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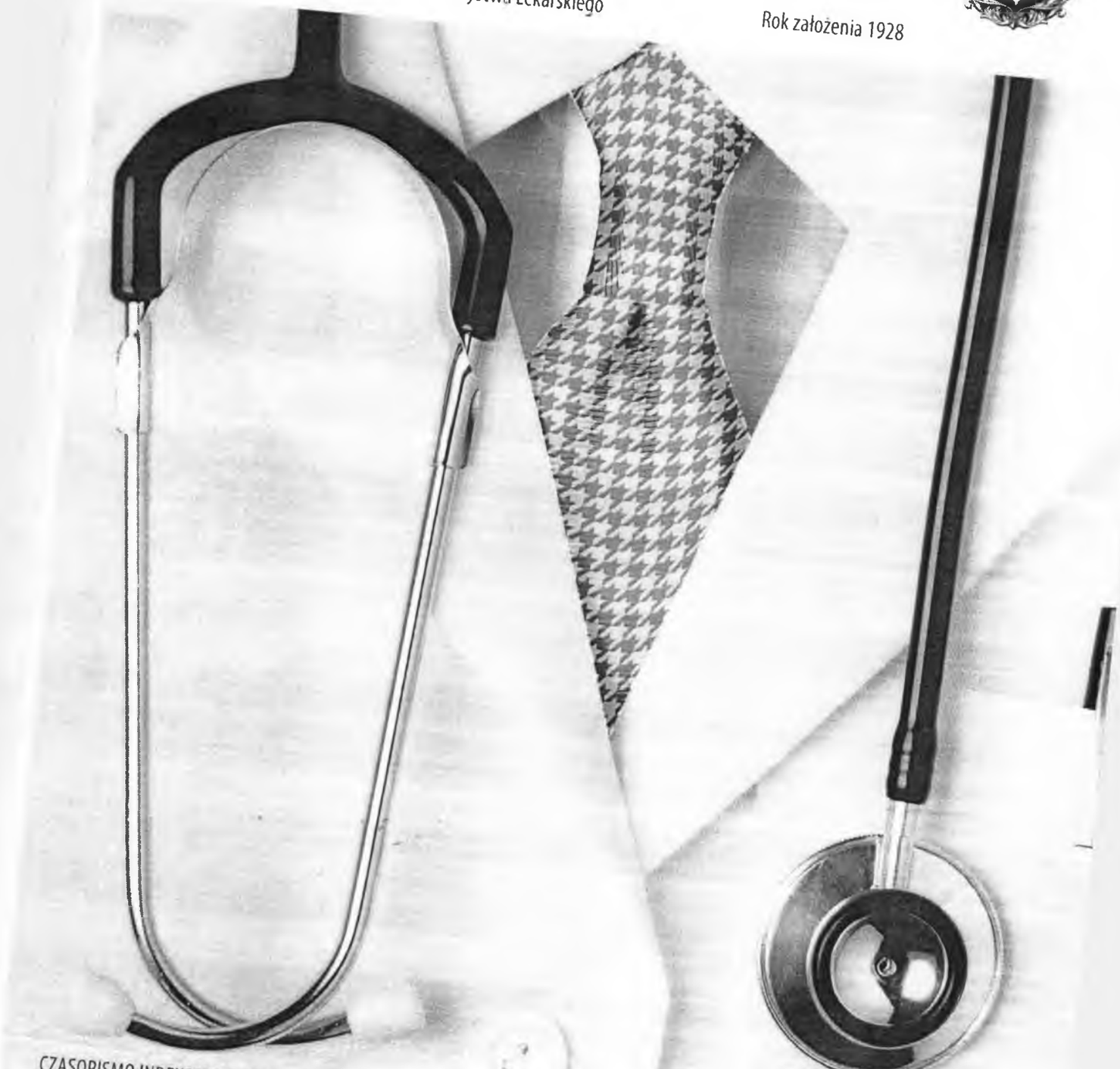

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CHANGES IN THE SECRETARY ABILITY OF MONONUCLEAR CELLS IN PATIENTS WITH ATHEROSCLEROSIS AND ACCOMPANYING PATHOLOGY, DEPENDING ON THE C-PEPTIDE BLOOD PLASMA

Olena O. Karlova[1], Olena V. Kuzminska[2], Alina I. Baryshnik[2]
[1]P.L. SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE
[2]O.O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Introduction: Atherosclerosis is a trigger in the development of cardiovascular disease. Complications of atherosclerosis give reason to search for new criteria, diagno concepts, treatment methods and active preventive measures.

The aim of our work is to study of the structural changes in the intima-media complex of the common carotid artery, pro-inflammatory cytokines (TNF-α, IL-6) secretec mononuclear cells; the level of the intercellular adhesion molecule (according to sICAM-1), the level of the C-peptide of the blood as well as the study of the relationship betw these factors affecting the development of atherosclerosis.

Materials and methods: In the group of 110 patients are studied the levels of secretion of TNF-α, IL-6, the soluble intercellular adhesion molecule-1, the le of blood C-peptide, performed of duplex scanning of the brachiocephalic vessels, studied of biopsy of the skin.

Results and conclusions: In the group of patients with atherosclerosis and the accompanying metabolic syndrome, endothelial activation is noted under the influence of factors (hyperinsulinemia, arterial hypertension, hypercholesterolemia), accompanied with the activation of mononuclear cells with marked hyperproduction of proinflammat cytokines (IL-6) and thickening of the intima-media complex of the common carotid artery with an increase in body weight. Patients with metabolic syndrome deve microangiopathy (edema of endothelial cells, thickening and reduplication of the basement membranes, focal reaction of the pericytes).

KEY WORDS: atherosclerosis cytokines, C-peptide, intima-media, microangiopathy, metabolic syndrome

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INTRODUCTION

Atherosclerosis (AS) is a trigger in the development of cardiovascular disease. The literature data indicate that atherosclerotic changes in the main arteries are more and more common at a young age, thereby justifying the need to identify these changes in the early stages of development. Complications of atherosclerosis, leading to disability of the working population, give reason to search for new criteria, diagnostic concepts, treatment methods and active preventive measures. In this regard, the development and implementation of effective prevention to identify risk factors for atherosclerosis, the implementation of preventive and therapeutic measures is quite relevant [1, 2, 3, 4, 5, 6].

One of the conditions causing a high risk of atherosclerosis is metabolic syndrome (MS). The components of this syndrome, on the one hand, are arterial hypertension (AH) and coronary heart disease (CHD), on the other -diabetes mellitus (DM) and obesity [1, 7, 8, 9].

The initiating moment of the development of MS is hyperinsulinemia and insulin resistance of tissues. In clinical practice, along with standard methods for evaluating hyperinsulinemia, the determination of the le of C-peptide in the blood plasma is used. C-peptide i stable fragment of endogenously produced proinsul which is cleaved from the proinsulin molecule to for insulin. Increasing the level of C peptide (hyper-C-pe tidemia) on an empty stomach or after glucose loadi may be evidence of insulin hypersecretion by the pa creas. The content of insulin in serum depends on t clearance of insulin by the liver. Unlike insulin, C-peptic having become detached from the proinsulin molecu is not extracted from the blood plasma by the liver a reflects the "true" insulin secretion by the pancreas 10, 11, 12, 13, 14].

In the pathological chain of formation of endothe al dysfunction, the first reaction is the reaction of t endothelium with the altering factors. The factors activation and prolongation of endothelial dysfunct can be hyperglycemia, reactive free radicals, oxidiz low-density lipoproteins, hypercholesterolemia, high h drostatic pressure arising from arterial hypertension. I is accompanied by an increase in the permeability of t endothelium for immunocompetent cells (lymphocyt

monocytes, neutrophils), their metabolic products, the expression of adhesive molecules (EIAM-1, ICAM-1, VCAM-1). There is a subsequent active proliferation of smooth muscle cells and the production of connective tissue, which forms the basis of the fibrous capsule, which in turn contributes to the progression of atherogenesis, which ultimately leads to the development of vascular thrombosis [3, 13, 15, 16].

The immunological markers of atherosclerosis, the clinical significance of which is the subject of the most intensive studies, include pro-inflammatory cytokines (interleukins IL-1, IL-6; tumor necrosis factor - TNF, etc.), affecting the nature, depth and duration of the immune-inflammatory process.

The nature of the atherosclerotic process in clinical practice is judged by studying the structure of the intima-media complex (IMC) of the common carotid artery [8, 17, 18]. Structural changes in the vascular wall of the common carotid artery are extrapolated to the vascular regions of the body, which indicates the presence of a morphological reorganization of the vascular wall.

A complex of interrelated changes (structural changes in the vascular wall, the formation and development of an immunoinflammatory reaction, hyperinsulinemia, obesity, morphogenesis of angiopathy) in MS and became the subject of our study.

THE AIM

The aim of our work is to study the structural changes in the intima-media complex of the common carotid artery; pro-inflammatory cytokines (TNF-α, IL-6) secreted by mononuclear cells; the level of the intercellular adhesion molecule (according to sICAM-1); the level of the C-peptide of the blood, as well as the study of the relationship between these factors affecting the development of atherosclerosis.

MATERIALS AND METHODS

The study was based on 110 patients who were divided into 3 groups. The first group - 40 patients with MS (mean age 53.2 ± 4.0 years); the second - 40 patients with coronary artery disease (stable angina II-III FC (functional class) in combination with hypertension (GB) II degree and MS (average age 55.0 ± 1.5 years); the third group - 30 patients with GB II degrees in combination with MS (mean age 45.4 ± 4.1 years). The control group consisted of 20 healthy individuals matched by age and sex with the main group.

All patients gave written consent to participate in the study, in accordance with the requirements of the WHO Ethics Committee.

The diagnosis of coronary heart disease (CHD), hypertensive heart disease (HD), metabolic syndrome (MS) was established on the basis of history, physical examination, laboratory and instrumental (ECG-electrocardiography, VEM-cycle ergometry) methods, in accordance with the recommendations of the expert group WHO and t Ukrainian Cardiology Association.

Duplex scanning of the brachiocephalic vessels was pe formed using a 7.5 mm linear transducer using an Alol 3500 apparatus using a standard technique.

To establish the functional ability of immune cel (monocytes, lymphocytes) that participate and initia immuno-inflammatory reactions, the levels of secretic of TNF-α, interleukin 6 (IL-6) in serum were determine using enzyme immunoassay PgoCon. The presence of soluble intercellular adhesion molecule-1 (sIAM-1) in tl serum was also studied using the enzyme immunoassa test system Diaclone (France).

The determination of the level of blood C-peptide wa carried out by immunoradiometric method using reagen of the company IMMUNOTECH (Czech Republic).

For histological examination, pieces of organs wer removed, which were fixed in 10% formalin solution an embedded in paraffin. Subsequently, paraffin sections 5–7 μm thick were stained with hematoxylin and eosi with picrofuchsin according to Van Gieson, Sudan blacl on Weigert elastic, with silver impregnation along Foo

Statistical data processing was performed using standar techniques. The calculation of the degree of deviation fror the norm (DDN) of each indicator was estimated usin the formula: DDN = (n / N-1) x100% (n - indicators c results in the group of subjects, N - indicators of results i the group of donors).

DDN was assessed by the following indicators: stage-a deviation from 0 to 33%, II stage-34-66%, II stage-67-100%, IV stage > 100%, V Art. > 150%, VI Art 200%. Correlation analysis was performed by the Pearson method.

RESULTS AND DISCUSSION

Our studies showed that the level of C-peptide in the tota group of patients with atherosclerosis was 1301 ±0 pM which is significantly higher than the results in the contro group (Table 1).

Given the diversity, heterogeneity and wide variation of indicators characterizing the amount of C peptide, this became the basis to analyze what characteristics were found in patients with hyperinsulinemia (high levels of C peptide in the blood) and without the presence of such (C-peptide is normal). In this regard, we divided all the studied patients with atherosclerosis into two groups: patients with elevated levels of C-peptide (1st group) and patients with normal levels of C peptide (2nd group).

We found that the first group consisted of patients with clinical manifestations of atherosclerosis and MS, who had a high level of IL-6 in vitro (VI DDN), a significant inhibition of the complex intima-media carotid complex with a high level of IL-6 arteries (III DDN) at normal levels of TNF-α. In this group, patients with obesity grade III predominate (70%). In patients of this group, the level of sICAM-1 was moderately elevated, which can be con sidered as depletion of the secretory ability of endothelial

Table I. The distribution of patients in groups depending on the level blood peptide

	Increased blood C-peptide	Normal level blood peptide	Control
TNF-α, in vitro	141,2±23,8	115,6±76,1	122,3±11,93
IL-6 In vitro, pg/ml	906,2±35,0*(VI)	573,2±41,9*(III)	293,2±13,49
IL-6 in vivo, pg/ml	4,8±0,4	4,8±1,3	5,5±0,39
sICAM-1, pg/mg	768,4±41,5*(I)	870,2±95,2*(II)	647,5±19,29
C-peptide, pmol	1802,1 ±40,9*(V)	675,8±92,3	601,7±86,6
Disease duration, years	9,4±1,5 (II)	7,7±1,96 (II)	-
Obesity,%	70% III st., 30% II st	25%, I st., II st., 30%, 45%	-
IMC, mm	0,7±0,14 (III)	0,5±0,22	0,37±0,02
Body weight, kg	116,8±25,2	80,2±22,4	69±12,4

Note. * significant differences between groups ($p < 0.05$)

cells relative to adhesive molecules, due to the long course of the disease (the disease is 5-10 years old).

In the second group of patients, we observed a statistically significant increase in the level of IL-6 in-vitro (III) and sICAM (II) at the normal thickness of the intima-media complex and the level of TNF-α. This group included patients with clinical manifestations of atherosclerosis, coronary heart disease, as well as atherosclerosis and hypertension. The duration of the disease in the group of these patients was up to 5 years.

To establish the relationship between the studied parameters characterizing the secretory ability of mononuclear cells (IL-6 and TNF-α), the degree of endothelial activation (sICAM-1) with the clinical manifestations of atherosclerosis (IMC, disease duration, C-peptide level, degree of obesity), we conducted a correlation analysis in the group of patients with AS.

Direct correlations were established between the number of intercellular adhesion molecules and the common carotid artery intima-media complex (IMC) ($r = 0.5$), the level of blood C-peptide and IMC ($r = 0.8$), C-peptide and disease duration ($r = 0.41$), IMC and the duration of the disease ($r = 0.57$) only in the group of patients with metabolic syndrome.

Thus, according to the literature and according to our data, in the group of patients with atherosclerosis and the accompanying metabolic syndrome, endothelial activation is noted under the influence of risk factors (hyperinsulinemia, hypertension, hypercholesterolemia), accompanied by activation of mononuclear cells with pronounced hyperproduction of proinflammatory cytokines (IL-6). These changes are accompanied by a thickening of the intima-media complex of the common carotid artery and an increase in body weight corresponding to the III degree of obesity. The established relationships between proinflammatory interleukin-6, blood C-peptide level, degree of obesity and IMC indicate not only the participation of these factors in the development of the pathological process, but also the possibility of using them as reliable markers for the development and progression of the atherosclerotic process.

These pathogenetic links in the pathogenesis of the metabolic syndrome with concomitant pathology can cause morphological, structural changes in various organs and systems, including the skin, which is the first to ensure communication of the organism with the environment. Therefore, morphological studies were aimed at identifying changes in skin vessels during MS.

Considering the important role of angiopathy as a pathogenetic component of MS, an in vivo histological examination of skin biopsy specimens was performed in 10 patients with this syndrome. Examples of skin biopsies are presented in Figures 1 and 2.

One can see swelling of the endothelial cells of the dermis, focal proliferation of pericytes around the vessels of the microvasculature, the beginning of the development of focal sclerosis.

It has been found that the skin of the skin has a thickness of about 10 cm (Fig. 1). Significant changes have been developed, which has swelled, prolobiet into the lumen of the blood vessels of the microcircular channel , often almost completely closed it.

Established significant thickening and branching layers of the basement membrane and proliferation of pericytes. Edema was observed in the surrounding connective tissue vessels. A microscopic examination revealed that sclerosis was attached to the above-described changes, which was noted in many vessels and had a focal character.

Histological examination in the capillaries of the dermis observed thickening and reduplication of the vascular wall basal membranes (Fig. 2), which are the result of the long term influence of the pathological effects of hyperinsulinemia, oxidized LDL, activation of lipid peroxidation (LPO), as well as the reaction of the microcirculation.

One can see a thickening and reduplication of the basement membranes of the capillaries of the skin.

Thus, the formation of microangiopathy in patients with MS begins already at the initial stages of development of the pathological process and is manifested by the presence of changes in the form of moderate proliferation of the

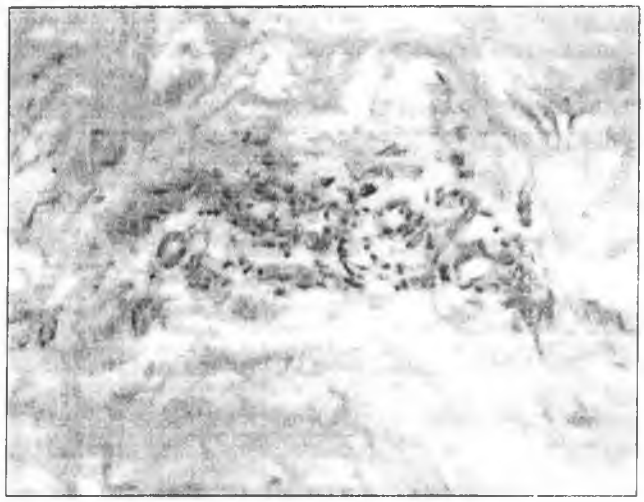

Fig. 1. Biopsy of the skin of the patient 55 years old with MS (light microscopy, Ch400, color by Van Gieson).

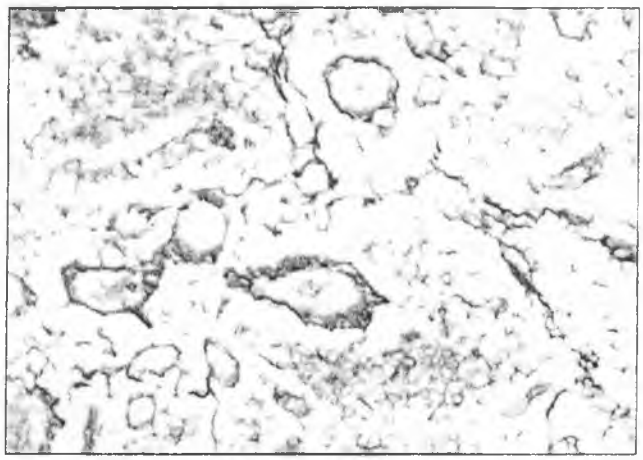

Fig. 2. Biopsy of the skin of the patient 45 years old with MS (light microscopy Ch 400, impregnation with silver on Foot).

endothelium, plasma impregnation of the vascular wall, thickening and branching of the basal membrane layers, and pericyte proliferation.

CONCLUSIONS

1. In the group of patients with atherosclerosis and the accompanying metabolic syndrome, endothelial activation is noted under the influence of risk factors (hyperinsulinemia, arterial hypertension, hypercholesterolemia), accompanied with the activation of mononuclear cells (with marked hyperproduction of proinflammatory cytokines (IL-6) and thickening of the intima-media complex of the common carotid artery with an increase in body weight (corresponding to grade III obesity).
2. Patients with metabolic syndrome develop microangiopathy, which manifests as edema of endothelial cells, thickening and reduplication of the basement membranes and focal reaction of the pericytes of th vascular wall as a result of the action of alteratin factor agents.

REFERENCES

1. Berezin A.E. Diagnostic informatively and prognostic value intestinal regulatory neuropeptides in patients with metabol syndrome. Ukrainian Medical Chronicle. 2013; 1 (93): 23–28 (I Ukrainian)
2. Bokova T.A. Modern view on the etiopathogenesis of metabol syndrome in children. The attending physician. 2013; 2: 74–77.
3. Diagnosis and treatment of metabolic syndrome, diabetes, prediabete cardiovascular diseases. Methodical recommendations of the work grou on metabolic syndrome problems, tactile diabetes and cardiovascula diseases of the Ukrainian Cardiovascular Cardiology Association an Ukrainian Associated Association of Endocrinologists. Kyiv, 2009. 40 (In Ukrainian).
4. Oslopov V.N. Metabolic syndrome in cardiovascular diseases. Th possibility of using the drug Cardionat (meldonium). Russian Medic Journal. 2013; 19: 956 (in Russian).
5. Unified clinical protocol of primary and secondary (specialized) medica aid "Type 2 diabetes mellitus". Order Ministry of Health of Ukraine. 201 1118. Access mode: http://www.dec.gov.ua/mtd/reestr_r.html.
6. Unified clinical protocol of primary, emergency and secondar (specialized) medical aid "Arterial hypertension". Mandate of th Ministry of Health Protection of Health of Ukraine. 2012; 384. Acce mode: http://www.dec.gov.ua/mtd/reestr_r.html
7. Militsia K.M. Multidisciplinary approach to therapy metabolic syndrom and obesity as a guarantee of their effectiveness treatment. Famil medicine. 2015; 4 (60): 38–40.
8. Miloslavsk Yu.O. Influence of biochemical markers on the cours of hypertonic disease of the II stage in conjunction with metabol syndrome. Ukrainian Medical Chronicle. 2012; 5 (91): 129–133.
9. Oshakbayev K.P. Metabolic syndrome: etiology, pathogenesis, diagnosi clinical management and prognosis. Author dis. ... Dr. med. 14.00.0 Internal disease. Almaty, 2010. (in Russian)
10. Goldibi S. Diabetes mellitus and alpha lipoic acid (review). Diabete Obesity. Metabolic syndrome. 2012; 1: 48–58.
11. Management of patients with diabetes mellitus. Recommendation of the American Association for the Study of Diabetes. Rationa Pharmacotherapy. 2010, 2 (15). P. 69–76.
12. Mankovsky B.N. Metabolic syndrome: is there a solution to the problen Medical aspects of women's health. 2007, 3: 4–12.
13. Recommendations of experts of the All-Russian Scientific Society Cardiology for the diagnosis and treatment of metabolic syndrome The second revision. Practical medicine. – 2010, 5 (44): 81–105.
14. Reytberg G.E. Metabolic syndrome.Ed. Corr. RAMS G.E. Roetberg. M MED press-inform. 2007. 224.
15. Michchenko O.I. Leptin resistance, profile of blood pressure and structural-functional characteristics of the myocardium in patients with hypertension and metabolic syndrome. Ukrainian Medical Chronicle 2015; 4 (108): 91–94 (In Ukrainian)
16. Piatnitsa-Gorpinchenko N. Maintaining patients with diabetes mellitu in outpatient practice. Zdorov'ya Ukrainy. 2013; 3 (304): 1–3.
17. Michchenko O.I., Romanov V.Yu. Optimization of treatment and correction of cardiovascular risk in patients with arterial hypertension and metabolic syndrome. Ukrainian Medical Chronicle. 2015; 2 (106): 667–670 (In Ukrainian).

18. Rahman S.T. Effect of α-lipoic acid on endothelial function and proteinuria when quinapril is taken by patients with diabetes and hypertension: results of treatment a QUALITY. Diabetes. Adiposity. Metabolism syndrome. 2013; 2: 65–71

Authors' contributions:
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CORRESPONDING AUTHOR
Olena O. Karlova
Mayakovskogo avenue 3 – 2?, 022?1, Kyiv, Ukraine
e-mail: el_kuz@ukr.net
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