The study of structural and biochemical changes in the muscular tissue of the oesophagus for solving the problem of diagnosing the prescription of death coming

LIDIYA CHERKASHYNA[1], NATALIYA KONOVAL[2], ANTON SHKLYAR[2], IGOR KYRYCHENKO[3], OLENA HAIDASH[4], NADIIA DEMIKHOVA[5]

[1]Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine; [2]Kharkiv National Medical University, Kharkiv, Ukraine; Military Medical Clinical Centre of the Northern Region, Kharkiv, Ukraine; [4]Lugansk State Medical University, Rubizhne, Ukraine; [5]Sumy State University, Sumy, Ukraine

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Cherkashyna L[1], Konoval N[2], Shklyar A[2], Kyrychenko I[3], Haidash O[4], Demikhova N[5].

[1]V.N. Karazin Kharkiv National University, Kharkiv, Ukraine; [2]Kharkiv National Medical University, Kharkiv, Ukraine; [3]Military Medical Clinical Centre of the Northern Region, Kharkiv, Ukraine; [4]Lugansk State Medical University, Rubizhne, Ukraine; [5]Sumy State University, Sumy, Ukraine

The modern practice and tactics of forensic medical diagnosis require scientific substantiation of informative diagnostic criteria for assessing the prescription of death coming (PDC), first of all during the early postmortem period (PMP).

***The aim** of the study was an evaluation of the concentration changes of biochemical markers (BCM) in muscular tissue (MT) of the oesophagus during the early postmortem period.*

***Material and methods.** MT of the oesophagus within the period of 3-13 hours after the coming of death was studied on 30 human corpses. MT was taken with use of special instruments, MT homogenates were prepared following the standard technique with subsequent determination of BCM content in MT homogenates by the kinetic method using for this purpose test systems.*

***Results.** Absolute and relative values of the concentrations of glycogen, acid phosphatase, lactate, lactate dehydrogenase, lipofuscin and cholinesterase in MT of the oesophagus in the early PMP (3-13 hours) were determined in 30 human corpses by results of study of the above tissue during different time intervals of the early PMP. Tendencies and regularities in the dynamics of these BCM of the state of the oesophagus were determined by relative values (beginning from 3 hours after the moment of death coming). It is proved that in the interval of 3-5 hours after the coming of death the content of lipofuscin increases by 16.0%, while the concentration of cholinesterase and glycogen decreases by 9.0%, and later – till 9 hours after death coming – these BCM concentrations continue 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 concentration of glycogen and cholinesterase by 41%, the content of lipofuscin goes on increasing by 57.0%, while the dynamics of changes in the concentration of acid phosphatase are not the same (they decrease).*

***Conclusion.** Absolute and relative values of the concentration of glycogen, acid phosphatase, lactate, lactate dehydrogenase, lipofuscin and cholinesterase in MT of the human oesophagus during different time intervals of the early PMP were determined. Tendencies and regularities in the dynamics of changes in BCM of the state of the oesophagus were determined by relative values. It is proved that determinations of such relationships as lipofuscin/glycogen or lipofuscin/cholinesterase within the terms of 9 hours after the moment of death coming are diagnostically significant (and of equal worth).*

Key words: *prescription of death coming, early postmortem period, muscular tissue, biochemical markers*

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Badanie zmian strukturalnych i biochemicznych w tkance mięśniowej przełyku jako rozwiązanie problemu rozpoznawania nadchodzącej śmierci

Cherkashyna L[1], Konoval N[2], Shklyar A[2], Kyrychenko I[3], Haidash O[4], Demikhova N[5].

[1]Narodowy Uniwersytet im. V.N. Karazyna w Charkowie, Ukraina; [2]Narodowy Uniwersytet Medyczny w Charkowie, Ukraina; [3]Wojskowe Medyczne Centrum Kliniczne Regionu Północnego, Charków, Ukraina; [4]Ługański Państwowy Uniwersytet Medyczny, Rubiżne, Ukraina; [5]Państwowy Uniwersytet w Sumie, Ukraina

Współczesna praktyka i taktyka sądowej oceny medycznej wymagają naukowego uzasadnienia informacyjnych kryteriów diagnostycznych dla nadchodzącej śmierci (PDC – prescription of death coming), przede wszystkim we wczesnym okresie pośmiertnym (PMP – postmortem period).

***Celem badań** była ocena zmian stężeń markerów biochemicznych (BCM – biochemical markers) w tkance mięśniowej (MT – muscular tissue) przełyku we wczesnym okresie pośmiertnym.*

***Materiał i metody.** MT przełyku w ciągu 3-13 godzin po śmierci badano na 30 ludzkich zwłokach. MT pobrano za pomocą specjalnych przyrządów, homogenaty MT przygotowano zgodnie ze standardową techniką, a następnie oznaczono zawartość BCM w homogenatach MT metodą kinetyczną, stosując do tego celu systemy testowe.*

***Wyniki**. Bezwzględne i względne wartości stężeń glikogenu, kwaśnej fosfatazy, mleczanu, degydrogenazy mleczanowej, lipofuscyny i cholinoesterazy w MT przełyku we wczesnym PMP (3-13 godzin) określono na 30 zwłokach ludzkich na podstawie wyników badań powyżej tkanki w różnych przedziałach czasowych wczesnego PMP. Tendencje i prawidłowości w dynamice tych BCM stanu przełyku określono na podstawie wartości względnych (począwszy od 3 godzin po śmierci). Udowodniono, że w ciągu 3-5 godzin po śmierci stężenie lipofuscyny ulega zwiększeniu o 16,0%, a zawartość cholinoesterazy i glikogenu zmniejsza się o 9,0%, a później – do 9 godziny po śmierci – te stężenia BCM nadal się zmieniają zgodnie z powyższymi prawidłowościami. Ale w 9 godzin po śmierci, wraz z dalszym zmniejszeniem stężenia glikogenu i cholinoesterazy o 41%, stężenie lipofuscyny ulega zwiększeniu o 57,0%, a dynamika zmian zawartości kwaśnej fosfatazy nie są takie same (zmniejszają się).*

***Wniosek.** Określono bezwzględne i względne wartości zawartości glikogenu, kwaśnej fosfatazy, mleczanu, degydrogenazy mleczanowej, lipofuscyny i cholinoesterazy w MT przełyku ludzkiego w różnych przedziałach czasowych wczesnego PMP. Tendencje i prawidłowości w dynamice zmian BCM stanu przełyku zostały określone przez wartości względne. Udowodniono, że oznaczenia takich związków jak lipofuscyna/glikogen lub lipofuscyna/cholinesteraza w ciągu 9 godzin po śmierci, są istotne diagnostycznie (i mają taką samą wartość).*

Słowa kluczowe*: recepta na nadchodzącą śmierć, wczesny okres pośmiertny, tkanka mięśniowa, markery biochemiczne*

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The modern practice and tactics of forensic medical diagnosis require scientific substantiation of informative diagnostic criteria for assessing the prescription of death coming (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 systems of the human organism [1,5,12,16,20].

At the present stage in the development of forensic medicine scientists suggest different differential diagnostic criteria for determining PDC that give positive results and help in receiving solutions for a number of problems, which by this time have not been solved yet. 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 forensic medical examinations [2,3,4,6,14,15,21].

In the above respect, the interest of scientists and practitioners in forensic medical examination (FME) is attracted by structural and biochemical changes in the muscular tissue (MT) of different morphological types [17]. 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 [7-9].

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]. Influence of environmental factors on the process of postmortem changes in MT requires further study too [2,10,11,16-19].

Postmortem structural and biochemical changes in their systemic relationships were not studied, this fact determining the purpose of the present research.

The purpose of the study was an evaluation of concentration changes of BCM in MT of the oesophagus during the early postmortem period.

MATERIALS AND METHODS

MT of the oesophagus within the period of 3-13 hours after the coming of death was studied on 30 human corpses. Six BCM in MT homogenates 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 [11] 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. Results of the research were statistically analysed with help of variation statistics and assessment of normality of distribution and reliability of findings [13].

RESULTS AND DISCUSSION

The level of glycogen content in oesophageal muscle homogenates (OMH) during the analysed time intervals of the early PMP ranged: from (5.249±0.038) mg/g in 3 hours after death coning to (2.178±0.016) mg/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 absolute content of glycogen during the early PMP in all time intervals was below 10%, it 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 OMH down to (4.754±0.030) mg/g, in 7 hours it decreased reliably ($p<0.01$) again down to (4.235±0.022) mg/g. During subsequent time intervals a further reduction of the absolute content of glycogen in OMH was registered too: in 9 hours down to (3.625±0.022) mg/g, in 11 hours down to (2.878 ±0.019) mg/g, and in 13 hours down to (2.178±0.016) mg/g. That is, with an increase of PDC terms the level of glycogen content in OMH continuously and progressively decreased; on the whole, during 10 hours of the early PMP the level of OMH glycogen reduced more than by a factor of 2.4.

The level of content of acid phosphatase in OMH during the analysed time intervals ranged from (4.039±0.036) U/g in 9 hours after death coming to (2.748±0.019) U/g after the coming of death, reliably ($p<0.001$) differing during different time intervals of the early PMP. 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 (3.111±0.023) U/h, in 7 hours it rose reliably ($p<0.01$) again up to (3.898±0.066) U/h and, having reached its maximum after 9 hours, during subsequent time intervals the content of acid phosphatase (versus 9 hours) decreased. That is, the level of content of acid phosphatase in OMH depended nonlinearly upon PDC terms.

A nonlinear regularity is also typical for the dynamics of the lactate content in OMH during 13 hours from the moment of death coming, as it was manifested by its increase in time intervals of 3-5-7 hours after the coming of death – respectively, up to (3.762±0.020) mmol/g and (4.429±0.043) mmol/g, – and later (beginning from 9 hours) reliably decreased down to (2.633±0.039) mmol/g without reaching its initial value. On the whole, in 10 hours of the early PMP the level of lactate content in OMH was 81.0% of its initial value.

The content of LDH in OMH during the analyzed time intervals ranged from (465.6±6.9) U/g in 3 hours after death coming to (308.0±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

Table 1. Concentrations of biochemical markers of the state of the muscular tissue in the oesophagus during the early postmortem period depending upon the prescription of death coming
Tabela 1. Stężenia markerów biochemicznych stanu tkanki mięśniowej w przełyku we wczesnym okresie pośmiertnym

Content of biochemical indicators	Postmortem time intervals (hours)					
	3	5	7	9	11	13
BCM_1 – glycogen, mg/g	5.249±0.038	4.754±0.030[a]	4.235±0.022[a,b]	3.625±0.022[a,b]	2.878±0.019[a,b]	2.178±0.016[a,b]
BCM_2 – acid phosphatase, U/g	2.748±0.019	3.111±0.023[a]	3.898±0.066[a,b]	4.039±0.036[a,b]	3.681±0.039[a,b]	3.338±0.023[a,b]
BCM_3 – lactate, mmol/g	3.266±0.031	3.762±0.020[a]	4.429±0.043[a,b]	3.740±0.028[a,b]	3.371±0.028[a,b]	2.633±0.039[a,b]
BCM_4 – lactate dehydrogenase, U/g	465.6±6.9	442.3±6.3[a]	390.1±3.5[a,b]	364.3±2.5[a,b]	319.2±1.8[a,b]	308.0±3.4[a,b]
BCM_5 – lipofuscin, U/g	3.199±0.022	3.701±0.032[a]	4.406±0.033[a,b]	4.933±0.047[a,b]	5.651±0.046[a,b]	6.140±0.042[a,b]
BCM_6 – cholinesterase, U/g	2717.1±37.1	2497.4±21.4[a]	2127.6±20.4[a,b]	1840.6±17.2[a,b]	1429.1±15.9[a,b]	1281.4±10.1[a,b]

[a] – reliable differences from the previous interval at the level of $p<0.05$;
[b] – reliable differences from the first interval at the level of $p<0.01$; OMH – oesophageal muscular tissue homogenate.

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 (442.3±6.3) U/g, in 7 hours it reliably ($p<0.01$) decreased again down to (390.1±3.5) U/g and went on decreasing down to the level of (308.0±3.4) U/g in 13 hours after the coming of death; on the whole, in 10 hours of the early PMP the level of LDH in OMH decreased and was equal to 66.0% of its initial value.

The level of lipofuscin content in OMH 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. The variation coefficient of the content of lipofuscin in OMH by time intervals was below 10.0%, it characterizing a low level of variation of a sign (within the limits of a particular time interval). On the whole, in 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 level of cholinesterase content in OMH ranged within the limits from (2717.1±37.1) U/g in 3 hours after death coming to (1281.4±10.1) U/g in 13 hours after the coming of death, reliably ($p<0.001$) differing in different time intervals of PMP, was characterized by a low level of the variation coefficient, continuously decreased and on the whole, in 10 hours of the early PMP, reduced and was equal to 47% of its initial value. It should be noted that the dynamics of changes in the content of glycogen and cholinesterase in OMH were actually the same.

Analysis of standardized values of BCM revealed that the dynamics of changes in the content of glycogen, acid phosphatase, cholinesterase and lipofuscin were the most significant. For example, within the interval of 3-5 hours after death coming the content of lipofuscin and acid phosphatase increased by 16,0%, the content of cholinesterase and glycogen decreased by 9.0% and later, till 9 hours after the coming of death, these 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 glycogen and cholinesterase by 41%, the content of lipofuscin went on increasing by 57.0%, while the dynamics of changes in the content of acid phosphatase were not the same (they decreased). So, determinations of such relationships as lipofuscin/glycogen or lipofuscin/cholinesterase within the terms of 9 hours after the moment of death coming are diagnostically significant and of equal worth. It is the above BCM that most manifestly demonstrate regularities of biochemical changes in OMH during the early PMP depending upon the prescription of the coming of death.

CONCLUSIONS

It was proved that in the interval of 3-5 hours after the coming of death the concentration of lipofuscin and lipofuscin increases by 16.0%, while the concentration of cholinesterase and glycogen decreases by 9.0%, and later – till 9 hours after death coming – these BCM concentrations continue 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 concentration of glycogen and cholinesterase by 41%, the concentration of lipofuscin goes on increasing by 57%, while the dynamics of changes in the concentration of acid phosphatase are not the same (they decrease).

Determinations of such relationships as lipofuscin/glycogen or lipofuscin/cholinesterase within the terms of 9 hours after the moment of death coming are diagnostically significant and of equal worth.

Prospects of further researches should be aimed to study the postmortem dynamics of BCM concentrations for the structural and biochemical state of MT of other morphofunctional types (myocardium, diaphragm, intercostal muscles) in order to scientifically provide accuracy in diagnosing terms of the prescription of death coming in the practice of forensic medical examinations.

REFERENCES

1. Bachinsky VT.: Advanced diagnostic capabilities of the previous prescription of death (problems and prospects). Materials of scientific conference – Achievement in the galuse of marine medicine and problem nutrition during medical and medical examinations at the forensic examination and at the forensic examination of corpses. Lviv, 2016; 302-304.
2. Bachinsky VT, Garazdyuk MS, Bezhenar IL, et al.: Could be the same day as death for the fluorescent liquor in the short-wave spectrum. Materials of 98th scientific conference in Bukovynsky state medical university, Chernivtsi 4-5, 2017.
3. Boichuk TM, Bachinskiy VT, Vanchuliak OY, et al.: Statistical and fractal analysis of autofluorescent myocardium images in posthumous diagnostics of acute coronary insufficiency. Biosensing and Nanomedicine. 2014;27. doi.org/10.1117/12.2061280
4. Cherkashyna L, Konoval N, Shklyar A, et al.: The dynamics of changes in biochemical markers of the state of tissue in intercostal muscles during the early postmortem period. Pol Merkur Lekarski. – in print.
5. Chernatska O, Demikhova N, Rudenko T, et al.: Assesment of the lipid profile correction in patients with arterial hypertension and type 2 diabetes mellitus. Azerb Med J. 2019; 1: 95-99.
6. Garazdyuk MS, Bachynsky VT, Vanchulyak OY, et al.: Determining the prescription of death by two-dimensional mapping of polarization-heterogeneous images of polycrystalline liquor films. Clin Exp Pathol. 2016; 15 (1): 36-42.
7. Gladkikh DB.: Forensic diagnostics of the prescription of the onset of death by the supravital reaction of the pupils. Abstract of the PhD dissertation, Kyiv, 2015; 19.
8. Gurov OM, Kozachenko IM, Kutsenko SV, et al.: Determining the prescription of the onset of death in the early post-mortem period by the supravital reaction of the pupils to the action of pilocarpine. Criminalistics and Forensic Medicine: An Interagency Scientific and Methodological Collection. 2014; 59: 443-451.
9. Kuzovkov AV, Vavilov AY.: Objectification of diagnostic algorithms for establishing the limitation of human death by the dynamics of tympanic temperature. Modern Probl Sci Edu. 2017; 2: 12-20.
10. Marushchak M, Krynytska I, Petrenko N, et al.: The determination of correlation linkages between level of reactive oxygen species, contents of neutrophils and blood gas composition in experimental acute lung injury. Georg Med News. 2016; 4(253): 98-103.
11. Marushchak M, Maksiv K, Krynytska I.: ACE gene I/D polymorphism and arterial hypertension in patients with COPD. Pneumologia. 2019; 68: 1-6.
12. Marushchak M, Maksiv K, Krynytska I.: The specific features of free radical oxidation in patients with chronic obstructive pulmonary disease and arterial hypertension. Pol Merkur Lekarski. 2019; 47(279): 95-98.
13. Moskalenko VM, Voronenko YV.: Social Medicine and Health Organization: textbook. Ternopil, 2002; 50-75.
14. Popov SV, Melekhovets OK, Demikhova NV, et al. Application of „reytoil" in prevention of atherosclerosis in diabetes patients. Likarska sprava/Ministerstvo okhorony zdorovia Ukrainy. 2012: 8: 119-126.
15. Prykhodko OA, Gulaya VI, Yarmolenko OS, et al.: Microscopic changes in the organs of rats under conditions of general dehydration of the organism. Azerb Med J. 2016; 4: 95-100.
16. Olkhovsky VO, Golubovich LL.: Determining the prescription of an onset of death. Kharkiv: Brovin OV, 2017; 168.
17. Olkhovsky VO, Golubovich LL, Khizhnyak VV.: Determining the duration of the post-mortem period in forensic expert practice: current state and prospects. Exp Clin Med. 2016; 4(73): 154-162.
18. Smiyan OI, Loboda AM, Manko YA, et al.: Dynamics of content of some minerals in teenagers with cardiovascular system pathology against the background of chronic tonsillitis. Wiadomosci lekarskie. 2018; 71(4): 861-864.
19. Smiyan OI, Smiian-Horbunova KO, Bynda TP, et al.: Optimization of the treatment of rotavirus infection in children by using bacillus clausii. Wiadomosci Lekarskie. 2019; 72(7): 1320-1323.
20. Yarmolenko O, Sikora V, Bumeister V, et al.: Age-dependent cardioprotective action of meldonium on heart remodeling under the experimental hypoosmolar hyperhydration. Bangladesh J Med Sci. 2019; 18 (2): 435-444.
21. Zhaldak DA, Melekhovets OK., Orlovskyi VF. CYP7A1 gene polymorphism and the characteristics of dyslipidemias in patients with nonalcoholic fatty liver disease concurrent with hypothyroidism. Ter Arkh. 2017: 89(10): 62-65. doi.org/10.17116/terarkh2017891062-65

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Address for correspondence:
Demikhova Nadiia
Sumy State University of Sumy
40007 Sumy, Rymsky-Korsakov St. 2, Ukraine
e-mail: n.demyhova@med.sumdu.edu.ua