 2
Correlation analysis showed a reverse relationship between the level of NOS and PAI-1 ($r = -0.79; p < 0.05$) on the first day of AMI in patients with concomitant type 2 diabetes mellitus (Fig. 2).

Consequently, patients with type AMI and type 2 DM were found to have an increase in PAI-1 levels and a decrease in NOS on the first day of AMI, which suggests that PAI-1 is a risk factor associated with diseases accompanied by the development of endothelial dysfunction.

Type 2 DM was associated with an increase in carbohydrate metabolism indices and atherogenic fractions of the lipid spectrum due to TC, LDLC, VLDLC, and TG in patients with AMI in proportion to the high content of PAI-1. An increase in hemostasis rates due to the increase in fibrinogen and Quick’s prothrombin and inflammation rates, namely CRP, was increased proportionally in the presence of concomitant type 2 diabetes mellitus, which confirms its inevitable effects and increased endothelial dysfunction, inflammation and, as

#### Table

<table>
<thead>
<tr>
<th>Index</th>
<th>Patients with AMI</th>
<th>Control group, n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With type 2 DM, n=73</td>
<td>Without type 2 DM, n=57</td>
</tr>
<tr>
<td>Fibrinogen, g/l</td>
<td>4.81 ± 0.06*</td>
<td>4.61 ± 0.10**</td>
</tr>
<tr>
<td>Quick’s prothrombin, %</td>
<td>91.32 ± 0.87*</td>
<td>70.79 ± 2.23**</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>6.38 ± 0.14*</td>
<td>5.05 ± 0.10**</td>
</tr>
<tr>
<td>LDLC, mmol/l</td>
<td>4.44 ± 0.12*</td>
<td>3.27 ± 0.06**</td>
</tr>
<tr>
<td>VLDLC, mmol/l</td>
<td>1.82 ± 0.08*</td>
<td>0.98 ± 0.04**</td>
</tr>
<tr>
<td>HDLC, mmol/l</td>
<td>1.08 ± 0.04*</td>
<td>1.41 ± 0.03**</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>2.68 ± 0.10*</td>
<td>1.97 ± 0.08**</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>12.7 ± 0.35*</td>
<td>4.56 ± 0.11**</td>
</tr>
<tr>
<td>Glycated hemoglobin, mcmol fructose/g Hb</td>
<td>11.6 ± 0.30*</td>
<td>5.80 ± 0.13**</td>
</tr>
<tr>
<td>Insulin, mcIU/ml</td>
<td>68.95 ± 1.66*</td>
<td>20.21 ± 0.69**</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>45.7 ± 0.89*</td>
<td>41.33 ± 0.77**</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>63.15 ± 1.48*</td>
<td>51.03 ± 1.72**</td>
</tr>
<tr>
<td>NOS, ng/ml</td>
<td>3.12 ± 0.13*</td>
<td>4.22 ± 0.11**</td>
</tr>
</tbody>
</table>

* is the probability of discrepancies as compared to the control group (p < 0.05);

** is the probability of discrepancies as compared to the group of AMI with type 2 DM.
a consequence, destabilization of atherosclerotic plaques and thrombosis.

Reduction of NOS in patients with AMI and type 2 DM indicates an increase in endothelial dysfunction in patients with ischemic myocardial damage.

Patients with AMI and type 2 DM were shown to have strong reverse correlation between PAI-1 and NOS, which confirms the involvement of this marker in the pathogenesis of endothelial dysfunction and suggests that it is a potential marker of severe course and development of possible thrombotic complications in this cohort of patients.

**CONCLUSIONS**

1. Patients with acute myocardial infarction and type 2 diabetes mellitus were found to have a significant increase in the levels of plasminogen activator inhibitor type 1 (63.15 ± 1.48 ng/ml) and the reduction of endothelial nitric oxide synthase NOS (3.12 ± 0.13 ng/ml) as compared to patients with acute myocardial infarction without concomitant type 2 diabetes mellitus (51.03 ± 1.72 ng/ml and 4.22 ± 0.11 ng/ml, respectively) and the control group (18.64 ± 1.05 ng/ml and 5.21 ± 0.19 ng/ml, respectively) (p < 0.05).

2. Levels of plasminogen activator inhibitor type 1 have direct correlation with lipid and carbohydrate metabolism, namely, cholesterol and its atherogenic fractions: total cholesterol (r = 0.72), low density lipoprotein cholesterol (r = 0.49), very low density cholesterol (r = 0.61), triglycerides (r = 0.39), glucose (r = 0.73), glycated hemoglobin (r = 0.65) and insulin (r = 0.83) (p < 0.05).

3. Indices of plasminogen activator inhibitor type 1 content reversely correlate with the indices of endothelial nitric oxide synthase — NOS (r = −0.79) (p < 0.05), which suggests...
that PAI-1 can be considered a marker of endothelial dysfunction in patients with acute myocardial infarction and concomitant type 2 diabetes mellitus.

4. The effect of type 2 diabetes mellitus on elevated levels of CRP, PAI-1 and hemostasis factors such as fibrinogen and Quick’s prothrombin in blood serum of patients with acute myocardial infarction is most pronounced in comparison with other indices, which confirms the fact that type 2 diabetes mellitus is one of the most important risk factors for atherothrombosis and, as a consequence, acute myocardial infarction.

5. The obtained findings suggest that plasminogen activator inhibitor type 1 is directly involved in the pathogenesis of type 2 diabetes mellitus. In practically healthy individuals, the risk of developing type 2 diabetes mellitus is associated with increased levels of plasminogen activator inhibitor type 1, fibrinogen, and CRP in serum.

REFERENCES


Acute myocardial infarction continues to occupy one of the leading places among the causes of mortality. Some indicators of endothelial dysfunction are also associated with a high risk of cardiovascular complications, which play a key role in the pathogenesis of cardiovascular complications, which makes it relevant to study the relationship between endothelial dysfunction markers and the development and progression of atherosclerotic vascular lesions. Dysfunction of the endothelial vasodilator system is accompanied by a decrease in the level of nitric oxide (NO), which is a characteristic feature of patients who are at risk of coronary artery disease, so coronary vasodilator dysfunction may involve prolonged progression of atherosclerosis and the frequency of cardiovascular events.

The high risk of vascular accidents is an incentive to study the pathogenetic mechanisms of thrombotic formation in type 2 diabetes. Plasminogen activator inhibitor type 1, which is a member of the superfamily of serine protease inhibitors and the main inhibitor of fibrinolysis in the plasminogen activator system, plays a key role in the pathogenesis of arterial and venous thrombosis and, therefore, contributes to the occurrence of thrombotic events.

The combination of acute myocardial infarction with type 2 diabetes mellitus is characterized by a significant increase in the content of the plasminogen activator inhibitor type 1 in serum compared with those without diabetes. As a result of the study, a close direct correlation between the increase of lipid, carbohydrate metabolism, hemostasis parameters proportional to the high content of the plasminogen activator inhibitor type 1 in serum of patients with acute myocardial infarction with concomitant type 2 diabetes mellitus was proved.

The inverse correlation between the level of type 1 plasminogen activator inhibitor and the reduced content of nitric oxide synthase, which increases endothelial dysfunction and atherothrombosis in patients with acute myocardial infarction with concomitant type 2 diabetes mellitus, is proved.

**Key words:** acute myocardial infarction, type 2 diabetes mellitus, plasminogen activator inhibitor type 1, NO synthase, endothelial dysfunction, carbohydrate and lipid metabolism parameters, coagulation parameters.
інгібітора активатора плазміногена 1 типу та зниженням вмісту синтази оксиду азоту, що посиллює ендотеліальну дисфункцію та процеси атеротромбозу у хворих на гострій інфаркт міокарда з супутнім цукровим діабетом 2 типу.

Ключові слова: гострий інфаркт міокарда, цукровий діабет 2 типу, інгібітор активатора плазміногену 1 типу, ендотеліальна дисфункція, показники углеводного та ліпідного обмінів, показники гемостазу.

РОЛЬ ИНГИБИТОРА АКТИВАТОРА ПЛАЗМИНОГЕНА 1 ТИПА В РАЗВИТИИ ЭНДОТЕЛІАЛЬНОЙ ДИСФУНКЦИИ У БОЛЬНЫХ ОСТРЫМ ИНФАРКТОМ МИОКАРДА И САХАРНЫМ ДИАБЕТОМ 2 ТИПА

Минухин Д. В., Бадаев В. Д., Кравчук П. Г., Дунаева И. П., Минухин Д. В., Краснояружский А. Г., Евтушенко Д. А.

1 Харьковский национальный медицинский университет, Харьков, Украина
2 Харьковская медицинская академия последипломного образования, г. Харьков, Украина

minukhinadv@ukr.net

Острый инфаркт миокарда продолжает занимать одно из ведущих мест среди причин смертности населения. С высоким риском сердечно-сосудистых осложнений ассоциируются некоторые показатели эндотелиальной дисфункции, которым принадлежит ключевая роль в патогенезе развития сердечно-сосудистых осложнений, что обусловливает актуальность изучения взаимосвязи маркеров дисфункции эндотелия с развитием и прогрессированием атеросклеротического поражения сосудов. Дисфункция эндотелиальной сосудорасширяющей системы сопровождается снижением уровня оксида азота (NO), что является характерной чертой пациентов, находящихся под угрозой поражения коронарных артерий, поэтому коронарная вазодилататорная дисфункция может предусматривать длительное прогрессирование атеросклероза и частоту сердечно-сосудистых событий.

Высокий риск сосудистых катастроф является побудительным мотивом изучения патогенетических механизмов тромбообразования при сахарном диабете 2 типа. Ингибитор активатора плазминогена 1 типа, который является членом суперсемейства ингибиторов сериновых протеаз и основного ингибитора фибринолиза в системе активатора плазминогена, играет ключевую роль в патогенезе артериального и венозного тромбоза и, следовательно, способствует возникновению тромботических событий.

Сочетание острого инфаркта миокарда с сахарным диабетом 2 типа характеризуется значимым повышением содержания ингибитора активатора плазминогена 1 типа в сыворотке крови больными без диабета. В результате проведенного исследования доказана тесная прямая корреляционная связь между увеличением параметров липидного, углеводного обмена, показателями гемостаза пропорционально высокому содержанию ингибитора активатора плазминогена 1 типа в сыворотке крови больных острым инфарктом миокарда с сопутствующим сахарным диабетом 2 типа. Доказана обратная корреляционная зависимость между уровнем ингибитора активатора плазминогена 1 типа и пониженным содержанием синтазы оксидазы азота, что усиливает эндотелиальную дисфункцию и процессы атеротромбоза у больных острым инфарктом миокарда с сопутствующим сахарным диабетом 2 типа.

Ключевые слова: острый инфаркт миокарда, сахарный диабет 2 типа, ингибитор активатора плазминогена 1 типа, эндотелиальная дисфункция, показатели углеводного и липидного обменов, показатели гемостаза.