Lipofuscin content in the muscle tissue during the early postmortem period: improvement of forensic diagnosis of the prescription of death coming

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Forensic medicine is naturally supported by fundamental sciences, its integration with them contributing to its improvement on the whole, particularly in diagnosis of the prescription of death coming. Scientific achievements and foreign specialists in forensic medicine during recent years have made it possible to significantly deepen our sound knowledge of postmortem phenomena.
The aim *of the research consisted in study of postmortem regularities in the content of lipofuscin in different types of the muscle tissue (MT) for improving accuracy of determination of prescription of death coming.*
Materials and methods. *The content of lipofuscin was determined in homogenates of the myocardial, oesophageal, diaphragm and intercostal muscles within the early postmortem period on 30 human corpses. MT was sampled in conditions of postmortem biopsy with use of special instruments; homogenates were prepared following the standard technique with subsequent determination of lipofuscin content in MT homogenates according. Presentation of revealed regularities in changes of lipofuscin content in each type of MT homogenates is provided by building dynamic lines with polynomials of different (2-5) stages and accuracy of reproduction R^2>0.95.*
Results. *By results of biochemical examination of lipofuscin in different types of the MT within different terms of the early postmortem period (PMP) it was proved that its content regularly changed in all types of the above tissue. The analytical and graphical dependences of the change in the content of lipofuscin in muscle tissue within the early PMP made it possible to substantiate relevant nomograms. Limitations for using the nomogram technique are as follows: prescription of death coming more than 13 hours, unknown conditions of the stay of a corpse after the coming of death. Advantages of the technique consist in the integrity of biochemical examination of different types of MT and simplicity in interpretation of findings.*
Conclusion. *The application of the nomogram technique for assessing PDC by lipofuscin content in MT makes it possible to improve the accuracy of diagnosis for terms of the coming of death up to 60 minutes.*

Key words: *forensic examination, prescription of death coming, muscle tissue, lipofuscin*

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Zawartość lipofuscyny w tkance mięśniowej we wczesnym okresie pośmiertnym: poprawa diagnostyki sądowej zagrożenia śmiercią

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Medycyna sądowa jest w naturalny sposób wspierana przez nauki podstawowe, a jej integracja z nimi przyczynia się do jej ogólnej poprawy, szczególnie w diagnozowaniu zagrożenia nadchodzącą śmiercią. Osiągnięcia naukowe i zagraniczni specjaliści medycyny sądowej w ostatnich latach umożliwili znaczne pogłębienie wiedzy na temat zjawisk pośmiertnych.
Celem badań *było zbadanie prawidłowości pośmiertnych zawartości lipofuscyny w różnych typach tkanki mięśniowej (MT) w celu poprawy dokładności określenia zgonu.*
Materiał i metody*. Zawartość lipofuscyny oznaczano w homogenatach mięśni mięśnia sercowego, przełyku, przepony i międzyżebrowych we wczesnym okresie pośmiertnym w 30 ludzkich zwłokach. Próbki MT pobrano w warunkach biopsji pośmiertnej przy użyciu specjalnych instrumentów; homogenaty przygotowano zgodnie ze standardową techniką, a następnie oznaczono zawartość lipofuscyny w homogenatach MT zgodnie. Prezentację ujawnionych prawidłowości zmian zawartości lipofuscyny w każdym typie homogenatów MT zapewnia budowanie linii dynamicznych z wielomianami o różnych (2-5) etapach i dokładność reprodukcji R^2> 0,95.*
Wyniki. *Na podstawie wyników badań biochemicznych lipofuscyny w różnych typach MT w różnych okresach we wczesnym okresie pośmiertnym (PMP) wykazano, że jej zawartość regularnie zmieniała się we wszystkich typach MT. Analityczne i graficzne zależności zmiany zawartości lipofuscyny w MT we wczesnym PMP umożliwiły uzasadnienie odpowiednich nomogramów. Ograniczenia w stosowaniu techniki nomogramu są następujące: zagrożenie śmiercią nadchodzącą dłużej niż 13 godzin, nieznane warunki pobytu zwłok po śmierci. Zaletą tej techniki jest integralność badania biochemicznego różnych rodzajów MT oraz prostota interpretacji wyników.*
Wniosek. *Zastosowanie techniki nomogramu do oceny PDC na podstawie zawartości lipofuscyny w MT pozwala poprawić dokładność rozpoznania pod kątem zagrożenia śmiercią do 60 minut.*

Słowa kluczowe*: badanie sądowe, zagrożenie śmiercią, tkanka mięśniowa, lipofuscyna*

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Forensic medicine is naturally supported by fundamental sciences, its integration with them contributing to its improvement on the whole, particularly in diagnosis of the prescription of death coming (PDC) [2,3,7,8,13]. Scientific achievements of Ukrainian and foreign specialists in forensic medicine during recent years have made it possible to significantly deepen our sound knowledge of postmortem phenomena and processes [1,14,15,17]. But despite the above fact the problem of deter-

mination of PDC remains one of the most important problems in forensic medicine. The modern development of the medical science and practice of determination of PDC demands from specialists a significant reduction in the error down to ± 1 hour and less because high technological capabilities exist for that [4,5,6,9,10,11,14,16]. It is for this reason that an interest in studying informative criteria in the context of determination of PDC is natural.

The purpose of the research consisted in study of postmortem regularities in the content of lipofuscin in different types of the muscle tissue (MT) for improving accuracy of determination of PDC.

MATERIALS AND METHODS

The content of lipofuscin was determined in homogenates of the myocardial (MMH), oesophageal (OMH), diaphragm (DMH) and intercostal muscles (IMH) within the early postmortem period (PMP) (3-13 hours after the coming of death) on 30 human corpses. MT was sampled in conditions of postmortem biopsy with use of special instruments; homogenates were prepared following the standard technique [2,3] with subsequent determination of lipofuscin content in MT homogenates according to *I.A. Volchegorsky's* procedure [16].

The research findings were also analyzed statistically with help of variation statistics and assessment of the normality of distribution and reliability of findings [12]. Information analysis of the pathometric sign (lipofuscin content) was made by calculation of its comparative informativeness (I, bit) for each time interval as $I = -p \times \log_2 p$, where p is the relation between MT homogenates after 3 hours and its content in the relevant postmortem time interval [7,8]. Presentation of revealed regularities in changes of lipofuscin content in each type of MT homogenates is provided by building dynamic lines with polynomials of different (2-5) stages and accuracy of reproduction $R^2>0.95$ [12]. The tabular nomogram was devised by dynamic extrapolation of polynomial dependencies with an interval of 30 minutes.

RESULTS AND DISCUSSION

The analysis of postmortem changes in the content of lipofuscin in MT depending upon time periods of PDC revealed that after 3 hours from the moment of death coming its highest content was in MT of the oesophagus, the least one being in MT of the thoracic diaphragm (respectively, (3.199±0.022) and (2.121±0.024) U/g, p<0.001 (tab. 1).

The level of lipofuscin content in homogenates of MT of the oesophagus during the analyzed time intervals of the early PMP progressively rose from (3.199±0.022) U/g in 3 hours after death coming to (6.140±0.042) U/g in 13 hours after the coming of death, reliably (p<0.001) differing in different time intervals of the early PMP (tab. 1, tab. 2). The variation coefficient of the content of lipofuscin in OMH by time intervals was below 10%, it characterizing a low level of variation of the sign (within the limits of a particular time interval). On the whole, during 10 hours of the early PMP the level of lipofuscin content in OMH rose actually twice and was equal to 192.0% of its initial value.

The levels of lipofuscin content in MMH within the analyzed time intervals significantly ranged from (2.564±0.042) U/h in 3 hours after death coming to (5.026±0.038) 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 coefficient of the content of lipofuscin in MMH during all time intervals was below 10%, it being characterized as a low level of variation of the sign. In homogenates of MT of the intercostal muscles the level of lipofuscin content within the analyzed time intervals of the early PMP progressively rose from (2.258±0.031) U/h in 3 hours after death coming to (5.589±0.030) U/g in 13 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 during time intervals was below 10%, it characterizing a low level of variation of the sign.

The level of lipofuscin content in DMT during the analyzed time intervals of the early PMP progressively rose from (2.121±0.024) U/g in 3 hours after death coming to (5.982±0.050) U/g in 13 hours after the coming of death, reliably

Table 1. Levels and quantitative-analytical regularities in the dynamics of lipofuscin content in different morphological types of the muscle tissue during the early postmortem period depending upon the prescription of death coming
Tabela 1. Poziomy i prawidłowości ilościowo-analityczne w dynamice zawartości lipofuscyny w różnych typach morfologicznych tkanki mięśniowej we wczesnym okresie pośmiertnym, w zależności od zagrożenia śmiercią

Content (Y_5) of lipofuscin and its informativeness	Postmortem time intervals (hours)					
	3	5	7	9	11	13
In homogenates of the myocardial muscles, MMH, U/g I_{M-5} = 5.688 bits	2.564±0.042[a]	3.423±0.022[a]	3.707±0.027[a]	4.147±0.036[a]	4.401±0.038[a]	5.026±0.038[a]
	0.000	0.556	0.769	1.122	1.338	1.903
	$Y_{M-5} = -0.003x^4 + 0.084x^3 - 0.62x^2 + 2.138x + 0.973$; $R^2 = 0.995$					
In homogenates of the intercostal muscles, IMH, U/g I_{R-5} = 1.040 bits	2.258±0.031	2.489±0.046	2.924±0.038	3.439±0.032	4.990±0.046	5.589±0.035
	0.000	0.127	0.198	0.199	0.370	0.146
	$Y_{R-5} = 0.112x^2 - 0.082x + 2.198$; $R^2 = 0.997$					
In homogenates of the diaphragm muscles, DMH, U/g I_{D-5} = 1.877 bits	2.121±0.024	2.468±0.023	3.243±0.044	4.329±0.033	5.007±0.048	5.982±0.050
	0.000	0.254	0.518	0.556	0.242	0.307
	$Y_{D-5} = 0.049x^2 + 0.456x + 1.515$; $R^2 = 0.992$					
In homogenates of the oesophageal muscles, OMH, U/g I_{O-5} = 1.081 bits	3.199±0.022	3.701±0.032	4.406±0.033	4.933±0.047	5.651±0.046	6.140±0.042
	0.000	0.243	0.300	0.183	0.225	0.130
	$Y_{O-5} = -0.009x^3 + 0.103x^2 + 0.291x + 2.809$; $R^2 = 0.997$					

Note: [a] – reliable differences from the previous interval at the level of p<0.05

The studies were conducted following the basic regulations of *Ethical Principles for Medical Research Involving Human Subjects* approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEU Directive No. 609 (dated November 24, 1986), Orders of the Ministry of Health of Ukraine No. 690 (dated September 23, 2009), 944 (dated December 14, 2009) and 616 (dated August 03, 2012).

(p<0.001) differing in different time intervals of the early PMP. The variation coefficient of the content of lipofuscin in DMH during time intervals was below 10%, it characterizing a low level of variation of this sign; on the whole, within 10 hours of the early PMP the level of lipofuscin content in DMH rose more than 1.8 times and was equal to 282.0% of its initial value (tab. 2).

Thus, a common pattern for the content of lipofuscin in different types of MT was characterized by an increase of this content with an increase in PDC terms; besides, the dynamic lines of its changes, that we obtained (tab. 1), became basic ones for substantiating quantitative time dependencies and construction of relevant nomograms for forensic diagnosis of PDC.

The quantitative dependencies between the content of lipofuscin and PDC, that we statistically justified, have the analytical form (polynomial stages 2-5) and their use enabled us to represent the revealed regularity and determine "intermediate" (between time intervals, with the accuracy of at least $p<0.01$) values of lipofuscin content, thereby in its turn making it possible to increase the accuracy in diagnosing PDC.

Besides, using methods of clinical informatics, we calculated informational values for dynamic changes in the content of lipofuscin for each time period and each type of MT; in particular, it was revealed that the total informativeness of determination of lipofuscin for diagnosing PDC by MT of the myocardium was $I_{M-5} = 5.688$ bits, by MT of the intercostal muscles $I_{R-5} = 1.040$ bits, by MT of the diaphragm $I_{D-5} = 1.877$ bits, by MT of the oesophagus $I_{O-5} = 1.081$ bits. It should be noted (tab. 1) that the diagnostic value of determination of lipofuscin content depends upon the type of MT and the term of PDC (time interval of PMP).

In order to apply to practice of forensic examination the regularities, revealed by us in the process of this investigation, and to introduce them into the work of medical examiners we constructed a graphic nomogram and made its simplified (traditional), tabular form (tab. 2) for determining PDC by the level of lipofuscin in different types of MT. The presented nomograms make it possible to determine PDC by both a single diagnostic criterion and several ones; in order to provide accuracy at the level of $p<0.05$ it is enough to use one criterion (for example, "lipofuscin content in MT of the myocardium"), but for improving the accuracy (and in conditions of presence of morphological material) it is necessary to use several criteria.

An example of forensic determination of PDC by the value of lipofuscin content in different types of MT. In natural conditions of examination of a corpse the following morphological material (in the amount of 100 mg) was isolated by means of postmortem biopsy: MT of the myocardium, MT of the oesophagus, MT of the diaphragm, MT of the intercostal muscles. In conditions of biochemical laboratory the above MT fragments (100 mg) were homogenized in chloroform and methanol mixture (2:1) at a temperature of 18÷20°C, after that water-soluble components were extracted by adding the proper amount of distilled water to the homogenate; after the above mixing the samples were centrifuged during 90 seconds at a speed of 2,000 rpm. Following separation of the chloroform (lower) layer 0.2 ml of methanol were added to each sample (for reducing their turbidity); the samples were exposed to ultraviolet irradiation (enhanced photooxidation, removal of retinol residues) during three minutes. Then absorbance was measured using a CF-46 spectrophotometer at a wavelength of 450 nm, and the content of lipofuscin was expressed in absorbance units per gram of MT (U/g). The following values of lipofuscin content were obtained: $MMH_L = 4.53$ U/g, $OMH_L = 5.32$ U/g, $DMH_L = 4.41$ U/g, $IMH_L = 3.79$ U/g. Proceeding from results of biochemical determination of lipofuscin activity in MT homogenates and using the nomogram (tab. 2), one can conclude that PDC varies and corresponds to the following terms: 1) by lipofuscin content in MT of the myocardium – from 10 hours to 10 hours 30 minutes, 2) by lipofuscin content in MT of the oesophagus – from 9 hours 30 minutes to 10 hours, 3) by lipofuscin content in MT of the diaphragm – from 9 hours 30 minutes to 10 hours, 4) by lipofuscin content in MT of the intercostal muscles – from 9 hours to 9 hours 30 minutes. Hence, by data of biochemical examination of the content of lipofuscin in different types of MT, PDC ranged from 9 hours to 10 hours 30 minutes from the moment of sampling of biopsy material. It should be noted that extrinsic factors (factors of the environment, where a corpse

Table 2. The quantitative nomogram for determining the term of PDC depending upon the content of lipofuscin in different morphological types of the human muscle tissue with different localizations.
Tabela 2. Nomogram ilościowy do określania terminu PDC w zależności od zawartości lipofuscyny w różnych typach morfologicznych ludzkiej tkanki mięśniowej o różnych lokalizacjach

Prescription of coming		**Lipofuscin content in homogenate of muscles (Y, U/g)**							
Minutes	**Hours**	**Myocardium, Y_M**		**Oesophagus, Y_O**		**Diaphragm, Y_D**		**Intercostal, Y_R**	
1	2	3	$Y_{M-5} = -0.003x^4 + 0.084x^3 - 0.62x^2 + 2.138x + 0.973$; $R = 0.995$	4	$Y_{O-5} = -0.009x^3 + 0.103x^2 + 0.291x + 2.809$; $R = 0.997$	5	$Y_{D-5} = 0.049x^2 + 0.456x + 1.515$; $R = 0.992$	6	$Y_{R-5} = 0.112x^2 - 0.082x + 2.198$; $R^2 = 0.997$
180	**3 hours**	**2.57**		**3.19**		**2.01**		**2.23**	
210	3 h 30 min.	2.83		3.32		2.15		2.27	
240	**4 hours**	**3.05**		**3.45**		**2.30**		**2.33**	
270	4 h 30 min.	3.24		3.59		2.45		2.40	
300	**5 hours**	**3.39**		**3.73**		**2.61**		**2.48**	
330	5 h 30 min.	3.52		3.88		2.78		2.58	
360	**6 hours**	**3.64**		**4.04**		**2.95**		**2.69**	
390	6 h 30 min.	3.74		4.20		3.12		2.82	
420	**7 hours**	**3.83**		**4.37**		**3.31**		**2.96**	
450	7 h 30 min.	3.92		4.53		3.50		3.11	
480	**8 hours**	**4.01**		**4.70**		**3.69**		**3.28**	
510	8 h 30 min.	4.11		4.87		3.89		3.47	
540	**9 hours**	**4.21**		**5.05**		**4.10**		**3.66**	
570	9 h 30 min.	4.33		5.22		4.31		3.87	
600	**10 hours**	**4.46**		**5.38**		**4.53**		**4.10**	
630	10 h 30 min.	4.61		5.55		4.76		4.34	
660	**11 hours**	**4.79**		**5.71**		**4.99**		**4.59**	
690	11 h 30 min.	4.98		5.87		5.23		4.85	
720	**12 hours**	**5.21**		**6.03**		**5.47**		**5.14**	
750	12 h 30 min.	5.46		6.18		5.72		5.43	
780	**13 hours**	**5.74**		**6.32**		**5.98**		**5.74**	

is after death), which can affect the dynamics of changes in the content of lipofuscin in different types of MT, were not taken into account; the studies were conducted in usual conditions for preservation of corpses. Using morphological data from 30 corpses and PDC, which was verified in them before, we carried out inverse approbation of the nomogram technique for determination of PDC and revealed that the accuracy of determination for the term of PDC ranged within ±(1.0÷1.5) hours, with diagnostic inaccuracies of the first (α) and second (β) type at the level of 10.0-12.0%.

CONCLUSIONS

It was proved that the content of lipofuscin in all MT homogenates changed regularly (and nonlinearly), but the initial and final levels of lipofuscin content differed depending upon the type of MT. Besides, the dynamics in changes of the content of lipofuscin within the time period of 3÷13 hours from the moment of death coming differed upon the type of MT too. The quantitative analytical and graphical dependences of the change in the content of lipofuscin in MT within the early PMP, revealed during the research, made it possible to substantiate relevant nomograms. Limitations for using the nomogram technique are as follows: PDC more than 13 hours, unknown conditions of the stay of a corpse after the coming of death (influence of environmental factors). Advantages of the technique consist in the integrity of biochemical examination of different types of MT and simplicity in interpretation of findings. The application of the nomogram technique for assessing PDC by lipofuscin content in MT makes it possible to improve the accuracy of diagnosis for terms of the coming of death up to 60 minutes.

Prospects of further researches regarding improvement in the accuracy of diagnosis of PDC are related to study of informativeness of other structural-biochemical markers of MT.

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