THE ROLE OF A NEW BIOMARKER GROWTH DIFFERENTIATION FACTOR 15 IN PROGNOSIS OF PATIENTS WITH ACUTE CORONARY SYNDROME AND TYPE 2 DIABETES MELLITUS

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Numerous studies confirm worse results in diabetic patients with acute coronary syndrome (ACS) compared with non-diabetic patients. Different mechanisms underlie the adverse outcomes of ACS and diabetes mellitus. In this connection, a special place is occupied by the study of new biomarkers that reflect the complex pathogenic processes in these patients. Purpose: to investigate the role of the biomarker GDF 15 in prognosis of adverse outcomes in type 2 diabetes mellitus (DM2T) patients with ACS. Materials and methods: 73 patients with different forms of ACS were screened. Levels of biomarkers: GDF 15, N-terminal pro brain natriuretic peptide (NT-pro BNP) and C-reactive protein (C-RP) were determined. The follow up period was 1 year. Endpoint was defined as lethal outcome. Results: significant differences in GDF 15 level has been found, prognostic value of GDF 15 was estimated in patients with DM2T, using a ROC-analysis. Threshold level of GDF 15 has been determined as 3894 pg/ml, with sensitivity of 64 % and specificity of 75 %. Conclusion: Patients with ACS and DM2T more often had a history of different cardiovascular diseases and risk factors compared to patients without diabetes. GDF 15 level was significantly higher in patients with ACS who had history of DM2T.

KEY WORDS: biomarkers, GDF 15, diabetes mellitus, acute coronary syndrome, prognosis

РОЛЬ НОВОГО БІОМАРКЕРУ GROWTH DIFFERENTIATION FACTOR 15 У ПРОГНОЗІ ХВОРИХ НА ГОСТРИЙ КОРОНАРНИЙ СИНДРОМ ТА ЦУКРОВИЙ ДІАБЕТ ДРУГОГО ТИПУ

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Численні дослідження підтверджують гірші результати у хворих на цукровий діабет при гострому коронарному синдромі (ГКС) в порівнянні з пацієнтами без діабету. Різні механізми лежать в основі несприятливих наслідків ГКС на фоні цукрового діабету. У зв'язку з цим особливе місце займають дослідження нових біомаркерів, що відображають складні патогенетичні процеси у цих хворих. Мета роботи: вивчити роль нового біомаркери GDF 15 в прогнозуванні перебігу та результатів ОКС на тлі цукрового діабету 2-го типу (ЦД2Т). Матеріали і методи: обстежено 73 хворих з різними формами ГКС. Було визначено рівень біомаркерів: GDF 15, N-terminal pro brain natriuretic peptide (NT-pro BNP) та С-реактивний протеїн (С-РП). Період спостереження склав 1 рік. Кінцева крапка визначена як летальний вихід. Результати: з огляду на виявлені достовірні відмінності рівня GDF 15, була проведена спроба оцінити прогностичну цінність GDF 15 у хворих на ЦД2Т за допомогою ROC-аналізу. Граничним значенням був визначений рівень GDF 15 3894 пг/мл, з чутливістю 64 % і специфічністю 75 %. Висновки. Хворі з ГКС і ЦД2Т частіше мали в анамнезі різні серцево-судинні захворювання та фактори ризику в порівнянні з хворими без ЦД. Рівень GDF 15 був достовірно підвищений у групі хворих на ГКС, які мали в анамнезі ЦД2Т.

КЛЮЧОВІ СЛОВА: біомаркери, GDF 15, цукровий діабет, гострий коронарний синдром, прогноз

РОЛЬ НОВОГО БИОМАРКЕРА GROWTH DIFFERENTIATION FACTOR 15 В ОЦЕНКЕ ПРОГНОЗА У БОЛЬНЫХ ОСТРЫМ КОРОНАРНЫМ СИНДРОМОМ И САХАРНЫМ ДИАБЕТОМ ВТОРОГО ТИПА

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Многочисленные исследования подтверждают худшие результаты у больных сахарным диабетом при остром коронарном синдроме (ОКС) по сравнению с недиабетическими пациентами. Различные механизмы лежат в основе неблагоприятных исходов ОКС на фоне сахарного диабета. В связи с этим особое место занимают исследования новых биомаркеров, отражающих сложные патогенетические процессы у этих больных. Цель работы: изучить роль нового биомаркера GDF 15 в прогнозировании течения и исходов ОКС на фоне сахарного диабета 2-го типа (СД2Т). Материалы и методы: обследовано 73 пациента с различными формами ОКС. Был определен уровень биомаркера – GDF 15, N-terminal pro brain natriuretic peptide (NT-pro BNP) и С-реактивній протеин (С-РП). Наблюдательный период составил 1 год. Конечная точка определена как летальный исход. Результаты: учитывая выявленные достоверные отличия уровня GDF 15, была проделана попытка оценить прогностическую значимость ценность GDF 15 у больных СД2Т с помощью ROC-анализа. Пороговым значением был определен уровень GDF 15 3894 пг/мл, с чувствительностью 64 % и специфичности 75 %. Выводы. Больные с ОКС и СД2Т чаще имели в анамнезе различные сердечно-сосудистые заболевания и факторы риска по сравнению с больными без СД. Уровень GDF 15 был достоверно повышен в группе больных ОКС на фоне СД2Т.

КЛЮЧЕВЫЕ СЛОВА: биомаркеры, GDF 15, сахарный диабет, острый коронарный синдром, прогноз

INTRODUCTION

Diabetes mellitus (DM) is significant problem today. According to the WHO data, in 2014 9 % of world population over 18 years has DM. According to the International Diabetes Federation data (Atlas Diabetes, 6th Edition, 2013), number of people with diabetes is 382 million. By 2035 it will increase to 592 million patients.

Type 2 diabetes mellitus (DM2T) is the most common type. The disease is associated with cardiovascular pathology and it significantly impairs patients` survival. Furthermore, DM2T is frequent condition in patients with acute coronary syndrome (ACS). Various international registers have shown that people with DM2T make up 22–34 % of patients with ACS [1–2]. It is known that DM generally and DM2T particularly is closely related to overweight (abdominal obesity), that is confirmed by elevated levels of adipokines such as resistin, leptin, adiponectin, ghrelin [3].

The relationship between levels of Growth differentiation factor 15 (GDF 15) and DM2T is discussed in recent world researches. GDF 15 serum level was increased in women with obesity and DM2T, and it was correlated with body mass index (BMI), level of visceral fat, blood glucose level and C-reactive protein (CRP) [4].

Transforming growth factor β GDF 15 was originally introduced as a marker, that inhibits tumor necrosis factor-α (TNF-α) in Lipopolysaccharide-stimulated macrophages, and thus it was classified as macrophage-inhibitory cytokine-1 (MIC-1) [5].

Today it is known that GDF 15 is an independent predictor of total and cardiovascular mortality. GDF 15 is produced by cardiomyocytes, adipocytes, macrophages, endothelial cells and vascular myocytes, in normal conditions and under stress. But its role as a marker of concomitant pathology has been insufficiently studied.

OBJECTIVE

To investigate the role of the biomarker GDF 15 in prognosis of adverse outcomes in DM2T patients with ACS.

MATERIALS AND METHODS

In Government institution «L. T. Malaya Therapy national institute of the National Academy of Medical Science of Ukraine» 73 patients with different forms of ACS were examined, average age of the studied patients was 61,8 ± 1,3 years. Patients with hemodialysis, terminal liver failure, active cancer and also people who refused to sign the informed consent and to comply the study protocol were excluded.

According to the clinical picture, ECG changes and troponin I 18 patients had unstable angina (UA), 14 patients – non Q wave myocardial infarction (non-Q-MI), in 38 patients – Q wave myocardial infarction (Q-IM), 3 patients were excluded from the study because of diagnosis mismatch. As was listed in the medical history 11 patients had DM2T, 60 patients – hypertension, 36 patients – stable angina, 17 patients – myocardial infarction. Blood serum was taken from patients` vein on admission. All patients underwent standard protocol examination. For an acute heart failure diagnosis was used Killip-Kimball classify-cation. Additionally, biomarkers level GDF 15 and N-terminal pro brain natriuretic peptide (NT-pro BNP), C-reactive protein (C-RP) were identified.

Follow up period was 1 year. Endpoint was defined as lethal outcome. After 1 year ($\pm$ 1 month) patients underwent the six minute walk test (6-MWT) in order to estimate functional class of heart failure according to the New York Heart Association classification. During follow up period 9 people have reached endpoint.

We have received and processed 95 % of information from patients that were included in the study. Statistical data manipulation was carried out using the program «Statistics» (version 10.0). Assessments of significant differences between paired random samples were evaluated by Student's t-test. Continuous variables are presented as $M \pm SD$ (SD – standard deviation, mean $\pm$ standard error of mean) or Me, depending on distribution type (parametric or non-parametric). Also, assessment of differences between the groups was performed using non-parametric statistics methods: chi-square test, Fisher's exact test. Receiver operating characteristic analysis (ROC-analysis) was used for comparative assessment of parameters influence, characteristic curves tracing was done. For comparison area under curve ratio was used. Considered parameters had a prognostic significance at the borderline of confidence interval area > 0.5 and $p < 0.05$. The larger the area is, the higher the accuracy of the model.

RESULTS AND DISCUSSION

Average glucose level of studied patients was $7,80 \pm 0,5$ mmol/l. Patients with Q-MI had average blood glucose level of 8.03 ± 0.72 mmol/L, with non-Q-MI – 7.30 ± 0.63 mmol/l, UA – $6,38 \pm 0.52$ mmol/l; there wasn't a significant difference in blood glucose level in these groups. 11 patients had established diagnosis DM2T on admission to the hospital, glucose level in this group was $9,66 \pm 1,40$ mmol/l, and differed significantly ($p < 0.05$) compared to the group without DM2T ($7,03 \pm 0,44$ mmol/l).

Family history of coronary artery disease was more frequent in group with DM than group without diabetes (64 % and 49 %, respectively), the same was for patients with myocardial infarction – 36 % and 22 %, respectively.

In detailed assessment of parameters that have been identified during hospitalization in patients with DM2T and without in history, significant differences were identified in the level of high-density lipoproteins (HDL) ($p < 0.05$), C-RP ($p < 0.007$), GDF 15 ($p < 0.01$). There was no significant difference in level of NT-pro BNP. There was no significant difference of BMI parameters in both groups. Detailed characteristic of estimated parameters is presented in table 1.

Considering identified significant differences of GDF 15 level, an attempt has been done to evaluate prognostic value of GDF 15 in patients with DM2T. ROC-analysis was performed to determine threshold level of GDF 15 that allows us to identify DM2T patients with the most sensitivity and specificity. Threshold level of GDF 15 was determined as 3894 pg/ml, with sensitivity of 64 % and specificity of 75 % (95 % Confidence interval (CI) 0.49 – 0.88; Area under curve (AUC) 0.68). Unfortunately, relationship between GDF 15 and diabetes did not reach significant level of ($p = 0.06$), despite significant differences of biomarker in both groups (Fig. 1).

Table 1

Comparative characteristic of parameters in examined groups of patients (M ± sd)

Parameters	n	Without DM2T	N	DM2T	p
Age, years	58	62,17±1,38	11	66,27±2,71	0,2
SBP, mmHg	58	147,9±3,9	11	155,0±7,8	0,46
DBP, mmHg	58	88,34±1,91	11	88,27±3,55	0,98
HR, beats per 1 minute	58	80,05±2,86	11	83,18±8,89	0,68
Creatinine, μmol/l	56	116,5±6,9	11	113,9±13,8	0,87
Cholesterol, mmol/l	55	6,78±1,73	10	4,35±0,47	0,55
HDL, mol/l	55	1,327±0,049	10	1,057±0,081	0,028
Triglycerides, mmol/l	55	1,299±0,089	10	1,223±0,135	0,73
LDL, mmol/l	55	3,16±0,14	10	2,74±0,41	0,27
GDF 15, pg/ml	58	3 565±456	11	7 468±2 073	0,0058
C-RP	34	7,27±1,06	6	20,00±8,96	0,007
Blood glucose, mmol/l	55	7,03±0,44	11	9,66±1,40	0,025
NT-pro BNP, pg/ml	38	825.8±175.9	9	706.5±397.1	0.77

Note: SBP – systolic blood pressure, DBP – diastolic blood pressure, HR – Heart rate, HDL – high-density lipoproteins, LDL – Low density lipoproteins.
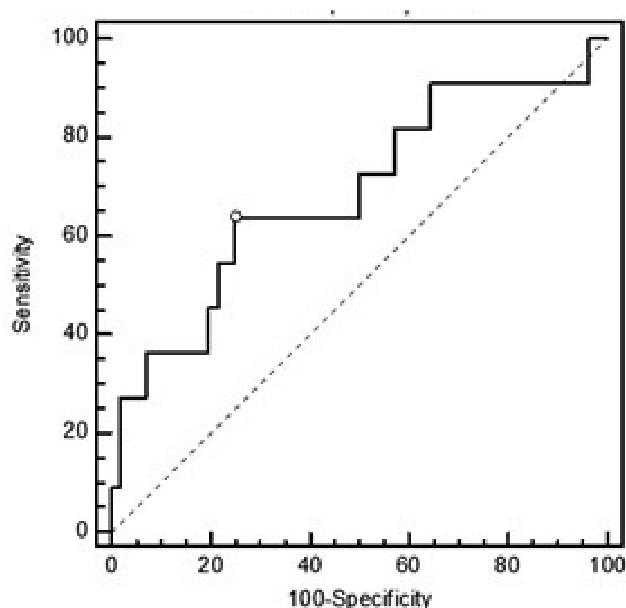

Fig. 1. ROC-curve values GDF 15 from DM2T in patients with ACS history

A significant relationship was identified ($p \leq 0.0001$, sensitivity of 72 % and specificity of 73 %) between GDF 15 biomarker and narcotic drugs prescription frequency in hospital period (95 % CI 0.19 to 0.59; AUC 0.738, level of > 2508 pg/ml) (Fig. 2).

More detailed information about treatment in both groups at the hospital stage, is presented in table 2.

A relationship between the presence of diabetes mellitus and 6-MWT that was conducted one year after coronary events ($p < 0.05$) was found. Significant association was not identified in assessing the relationship between DM2T and class of heart failure by Killip-Kimball. Conversely, elevated level of GDF 15 marker significantly depends on class of heart failure by Killip-Kimball (95 % CI 0.57 – 0.86; AUC 0,714, significant level > 2910 pg/ml), with sensitivity of 80 % and specificity of 65 % ($p \leq 0.005$) (Fig. 3).
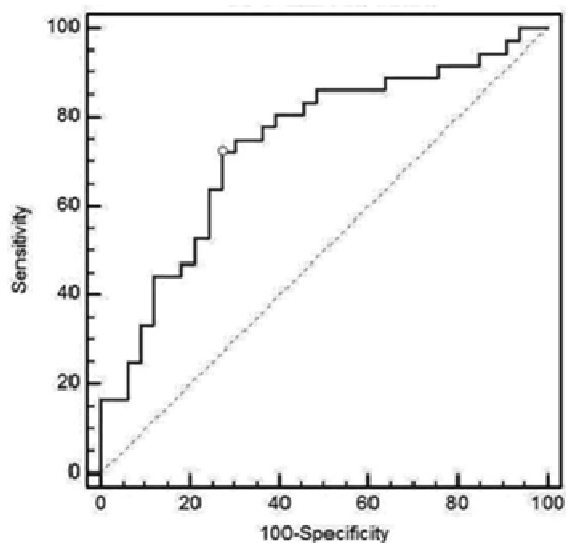
Fig. 2. ROC-curve of GDF 15 and narcotic drugs prescription during hospitalization period

Table 2

Frequency of drugs prescribing in patients with and without DM2T - hospital period (%)

| Drugs	DM2T n=11	without DM2T n=59
Acetylsalicylic acid (loading dose)	36	57
Acetylsalicylic acid	91	100
Inhibitors of P2Y12, (clopidogrel, ticagrelor)	100	100
Low molecular weight heparins/pentasaccharides (Enoxaparin/fondaparinux)	91	95
Angiotensin-converting-enzyme inhibitors	64	86
B-blockers	73	85
Narcotic analgesics	73	47
Loading dose of inhibitors of P2Y12	64	73
Nitrates	64	49
Fibrinolytic therapy	9	27
Diuretics	9	20
Statins	91	95
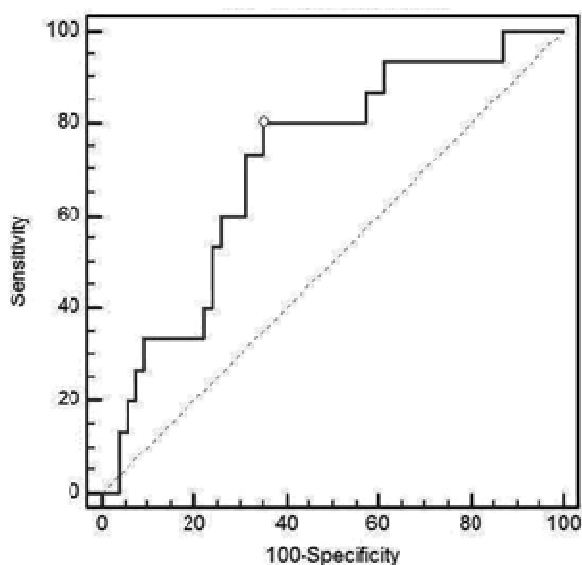
Fig. 3. ROC-curve GDF 15 and class of HF by Killip-Kimball

A significant difference was found in NT-pro BNP among groups of patients with ACS and DM2T who died and survived ($p < 0.05$)

and there was no significant difference in GDF 15 (tab. 3).

Table 3

Average values of biomarkers in groups of patients by prognosis (M ± sd)

Parameters	Had reached endpoint	Had not reached endpoint	p
GDF 15, pg/ml	11212,96 ± 4437	6064,15 ± 1157	0,29
NT-pro BNP, pg/ml	1910,6 ± 894	104,5 ± 34,5	0,018

In 2010 T. Kempf et al. [6] have conducted a study where an association between MIC-1 and incident of DM2T was first presented. The level of MIC-1 was significantly increased in the group of patients where DM2T subsequently has developed compared to DM2T-free group. However, there was a borderline significant relationship with modification in gender and age; also this relationship was significantly decreased with modification in indicators such as waist circumference, cardiovascular risk factors, proinflammatory mediators and glycaemia. This study was the first to predict development of DM2T 11 years before manifestation. However, as a result, it was found that an elevated level of MIC-1 was not independently associated with DM2T, although it was significantly increased in the group.

Large number of studies had been conducted to estimate GDF 15 in DM2T patients with concomitant cardiovascular pathology and without it. In XENDOS study, a relationship of obesity and insulin resistance with GDF 15 for patients with pre-diabetes was detected, where the biomarker was an independent predictor. Also, over 4 years follow-up, inadequate glucose control resulted in elevated levels of GDF 15. Besides, only GDF 15 and pre-diabetes, identified initially, were independent predictors of inadequate glucose control over 4-year period [7].

In a study conducted by Greisa Vila et al. [8] a cohort of patients with obesity was examined. All patients were divided into groups based on glucose-tolerance test results: a normal glucose level, with insulin resistance and DM. GDF 15 was significantly increased in all groups compared to control

group (healthy population). The main finding was that GDF 15 is related to all parameters that characterize glucose metabolism: it was significantly correlated with glucose, insulin, C-peptide, Hb A_{1C} index and HOMA. GDF 15 level was significantly higher in group of patients with obesity and new onset diabetes mellitus than in group with obesity and normal glucose tolerance.

In 2014, a review about relationship of depending cardiovascular pathology in patients with DM2T and GDF 15 role was published. Level of GDF 15 – 3812 pg/ml was indicated as an independent predictor of patients with diabetic cardiomyopathy (sensitivity of 82.2 %, specificity of 70.2 %) [9].

Although there are a large number of studies of GDF 15 in patients with diabetes, its role in acute coronary pathology is still insufficiently studied.

As noted above, percentage of patients with DM2T and ACS is 20–35 %, but in our study, this figure was 15 %. Patients with DM are initially patients of high risk for ACS complications. In our study adverse outcome was observed in 27 % in the group of patients with DM2T and 10.1 % – in the group of patients without DM2T, and 12.9 % in total group. There was no significant difference in GDF 15 among the group of dead and surviving patients. Probably, this is due to the small number of samples, so this issue needs to be improved by recruiting a larger number of patients.

CONCLUSIONS

GDF 15 levels' was significantly increased in patients with ACS who had DM2T history, but had not reached significant point in the group of patients who died. The final decision about prognostic

value of GDF 15 in patients with ACS and diabetes can be found after we reach the necessary number of patients.

PROSPECTS FOR FUTURE STUDIES

We are going to include more patients with such combined pathology and find the connection between high level of GDF 15 and prognosis for the new algorithm of risk stratification.

The review is a fragment of scientific work «Investigation of new biomarkers for high quality prognosis and treatment of patients with acute coronary syndrome» № 0113U001141.

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