CARBOHYDRATE EXCHANGE IMPACT ON EXTRACELLULAR MATRIX IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND TYPE 2 DIABETES MELLITUS*

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Type 2 diabetes mellitus (DM) is a significant factor that determines the unfavorable course of acute myocardial infarction (AMI) [1]. OASIS (Organization to assess strategies for ischemic syndromes) research showed that hospital mortality of patients with type 2 DM from cardiovascular complications was 2.9 % versus 2.0 % in patients without type 2 DM, respectively ($r < 0.033$) [2]. According to SHOCK (Should we emergently revascularize occluded coronaries for cardiogenic shock?) Trial register hospital mortality in AMI patients with type 2 DM was higher and amounted to 67 % compared to AMI patients without type 2 DM with 58 % [1]. Thus, mortality rate of AMI patients with type 2 diabetes mellitus was shown to increase, which may be due to the severity of acute myocardial infarction with concomitant abnormalities.

It is commonly known that in health the energy, necessary to myocardial cells develops due to metabolism of free fatty acids and glucose. Myocardial ischemia results in an increased demand for energy that is derived from glucose. However, in patients with diabetes mellitus this mechanism is impaired. This is due to the depletion of glucose transporters GLUT-1 and GLUT-4, causing oxidation of free fatty acids and dyslipidemia, namely an increase in the level of triglycerides; low density lipoproteins and a reduction in high density lipoproteins in patients with type 2 DM [3].

Atherosclerosis is considered to be one of the factors of AMI development in patients with type 2 DM, as it causes occlusion of coronary arteries. Metabolic disorders (chronic hyperglycemia, dyslipidemia and insulin-resistance) in patients with type 2 DM results in the involvement of the arterial wall. Endothelial cells of vascular endothelium synthesize nitric oxide, acting as a vasodilator. It inhibits platelets synthesis, limits inflammation by reducing adhesion and migration of leukocytes to the endothelium and reduces proliferation of smooth muscle cells. Patients with type 2 DM were shown to have inhibited endothelium-dependent (NO-mediated) vasodilation. There

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are several mechanisms that reduce the production of nitric oxide by vascular endothelium. First, hyperglycemia blocks the activation of endothelial NO-synthase (eNOS) and increases the production of reactive oxygen intermediates (particularly superoxide-anion O2-) in smooth muscle cells of vascular walls and endothelial cells, which reduces the production of nitric oxide [4]. Superoxide-anion directly inactivates nitric oxide, combining with it and forming toxic peroxynitrite ion (ONOO-), which disrupts eNOS, oxidizing its cofactor with tetrahydrobioptherine. Secondly, insulin resistance results in an excessive release of free fatty acids from adipose tissue, activating the signal enzyme protein kinase-C, inhibiting the activity of phosphatidylinositol-3-kinase and stimulating the production of reactive oxygen species, all of which impairs the production of nitric oxide or decreases the activity of existing nitric oxide molecules [4].

Nitric oxide [5] was established to be involved in the regulation of lipid peroxidation, namely inhibiting the development of radical oxidation reactions by binding to divalent iron ions, which are part of heme and inhibiting decomposition of peroxides. Hyperproduction of reactive oxygen intermediates causes the destruction of nitric oxide and promotes the formation of oxidized low density lipoproteins. An increase in the rate of reactive oxygen intermediates leads to oxidative stress and as a consequence, disruption of endothelium structure.

It could be explained by the impact of oxidative stress on the state of intercellular matrix, which has such markers as matrix metalloproteinases, tissue inhibitors of metalloproteinases, tenascin C, but this process is still poorly understood. Atherosclerotic plaques are known to undergo binding of oxidized lipoproteins to the extracellular matrix at early stages of their formation. Modified lipoproteins induce the production of chemokines and cytokines which stimulate the migration of leukocytes into the intima, secretion of matrix metalloproteinases by macrophages and smooth muscle cells, causing local inflammation. Increased content of metalloproteinases results in the destruction of intima collagen and inner basal membrane. Thus, atherosclerotic plaque becomes unstable and can cause coronary artery occlusion [5].

On the one hand, tenascin C stimulates metalloproteinases synthesis in patients with AMI and is a predictor of cardiovascular complications, namely myocardial rupture. On the other hand, it prevents the development of acute aneurysm after myocardial infarction. Up to this day the impact of tenascin C in patients with AMI remains disputable. Tissue inhibitors of metalloproteinases take part in preventing excessive activation of metalloproteinases. A balance between the activity of metalloproteinases and their inhibitors is essential for normal reorganization of extracellular matrix [6, 7]. Consequently, intercellular matrix activity may be considered to be an additional factor when evaluating atherosclerotic progression.

The purpose of the research is to study the state of extracellular matrix in patients with acute myocardial infarction depending on the presence and absence of type 2 diabetes mellitus in relation to matrix metalloproteinase-13, tissue inhibitor of metalloproteinase-4, tenascin C and to analyze the relationship between extracellular matrix components and carbohydrate exchange indices (blood glucose and glycosylated hemoglobin).

MATERIALS AND METHODS

The study involved examination of 120 patients, including 46 women (42.6 %) and 74 men (57.4 %) who underwent out-patient treatment at myocardial infarction department of Kharkiv City Clinical Hospital № 27 (clinical center of the Department of Internal Medicine № 2, Clinical Immunology and Allergology of Kharkiv National Medical University, Ministry of Health of Ukraine) and in the first Cardiology department of the Central Clinical Hospital «Ukrzaliznytsia». All patients were divided into the following groups: trial group of 60 patients with AMI and concomitant type 2 diabetes mellitus (among them 31 men,

29 women, aged from 45 to 88); comparison group — 40 patients with AMI but without type 2 diabetes mellitus (32 men and 8 women aged from 45 to 75); control group — 20 patients (including 11 men and 9 women, aged from 22 to 27). The control group involved substantially healthy subjects.

Diagnosis of acute myocardial infarction was established according to the Order № 455 issued by the Ministry of Health on 02.07.2014, «Unified clinical protocol of emergency, primary, secondary (specialized) and tertiary (highly specialized) health care and medical rehabilitation of patients with acute coronary syndrome and elevation of ST segment, basing on clinical, biochemical and electrocardiographic criteria»; Order № 436 issued by the Ministry of Health on 03.07.2006, «Protocol of providing medical care to patients with acute coronary syndrome without ST segment elevation».

Duration of type 2 diabetes mellitus amounted for one to thirty years. The diagnosis of type 2 diabetes mellitus was established in accordance with recommendations of American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD).

Exclusion criteria included rheumatologic diseases, cancer, diffuse diseases of connective tissue, diseases of the pituitary gland and hypothalamus, thyroid diseases, symptomatic hypertension.

Glycosylated hemoglobin (HbA_{1c}) content in whole blood was determined by photometric method with thiobarbituric acid using a commercial test system «Reagent» (Ukraine). Glucose level was determined by glucose oxidase test in capillary fasting blood (normal range: 3.3–5.5 mmol/l).

Matrix metalloproteinase-13 (MMP-13) was determined by enzyme immunoassay using a set of reactants «Human MMP-13» (RayBiotech, Norcross, USA). Tissue inhibitor of metalloproteinase-4 (TIMP-4) was evaluated by enzyme immunoassay using a set of reactants «Human TIMP-4» (R&D Systems, Minneapolis, USA), tenascin C — by enzyme immunoassay using a set of reactants «Human Tenascin-C Large (FNIII-C)» (Immuno-Biological Laboratories Co. Ltd. (IBL), Takasaki-Shi, Japan), troponin I — by enzyme immunoassay using a set of reactants «Troponin I» (KHEMA, Moscow, Russia).

Study design was approved by the Ethics Committee of Kharkiv National Medical University. All the patients, involved in the study, voluntarily signed Patient Informed Consent.

Obesity was characterized by body mass index (BMI) (Quetelet index), which was calculated using the formula:

$$\frac{\text{Body mass, kg}}{\text{Height, m}^2}.$$

Assessment of mortality risk in patients with AMI depending on the presence or absence of type 2 diabetes mellitus was carried out using Grace Scale (Global registry of acute coronary events).

Clinical characteristics of data concerning patients with acute myocardial infarction and concomitant type 2 diabetes mellitus are shown in Table 1.

Statistical processing of the results was performed by computer software Microsoft Office Excel 2003 and Statistica 10.0. Comparative analysis of samples was conducted by calculation of mean arithmetical value and standard deviation. Assessment of differences between the groups at distribution close to normal was carried out using Student's t-test. Differences with $p < 0.05$ were considered statistically significant. Pearson criterion was employed to analyze frequency differences in two independent samples.

RESULTS AND DISCUSSION

Analysis of the survey results showed (Table 2) an increase in glucose content by 117 % and glycosylated hemoglobin by 21 % in patients with AMI and type 2 diabetes mellitus on the 1^{st}–2^{nd} day ($p < 0.05$). In comparison group with AMI patients without type 2 diabetes mellitus the level of the aforementioned indices did not correspond to that of the control group ($p < 0.05$).

The study of MMP-13 content in patients with AMI with or without type 2 diabetes showed differences in comparison with the con-

trol group. There was an increase in MMP-13 in patients with AMI and type 2 diabetes mellitus by 94 % and 48 % without type 2 diabetes mellitus when compared with the control group ($p < 0.05$). TIMP-4 level on the 1^{st}–2^{nd} day was significantly higher in patients with AMI by 19 % with type 2 diabetes mellitus and by 21 % without type 2 diabetes mellitus when compared with the control group ($p < 0.05$). As for tenascin C level, the level of this marker was significantly increased in patients with AMI without type 2 diabetes mellitus by 34 % when compared with the control group ($p < 0.05$). Patients with AMI and type 2 diabetes mellitus were found to have a trend towards an increase in tenascinemia that did not reach the level of probability ($p = 0.07$).

Comparison of carbohydrate metabolism in patients with AMI depending on the presence or absence of type 2 diabetes mellitus showed that in type 2 DM blood glucose and glycosylated hemoglobin increased significantly by 81 % and 23 % respectively ($p < 0.05$). There was a probable increase in MMP-13 concentration in patients with AMI and concomitant type 2 DM by 36 % compared with patients without type 2 DM ($p < 0.05$). There were no probable differences in terms of TIMP-4 and tenascin C when comparing patients with AMI in the presence or absence of type 2 DM ($p < 0.05$). According to literature data the content of MMP-13, TIMP-4 and tenascin C in patients with AMI remains elevated for 1–7 days, then

Table 1

Clinical characteristics of data concerning patients with acute myocardial infarction depending on the presence or absence of type 2 diabetes mellitus, ($\overline{X} \pm S_{\overline{X}}$), *n*, %

Indices	AIM with type 2 DM	AIM without type 2 DM
Sex:		
Men	31 (52)	32 (80)
Women	29 (48)	8 (20)
Average age, years	64.3 ± 3.5	61.1 ± 4.3
Risk factors:		
AH	37 (62)	15 (37.5)
Smoking	2 (3)	6 (15)
Obesity	24 (40)	14 (35)
revious infarction	16 (27)	2 (5)
Previous strokes	6 (10)	1 (2,5)
Atrial fibrillation	11 (18)	2 (5)
SBP, mm Hg	145.9 ± 7.7	136.3 ± 9.8
DBP, mm Hg	84.0 ± 4.3	82.1 ± 5.7
Heart rate, bpm	82.6 ± 4.6	77.3 ± 5.6
BMI, kg/m^2	29.3 ± 1.4	29.4 ± 2.0
MI with Q wave	24 (40)	17 (42)
MI without Q wave	36 (60)	23 (57)
Anterior MI	39 (65)	26 (65)
Posterior MI	21 (35)	14 (35)
Grace scale at hospital stage, %	60 (3.9)	40 (2.7)
Grace scale in 6 months, %	60 (7.5)	40 (4.9)
Grace scale in 1 year, %	60 (8.4)	40 (5.2)

Note. *n* — number of examined subjects, ($\overline{X} \pm S_{\overline{X}}$) — mean arithmetical value ± standard deviation, AH — arterial hypertension, MI — myocardial infarction, SBP — systolic blood pressure, DBP — diastolic blood pressure, heart rate, bpm — beats per minute, Grace — Global registry of acute coronary events.

Table 2

Indices of carbohydrate exchange, MMP-13, TIMP-4, tenascin C in patients with acute myocardial infarction with or without type 2 diabetes mellitus, ($\overline{X} \pm S_{\overline{X}}$)

Indices	AMI with type 2 DM ($n = 60$ subjects)	AMI without type 2 DM ($n = 40$ subjects)	Control group ($n = 20$ subjects)
Glucose, mmol/l	9.80 ± 0.71*#	5.41 ± 1.04	4.50 ± 0.18
HbA_{1c}, %	5.97 ± 0.11*#	4.83 ± 0.12	4.9 ± 0.11
MMP-13 on the 1^{st}–2^{nd} day, pg/ml	42.6 ± 2.5*#	47.9 ± 3.8*	32.2 ± 2.6
TIMP-4 on the 1^{st}–2^{nd} day, pg/ml	1518 ± 136*	1540 ± 113*	1269 ± 75
Tn C on the 1^{st}–2^{nd} day, ng/ml	18.64 ± 1.28	20.12 ± 1.48*	14.93 ± 0.97

N o t e. n — number of examined subjects, ($\overline{X} \pm S_{\overline{X}}$) — mean arithmetic value ± standard deviation, HbA_{1c} — glycosylated hemoglobin, MMP-13 — metalloproteinase-13, TIMP-4 — tissue inhibitor of metalloproteinase-4, Tn C — tenascin C, * — $p < 0.05$ in comparison of patients with AMI and type 2 DM, # — $p < 0.05$ in comparison of patients with AMI depending on the presence or absence of type 2 DM.

the level of MMP-13 is gradually reduced and the content of tenascin C stays increased [6, 7].

Correlation between MMP-13 on the 1^{st}–2^{nd} day and blood glucose ($r = 0.36$; $p < 0.05$) and glycosylated hemoglobin ($r = 0.42$; $p < 0.05$) in patients with AMI with type 2 DM was used to analyze the relationship. The study showed correlation relationship between TIMP-4 on the 1^{st}–2^{nd} day and glucose ($r = 0.22$; $p < 0.05$). Correlation analysis showed inverse association between tenascin C level on the 1^{st}–2^{nd} day and glycosylated hemoglobin ($r = -0.28$; $p < 0.05$), and glucose on the 1^{st}–2^{nd} day ($r = -0.24$; $p < 0.05$). That is, AMI patients with type 2 DM were found to have an increase in MMP-13, which is indicative of an induction of extracellular matrix activity in hyperglycemia. The obtained results do not contradict the literature data. According to the literature [8], glycemia and glycosylated hemoglobin are accompanied by an increase in matrix metalloproteinases. Patients with AMI and type 2 DM were shown to have elevated TIMP-4 content along with an increase in matrix metalloproteinase-13, which is indicative of a compensatory response from the tissue inhibitors of metalloproteinases. Reverse nature of the relationship between carbohydrate metabolism and tenascin C reflects its reduction in response to the presence of type 2 DM. Taking into account the possible impact of tenascin C, tissue inhibitors of metalloproteinases for suppression of extracellular matrix system [6, 7], the results of the study showed that the presence of type 2 DM is associated with compensatory activity of TIMP-4 in the absence of such in tenascin C in patients with AMI.

CONCLUSIONS

1. Patients with acute myocardial infarction in the presence or absence of type 2 diabetes mellitus were found to have a significant increase in the activity of extracellular matrix system at the expense of matrix metalloproteinase-13 and tissue inhibitor of metalloproteinase-4.
2. The presence of type 2 diabetes mellitus is associated with compensatory tissue activity of tissue inhibitor of metalloproteinase-4 in the absence of such in tenascin C in patients with acute myocardial infarction.
3. Patients with acute myocardial infarction and type 2 diabetes mellitus were shown to have a more probable hyperproduction of matrix metalloproteinase-13 compared to patients with acute myocardial infarction without type 2 diabetes mellitus, which may be due to hyperglycemia.

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ВПЛИВ ВУГЛЕВОДНОГО ОБМІНУ НА ПОЗАКЛІТИННИЙ МАТРИКС У ХВОРИХ НА ГОСТРИЙ ІНФАРКТ МІОКАРДУ ТА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ

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У дослідженні було обстежено 60 хворих на гострий інфаркт міокарду з цукровим діабетом 2 типу, 40 хворих на гострий інфаркт міокарда без цукрового діабету 2 типу та 20 практично-здорових пацієнтів. Оцінено вплив вуглеводного обміну на компоненти позаклітинного матриксу у хворих на гострий інфаркт міокарда з наявністю та відсутністю цукрового діабету 2 типу. Визначено кореляційні зв'язки у хворих на гострий інфаркт міокарда та цукровий діабет 2 типу: між матриксною металопротеїназою-13 на 1–2 добу та глюкозою ($r = 0{,}36$; $p < 0{,}05$), глікозильованим гемоглобіном ($r = 0{,}42$; $p < 0{,}05$); тканинним інгібітором металопротеїназою-4 та глюкозою ($r = 0{,}22$; $p < 0{,}05$); тенасцину С та глікозильованим гемоглобіном ($r = –0{,}28$; $p < 0{,}05$), глюкозою — ($r = –0{,}24$; $p < 0{,}05$).

К л ю ч о в і с л о в а: гіперглікемія, гострий інфаркт міокарда, тенасцин С, позаклітинний матрикс, цукровий діабет 2 типу.

ВЛИЯНИЕ УГЛЕВОДНОГО ОБМЕНА НА ВНЕКЛЕТОЧНЫЙ МАТРИКС У БОЛЬНЫХ ОСТРЫМ ИНФАРКТОМ МИОКАРДА И САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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В исследовании было обследовано 60 больных острым инфарктом миокарда с сахарным диабетом 2 типа, 40 больных острым инфарктом миокарда без сахарного диабета 2 типа и 20 практически здоровых пациентов. Оценено влияние углеводного обмена на компоненты внеклеточного матрикса у больных острым инфарктом миокарда с наличием и отсутствием сахарного диабета 2 типа. Определены корреляционные связи у больных острым инфарктом миокарда и сахарным диабетом 2 типа: между матриксной металлопротеиназой-13 на 1–2 сутки и глюкозой ($r = 0{,}36$; $p < 0{,}05$), гликозилированным гемоглобином ($r = 0{,}42$; $p < 0{,}05$); тканевым ингибитором металлопротеиназы-4 и глюкозой ($r = 0{,}22$; $p < 0{,}05$); тенасцином С и гликозилированным гемоглобином ($r = –0{,}28$; $p < 0{,}05$), глюкозой ($r = –0{,}24$; $p < 0{,}05$).

К л ю ч е в ы е с л о в а: гипергликемия, острый инфаркт миокарда, тенасцин С, внеклеточный матрикс, сахарный диабет 2 типа.

CARBOHYDRATE EXCHANGE IMPACT ON EXTRACELLULAR MATRIX IN PATIENTS WITH ACUTE MYOCARIAL INFARCTION AND TYPE 2 DIABETES MELLITUS

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The study involved examination of 60 patients with acute myocardial infarction and type 2 diabetes mellitus, 40 patients with acute myocardial infarction and without type 2 diabetes mellitus and 20 substantially healthy subjects. The research allowed the authors to evaluate the effect of carbohydrate metabolism on extracellular matrix components in patients with acute myocardial infarction with the presence or absence of type 2 diabetes mellitus. The correlation relationship in patients with acute myocardial infarction and type 2 diabetes mellitus was found to be as follows: between matrix metalloproteinase-13 on the 1^{st}–2^{nd} day and glucose ($r = 0.36$; $p < 0.05$), glycosylated hemoglobin ($r = 0.42$; $p < 0.05$); tissue inhibitor of metalloproteinase-4 and glucose ($r = 0.22$; $p < 0.05$); tenascin C and glycosylated hemoglobin ($r = –0.28$; $p < 0.05$), glucose ($r = –0.24$; $p < 0.05$).

K e y w o r d s: hyperglycemia, acute myocardial infarction, tenascin C, extracellular matrix, type 2 diabetes mellitus.