INTRODUCTION

Chronic kidney disease (CKD) is a global public health problem. The number of people with CKD who receive dialysis therapy and patients after kidney transplantation has increased from 340,000 in 1999 to 651,000 in 2010, and this amount is increasing each year [1, 2]. Despite success in researches for effective ways of treatment in recent years, CKD remains one of the most severe and prognostically unfavorable diseases [3, 4].

Vascular endothelium function interruption has the main role among mechanisms of development and progression of CKD, as in kidney, so in all vessels. In numerous experimental and clinical studies, it was proved that activated vascular endothelium is a structural and functional unit that matches processes of inflammation with intravascular coagulation, fibrinolysis and haemorheological disorders [5, 6].

In modern literature, endothelium is considered not only as a barrier between blood and tissues, but also as a neuroendocrine organ that performs many functions, by developing pro- and anti-inflammatory factors, vaso-dilating and vasoconstrictor substances, pro- and antiaggregants, pro- and anticoagulants, - and antifibrinolytic agents, proliferation factors and growth inhibitors. In addition, endothelium has its own renin-angiotensin system [7, 8, 9].

Thus, endothelium can affect systemic arterial pressure (SAP). Under physiological conditions, vasodilation is the synthesis of aggregation inhibitors, coagulation and activators of fibrinolysis, antiadhesive substances predominate. Vascular cell dysfunction disrupts this balance and predisposes vessels to vasoconstriction, leukocyte adhesion, platelet activation, mitogenesis, inflammation [10, 11, 12].

Endothelial dysfunction was originally defined as a violation of vasodilation in response to specific stimuli, such as acetylcholine and bradykinin. In a broader sense, this term may include not only violation of va-
sodilation, but also pro-inflammatory and pro-thrombogenic states associated with endothelial dysfunction [13, 14, 15].

The study of morphological features of CKD has great medical and social importance and requires further scientific research.

THE AIM
To identify special features of endothelium morphological structure in kidney vessels, coronary arteries and aorta during chronic kidney disease.

MATERIALS AND METHODS
Based on autopsy materials, we conducted a morphological study of patients (n = 20) aged 45 to 55 years who were observed in cardiac and neurological hospitals for 5-7 years. The main diagnosis was ischemic heart disease on the background of signs of chronic glomerulonephritis and pyelonephritis. The main cause of death was acute heart failure (n = 15), acute impairment of cerebral circulation in form of ischemic infarcts (n = 2), acute myocardial infarction (n = 3). We removed kidney, heart and aorta samples from patients, then samples were fixed in 10% formalin, and then poured into paraffin. For the study, a histological method was used: staining with hematoxylin and eosin for observational microscopy.

To identify characteristics of immune cell reactions in inflammatory process zones we used primary monoclonal antibodies (MCAB) from DAKO (Denmark), Ready-to-Use. We revealed expression of T- and B-cell differentiation clusters (CD3, CD20), marker of plasma cells (CD38), macrophage marker (CD68) using immunohistochemical method. Features of endothelialization of intimal vessels were studied by expression of endothelial cells marker (CD31 JC 70A), tendency to vascularization was assessed using vascular endothelial growth factor (VEGF (VG1)). As marker of apoptosis, bcl-2 (124) was used.

Material for immunohistochemistry study was fixed with 10% neutral formalin for 24 hours, poured into paraffin, 4 microns thick sections were prepared which were applied to Super Frost high-adhesive slides and dried at 37 °C for 18 hours. Demasking heat treatment was performed by method of boiling sections in citrate buffer (pH 6.0). To visualize the primary antibodies, the UltraVision Quanto Detection Systems HRP Polymer (Thermo scientific) was used. We also used DAB (diaminobenzidine) as chromogen.

To mark severity of the immunohistochemical label, a semiquantitative scale was used: + - weak, ++ - moderate, +++ - severe reaction. The complex of morphological studies was carried out using the Primo Star microscope (Carl Zeiss) using the AxioCam (ERc 5s). Our study was conducted according to conditions specified in the methodical recommendations of Ministry of Health of Ukraine, and the ethical principles of WMA Declaration of Helsinki ethical principles for medical research involving human subjects. (Conclusion of ethical commission of KhMAPO №1 from 9.01.2014).

RESULTS
In each observation, histological structure of kidneys had its own features. In most cases, on the background of diffuse congestion of cortical and cerebral layers we found erythroptosis, diapededic hemorrhages and moderate edema. There was also congestion of glomeruli and areas of shrinkage in kidneys’ parenchyma in form of atrophy and sclerosis. In tubules epithelium protein dystrophy, zones of necrobiosis of individual epithelial cells and small groups of cells were observed. We also observed focal and, in places, diffuse lymphohistiocytic infiltration of stroma. We also found groups of tubules in state of thyroidization.

The walls of most vessels were defibrated and unevenly thickened due to foci of moderate and severe sclerosis. The intimas’ endotheliocytes of vessels and capillaries were flattened. In some of the vessels we revealed detachment of endotheliocytes and destruction of vessels’ wall. In some cases, desquamation and focal necrosis of the endothelium with poorly expressed polynucleic infiltration were revealed. There was also an increase in heterogeneity and a decrease in ordering of cells location, which, in our opinion, caused a low degree of endotheliocyte binding among themselves and, in turn, leads to an increase in permeability of the vascular wall.

We observed presence of pycnotic nuclei, nuclei with karyorexis and karyolysis, a microplasmatosis in many cells. Besides, the expression of bsl-2 could be assessed as moderate and even severe (+++), though in tubule epithelial cells cytoplasm, in the kidney vessels intima, endotheliocytes of the glomerular capillaries, it was negative (-). We regarded this data as a manifestation of oxidative stress and inability to restore the endothelial layer integrity (Figure 1).

The level of VEGF expression in vessels walls structures of the kidneys showed a similar pattern: the endothelial growth factor either did not appear at all, or showed as weak (+), and in isolated cases, moderate (++) immune response.

In our study, the inflammatory infiltrate was defined as moderate and, in some places, severe - from 4-8 to 10-12 mononuclears in the x400 field of view. In some observations, lymphoid cells predominated, with CD3 + status. In some cases, a large number of plasma CD38 + cells were observed in the interstitium. Many of them demonstrated holocrine type secretion and we observedclasmatosis, which is a morphological reflection of humoral immunity activity. CD20 + lymphocytes, CD68 + macrophages and mast cells, as well as plasmocytes in the kidney stroma were detected.

In myocardium samples, we observed signs of acute cardiac death. In all cases, uneven coloration of cardio-myocytes was detected, some muscle fibers lost transverse
striation and expressed eosinophilia. Multiple contractures and sections of decomposition of myofibrils, small foci of fragmentation, areas of undulating deformation of myocardial muscle fibers were observed. Interstitium showed edema, with development of its basophilia. There was observed protein dystrophy of cardiomyocytes, subtotal moderate-expressed hypertrophy, mild lipofuscinosis of cardiomyocytes' cytoplasm.

Signs of spasm and endothelial dysfunction were observed in main trunks of coronary arteries and in their large branches showed. They manifested themselves in the "chalky" location of the endothelium, contours falsity of arteries, infringement of smooth muscle fibers drawn in them, plasma penetration, and thrombus formation zones. We could see narrowing of arteries, exfoliation of the endothelium in small branches; sometimes under it were

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**Fig. 1.** Severe cytoplasmic expression of bcl-2 in the renal tubular epithelial cell group (A), absence of bcl-2 expression in vessel wall structures (B). Reaction with bcl-2 (124), x400

**Fig. 2.** Lipoidosis in small branch of coronary artery with disseminated inflammatory infiltration, frequent conduction of endothelium. Stained with hematoxylin and eosin, x400.

**Fig. 3.** Weak, uneven expression of CD31 in coronary arteries endothelium. Reaction with MKAT CD31 JC 70A, x400.
found erythrocytes or clusters of plasma. In a number of myocardium areas, vessels, especially capillaries and small veins, had different degrees of blood filling: some - sharply expanded, full of blood, others - not. In dilated capillaries, stasis, erythrocyte sludge was found, and in diapedemic hemorrhages were identified perivascularly.

In some observations, small and single-sized coronary arteries with uneven wall thickening were presented due to initial (at the stage of lipidosis) coronarosclerosis. It is narrowed, in the wall - with moderate perivascular productive inflammation (Figure 2).

Reaction with endothelial cells marker showed disturbances in endothelialization of coronary arteries intima. It was weak, uneven, with the exposure of wide intercellular contacts (Figure 3), while the myocardial vascularization was sufficient - 3-5 capillaries per cardiomyocyte (Figure 3).

Cellular infiltration both in arteries walls and in myocardium stroma was extremely meager. In myocardium, positively stained cells were located singly in the interstitium, sometimes perivascularly and showed plasma cell differentiation (CD38 +). In the coronary arteries intima and their branches, the inflammatory infiltrate was detected in the zones of edema, destruction of the vessel wall, and in it CD68 + granulocytes-macrophages and mast cells predominated.

Endothelium, subendothelial layer, plexus of elastic fibers were clearly visualized in aorta inner wall. The endothelium consisted large (up to 500 μm in length and 150 μm in width) flat single-nucleated, often multi-nucleated, polygonal cells located on the basal membrane. The areas of vascular cells enlargement, pockets of desquamation were found. The subendothelial layer was well developed, formed by a loose fibrous connective tissue, which contained thin collagen and elastic fibers, many amorphous substances and non-differentiated cells such as smooth muscle fibroblasts, macrophages. Almost all observations revealed areas of edema, deformation, deposition of cholesterol and fatty acids. Vasa vasorum in this layer were expanded, full of blood, others - not. In dilated capillaries, stasis, erythrocyte sludge was found, and in diapedemic hemorrhages were identified perivascularly.

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The reaction of endothelial cells with CD31 was quite severe, was detected in almost every cell, which differed in different sizes and shapes, formed protrusions and indentations and showed extended intercellular contacts.

The number of inflammatory cell elements in aortic wall was small, the qualitative composition of infiltrate, its location was similar to the coronary arteries intima.

It was difficult to judge the nature of apoptosis only indirectly by reaction with the vascular endothelial growth factor and bcl-2, since apoptotic bodies in coronary arteries and aorta cells intima were not detected. Features of the reaction with the growth factor of the endothelium resembled those in the arterioles and large vessels of kidneys - expression was moderate, in some vessels it was weak, it was detected as tender granules, clumps in endothelial cells, fibroblasts, macrophages. The anti-apoptosis protein bcl-2 in these structures was not detected.

**DISCUSSION**

Immunocompetent cells, which are main components of immune system, are involved in implementation of inflammation and formation of immune response in CKD patients [16]. In our material, single CD20 + lymphocytes, CD68 + macrophages and mast cells and more numerous plasma cells were found mainly in perivascular space. The very similar data were observed in Spatola L. et al. study in 2019 [17].

In myocardium samples with hematoxylin and eosin staining, we observed signs of acute cardiac death, with presence of focal damage of cardiomyocytes. Signs of spasm and endothelial dysfunction were found in main trunks of coronary arteries and in their large branches. The reaction with the CD31 endothelial cell marker more clearly demonstrated uneven intimal endothelialization in coronary arteries. Morgado-Pascual J.L. et al (2018) observed, that common mechanisms involved in CKD, including oxidative stress, inflammation, and uremic toxins, can contribute to renal damage progression by inducing epigenetic modifications [18].

Vascular endothelial growth factor (VEGF) is a biologically active substance, which binds and activates membrane receptors; triggers a signaling cascade that stimulates growth and proliferation of endothelial cells [19]. After new vessels formation, VEGF acts as a survival factor through suppression of endotheliocytes apoptosis. Level of VEGF expression in vascular walls showed a similar picture: either it was not detected at all, or showed a weak, and in isolated cases, a moderate response [20].

Apoptosis plays a decisive role in pathogenic factors that affect cell viability under traumatic effects [21]. One of main participants in cell death program implementation is bcl-2 protein, which blocks mitochondrial pathway for start of apoptosis. The cell is retained at certain points of cell cycle for possible repair of damage or undergoes apoptosis due to a violation of mitochondrial and nuclear membranes permeability. Agents such as calcium ions, inflammation factors, free radicals, and nitrous oxide can also “turn on” genes that initiate apoptosis [22]. In our study bcl-2 expression in vascular intima was weak or negative, which we regarded as a manifestation of oxidative stress and inability to restore the integrity of endothelial layer.
CONCLUSIONS
Morphological study of vessels endothelium of kidneys, heart and aorta demonstrated that in the majority of observations intima underwent profound pathological changes, manifested by different degrees of disorganization of endothelial lining and violations of structural and functional organization of the endotheliocytes, subendothelial layer, basal membrane.

These pathological processes in all cases had similar features with the development of immune inflammation. Inflammatory infiltration was represented by macrophages, mast cells, plasma cells. Biological mediators of the presented cells can aggravate the damage to endothelial cells. Indirect signs of low ability to restore the structure of the vessel wall and endothelial lining may be a weak expression of the VEGF and bcl-2 vascular endothelial growth factor. In conditions of their lack, prerequisites for the activation of apoptosis are created.

REFERENCES

The theme of research: «Improve of diagnostic methods and effectiveness of treatment of chronic glomerulonephritis (CGN) and chronic pyelonephritis (CPN) on basis of studying markers of functional state disturbances and damage of vascular endothelium of kidneys». Code 02/12, State registration number 0112U001097. 01.12-12.14. Head of Research Laboratory: professor Topchiy I.I., PhD, MD.

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