

# Seasonal Forensic Medical Aspects of Myocardial Histological Changes in Individuals Who Died of Ischemic Heart Disease

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**Abstract** This article analyzes the forensic aspects of histological changes observed in the myocardium of individuals who died from ischemic heart disease (IHD) during the winter season. Cases with a preliminary diagnosis of IHD were examined during the forensic medical examination process. According to the histological analysis results, dystrophic and necrobiotic changes in cardiomyocytes, increased cytoplasmic eosinophilia, nuclear hyperchromia and polymorphism, interstitial edema, and perivascular congestion predominated in the winter. Additionally, muscle fiber disorganization and increased connective tissue were assessed as signs of remodeling in the context of a chronic ischemic process.

**Keywords** Ischemic heart disease, Winter season, Myocardium, Histological changes, Ischemic-dystrophic process, Interstitial edema, Perivascular congestion, Forensic medical examination, Sudden death

## 1. Introduction

Global climate change is not only raising average air temperatures but also increasing the frequency and intensity of extreme weather events—such as heavy precipitation, thunderstorms, and cyclones. These processes negatively affect human health in several ways and are causing increases in morbidity and mortality [9].

Furthermore, it is projected that the number of heat-related deaths will increase further with future rises in climate temperatures. The 2024 edition of the Lancet Countdown report noted an excessively high number of heat-related deaths among people aged 65 and older in 2023, further highlighting the importance of adaptation measures [9]. It is now recognized that climate change is causing over 150,000 premature deaths globally each year, through direct effects and the exacerbation of environmental problems [5,6,7,8].

A hygienic assessment of the impact of weather conditions on public health not only allows for an understanding of the objective laws of nature but also makes it possible to identify the mechanisms by which environmental factors affect the human body and public health. Through these approaches, it will be possible to develop standards and sanitary and hygienic measures aimed at optimizing living conditions, quality of life, and human activity [2,6].

Furthermore, the implementation of hygienic monitoring and forecasting systems to minimize the negative impacts of weather and environmental conditions is of great importance.

For example, sudden temperature changes, increased humidity, or high concentrations of harmful substances in the air can all lead to an increase in cardiovascular, respiratory, and allergic diseases among the population [3,7]. In this regard, modern sanitary and hygienic measures must be aimed not only at preventing the spread of infections but also at preventing diseases related to atmospheric conditions [1].

Thus, international research conducted in recent years has highlighted the need for modern morphological techniques, such as confocal microscopy, that expand forensic diagnostic capabilities in sudden death cases and increase the accuracy and reliability of expert conclusions.

**Study objective:** To determine the macroscopic and microscopic morphological changes in the myocardium of individuals who died from ischemic heart disease during the winter season, to assess their distinctive characteristics and to scientifically substantiate, from a forensic medicine perspective, the ischemic-hypertrophic and interstitial processes characteristic of the winter season.

## 2. Materials and Methods

Materials from forensic medical autopsies performed on cases of sudden death associated with ischemic heart disease were obtained. A total of 196 cases of sudden death from various seasons were included in the study. All cases were studied at the Samarkand branch of the Republican Scientific and Practical Center for Forensic Medicine from 2021 to 2025.

The material obtained for examination was fixed in a 10% neutral formalin solution and stored at a temperature of +7 °C to +20 °C in conditions protected from the adverse effects of air, moisture, and light. Subsequently, the samples were dehydrated in a water bath at 37 °C, passed through alcohol and toluene, embedded in paraffin, and stained using standard methods. Microslides were examined using a NanoZoomer scanner microscope (REF C13140-21, S/N 000198, Hamamatsu Photonics, Japan). Histomorphometric analyses were performed using the NDP. View 2.0 and Qu00.0url software packages on a scanner with a 7× ocular and objectives ranging from 8× to 40×. Micrographs of the histological specimens were also obtained with this scanner using NDP. Microphotographs of the histological preparations were obtained using NDP. View 2.0 and Qu00.0url software. Statistical analysis of the results was performed on a Pentium-IV computer using Microsoft Office Excel 2021, and Student's t-test was applied.

### 3. Research Results

In this study, through morphometric analysis of histological sections of the left and right ventricles of the heart, the parameters of the nuclei, the thickness of the cardiomyocytes, and the distance between muscle fibers were determined. Based on the obtained data, the relationships between nuclear area and cardiomyocyte thickness and interstitial distance were calculated. In the left ventricle, significant differences were identified in the cardiomyocyte nuclear domain in cases of IHD (Table 1).

In winter, a three-dimensional reconstruction obtained by confocal laser-scanning microscopy showed an increase in structural density in myocardial tissue. In the optical density profile, high-amplitude fluctuations in signal intensity were observed, indicating the heterogeneous nature of the tissue. In vertical profiling, the sharp change in the density gradient coincided with disorganization along the fiber direction and an expansion of the interstitial component. The disorganization index was  $0.62 \pm 0.04$ , which was found to be significantly higher than in the summer ( $p < 0.01$ ) (Figure 1).

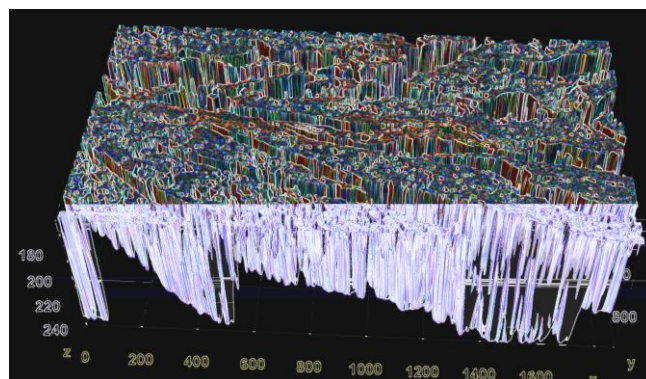
An expansion of the interstitial space up to  $6.1 \pm 0.3 \mu\text{m}$  was assessed in association with microcirculatory disturbances and interstitial edema. A localized enhancement of the fluorescent signal was observed in the perivascular zones, indicating increased metabolic activity or fluid

accumulation around the blood vessel walls. The mean fluorescence intensity was  $1.48 \pm 0.09$  arbitrary units. Overall, in winter, myocardial tissue was characterized by hypertrophic and ischemic-dystrophic changes, with a pronounced uneven distribution and reorganization of structural elements.

In spring, confocal images showed a relatively stable state of myocardial structure. Cardiomyocyte fibers were mostly parallel and orderly, and the signal distribution was uniform. The interstitial distance was  $5.4 \pm 0.3 \mu\text{m}$ , approximately 11% lower than in winter ( $p < 0.01$ ). The disorganization index was  $0.53 \pm 0.03$ , which was significantly lower than in winter ( $p < 0.01$ ) but remained higher than in summer ( $p < 0.05$ ).

Optical profiling showed a stable signal amplitude without sharp fluctuations. Local increases in perivascular intensity were observed less frequently. This indicates a partial recovery of structural elements associated with a reduction in hemodynamic load. However, the incomplete normalization of the interstitial component and the persistence of mild disorganization in some areas suggest the ongoing chronic ischemic process.

In the summer, confocal microscopy revealed the most stable myocardial architecture. Cardiomyocytes were arranged in a clearly parallel orientation, and in the three-dimensional reconstruction, virtually no vertical discontinuities of the fibers were observed. The interstitial distance was recorded at a minimal value of  $4.8 \pm 0.2 \mu\text{m}$ , which was significantly lower than in winter ( $p < 0.01$ ). Fluorescence intensity was  $1.21 \pm 0.06$  arbitrary units, which is the lowest value among all seasons.



**Figure 1.** Confocal multiplex morphometric imaging of the left ventricular myocardium, showing the volumetric measurements of muscle bundles and interstitial stroma. Stain: Shiff. Size:  $10 \times 10$

**Table 1.** Left ventricular myocardial morphometric parameters ( $M \pm m$ )

Indicator	Winter (n=42)	Spring (n=51)	Summer (n=59)	Fall (n=44)	p
Cardiomyocyte diameter, $\mu\text{m}$	$19.3 \pm 0.8$	$18.4 \pm 0.7$	$17.6 \pm 0.6$	$18.8 \pm 0.7$	<0.01
Myodone nucleus, $\mu\text{m}^2$	$44.1 \pm 1.9$	$41.8 \pm 1.7$	$39.6 \pm 1.6$	$42.7 \pm 1.8$	<0.01
Interstitial mass, $\mu\text{m}$	$6.1 \pm 0.3$	$5.4 \pm 0.3$	$4.8 \pm 0.2$	$5.7 \pm 0.3$	<0.01
Fluorescence intensity (short scale)	$1.48 \pm 0.09$	$1.32 \pm 0.07$	$1.21 \pm 0.06$	$1.41 \pm 0.08$	<0.05
Disorganization index	$0.62 \pm 0.04$	$0.53 \pm 0.03$	$0.45 \pm 0.03$	$0.58 \pm 0.04$	<0.01

**Table 2.** Micromorphometric measurements of the right ventricular myocardium (M±m) by season

Indicators	Winter (n=42)	Spring (n=51)	Summer (n=59)	Fall (n=44)
Nuclear area, $\mu\text{m}^2$	38.7 ± 1.6	36.9 ± 1.5	35.4 ± 1.4	37.8 ± 1.5
Cardiomyocyte thickness, $\mu\text{m}$	15.8 ± 0.6	14.9 ± 0.5	14.2 ± 0.5	15.3 ± 0.6
Interstitial distance, $\mu\text{m}$	5.4 ± 0.3	4.9 ± 0.2	4.4 ± 0.2	5.1 ± 0.3
Cardiomyocyte swelling, %	61 ± 3	54 ± 3	46 ± 3	58 ± 3
Presence of ischemic foci, %	69 ± 4	60 ± 3	52 ± 3	65 ± 4

The disorganization index was  $0.45 \pm 0.03$  and was found to be statistically significantly lower than in winter and autumn ( $p < 0.01$ ). Optical profiling revealed a uniform density distribution and stable signal amplitude. A minimal localized intensity increase was observed in the perivascular zones. This indicates a decrease in the intensity of ischemic-dystrophic processes and a relative physiological stability of myocardial structure.

In the fall, the confocal parameters showed a tendency to increase again. The interstitial distance increased to  $5.7 \pm 0.3 \mu\text{m}$ , which was significantly higher than in the summer ( $p < 0.05$ ), but the difference compared to the winter was not statistically significant ( $p > 0.05$ ). Fluorescence intensity was  $1.41 \pm 0.08$  arbitrary units, which was higher than in summer ( $p < 0.05$ ) but not significantly different from winter ( $p > 0.05$ ).

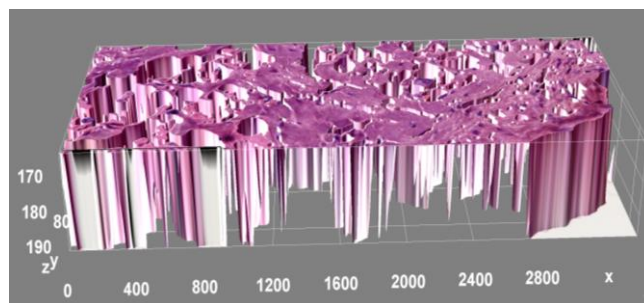
The disorganization index was  $0.58 \pm 0.04$ , significantly higher than in summer ( $p < 0.01$ ). In the three-dimensional reconstruction, partial disorganization of fiber orientation and uneven distribution of the signal gradient were observed. In the perivascular zones, increased intensity was again observed, indicating activation of the microvascular component. Confocal imaging in autumn confirmed that structural elements were approaching the state characteristic of winter.

Confocal microscopy results showed clear dynamic changes in myocardial tissue across the seasons. The winter season was characterized by structural disorganization, interstitial expansion, and high signal intensity, whereas the summer season exhibited the lowest morphofunctional changes. Spring and autumn were manifested as transitional phases, with a gradual redistribution of structural elements observed. Statistical analysis confirmed a significant difference between the seasons for all key parameters ( $p < 0.05$ ).

In cross-sectional analysis, it was found that the micromorphometric parameters of the right ventricular myocardium exhibit dynamic characteristics. In winter, all parameters were recorded at maximum values: nuclear area  $38.7 \pm 1.6 \mu\text{m}^2$ , cardiomyocyte thickness  $15.8 \pm 0.6 \mu\text{m}$ , and interstitial distance  $5.4 \pm 0.3 \mu\text{m}$ . Compared to the summer season, the nuclear area was approximately 9% higher, the cardiomyocyte thickness 11% higher, and the intermyofibrillar distance 23% higher. Cardiomyocyte swelling was observed in 61% of cases in winter, and ischemic foci in 69%, which were significantly higher than the summer figures of 46% and 52%, respectively ( $p < 0.05$ ). This indicates that in winter, against a backdrop of increased hemodynamic load and microvascular disturbances, ischemic-dystrophic processes

intensify.

In spring, the indicators remained at an intermediate level, decreased compared to winter ( $p < 0.05$ ), but were recorded at higher values than in summer. In the summer season, the micromorphometric parameters were at a minimal level, and the structural elements exhibited a relatively stable state. In autumn, however, the indicators showed a renewed upward trend: the nuclear area was  $37.8 \pm 1.5 \mu\text{m}^2$  and the cardiomyocyte thickness was  $15.3 \pm 0.6 \mu\text{m}$ . The differences in autumn compared to summer were statistically significant ( $p < 0.05$ ), but the difference with winter did not reach statistical significance ( $p > 0.05$ ). Overall, the seasonal dynamics in the right ventricular myocardium also exhibit a pattern of “high in winter - low in summer - renewed increase in autumn,” but the changes are somewhat less pronounced compared to the left ventricle (Figure 2).



**Figure 2.** Confocal multiplex morphometric imaging of the tangential section of the anterior lateral wall of the right ventricle showing the volumetric measurements of muscle bundles and interstitial stroma. Stain: G.E. Scale:  $10 \times 10$

## 4. Conclusions

The macroscopic and morphometric indicators of the heart in cases of sudden death associated with ischemic heart disease were found to be variable according to the seasons. In winter, the mean heart weight was 420 g, while in summer it was 380 g ( $p < 0.05$ ). Additionally, the left ventricular wall thickness was 1.6 cm in winter and 1.4 cm in summer, indicating that hypertrophic remodeling processes are more pronounced in winter.

Micromorphometric analysis of the left and right ventricular myocardium revealed a clear seasonal pattern. In winter, the nuclear area was  $44.1 \mu\text{m}^2$  compared to  $39.6 \mu\text{m}^2$  in summer ( $p < 0.01$ ), and ischemic foci were observed in 74% and 55% of cases, respectively. This confirms that the ischemic-dystrophic changes were more pronounced in winter.

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