

## FORENSIC MEDICAL ASSESSMENT OF MORPHOLOGICAL CHANGES IN THE MYOCARDIUM, AFFECTING ITS CONTRACTILE CAPACITY IN CASES OF DEATH FROM ALCOHOLIC CARDIOMYOPATHY

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*For citation:* Sokolova OV, Yagmurov OD, Nasyrov RA. Forensic medical assessment of morphological changes in the myocardium, affecting its contractile capacity in cases of death from alcoholic cardiomyopathy. *Pediatrician (St. Petersburg)*. 2018;9(1):23-28. doi: 10.17816/PED9123-28

Received: 14.12.2017

Accepted: 12.02.2018

A retrospective analysis of acts of forensic medical autopsies from the archive of BSME and a histological study of myocardial tissue in 180 cases (87 women and 93 men) were carried out with statistical processing of the obtained results for the purpose of studying and assessing the morphological changes in the main components of the histohematological barrier of myocardium, affecting the contractility of the cardiac muscle in cases of the death from alcoholic cardiomyopathy. As a result of the study, it was found that the occurrence of metabolic disturbances due to the toxic effects of ethanol and its metabolites contribute to the development of hypoxia of the heart muscle with the development of dystrophic and irreversible necrobiotic processes in it, which in its turn play a direct role in the formation of excitability processes, contractile function of myofibrils with the development of fatal rhythm disorders. The morphological changes in the contractile apparatus of the myocardium were discovered in the study in polarized light and can be used to diagnose alcohol damage to the heart. During the study of deaths from alcoholic cardiomyopathy in forensic medicine, it is recommended to use methods of polarization microscopy.

**Keywords:** alcoholic cardiomyopathy; contracture damages to cardiomyocytes.

## СУДЕБНО-МЕДИЦИНСКАЯ ОЦЕНКА МОРФОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ В МИОКАРДЕ, ВЛИЯЮЩИХ НА ЕГО СОКРАТИТЕЛЬНУЮ СПОСОБНОСТЬ В СЛУЧАЯХ СМЕРТИ ОТ АЛКОГОЛЬНОЙ КАРДИОМИОПАТИИ

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*Для цитирования:* Соколова О.В., Ягмуров О.Д., Насыров Р.А. Судебно-медицинская оценка морфологических изменений в миокарде, влияющих на его сократительную способность в случаях смерти от алкогольной кардиомиопатии // Педиатр. – 2018. – Т. 9. – № 1. – С. 23–28. doi: 10.17816/PED9123-28

Поступила в редакцию: 14.12.2017

Принята к печати: 12.02.2018

Проведены ретроспективный анализ актов судебно-медицинских вскрытий из архива СПбГБУЗ «БСМЭ» и гистологическое исследование ткани миокарда в количестве 180 случаев (87 женщин и 93 мужчины) со статистической обработкой полученных результатов с целью изучения и оценки морфологических изменений основных компонентов гистогематического барьера миокарда, влияющих на сократительную способность сердечной мышцы в случаях смерти от алкогольной кардиомиопатии. В результате исследования выявлено, что возникновение метаболических нарушений вследствие токсического воздействия этанола и его метаболитов способствует развитию гипоксии сердечной мышцы с развитием в ней дистрофических, а также необратимых некробиотических процессов, которые, в свою очередь, играют непосредственную роль в формировании процессов возбудимости, а также в нарушении

сократительной функции миофибрилл с развитием фатальных нарушений ритма. Обнаруженные при исследовании в поляризованном свете морфологические изменения сократительного аппарата миокарда в совокупности могут быть использованы для диагностики алкогольного поражения сердца. При исследовании случаев смерти от алкогольной кардиомиопатии в судебно-медицинской практике следует рекомендовать использовать методы поляризационной микроскопии.

**Ключевые слова:** алкогольная кардиомиопатия; контрактурные повреждения кардиомиоцитов.

## 1. INTRODUCTION

The primary properties of the muscle fibers of the myocardium are their excitation and contractility, which determine the performance of the heart as a pump in order to ensure the circulation of blood in the body. Of note, the contractile function of the heart is an energetically dependent process. Alterations in myocytes caused by ethanol and its metabolites may disrupt this function (metabolic myocardial damage) [1–7].

Undoubtedly, the long-term toxic effects of ethanol and its metabolites lead to the inhibition of cellular energy metabolism and metabolic processes, causing severe dystrophic and necrobiotic changes in cardiomyocytes. Although these changes are not associated with impaired blood circulation in the coronary arteries, they lead to an electrical instability in the myocardium and development of sudden cardiac death [5–9].

The purpose of the present study was to analyze and evaluate morphological changes in the primary components of the histochematic barrier of the myocardium, affecting the contractility of the cardiac muscle in cases of death resulting from alcoholic cardiomyopathy.

## 2. MATERIALS AND METHODS

In this study, paraffin blocks of autopsy material obtained from the St. Petersburg State Budgetary Healthcare Institution Office of the Chief Medical Examiner (SPbSBHI OCME) were used. This material included samples of myocardial tissues (subepicardial, subendocardial, interventricular septum, and papillary muscle) from 180 subjects (87 females and 93 males) collected from 2013 to 2017. The average age of the deceased females and males was  $45 \pm 2$  and  $42 \pm 2$  years, respectively. According to forensic medical research conducted at the SPbSBHI OCME, the immediate cause of death in all the cases under study was acute heart failure induced by alcoholic cardiomyopathy with characteristic morphological features. Concomitant diseases included chronic indurative pancreatitis, chronic bronchitis beyond exacerbation, and portal hypertension with the formation of hepatic cirrhosis. Ethanol (levels ranging between 0.3% and

1.5%) was detected in the blood and urine of all the cases under study.

The histological examination included the preparation of paraffin sections with a thickness of 5  $\mu\text{m}$ , mounted on prepared glass slides. Histological preparations were stained with hematoxylin and eosin, picrofuchsin (van Gieson's method), hematoxylin-basic fuchsin-picric acid (HBFP) (Lee's method), and iron hematoxylin (Regaud's method). The prepared frozen (cryostat) sections were stained using Sudan III dye. Examination via light microscopy was conducted at 20-fold magnification (DP2-BSW microscope, Olympus, Tokyo, Japan). The degree of reduction of the sarcomere and its pathological changes were studied and evaluated using polarized light microscopy.

Statistical processing was performed using the SPSS Statistics software (version 20; IBM, Armonk, NY, USA). Following the statistical analysis, the data obtained were presented as means with half the width of the confidence interval as the margin of error ( $M \pm m$ ). Differences between samples were analyzed using the Mann–Whitney  $U$  test.  $p < 0.01$  denoted statistical significance.

## 3. RESULTS

Plain light microscopy revealed marked proliferation of epicardial adipose tissue with slight spasm of unevenly full-blood arteries, in all samples of myocardial tissue (subepicardial regions), irrespective of the gender and age of the subject. This proliferation was expressed by a plethora of veins and capillaries with the presence in the lumen of individual vessels of the microcirculatory bed of erythrocyte stasis with the sludge phenomenon.

Throughout the study, the myocardium was shown to have a heteromorphic structure. Areas of hypertrophic cardiomyocytes alternated with those of atrophic cardiomyocytes and contained rod-, ovoid-, or round-shaped nuclei (some of which were pyknotic). The nuclei of certain cardiomyocytes had an unusual polygonal shape with perinuclear sarcoplasm clarification and accumulation of lipofuscin grains observed at the poles of the cardiomyocyte nuclei and in the sarcoplasm in the form of thin and wedge-shaped bands.

In the subepicardial, intramural, and subendocardial regions, characterized by pronounced parenchymal protein (granular and vacuolar), fatty, and mesenchymal fatty degeneration, foci of fragmented cardiomyocytes with hyperextension and wavy deformity of muscle fibers with uneven disappearance of transverse striation in them were noted. Moreover, in certain fields of vision in the intramural regions of the myocardium, giant multinuclear cardiomyocytes with regions of muscle fiber branching and excessive formation of randomly located bridges between myocytes were noted.

HBFP staining indicated fuchsinophilia of both individual cardiomyocytes and groups of cardiomyocytes diffusely located in the subendocardial regions of the left ventricle, interventricular septum, and papillary muscle. Intact cardiomyocytes were stained yellowish/greenish. Of note, fuchsinophilia of individual cardiomyocytes was observed in the tissues of the subepicardial regions.

Polarized light microscopy of HBFP-stained samples demonstrated luminescence fields in cardiomyocytes, coinciding with the fuchsin-positive foci in these cells. Subsegmentary contracture damage in cardiomyocytes was observed in the subendocardial regions, interventricular septum, and papillary muscles. This damage was expressed through the repeated reduction of myofibril segments, with uniform luminescence in the regions of the sarcoplasm, and the disappearance of transverse striation in them. Furthermore, in these areas, there were signs of myocyte dissociation, visualized in the form of muscle fiber division into cells or groups of cells through the end plates while maintaining the integrity of the basal plate.

In addition to the observed subsegmental lesions in the cardiomyocytes, segmental contracture foci were revealed in the subendocardial regions and interventricular septum, involving the entire muscle cell. Polarized light microscopy visualized these foci as uniform luminescence of the entire cardiomyocyte. In cases of segmental contractures of degree I, the length of the sarcomeres decreased slightly and the A-disks came slightly closer. In cases of segmental contractures of degree II, there was a noticeable shortening of the sarcomeres and a decrease in the height of the I-disks. Accordingly, with segmental contractures of degree III, the A-disks merged into a single anisotropic conglomerate, the I-disks completely disappeared, and the transverse striation of the myocytes was not observed. In individual muscle fibers, a lumpy decomposition of myofibrils was observed, characterized by a mosaic repeated reduction of sarcomeres,

lysis of nonreduced areas of myofibrils, and focal coagulation necrosis, which was manifested with the histological examination of the uneven staining of the sarcoplasm with alternating dense and light areas. Using polarized light microscopy, the lumpy decomposition of myofibrils was determined through the disappearance of the transverse striation in myocytes, with the presence of multiple lumps of anisotropic substances and pronounced bright luminescence.

Regaud's stain stained the myocardium brown, and muscle fibers were partially or completely stained black or dark gray, which were diffusely located in the subendocardial and interventricular septum regions. Positively stained foci were observed in cardiomyocytes in a state of both hypertrophy and atrophy. In the samples of the subepicardial regions and papillary muscle, the cardiomyocytes were stained black in a single amount.

Changes were noted in the vascular-stromal component of the heart muscle. The dilated myocardial and prominent full-blood veins were determined in all samples of the myocardium in the moderately edematous stroma, combined with unevenly full-blood intramural arteries with signs of moderate spasm, plasmorrhagia of the vascular wall, and presence of small-focus perivascular hemorrhages. Changes were also noted in the vessels of the microcirculatory bed, manifested as swelling, proliferation, dystrophic changes in the endothelium, and development of erythrocyte stasis with the sludge phenomenon in the lumen of individual capillaries with their pronounced plethora with small-focus perivascular hemorrhages.

Using picrofuchsin staining (van Gieson's method), changes in the stromal component were visualized in the form of perimuscular cardiosclerosis, pleximorphic cardiosclerosis, and perivascular cardiosclerosis with manifestations of precapillary fibrosis.

Correlation analysis showed that the morphological changes observed in the parenchymal and stromal-vascular components of myocardial tissue were not statistically significant between the male and female groups ( $p > 0.01$ ). This finding suggests that morphological changes in these components of myocardial tissue present in cases of death caused by alcoholic cardiomyopathy are not directly correlated with age or gender.

The above morphological changes reflect the toxic effects of prolonged consumption of ethanol and its metabolites, leading to severe and irreversible changes in the cardiac muscle [10–12]. As previously shown, ethanol and acetaldehyde suppress the activity of  $\text{Na}^+\text{K}^+\text{-ATPase}$ , resulting in  $\text{Na}^+$  ion

accumulation in cardiomyocytes and loss of  $K^+$  ions. As a result of the disruption of  $Ca^{2+}$ -ATPase activity,  $Ca^{2+}$  ions are supplied in myocytes in large amounts. The resulting disorder of electrolyte-ion homeostasis contributes to the incoordination of the processes of excitation and contractility of cardiomyocytes, consequently leading to impaired contractile function of the heart muscle [10, 11]. In this regard, changes in the properties of contractile proteins of cardiomyocytes, namely, a disruption of  $Ca^{2+}$  and troponin interaction and a decrease in the ATPase activity of myosin, also lead to a disorder of the contractile function of cardiomyocytes. In this case, the disruption of protein and glycogen synthesis in cardiomyocytes caused by ethanol metabolites plays a significant role in the development of myocardial dysfunction. In addition, ethanol and its metabolites cause hypercatecholaminemia through increased synthesis and release of a large amount of catecholamines from the adrenal glands. As a result, the myocardium is under conditions of catecholamine stress. The high levels of catecholamines increase the demand for oxygen in the myocardium, stimulate the metabolism of free fatty acids by free radical (peroxidic) oxidation, induce a cardiotoxic effect, and promote cardiac rhythm disturbance and myocardial overload with  $Ca^{2+}$  ions. Free fatty acids are the main source of energy in the myocardium. Under the influence of ethanol and its metabolites, the process of metabolic oxidation of free fatty acids is inhibited, unlike the process of free fatty acid peroxidation which is activated with the formation of peroxides and free radicals. The latter induces a pronounced damaging effect on the membranes of myocytes, thereby contributing to the development of myocardial dysfunction. Of note, ethanol and its metabolites inhibit the activity of mitochondrial oxidizing enzymes and those of the Krebs cycle and oxidative phosphorylation, leading to a decrease in energy production in the cardiac muscle. The enhancement of peroxide oxidation of free fatty acids also contributes to the impairment of the formation and accumulation of energy in the myocardium. Thus, a decrease in energy production in the myocardium along with a decrease in the activity of  $Ca^{2+}$ -ATPase of the sarcoplasmic reticulum ( $Ca^{2+}$  pump) may lead to a disruption in the contractile function of the myocardium. The resulting changes in the water-electrolyte balance, coupled with disturbances in the processes of energy metabolism in the main components of the histochemical barrier, play a direct role in the formation of excitation processes and, consequently, impact the state of the contractile apparatus of the heart.

The contractile lesions observed in cardiomyocytes, reflecting the pathological total and focal reduction of myofibrils, as well as the lumpy decomposition of the cytoplasm of cardiomyocytes, resulting from the simultaneous reduction of various groups of sarcomeres and lysis of the nonreduced areas of myofibrils, were mosaic in nature. Accordingly, these lesions played a direct role in the emergence of cardiac arrhythmias manifested in the form of focal fragmentation of cardiomyocytes with hyperextension and a wavy deformity of muscle fibers. Moreover, the detected damage to the vascular component of the histochemical barrier is most likely caused by the direct toxic effects of ethanol and its metabolites, increased hypoxia (manifested as increased vascular permeability, perivascular hemorrhages, dystrophic changes, and focal proliferation of endotheliocytes), and the development of erythrocyte stasis with the sludge phenomenon with the discirculatory disorders of microcirculation.

#### 4. CONCLUSIONS

The histological examination of the parenchymal and stromal-vascular components of the histochemical barrier of the myocardium established that the occurrence of metabolic disorders, caused by the toxic effects of ethanol and its metabolites, promotes the development of cardiac muscle hypoxia with the development of dystrophic and irreversible necrobiotic processes. In turn, this effect plays a direct role in the induction of excitation processes and the disruption of the contractile function of myofibrils, leading to the development of fatal rhythm disorders.

The morphological changes in the myocardium contractile apparatus, revealed in the present study using polarized light microscopy, may be used to diagnose alcoholic damage to the heart. In this regard, the use of polarized light microscopy is recommended for the study of mortality caused by alcoholic cardiomyopathy in forensic medicine.

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