The dynamics of changes in biochemical markers of the state of tissue in intercostal muscles during the early postmortem period

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***The aim** of study was to evaluate structural and biochemical changes in the tissue of intercostal muscles during the early postmortem period (PMP) – 3-13 hours.*
***Material and methods**. Absolute and relative values of the concentration of glycogen, acid phosphatase, lactate, lactate degydrogenase, lipofuscin and cholinesterase during the early PMP were determined on 30 human corpses by results of study of the tissue of intercostal muscles.*
***Results**. It was proved that the early PMP was characterized by proper biochemical and biophysical changes of the muscular tissue, the most demonstrative of them were as follows: a reduction in the concentration of glycogen and dynamic increases in the concentrations of lactate dehydrogenase and lipofuscin. For all six biochemical markers, representative absolute and relative values of their popstmortem content in homogenates of intercostal muscles depending upon the prescription of death coming were obtained. It was found out that the concentration of glycogen during the analysed time intervals ranged from (7.821±0.0649) mg/g in 3 hours after death coning to (3.204±0.030) mg/g in 13 hours after the coming of death, reliably (p<0.001) differing every 2 hours of PMP. The dynamics in the concentration of lactate were found to be demonstrative and characterized by its progressive (p<0.01) increase within the period of 9 hours from the moment of death coming: from (6.847±0.042) mmol/g after 3 hours to (12.960±0.085) mmol/g after 9 hours. The level of lipofuscin concentration in the analysed time intervals progressively rose too: from (2.258±0.031) U/g in 3 hours to (5.589±0.030) U/g in 13 hours, reliably (p<0.001) differing every 2 hours of PMP.*
***Conclusions.**Paired correlative indices between biochemical and biophysical markers of the state of tissue of intercostal muscles were examined in their systemic relationships and proper system-creating coefficients were determined by six time intervals of the early PMP, in its turn making it possible to substantiate those of them that were criterially significant for increasing the accuracy of diagnosis of prescription of death coming.*

Key words: *muscular tissue, structural and biochemical markers, postmortem period, diagnosis, forensic medicine*

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Dynamika zmian markerów biochemicznych stanu tkanki mięśni międzyżebrowych we wczesnym okresie pośmiertnym

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***Celem** badań była ocena zmian strukturalnych i biochemicznych w tkance mięśni międzyżebrowych podczas wczesnego okresu pośmiertnego (PMP – postmortem period) – 3-13 godzin.*
***Materiał i metody.** Bezwzględne i względne wartości stężeń glikogenu, kwaśnej fosfatazy, mleczanu, degydrogenazy mleczanowej, lipofuscyny i cholinoesterazy we wczesnym PMP określono w 30 ludzkich zwłokach na podstawie wyników badań tkanki mięśni międzyżebrowych.*
***Wyniki**. Wykazano, że wczesny PMP charakteryzował się właściwymi zmianami biochemicznymi i biofizycznymi tkanki mięśniowej, z których najbardziej demonstracyjnymi były: zmniejszenie stężenia glikogenu i dynamiczne zwiększenie stężeń dehydrogenazy mleczanowej i lipofuscyny. Dla wszystkich sześciu markerów biochemicznych uzyskano reprezentatywne wartości bezwzględne i względne ich stężeń popstmortem w homogenatach mięśni śródżebrowych w zależności od nadchodzącej śmierci. Stwierdzono, że stężenie glikogenu w analizowanych przedziałach czasowych wahał się od (7,821 ± 0,0649) mg/g w ciągu 3 godzin po śmierci do (3,204 ± 0,030) mg/g w 13 godzin po nadejściu śmierci, niezawodnie (p <0,001) różniące się co 2 godziny PMP. Stwierdzono, że dynamika zawartości mleczanu jest wyraźna i charakteryzuje się postępującym zwiększeniem (p<0,01) w ciągu 9 godzin od momentu śmierci: od (6,847 ± 0,042) mmol/g po 3 godzinach do (12,960 ± 0,085) mmol/g po 9 godzinach. Stężenie lipofuscyny w analizowanych przedziałach czasowych również stopniowoulegało zwiększeniu: z (2,258 ± 0,031) U / g w 3 godziny do (5,589 ± 0,030) U/g w 13 godzin, znamiennie (<0,001) różniąc się co 2 godziny PMP*
***Wnioski**. Sparowane wskaźniki korelacji między markerami biochemicznymi i biofizycznymi stanu tkanki mięśni międzyżebrowych zbadano w ich układach relacji systemowych, a właściwe współczynniki tworzenia układu określono na podstawie sześciu przedziałów czasowych wczesnego PMP, co z kolei umożliwia uzasadnić te z nich, które były istotne dla zwiększenia dokładności rozpoznania przedawnienia śmierci.*

Słowa kluczowe*: tkanka mięśniowa, markery strukturalne i biochemiczne, okres pośmiertny, rozpoznanie, medycyna sądowa*

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The development of technologies in the fields of modern forensic medical diagnosis, pathomorphology, biochemistry and medicine stimulates scientists to develop informative postmortem criteria for determination of the prescription of death coming (PDC) and to solve urgent problems in the practical work of doctors engaged in forensic medical examinations (FME). But the overwhelming majority of the suggested modern methods of investigation cause difficulties in their introduction into everyday practice, most frequently due to problems with material and technical support for FME [2-6,13,14]. The modern practice

and tactics of forensic medical diagnosis require scientific substantiation of obtainable informative diagnostic criteria at minimum cost for assessing PDC, first of all during the early postmortem period (PMP). The above substantiation is caused by a need for an increased accuracy and can be realized by means of objectification of structural and biochemical changes in different organs and tissues of the human organism [1,10,16,18,19,20]. In particular, the interest of scientists and practitioners in FME is attracted by structural and biochemical changes in the muscular tissue (MT) of different morphological types [9,12,15]. The known methods for diagnosing PDC are based on registration of the available postmortem rigidity of body, appearance of cadaveric lividity and some other diagnostic signs [7,8,11].

Nevertheless the use of the above criteria for diagnosing PDC in the majority of cases is not able to form forensic medical conclusions about PDC with a high reliability and accuracy. At the same time, time-dependent regularities in changes of biochemical (BCM) and biophysical markers (BPM) of the state of MT within the first hours after the coming of death remain insufficiently studied [17].

Postmortem structural and biochemical changes in the tissue of intercostal muscles from the positions of FME of PDC were not studied systematically, this fact determining the purpose of the present research.

The purpose of research consisted in study of structural and biochemical changes in the tissue of intercostal muscles during the early PMP.

MATERIALS AND METHODS

The tissue of intercostal muscles within the early period (3-13 hours) after the coming of death was studied on 30 human corpses. Six BCM in intercostal muscle homogenates (IMH) were determined: BCM_1 – the content of glycogen, BCM_2 – the content of acid phosphatase, BCM_3 – the content of lactate, BCM_4 – the content of lactate dehydrogenase (LDH), BCM_5 – the content of lipofuscin, BCM_6 – the content of cholinesterase. MT was taken with use of special instruments, MT homogenates were prepared following the standard technique [10] with subsequent determination of BCM content in MT homogenates by the kinetic method using for this purpose such test systems as SpineLab (Ukraine), DAC-SpeńtroMed and Vital Development (Russia) on a biochemical analyzer Labline-80 (Austria); the content of lipofuscin was determined according to *I.A. Volchegorsky's* procedure.

Cytological studies of MT preparations of intercostal muscles as well as their photographic recording were made on an Axiostar microscope (Zeiss, FRG). The optic density of nuclei and cytoplasm in conventional units of optic density was measured using VideoTest program (Russia).

Results of the research were statistically analysed with help of variation statistics and assessment of normality of distribution and reliability of findings [15].

RESULTS AND DISCUSSION

The level of glycogen content in IMH during the analysed time intervals significantly ranged: from (7.821±0.0649) mg/g in 3 hours after death coning to (3.204±0.030) mg/g in 13 hours after the coming of death, reliably ($p<0.001$) differing in different time intervals of the early PMP. It should be noted that the variation coefficient of the absolute content of glycogen during the early PMP in all time intervals was below 10%, this fact being characterized as a low level of variation of a sign.

For example (tab.1), as early as in 5 hours after death coming there was a reliable ($p<0.01$) decrease of glycogen content in IMH down to (7.071±0.097) mg/g, in 7 hours it decreased reliably ($p<0.01$) again down to (6.172±0.073) mg/g. During subsequent time intervals a further reduction of the absolute content of glycogen in IMH was registered too: in 9 hours down to (5.365±0.046) mg/g, in 11 hours down to (4.229±0.025) mg/g, and in 13 hours down to (3,204±0,030) mg/g. That is, with an increase of PDC terms the level of glycogen content in IMH continuously and progressively decreased

The level of content of acid phosphatase in IMH during the analysed time intervals ranged significantly from (2.409±0.027) U/g in 3 hours after the coming of death (3.216±0.033) U/g to in 9 hours after death coming, reliably ($p<0.001$) differing during different time intervals of the early PMP. It should be noted that the variation of the absolute value in the content of acid phosphatase was characterized by its increase in time intervals of 3÷9 hours. For example (tab.1), as early as in 5 hours after death coming there was a reliable ($p<0.01$) increase of its content up to (2.662±0.028) U/h, in 7 hours it rose reliably ($p<0.01$) again up to (3.083±0.041) U/h and, having reached its maximum after 9 hours, during subsequent time intervals the content of acid phosphatase decreased. That is, the level of content of acid phosphatase in IMH depended nonlinearly upon PDC terms.

Rather demonstrative were the dynamics of the content of lactate in IMH that was characterized by its progressive ($p<0.01$) increase within the period of 9 hours from the moment of death coming: from (6.847±0.042) mmol/g after 3 hours to (12.960±0.085) mmol/g after 9 hours; after that the above content gradually decreased, reaching the level of (9.088±0.081) mmol/g in 13 hours after the coming of death.

The content of LDH in IMH during the analysed time intervals ranged from (493.0±3.0) U/g in 3 hours after death coming to (235.0±3.4) U/g in 13 hours after the coming of death, reliably ($p<0.001$) differing in different time intervals of the early PMP. It should be noted that the variation of the absolute value in the content of LDH by time intervals was characterized by its continuous decrease (tab.1): as early as in 5 hours after death coming down to (397.2±2.7) U/g, in 7 hours it reliably ($p<0.01$) decreased again down to (353.5±3.5) U/g and sharply decreased down to (299.2±4.7) U/g in 11 hours after the coming of death

The level of lipofuscin content in IMH during the analysed time intervals of the early PMP progressively rose from (2.258±0.031) U/g in 3 hours after death coming to (5.589±0.030) U/g in 13

Table 1. Levels of the content of biochemical markers of the state of intercostal muscles during the early postmortem period depending upon the prescription of death coming
Tabela 1. Zawartość markerów biochemicznych stanu mięśni międzyżebrowych we wczesnym okresie pośmiertnym w zależności od nadchodzącej śmierci

Content of biochemical indicators	Postmortem time intervals (hours)					
	3	5	7	9	11	13
BCM_1 – glycogen, mg/g	7.821±0.064	7.071±0.097[a]	6.172±0.073[a, b]	5.365±0.046[a, b]	4.229 ±0.025[a, b]	3.204±0.03[a, b]
BCM_2 – acid phosphatase, U/g	2.409±0.027	2.662±0.028[a]	3.083±0.041[a, b]	3.216±0.033[a, b]	2.797±0.046[a, b]	2.601±0.029[a, b]
BCM_3 – lactate, mmol/g	6.847±0.042	10.751±0.075[a]	12.155±0.088[a, b]	12.960±0.085[a, b]	10.881±0.11[a, b]	9.088±0.081[a, b]
BCM_4 – lactate dehydrogenase, U/g	493.0±3.0	397.2±2.7[a]	353.5±3.5[a, b]	299.2±4.7[a, b]	256.8±3.6[a, b]	235.0±3.4[a, b]
BCM_5 – lipofuscin, U/g	2.258±0.031	2.489±0.046[a]	2.924±0.038[a, b]	3.439±0.03[a, b]	4.990±0.046[a, b]	5.589±0.03[a, b]
BCM_6 – cholinesterase, U/g	883.5±6.2	790.4±7.3[a]	707.6±10.2[a, b]	645.9±5.0[a, b]	544.9±5.1[a, b]	525.3±5.3[a, b]

Note: [a] – reliable differences from the previous interval at the level of $p<0.05$; [b] – reliable differences from the first time interval at the level of $p<0.01$.

hours after the coming of death, reliably (p<0.001) differing in different time intervals of the early PMP. The variation coefficient of the content of lipofuscin in IMH by time intervals was below 10.0%, it characterizing a low level of variation of a sign.

The level of cholinesterase content in IMH ranged within the limits from (883.5±6.2) U/g in 3 hours after death coming to (525.3±5.3) U/g in 13 hours after the coming of death, reliably (p<0.001) differing in different time intervals of the early PMP, and was characterized by a low level of the variation coefficient.

Analysis of standardized values of BCM revealed that the dynamics of changes in the content of glycogen, lactate and lipofuscin in IMH were the most significant. For example, during the first 3 hours after death coming the content of lactate increased by 57,0%, the content of LDH decreased by 19.0% and later, till 9 hours after the coming of death, these two BCM concentrations continued to change according to the above regularities. But in 9 hours after the moment of death coming, side by side with a further decrease in the content of LDH by 48.0%, the content of glycogen increased (actually twice). So, determinations of such relationships as lactate/LDH within the term of 9 hours after the moment of death coming and glycogen/LDH after 9 hours are diagnostically significant.

It is the above BCM that most manifestly demonstrate regularities of biochemical changes in IMH during the early PMP depending upon PDC.

The conducted research made it possible to objectify changes in myocytes of intercostal muscles (tab.2) by measuring their optic density (OD), determining quantitative regularities and diagnostic informative value (I, bit) of these indices depending upon PDC.

As it can be concluded from the above findings, a change (p<0.01) in OD of myocyte nuclei of intercostal muscles was registered as early as after the 5th hour from the moment of death coming, when it decreased more than by 10% versus the 3rd hour from the moment of death coming and was, respectively, (140.6±0.9) U and (125.9±0.7) U. A similar regularity characterized changes (p<0.01) in OD of cytoplasm: its reduction was registered by the 5th hour after the coming of death, when it decreased from (164.1±1.1) U to (149.4±0.8) U. Taking into consideration the revealed changes of OD in nuclei and cytoplasm that were nonlinear in time, we found out statistical regularities of these processes in intercostal muscles, which are presented in the form of polynomes.

Indeed, pursuing the aim of substantiation of criterial significance for certain morphological, biochemical and biophysical markers of changes in the tissue of intercostal muscles during the early PMP and taking into consideration the consistency of their postmortem changes, we studied correlative relationships between diagnostic signs (markers of changes) and determined the most diagnostically valuable of them (tab. 3).

For example, the most significant indices for diagnosis of PDC by data from intercostal muscles during the early PMP by the system-creating coefficient (SCC) were as follows: BCM_4 – the content of LDH, BCM_6 – the content of cholinesterase, BCM_1 – the content of glycogen and BPM_8 – relative background OD of nuclei (the first four ranks). It should be noted that these four markers of the process of postmortem changes in intercostal muscles (three characterize biochemical changes, and one is for biophysical changes in cytoplasm) are actually equally able to be criteria for assessing PDC.

Consequently, tissues of intercostal muscles during the early PMP are characterized by morphological, biochemical and biophysical regularities, which we found out; the basic of them are: a gradual and continuous reduction of the relative density of cytoplasm (Y_{M-8}) and nuclei (Y_{M-7}); the rate and extent of these dynamics depend nonlinearly upon PDC; we substantiated and received the following quantitative regularities for these biophysical indicators of morphological postmortem changes in intercostal muscles . A comparative morphological study of the tissue of intercostal muscles during the early PMP depending upon PDC was made.

Table 2. Biophysical markers and quantitative-analytical regularities of changes in the optic density of cell nuclei and cytoplasm in intercostal muscles during the early postmortem period depending upon the prescription of death coming
Tabela 2. Markery biofizyczne i prawidłowości ilościowo-analityczne zmian gęstości optycznej jąder komórkowych i cytoplazmy w mięśniach międzyżebrowych we wczesnym okresie pośmiertnym w zależności od nadchodzącej śmierci

Morphological indicators		Postmortem intervals (hours)					
		3	5	7	9	11	13
Optic density of nuclei (BPM_7)	OD, U	140.6±0.9	125.9±0.7	123.4±0.7	114.6±0.6	92.1±0.6	83.5±0.6
	I, bit	0.330	0.254	0.329	0.371	0.403	0.401
Optic density of cytoplasm (BPM_8)	OD, U	164.1±1.1	149.4±0.8	142.2±0.8	132.0±0.6	113.9±0.7	102.7±0.6
	I, bit	0.194	0.102	0.206	0.266	0.251	0.255

Table 3. Correlative relationships between biochemical and biophysical indicators of the state of intercostal muscles during the early postmortem period
Tabela 3. Zależności korelacyjne między wskaźnikami biochemicznymi i biofizycznymi stanu mięśni międzyżebrowych we wczesnym okresie pośmiertnym

Indicators	BCM_1	BCM_2	BCM_3	BCM_4	BCM_5	BCM_6	BPM_7	BPM_8
BCM_1		-0.198	-0.235	0.964	-0.977	0.983	0.990	0.996
BCM_2	-0.198		0.941	-0.407	0.010	-0.328	-0.104	-0.176
BCM_3	-0.235	0.941		-0.476	0.055	-0.380	-0.170	-0.243
BCM_4	0.964	-0.407	-0.476		-0.901	0.990	0.946	0.968
BCM_5	-0.977	0.010	0.055	-0.901		-0.944	-0.992	-0.978
BCM_6	0.983	-0.328	-0.380	0.990	-0.944		0.973	0.984
BPM_7	0.990	-0.104	-0.170	0.946	-0.992	0.973		0.996
BPM_8	0.996	-0.176	-0.243	0.968	-0.978	0.984	0.996	
SCC	0.763	0.328	0.378	0.781	0.647	0.767	0.697	0.724
p	3	8	7	1	6	2	5	4

Note: BCM_1 – the content of glycogen, BCM_2 – the content of acid phosphatase, BCM_3 – the content of lactate, BCM_4 – the content of lactate dehydrogenase, BCM_5 – the content of lipofuscin, BCM_6 – the content of cholinesterase, BPM_7 – relative optic density of nuclei, BPM_8 – relative background optic density of cytoplasm, SCC – system-creating; p – rank of a diagnostic sign (marker)

CONCLUSIONS

It was found out that the early PMP was characterized by proper biochemical changes in the tissue of intercostal muscles, the most demonstrative of them were as follows: a reduction in the content of glycogen and a dynamic increase of the content of LDH and lipofuscin. For all six BCM, representative absolute and relative values of their content in IMH depending upon PDC were obtained. Paired correlative indices between biochemical and biophysical markers of the state of the tissue of intercostal muscles were examined in their systemic relationships and proper SCC were determined by six time intervals of the early PMP, thereby making it possible to substantiate those of them that were criterially significant for increasing the accuracy of diagnosis of PDC.

Prospects of further researches should be aimed to study the postmortem dynamics of BCM for the structural and biochemical state of MT of other morphofunctional types in order to scientifically provide accuracy in diagnosing terms of PDC in the practice of FME.

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