Heart pathomorphological changes in the long-term alcohol consumption

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Alcohol is one of the leading causes of death worldwide, annually taking 3 million lives. Alcohol abuse to a greater degree negatively affects the functioning of the cardiovascular system.

**Aim.** Studying the severity of pathomorphological changes in the heart of deceased patients with alcoholism.

**Materials and methods.** The heart sections of 35 deceased alcoholic patients (38–60 years old) were taken for histomorphometric analysis. As a comparison group, the material of deceased patients (n = 15), suffering from ischemic heart disease (IHD) and hypertension disease (HD) was studied.

**Results.** In conditions of alcoholism in 100 % of cases myocardium showed marked microcirculatory disorders, increased vascular permeability. In all cases sclerotic changes similar in topography to the comparison group were noted, however, sclerosis of large arteries was 1.9 times less pronounced than in IHD group. In the myocardium of alcoholics, edema, atrophy of cardiomyocytes bundles and areas of fatty stroma are common. In 20 % of cases, there were mosaic necrosis of individual cardiomyocytes and small focal necrosis of small groups of cells, fibrinoid necrosis of small branches of coronary arteries. Compared with IHD group the number of fatty degenerated cardiomyocytes increased by 1.23 times, fuchsinophilic cardiomyocytes – by 1.38 times with simultaneous reducing severity of sclerotic changes in vessels by 1.9 times. Sclerotic changes in stroma are significantly higher in relation to IHD and HD groups (by 1.88 and 6.94 times, respectively).

**Conclusions.** The morphogenesis of heart damage during alcoholism consists of combination of dystrophic, necrotic, atrophic and sclerotic changes – morphological substrate of alcoholic cardiomyopathy. These signs, based on quantitative analysis, are important criteria necessary for the thanatogenetic analysis in cases of death on the background of alcoholism.
Alcoholism continues to be one of the main causes of preventable mortality worldwide, causing 3 million deaths annually [1]. In terms of mortality, alcoholism currently ranks third place in world statistics, yielding to ischemic heart disease (IHD) and neoplasms. According to WHO statistics for 2012, Ukraine ranked fifth in the world in terms of alcohol consumption and second in terms of mortality from alcoholism. Also, according to WHO, Ukraine ranks first in the world in terms of child alcoholism. In Ukraine, about 40 thousand people die from alcoholism every year.

The damaging effect of long-maintained high doses of alcohol is most directed to the cardiovascular system [8,9]. Alcoholism is often a direct cause of hypertension [2,3], non-ischemic dilated cardiomyopathy [4], atrial fibrillation [5,6], and stroke [7]. The effect of ethanol and its metabolites on the myocardium leads to the formation of alcoholic cardiomyopathy, which is detected, as a rule, at the late stages of alcoholism [10]. It is known that in sudden death on the background of alcohol intoxication, morphological signs of myocardial dystrophy were detected in 35–50 % of cases [10].

At present, the pathogenetic mechanisms of myocardial damage in chronic alcohol intoxication have been studied quite well [11,12], however, the description of pathomorphological changes in the heart of those who died in alcoholism is limited – in a few works of recent years [13].

Aim
To determine the severity of pathomorphological changes in the heart of deceased patients with alcoholism.

Material and methods
The pathomorphological study based on the results of 35 autopsies of deceased patients with alcoholism (30 men and 5 women) aged 38 to 60 years who were treated in Zaporozhzhia Regional Neuropsychiatric Dispensary. The inclusion criterion was addiction syndrome, established in accordance with ICD-10 diagnostic guidance and recorded in the medical histories of patients. As a comparison group, pathomorphological study of heart of deceased patients (n = 15), suffering from IHD (n = 8) and hypertension disease (HD) (n = 7) was carried out. In the group of alcoholism, 31 observations presented by cases of chronic course of the disease, and 4 cases – by death of alcoholic patients on the background of acute alcohol excess. Cardiopulmonary insufficiency was the cause of death in 74.28 % of patients (26 cases). In 22.85 % of patients (8 cases) death occurred suddenly, as the result of acute left ventricular failure on the background of alcoholic cardiomyopathy. In 5 cases death occurred as the result of complications of liver cirrhosis against the background of acute hepaticenol insufficiency.

An autopsy was performed within 12–24 hours after death according to standard technique. Myocardial parts for histological examination were taken from the region of the apex, the wall of left ventricle with papillary muscles, and the interventricular septum. Fragments of coronary artery branches were also dissected. The sampling was carried out in the amount necessary for standard histological examination. Material was fixed in 10 % formalin solution on 0.1 pH 7.4–7.6 phosphate buffer, embedded in paraffin; sections were prepared with a thickness of 5 μm. Preparations were stained with hematoxylin and eosin, picrofuchsin according to van Gieson, according to Masson. In addition, PAS reaction was used, which allows detecting weak fuchsinophilia of the damaged myocardium. To detect the loss of transverse striation in cardiomyocytes, polarization microscopy was used.

The calculation of the relative area occupied by the studied structural objects was carried out in each case in 5 standard fields of view of the Scope.A1 Carl Zeiss microscope (Germany) using Progres Gryphax Jenoptik 60N-C1*1.0 × 426114 camera (Germany) with magnification ×200 and the morphometric program Vid- eotest-Morphology 5.2.0.158 (VideoTest LLC, Russia). Statistical processing of the data was carried out using the Statistica® for Windows 13.0 package (StatSoft Inc., license No. JPZ8041382130/ARC110-J). The median (Me) and the lower and upper quartiles (Q1; Q3) were calculated; the comparison between the two groups of observations was carried out using the Mann-Whitney U-test. Results were considered statistically significant at 95 % (P < 0.05).
Results

Analyzing macroscopic changes of the comparison group where deceased suffered from IHD and HD, the heart had a characteristic macro- and microscopic picture. The myocardial consistency was tightly elastic, epicardium contained a small amount of fatty tissue; the heart cavities were often dilated, changes in the valve apparatus corresponded to the duration of the disease and the nature of the lesion. In hypertension, the valves were most often without change. With concomitant atherosclerosis, the valve cusps, especially along the trailing edge, were thickened, the chordal filaments were thickened and shortened, but there was no significant deformation of the valve cusps.

In IHD, the heart mass averaged 450.0 ± 20.0 g. The coronary arteries were characterized by narrowed lumens due to roundish whitish-yellow fibrous plaques, located more often segmentally, less often – in a circular character. The degree of stenosis ranged from 50 % to 90 %. Microscopic examination revealed small focal diffuse or large focal postinfarction fibrosis. At the periphery of the sclerosis sites, myocardial muscle fibers were in a state of fatty degeneration and contractionally altered cardiomyocytes. Identified single cardiomyocytes in vessels led to the development of dystrophic changes in arteries, which was typical for IHD/HD group. In the arteries, the inner elastic membrane was clearly traced, the thickness of the muscle layer was slightly thinned, and the adventitia membrane was with areas of excision.

There were signs of increased vascular permeability: perivascular edema of varying severity, minor perivascular hemorrhages, plasmorrhagia with the accumulation of PAS-positive substances in the perivascular space. In 5 cases (14.28 %), perivascular and intramural hemorrhages, contracture lesions of varying severity and multiple foci of myocytolysis were noted (Fig. 2A). In single microvessels fibrin thrombi were found. In the history of the disease in such patients, more pronounced manifestations of heart failure were observed in vivo. In cases of death from acute coronary insufficiency, acute microcirculatory disorders were pronounced.

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The presence of interstitial edema in the myocardium and atrophy of the bundles of cardiomyocytes often led to exposure of the vascular bundles, which often could be found in the loose stroma (Fig. 2B). Such microvessels had a sinuous course, often with multiple kinks. The density of adventitious cells on the surface of such vessels was uneven.

Disorders in the microvasculature were often accompanied by flattening in microvessels. A large number of fibroblasts and histiocytes appeared in such areas. Over time, obliteration of the microvessels took place, in the circumference of which the number of collagen fibers increased, spreading between myocytes over considerable distances. Dystrophic changes were observed in myocytes, which were accompanied by the loss of transverse striation, uneven staining of the cytoplasm, and fuzzy borders of individual cells, which was well detected by polarization microscopy (Fig. 3).

In the walls of the coronary arteries and their branches, one could find foci of fibrinoid necrosis, desquamation of the endothelium, plasmorrhagia, perivascular edema.

Along with microcirculatory disorders, sclerotic changes similar in topography as in the comparison group were observed in all cases. However, in the group of IHD foci of atheromatosis were detected in vessels often with peareance, with a few whitish streaks. The wall thickness of the right ventricle was 0.3–0.5 cm, the left – 1.4–1.7 cm. The heart cavities were dilated. The endocardium, especially of the left ventricle moderately thickened, with patches of whitish color. The walls of the coronary arteries, in those who died over the age of 50 years, in most cases were thin, elastic; flat fibrous plaques, revealed in vessels, stenosed the lumen up to 20–30 %.

Microscopic examination of the myocardium in 100 % of cases showed pronounced microcirculatory disorders. Circulatory disorders affected both the arterial and venous circulatory system. Marked dyscirculatory disorders were noted: a complete decline of the lumens in some vessels and overfilling by blood the others. Part of the microvessels was filled with plasma, erythrocyte or leukocyte aggregates, hemolized erythrocytes. Meanwhile, severe circulatory disorders, their combination with pronounced changes in the perivascular space in the form of small-droplet fatty degeneration of cardiomyocytes, sclerotic changes, not accompanied by atherosclerotic changes in arteries, which was typical for IHD/HD group. In the arteries, the inner elastic membrane was clearly traced, the thickness of the muscle layer was slightly thinned, and the adventitia membrane was with areas of excision.
the accumulation of cholesterol crystals and large number of foamy macrophages (mainly in the middle membrane of the arteries and to a lesser extent in the intima), there was presence of dystrophic calcinosis, which was not typical for alcoholism group. The most pronounced sclerosis was in arterioles with a diameter of 30–50 microns. Vascular sclerosis of the microvasculature (diameter 7–15 microns) was also commonly observed. Sclerotic changes in large arterioles were by 1.9 times less pronounced than in patients with IHD. Sclerotic changes in the myocardium were diffuse; uniform perivascular and interstitial components of sclerosis were noted. Sclerotic processes were also expressed in the endocardium, especially in cases of a longer alcohol history. Endocardial thickness was uneven in different divisions (Fig. 4A). In areas of the myocardium that were in contact with the sclerosed sections of the endocardium, pronounced dystrophic changes were observed, since trophic support of these areas occurs from the side of heart cavities. The muscle fibers adjacent to the endocardium were hypertrophied, of different thickness. In hypertrophied myocytes, along with an increase of the cytoplasmic sector and the size of the nucleus, dystrophic changes were also noted, which manifested themselves in limited areas of disappearance of transverse striation along the fiber. Uneven staining of adjacent myocytes was noted due to a change in their physicochemical properties. Areas with severe cardiomyocyte hypertrophy alternated with areas of atrophy, which were located both under the endocardium and intramurally. Between separate bundles of atrophied cardiomyocytes there was a loose connective tissue stroma with alternating areas of more pronounced collagenization.

Accumulations of adipose tissue often found in stroma, which formed small, optically empty cavities with an area of 80–130 μm², were located at considerable distance from each other. Patches of adipose tissue were more often located in close proximity to blood vessels. With a long history of alcoholism, adipose tissue formed significant areas or diffusely penetrated myocardium with stratification of cardiomyocytes bundles into individual cells. The total area occupied by adipose tissue in some parts of the heart amounted to 32 % of the field of view of the microscope with magnification of 200 times (Fig. 5A), however, the average values of the fatty degeneration area/field of view did not exceed 6.5 %. Cardiomyocytes that were adjacent to adipocytes had a wavy course (Fig. 5A), which is regarded as a possible morphological sign of heart rhythm disturbance. Most of these myocytes were characterized by atrophic changes, some of cells were fragmented. Around the bundles and individual myocytes, a varying severity of the cell-mesenchymal reaction was observed with a predominance of histiocytes, macrophages, and lymphocytes.

The pronounced fatty degeneration was observed in papillary muscle cardiomyocytes. The number of such cardiomyocytes amounted to 60 % of the field of view. Severe fatty degeneration of these myocardium areas, apparently, determined valvular dysfunction in such patients, which was recorded in vivo by instrumental methods. Significant foci of karyocytolysis were noted in subendocardial areas (Fig. 5B).

In 20 % of cases, mosaic necrosis of individual cardiomyocytes and foci of necrosis of small groups of cardiomyocytes, as well as fibrinoid necrosis of small branches of the coronary arteries were found in the myocardium, which was well documented in Masson staining (Fig. 6). In such areas, the presence of stasis in small arterioles and capillaries, stromal edema were detected.
Morphometric analysis showed that in comparison with the deceased with IHD the number of cardiomyocytes in state of fatty degeneration increased by 1.23 times (P < 0.05) (average area 5.4 (4.37; 6.27) %) and by 1.38 times (P < 0.05) – the number of fuchsinophilic cardiomyocytes (average area 7.04 (6.16; 8.15) %). The area of sclerotic changes in stroma reached an average of 16.04 (14.4; 17.15) %, which is significantly higher in comparison with IHD and HD group (1.88 and 6.94 times, respectively (P < 0.05)). In conditions of prolonged alcohol intoxication, sclerotic changes were diffuse in nature and combined with significant atrophic changes in cardiomyocytes.

Based on the obtained data, morphological signs of alcoholic cardiomyopathy (ACMP) can be distinguished, which is characterized by arteriosclerosis and thickening of the walls of the coronary arteries without atherosclerotic changes in these vessels or with minimal signs of atherosclerosis, as well as subendocardial, perivascular and diffuse small focal cardiolsclerosis. Uneven hypertrophy and atrophy of muscle fibers, their fragmentation, the presence of adipose tissue accumulations in the stroma and under the endocardium, fatty degeneration of cardiomyocytes and their focal fuchsinophilia are characteristic for ACMP.

**Discussion**

The study showed that in IHD, HB and ACMP there are similar morphological changes in heart, and any specific diagnostic criteria associated with the effect of alcohol on the heart are quite difficult to distinguish. Thus, in addition to the direct action of ethanol on cardiomyocytes, chronic micro- and macroangiopathy, as well as increasing perivascular sclerosis, contribute to the development of ACMP. Due to the pathology of the histochemical barriers and the membrane-toxic action of ethanol to the myocardium, heterogeneous changes increase: atrophy, hypertrophy of muscle fibers and replacement sclerosis. In cardiomyocytes, we revealed pronounced fatty degeneration, both parenchymal and stromal, with an increased volume of adipose tissue in the myocardial interstitium (obesity of heart). These processes, as indicated by a number of authors, are progressing due to increasing changes in the metabolic, synthetic and hematopoietic functions of the liver, as well as the result of the neuropathic effect of ethanol on the conduction system of the heart. Our analysis also allows us to say that alcoholism does not exclude the development of atherosclerosis, but atherosclerotic vascular changes are much less pronounced in comparison with IHD and HD. Previous studies confirm that pathomorphological changes in alcoholic cardiomyopathy, IHD, idiopathic dilated cardiomyopathy are not much specific to allow differential diagnosis of these conditions [13]. In relation to hypertension, it should be noted, that HD is a practically obligate component of the alcoholic lesion of the cardiovascular system [14]. In the pathogenesis of alcoholic hypertension, violation of vascular tone regulation plays leading role, which is determined by toxic effect of ethanol on various parts of the nervous system [15].

Macroscopic changes in heart of patients with alcoholism are characterized by dilatation of heart cavities, excessive accumulation of fatty tissue in the epicardium, while the heart mass was less than in comparison groups.
The analysis of histological preparations revealed a combination of dystrophic, microcirculatory, sclerotic, atrophic and necrotic changes. Among the most characteristic pathomorphological features of alcoholic cardiomyopathy, microcirculation disorders in the form of aggregation and sludge of erythrocytes in venules, vascular plasmatization, as well as changes in cardiomyocytes such as cardiomyocytolysis and dissociation of cardiomyocytes were noted. Against the background of microcirculatory disorders, stromal edema develops, which is also a characteristic sign of ACMF.

Despite the absence of specific criteria for alcoholic myocardial damage, the most characteristic histological criteria can be distinguished: the alternation of hypertrophic and atrophied cardiomyocytes in a state of severe parenchymal dystrophy; pronounced mesenchymal fatty degeneration; plasmatization of the vascular walls, microcirculatory disorders in the form of erythrocytic stasis, sludge phenomenon and precapillary fibrosis.

Frequently recorded changes in alcoholic damage are sclerotic changes, which in our observations were diffuse, despite the fact that the coronary arteries most often remained intact. Apparently, the pathogenesis of sclerotic changes in alcoholism is not associated with insufficient blood circulation, which was observed in patients with IHD and hypertension, and depends mainly on toxic damage of the vascular wall with the subsequent replacement of irreversibly damaged structures with connective tissue. Most likely sclerosis of arterial network results in decrease of the tone of the intraorganic arteries and, accordingly, the arterial pressure in them also falls, which leads to a decrease in the volume of blood entering the microvasculature. Moreover, it is likely that for a long time the blood supply to organs and tissues is compensated by the formation of new capillaries. However, repeated alcoholic excesses eventually exhaust the compensatory reserves of the body. As a result, the number of capillaries and venules gradually decreases, which leads to progressive metabolic disorders, the progression of chronic hypoxia, followed by increased sclerotic processes in the myocardium. Given the nature of morphological changes in the myocardium, modern therapeutic strategies should be directed on preventing oxidative damage, myocyte hypertrophy, interstitial fibrosis, and cardiomyocyte death [16].

3. Pathomorphological changes in the myocardium that occur during alcoholism, based on qualitative and quantitative methods of analysis are important morphological criteria for establishing thanatogenesis and the immediate cause of death.

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