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NATRIURETIC PEPTIDE: DIAGNOSTIC, PROGNOSTIC, AND CLINICAL RELEVANCE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS, ARTERIAL HYPERTENSION AND OBESITY*

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Modern medicine is actively using the N-terminal fragment of B-type brain natriuretic peptide (NT-proBNP) as a biomarker for several cardiovascular diseases.

Above all, one of the main biomarkers of chronic heart failure (CHF) consists of natriuretic peptides. It is known that the group of natriuretic peptides (NPs) includes three biologically active peptides: atrial natriuretic peptide (ANP), BNP, and C-type natriuretic peptide (CNP). ANP and BNP are secreted by the heart and exert their actions by increasing intracellular cyclic guanosine monophosphate in target tissues [1, 2].

Here we will focus on NT-proBNP, which is secreted in cardiac fibroblasts as a prohormone. When exposed to relevant factors before release, natriuretic peptides (NPs) are metabolized and released into the bloodstream as hormonally active C- and N-terminal peptides in equivalent concentrations. Following the active conversion of NPs in the kidneys, urodilatin is formed, which has auto-paracrine activity. The BNP gene is located in the 1st chromosome in humans, which encodes a proBNP (prohormone) of 108 amino acids.

The biologically active hormone BNP circulating in the blood consists of 32 amino acids. It is separated from the N-terminal chain of the prohormone NT-proBNP. ProBNP is secreted mainly in the ventricles of the heart, forming physiologically active BNP and hormonally inactive NT-proBNP (N-terminal fragment) [3, 4].

The main mechanism of NP clearance is the renal excretion pathway, which is the main one for NT-proBNP and BNP to a lesser extent. It is removed from the blood plasma by binding to C-type receptors, followed by endocytosis and lysosomal degradation through proteolysis by peptidases. Neutral endopeptidase

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is the most studied enzyme containing zinc, which is absent in blood plasma and is found at the apical pole of epithelial cells of the nephron proximal tubules, in the lungs, and in vascular endothelium.

The clinical significance of NT-proBNP resides in the diagnosis of the myocardial dysfunction degree, that is, from clinically asymptomatic mild forms to decompensated stages of CHF, as well as in the assessment of changes in its severity under the treatment.

The NT-proBNP level can be used to assess the negative prognosis in patients with CHF, as well as to monitor the sensitivity of patients with left ventricular dysfunction to treatment.

The diagnostic significance of NPs in the assessment of cardiovascular dysfunction associated with left ventricular dysfunction has been proven by several studies [5–9]. The prognostic accuracy with NT-proBNP is very high with negative prognoses detected in 97 % of cases.

As of today, NT-proBNP is still being actively studied. It has been found that BNP is an independent predictor of cardiovascular disease. An increase in the level of this peptide may indicate the presence of chronic heart failure, myocardial ischemia, pulmonary hypertension, and arterial hypertension [10, 11].

NT-proBNP has been proven to be a marker inversely associated with mean arterial blood pressure, pulse blood pressure, and is a central prognostic biomarker for IHD, atrial fibrillation, myocardial infarction, and chronic renal failure.

Given the above reference literature on the clinical significance of NT-proBNP, our study was aimed at investigating this biomarker in comorbid patients with arterial hypertension (AH), obesity, and type 2 diabetes mellitus (T2DM) and determining the relationship with several hormonal and biochemical parameters.

MATERIALS AND METHODS

These studies were conducted in compliance with the ethical and moral requirements of the Ukrainian Association for Bioethics and the norms of GCP (1992), GLP (2002), the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and approved by the Ethics and Bioethics Committee of Kharkiv National Medical University.

111 patients with hypertension (men/women — 50/61) and 20 control subjects were examined. All patients with AH aged 54.37 ± 1.18 yrs were treated at the clinic of the Government Institution «L.T. Malaya Therapy National Institute» of the National Academy of Medical Sciences of Ukraine. Through a careful examination and follow-up, patients were classified into 4 groups depending on the comorbid pathology they had: patients with AH (group 1 with 22 patients); patients with AH in combination with obesity (group 2 with 30 patients); AH in combination with T2DM (group 3 with 31 patients); patients with AH, T2DM, and obesity (group 4 with 28 patients).

All the patients' body weight and height were measured, BMI = body weight/height^2 (m^2) was calculated, and systolic and diastolic blood pressure levels were measured.

The content of N-terminal brain natriuretic peptide (NT-proBNP) in the patient's serum was determined by enzyme-linked immunosorbent assay with a Labline-90 analyzer (Austria) using a commercial test system manufactured by Elabscience (ELISA, China) according to the instructions included in the kit.

Biochemical tests (creatinine, urea, lipid spectrum in the blood serum, and glycated hemoglobin level) were conducted with a Labline-90 analyzer (Austria). Serum urea levels were measured by the kinetic and enzymatic method with urease and glutamate dehydrogenase using Liquick Cor-UREA 30 kits (Cormay, Poland) according to the manufacturer's instructions. Serum creatinine level was measured by the modified Jaffe method without deproteinization using Liquick Cor-CREATININ 30 reagent kits (Poland) according to the manufacturer's instructions. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) were measured by the enzymatic method using «Cholesterol liquicolor», «HDL – Cholesterol», and «Triglycerides liquicolor» reagent kits (Human, Germany) according to the manufacturer's instructions. The content of VLDL (very low-density lipoprotein) was calculated using the formula TG/2.22; the

content of LDL (low-density lipoprotein) was calculated using the W. T. Friedewald formula, 2004:

LDL = TC – (HDL + TG / 2.22), mmol/L.

Exclusion criteria for the study were type 1 DM, congenital heart and urinary tract disorders, presence of an artificial pacemaker, presence of artificial heart valves, stage II B and III heart failure, acute myocardial infarction, infectious and severe inflammatory processes, and hematological diseases.

Statistical analysis was completed using the SPSS Statistics 17.0 package. Appropriate criteria for statistical analysis of the data were selected according to the research goals and the compliance of the obtained numerical values with the standard distribution law. Nonparametric methods were used when analyzing the samples: Wilcoxon signed-rank test for related populations, and Mann-Whitney U test for independent populations. Differences were considered significant at $p < 0.05$. The Pearson correlation coefficient and Spearman's rank correlation coefficient were used to assess the degree of relationship. As a descriptive statistic, the median was estimated with the values of 25 % and 75 % percentiles (Me [25 %; 75 %]).

Regression models were statistically processed using Statistica 10.0. Such indicators as arithmetic mean (X), mean-square deviation (Sx), and 95 % confidence interval (95 % CI) were calculated.

The diagnostic value of the tests and their impact on the disease outcome to optimize risk prediction and prognostic significance of the indicators were determined by using ROC curves (Receiver Operating Characteristic) and calculating the operating characteristics of ROC analysis.

RESULTS AND THEIR DISCUSSION

At the first stage of the study, we determined the NT-proBNP level among the examined groups of patients, as well as their comparative results with those of the control group. Thus, it was found that the NT-proBNP level in all patients significantly differed from the group of healthy individuals ($p < 0.05$) (Table 1).

The results of the study of indicators of carbohydrate metabolism in patients with T2DM are presented in Table 2.

We also evaluated the NT-proBNP level in different groups of patients and compared these groups with each other (Table 3).

Table 2 and the mathematical models (Fig. 1) show that the modern NT-proBNP biomarker has sufficient prognostic value for determining the risk of adverse cardiovascular outcomes in AH, which contributes to the implementation of a personalized approach to patients at risk of developing HF and enables

Table 1

Assessment of differences between groups by NT-proBNP level

Parameter	AH	AH + obesity	AH + T2DM	AH + obesity + T2DM	Control group
$\bar{X} \pm S_{\bar{X}}$	466.02 ± 35.24	459.81 ± 38.75	521.90 ± 40.58	526.13 ± 50.68	154.47 ± 9.49
CI, 95%	[449.5; 482.5]	[441.7; 477.9]	[502.9; 540.9]	[502.4; 549.8]	[136.7; 172.2]
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 2

Data on indicators of carbohydrate metabolism in examined patients (M ± m)

Indicator	AH	AH + obesity	AH + T2DM	AH + obesity + T2DM	Control group
Glucose, mmol/L	5.4 ± 0.5	5.52 ± 0.6	7.8 ± 0.8	7.01 ± 0.9	4.5 ± 0.5
Insulin, mIU/L	15.75 ± 7.50*	19.42 ± 9.44*	21.44 ± 16.55*	20.06 ± 11.60*	14.38 ± 2.69
HbA1c, %	5.85 ± 1.64	6.21 ± 0.91	7.46 ± 1.2*	7.12 ± 0.83*	4.80 ± 0.32

Note:

* $p < 0.05$ relative to the control group.

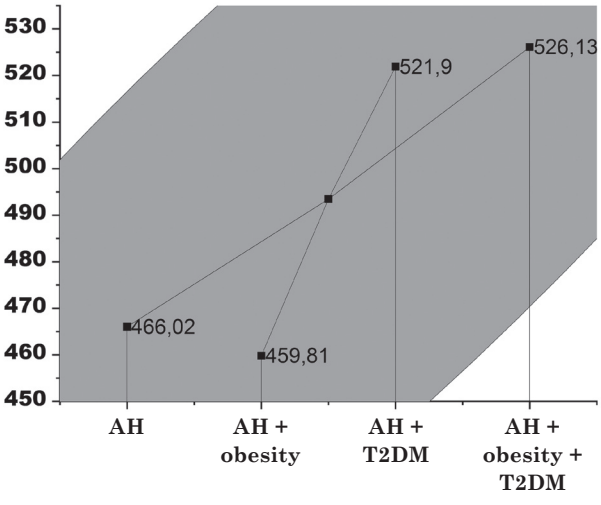

Fig. 1. Mathematical models for NT-proBNP as a biomarker.

timely selection of more effective management tactics for this category of patients aimed at improving the prognosis of the disease.

The regression analysis was used to build mathematical models that proved a significant increase in NT-proBNP levels with comorbidities associated with AH, namely, with T2DM (Fig. 1).

At the second stage of the study, a comparative analysis of the examined patients was performed (Table 4) and factors influencing the NT-proBNP level were determined (Table 5).

To establish the relationship between the NT-proBNP level and the studied parameters in the examined patients with AH, a correlation analysis was performed (Table 6).

Table 3

NT-proBNP levels in the blood serum of different patient groups

Parameter	AH	AH + obesity	AH + T2DM	AH + obesity + T2DM	p
NT-proBNP (pg/ml) Me [Q1;Q3]	466.02 [430.8; 501.3]	459.81 [421.1; 498.6]	521.90 [481.3; 562.5]	526.13 [475.5; 576.8]	H = 24.5 df = 2 p < 0,010* $p_{AH;\ AH + DM} < 0.010$ $p_{AH;\ AH + obesity;\ AH + DM;\ p_{AH + obesity + DM}} < 0.010$

Table 4

Comparative study of the examined patients with arterial hypertension with different comorbidities depending on the NT-proBNP level, $\bar{X} \pm S_{\bar{x}}$

Parameter	NT-proBNP < 464.02 pg/ml (n = 55)	NT-proBNP > 464.02 pg/ml (n = 56)	p
Age, years	54.49 ± 4.46	52.23 ± 5.04	0.425
BMI, kg/m²	31.62 ± 1.91	31.60 ± 2.43	0.135
SBP, mm Hg	147.24 ± 5.51	144.8 ± 6.35	0.356
DBP, mm Hg	90.16 ± 4.37	88.64 ± 4.50	0.131
Creatinine, μmol/L	93.03 ± 7.43	93.43 ± 8.35	0.193
Urea, mmol/L	5.85 ± 0.92	6.08 ± 0.88	0.128
EC, mmol/L	5.59 ± 0.75	5.85 ± 0.63	0.361
HDL, mmol/L	1.32 ± 0.19	1.30 ± 0.14	0.271
TG, mmol/L	1.81 ± 0.32	2.22 ± 0.68	0.139
VLDL, mmol/L	0.81 ± 0.13	0.93 ± 0.25	0.311
LDL, mmol/L	3.42 ± 0.62	3.646 ± 0.64	0.277
AC	3.46 ± 0.68	3.72 ± 0.73	0.145
CTF-1, pg/mL	1030.65 ± 117,	1067.74 ± 127,	0.145
CST, ng/mL	2.87 ± 0.56	2.83 ± 0.56	0.178
Cystatin C, ng/mL	137.39 ± 22.1	130.46 ± 23.01	0.299

Continuation of table 4

Parameter	NT-proBNP < 464.02 pg/ml (n = 55)	NT-proBNP > 464.02 pg/ml (n = 56)	p
Leptin, ng/mL	26.86 ± 6.43	27.38 ± 6.61	0.379
NGAL, ng/mL	18.65 ± 3.17	20.09 ± 2.47	0.425
NT-proBNP, pg/mL	359.13 ± 35.0	628.6 ± 61.7	0.303
Insulin, μU/mL	15.59 ± 4.59	15.26 ± 3.78	0.257
ß2-M, μg/mL	3.13 ± 0.47	3.12 ± 0.48	0.286
25(OH)D, ng/mL	38.61 ± 5.85	42.80 ± 5.88	0.355
HbA1c, %	6.03 ± 0.50	6.44 ± 0.46	0.371

Table 5

Factors influencing the NT-proBNP level

Parameters	Dependent component: NT-proBNP (Y)							
	Univariate linear regression analysis (χ^2 = 44.72; p = 0.006)				Multivariate linear regression analysis (χ^2 = 37.40; p = 0.0004)			
	β-coefficient	Mean	95 % CI	P	β-coefficient	Mean	95 % CI	P
X1 CTF-1, pg/mL	– 0.029	0.999	0.995–1.003	0.670				
X2 CST, ng/mL	0.041	1.042	0.647–1.680	0.865				
Cystatin C, ng/mL	– 0.004	0.996	0.984–1.007	0.444				
X3 AC	1.069	2.914	0.652–13.027	0.162	0.581	1.787	1.057–3.021	0.030
X4 Leptin, ng/mL	0.033	1.033	0.988–1.081	0.157				
X5 NGAL, ng/mL	0.076	1.078	0.986–1.180	0.100	0.083	1.086	0.999–1.181	0.053
X6 25(OH)D, ng/mL	0.054	1.055	1.009–1.104	0.019	0.057	1.059	1.015–1.105	0.008
X7 ß2-M, μg/mL	0.234	1.264	0.724–2.205	0.409				
X8 HbA1c, %	0.212	1.237	0.499–3.063	0.647				
X9 TC, mmol/L	12.222	0.640	0.0015–26.6E+012	0.200	0.792	2.208	0.722–6.753	0.165
Insulin, μU/mL	– 0.040	0.960	0.897–1.029	0.250				
Creatinine, μmol/L	– 0.021	0.979	0.941–1.020	0.323	– 0.028	0.972	0.939–1.006	0.108
Urea, mmol/L	– 0.006	0.995	0.747–1.323	0.969				
TG, mmol/L	– 8.894	0.235	0.000–3.360	0.085				
HDL, mmol/L	– 6.318	0.209	0.000–2283.698	0.263				
X10 VLDL, mmol/L	7.939	0.130	0.000–444E+009	0.409				
LDL, mmol/L	– 4.254	0.186	0.000–538.844	0.189	0.929	0.395	0.101–1.547	0.182

Table 6

Correlations of NT-proBNP levels found in the examined patients

Parameter	R	p level
Creatinine, μmol/L	0.328	0.045
TG, mmol/L	0.406	0.027
HDL, mmol/L	0.377	0.031
Urea, mmol/L	0.411	0.038
NGAL, ng/mL	0.282	0.004
HbA1c, %	0.202	0.038

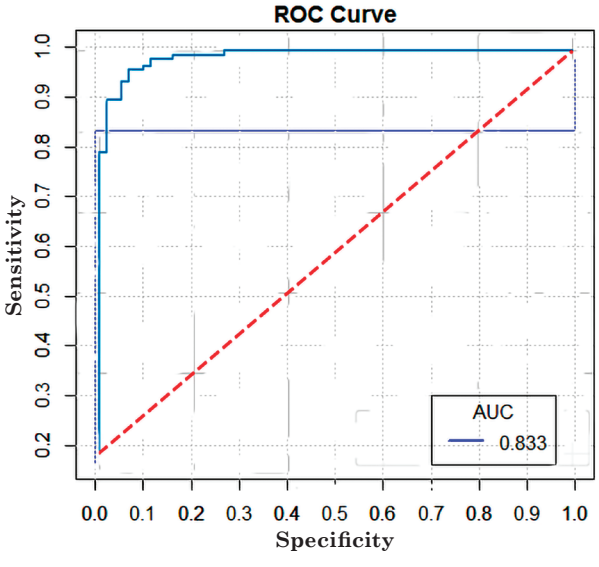

Fig. 2. ROC curve for predicting the risk of developing cardiovascular complications depending on the NT-proBNP level in the examined patients with AH (sensitivity: 83.6; specificity: 80.0; criterion 411.7).

Direct correlations of the average dependence of the NT-proBNP level with creatinine ($r = 0.328$; $p = 0.045$) and urea ($r = 0.411$; $p = 0.038$) were established. These parameters were likely to be observed in patients with organic changes in the kidneys, as evidenced by the association with the NT-proBNP levels. A direct correlation of the NT-proBNP levels with TG ($r = 0.406$; $p = 0.027$) and TC ($r = 0.377$; $p = 0.031$) was found. These correlations reveal that these indicators are markers for detecting a high risk of severe liver dysfunction in patients with AH. A direct correlation was found between NT-proBNP and HbA1c ($r = 0.202$; $p = 0.0038$). This correlation may be caused by the negative effect of hyperglycemia on the efficiency of cardiac mechanisms. A direct correlation was found between the concentration of NT-proBNP and NGAL ($r = 0.282$; $p = 0.004$) and the severity of tubular insufficiency.

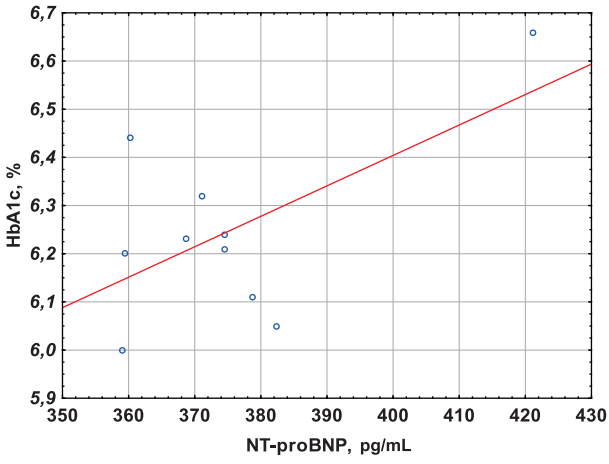

Fig. 3. Correlation analysis of NT-proBNP and glycated hemoglobin (HbA1c) concentration in patients with T2DM ($r = 0.207$; $p = 0.036$)

The NT-proBNP level has a predictive value regarding the risk of cardiovascular complications in patients with AH [11, 12]. To determine the prognostic value of NT-proBNP in comorbid patients under our supervision, the optimal value of the distribution point, an excess of which is associated with the risk of developing cardiovascular complications/events in patients with AH, was found by ROC analysis to be 411.7 pg/mL.

Our results confirm the importance of determining NT-proBNP in patients with comorbid pathology, namely, AH in combination with T2DM and obesity is a prognostically significant parameter of heart failure in this patient group. Our study is consistent with other authors who have studied the importance of NT-proBNP in the development of cardiovascular complications in patients with monopathology of AH, T2DM, and obesity [12–18].

CONCLUSIONS

We found that patients with AH, AH in combination with obesity, AH in combination with type 2 DM, and AH in combination with type 2 DM and obesity showed a significant increase in NT-proBNP level compared with the control group ($p < 0.05$). The most significant factors affecting NT-proBNP levels are cardiotrophin-1, catestatin, and cystatin C.

It has been proven that NT-proBNP has a direct correlation with creatinine ($p = 0.045$),

urea (p = 0.038), triglyceride (p = 0.027), and cholesterol (p = 0.031); direct correlation with HbA1c (r = 0.202; p = 0.0038).

NT-proBNP levels have a significant predictive value for the risk of developing cardiovascular complications in patients with AH.

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НАТРІЙУРЕТИЧНИЙ ПЕПТИД: ДІАГНОСТИЧНЕ, ПРОГНОСТИЧНЕ ТА КЛІНІЧНЕ ЗНАЧЕННЯ У ПАЦІЄНТІВ З ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ, АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ ТА ОЖИРІННЯМ

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Одним з головних біомаркерів хронічної серцевої недостатності (ХСН) є саме натрійуретичні пептиди. Рівень NT-proBNP дозволяє оцінити негативний прогноз у пацієнтів з ХСН, а також моніторувати чутливість пацієнтів з дисфункцією лівого шлуночка до призначення лікування. **Мета дослідження:** вивчення біомаркеру NT-proBNP у коморбідних хворих з АГ, ожирінням, ЦД 2 типу та визначення взаємозв'язків з рядом гормональних та біохімічних показників.

Матеріали та методи: всі хворі були розподілені на 4 групи в залежності від наявності в них коморбідної патології: хворі на АГ — 1 група — 22 особи; хворі на АГ в сполученні з ожирінням — 2 група – 30 осіб; АГ в сполученні з ЦД 2 типу — 3 група — 31 особа; пацієнти з АГ, ЦД 2 типу та ожирінням — 4 група — 28 осіб. Визначення вмісту N-термінального мозкового натрійуретичного пептиду (NT-proBNP) у сироватці крові пацієнтів проводили імуноферментним методом на аналізаторі «Labline-90» (Австрія) з використанням комерційної тест-системи виробництва фірми «Elabscience» (ELISA, Китай) згідно інструкції, яка входила до складу набору.

Висновки. Встановлено, що у хворих з АГ, АГ в сполученні з ожирінням, АГ в сполученні з ЦД 2 типу, а також з АГ в сполученні з ЦД 2 типу та ожирінням має місце значуще збільшення NT-proBNP у порівнянні з показником контрольної групи ($p < 0,05$). Доведено, що NT-proBNP має прямий кореляційний зв'язок з креатиніном ($p = 0,045$), сечовиною ($p = 0,038$), рівнем тригліцеридів ($p = 0,027$) та холестерина ($p = 0,031$); пряма кореляційна залежність з рівнем HbA1c ($r = 0,202$; $p = 0,0038$). Рівень NT-proBNP має значну предикторну цінність щодо ризику розвитку серцево-судинних ускладнень у хворих на АГ.

Ключові слова: натрійуретичний пептид, цукровий діабет 2 типу, артеріальна гіпертензія, ожиріння.

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Natriuretic peptides are one of the main biomarkers of chronic heart failure (CHF). The level of NT-proBNP makes it possible to evaluate the negative prognosis in patients with CHF, as well as to monitor the sensitivity of patients with left ventricular dysfunction to the appointment of treatment. The purpose of the study: to study the NT-proBNP biomarker in comorbid patients with hypertension, obesity, type 2 diabetes and determine the relationship with a number of hormonal and biochemical indicators.

Materials and methods: all patients were divided into 4 groups depending on the presence of comorbid pathology in them: patients with hypertension — 1 group — 22 people; patients with hypertension combined with obesity — group 2 — 30 people; Hypertension in combination with type 2 diabetes — group 3 — 31 people; patients with hypertension, type 2 diabetes and obesity — group 4 — 28 people. Determination of the content of N-terminal brain natriuretic peptide (NT-proBNP) in the blood serum of patients was carried out by the immunoenzymatic method on the analyzer «Labline-90» (Austria) using a commercial test system manufactured by the company «Elabscience» (ELISA, China) according to the instructions, which was part of the set.

Conclusions. It was established that in patients with hypertension, hypertension in combination with obesity, hypertension in combination with type 2 diabetes, as well as hypertension in combination with type 2 diabetes and obesity, there is a significant increase in NT-proBNP compared to the indicator of the control group ($p < 0.05$). It has been proven that NT-proBNP has a direct correlation with creatinine ($p = 0.045$), urea ($p = 0.038$), triglyceride level ($p = 0.027$) and cholesterol ($p = 0.031$); direct correlation with the level of HbA1c ($r = 0.202$; $p = 0.0038$). The level of NT-proBNP has a significant predictive value for the risk of developing cardiovascular complications in patients with hypertension.

Keywords: natriuretic peptide, type 2 diabetes, arterial hypertension, obesity.