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COMPARATIVE ANALYSIS OF THE EFFECTIVENESS OF USING HISTOLOGICAL AND PHYSICAL-OPTICAL METHODS OF INVESTIGATING OF THE TIME OF HEMORRHAGE FORMATION IN THE SUBSTANCE OF THE HUMAN BRAIN

M. S. Garazdiuk

Bukovinian State Medical University
(Chernivtsi, Ukraine)

Summary

Important attention in forensic medical practice is given to the solution of issues that arise during the examination in the case of traumatic brain injury (TBI), as this type of injury leads to high mortality and disability. Conducting an autopsy of the body of the deceased, whose death occurred from a TBI, there are cases when the injury occurs as a result of a fall of a person who previously developed a cerebral infarction, and sometimes vice versa. In this case, it is important to accurately verify the primary cause of death, therefore, timely and objective determination of the time of formation of a hemorrhage in the human brain is necessary.

The purpose and tasks of the research. *To evaluate the possibility of detecting time of formation of a hemorrhage of traumatic and non-traumatic genesis by the generally accepted histological method and polarization phase microscopy, and to compare their effectiveness.*

Research materials and methods. *For the study, human brain samples were selected from 140 deceased individuals whose deaths occurred between 1 and 3 days after the onset of bleeding, according to medical records. As a control group, human brain samples were selected from 40 individuals who died of coronary heart disease. Light microscopy was performed by preliminary staining of histologic specimens according to the Perls method. Phase polarization tomography of the samples was performed using a Stokes polarimeter.*

Research results. *Stained human brain preparations of the experimental and control groups were examined and analyzed and it was found that hemosiderin was not present in all experimental samples (only in 31 out of 40 samples were present). It was not possible to establish the corresponding time dependences of the age of the formation of hemorrhages, regardless of the genesis, since the random appearance of the pigment in the experimental samples is noted at different time intervals. Analytical processing of the results of the statistical processing of the topographic structure of tomograms of the anisotropy of linear dichroism of the fibrillar networks of samples of the deceased from the experimental and control groups revealed a wider time range of sensitivity of this method to destructive changes in nervous tissue, in comparison with the polarimetric methods used in previous studies. As a result, there is an accelerated temporal decrease in the absolute values and the range of dispersion of the anisotropy of linear dichroism value with increasing time since the formation of hemorrhage.*

Conclusions. *Polarization-phase microscopy has shown a significant advantage in its use in comparison with the gold standard – the Perls' staining method for determining the time of hemorrhage formation in the human brain.*

Key words: *Craniocerebral Injury; Forensic Medicine; Polarization-Phase Microscopy; Time of Formation of Hemorrhage.*

Introduction

In forensic medical practice, great importance is attached to the solution of issues arising during the examination in the case of traumatic brain injury (TBI), as this type of injury leads to high mortality and disability [1]. According to scientists, the number of TBI in the world is approximately sixty-nine million annually [2]. Since investigators ask forensic experts about the age of the injury and the cause of death, it is necessary to answer these questions as accurately as possible, since the definition of the circle of suspects in the commission of the crime, if such a crime occurred, depends on this. When conducting an autopsy of the body of the deceased, whose death occurred as a result of TBI, there are cases when the injury occurs as a result of a fall of a person who previously developed a cerebral infarction, and sometimes vice versa [3]. In such a case, it is important to accurately verify the primary cause of death, therefore, timely and objective determination of the age of formation of a hemorrhage in the human brain substance is necessary.

For a long time, forensic histological examination remained the gold standard of forensic medical studies of the time of hemorrhage formation [4]. It was taken into account that in the case of primary damage, cell death occurs by necrosis/apoptosis, and later, with the development of secondary damage, the main mechanisms are inflammation and ischemia. In head injury, the blood-brain barrier undergoes both functional damage and more subtle structural changes. Scientific data have shown changes in the endothelial system that promote the extravasation of immunocompetent cells [5]. Some authors have analyzed the benefits of using markers of secondary damage in human brain substance [6-8]. Forkhead Box class O (FOXO) 3a is a transcription factor involved in various molecular processes such as regulation of cell apoptosis, neuroinflammation, and oxidative stress response. This is the first study to evaluate the postmortem immunohistochemical positive dynamics of FOXO3a expression in human TBI deaths. In addition, it was found that the longer the survival time of an individual after TBI, the more pronounced the positive reaction with FOXO3a. However, these methods do not provide an accurate hourly estimate of time of formation of a hemorrhage.

Computed tomography is successfully used for the diagnosis of TBI, but practically does not provide data on establishing the time of formation of hemorrhages [9-10]. Good temporal dynamics of degenerative changes in nerve tissue after the onset of death are demonstrated by physico-

optical research methods, which indicates the possibility of their use for the diagnosis of time of formation of a hemorrhage in human brain substance [11].

The purpose and tasks of the research. To assess the possibility of establishing of the time of formation of a hemorrhage of traumatic and non-traumatic genesis by the generally accepted histological method and polarization-phase microscopy, as well as to compare their effectiveness.

Research materials and methods

For the study, histological preparations stained by the Perls method and native sections of human brain substance on glass from 140 deceased persons with a known time of hemorrhage formation from 1 to 3 days, according to the medical documents, were used in the case of death from: hemorrhages of non-traumatic origin – 50 histological samples each (group 2), from cerebral infarction of ischemic origin – 40 histological samples each (group 3), death from hemorrhages of traumatic genesis – 50 histological samples each (group 4). For control, human brain substance samples were taken from 40 deceased in case of death from coronary heart disease – 40 samples each (group 1). As a research method, light microscopy of histological preparations of human brain substance at a magnification of 400 times, stained according to the Perls method (reaction to Berlin azure) was used. For a detailed description of the method, see source [12]. Statistical evaluation of the obtained data was carried out by using the non-parametric Kruskal-Wallis test.

Polarization-phase tomography of the studied samples was studied as follows [13-15]:

1) Mueller-matrix mapping of test samples and obtaining a series of Mueller-matrix images of their polycrystalline structure;

2) calculation of coordinate distributions of the elements of the differential matrix of the 1st order;

3) algorithmic reconstruction of distributions of linear birefringence of the polycrystalline structure of histological brain sections;

4) within the map of each parameter of optical anisotropy, criteria for objective differentiation of human brain substance samples of the deceased from all groups were identified;

5) Statistical evaluation was carried out by calculating statistical moments of the 1st-4th orders, namely: SM1 – average; SM2 – dispersion; SM3 – asymmetry; SM4- – kurtosis.

Research results

After the formation of a hemorrhage in human brain substance, a massive decay of red blood cells occurs in it, which leads to the accumulation of iron-containing decay products[16]. As a result of such processes, iron in the form of a blue hemosiderin pigment appears in areas where it should not normally be.

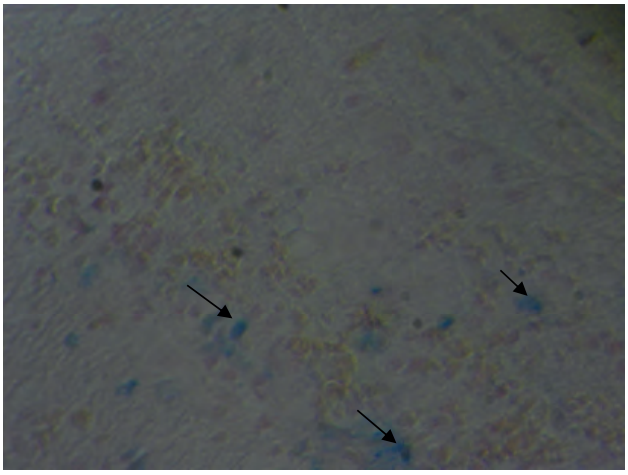

Fig. 1. Hemorrhages of traumatic genesis (10 days), microscope magnification 400x, Perls staining; arrows indicate accumulations of hemosiderin in the foci of hemorrhages.

Human brain substance stained preparations of experimental and control groups were examined and analyzed and we found that hemosiderin was not present in all experimental samples (only 31 out of 40 samples were present). It was not possible to establish the corresponding time dependences of the age of the formation of hemorrhages, regardless of the genesis, since the random appearance of the pigment in the experimental samples is noted at different time intervals. For example, in the case of death due to hemorrhages of traumatic genesis after 5 and 14 hours, the appearance of hemosiderin is noted, but in similar samples with time of formation of a hemorrhage 8 and 16 hours, it is not present at all.

Differential diagnosis of the time of formation of a hemorrhage of traumatic genesis, cerebral infarction of ischemic and hemorrhagic genesis by the method of reproduction of distributions of anisotropy of linear dichroism (ALD)[17].

In fig. 2,3 show maps (fragments (1, 3) and distribution histograms (fragments (2, 4) of linear dichroism values of samples of histological sections of human brain substance of deceased 2nd and 4th groups with different time of formation of a hemorrhage (6 hours and 24 hours).

Tables 1 and 2 show the results of a statistical analysis of the temporal change of necrotic changes in the structure of the ALD human brain substance maps of the deceased within representative samples of the studied sections of group 2 (table 1) and group 4 (table 2) with different time of formation of a hemorrhage.

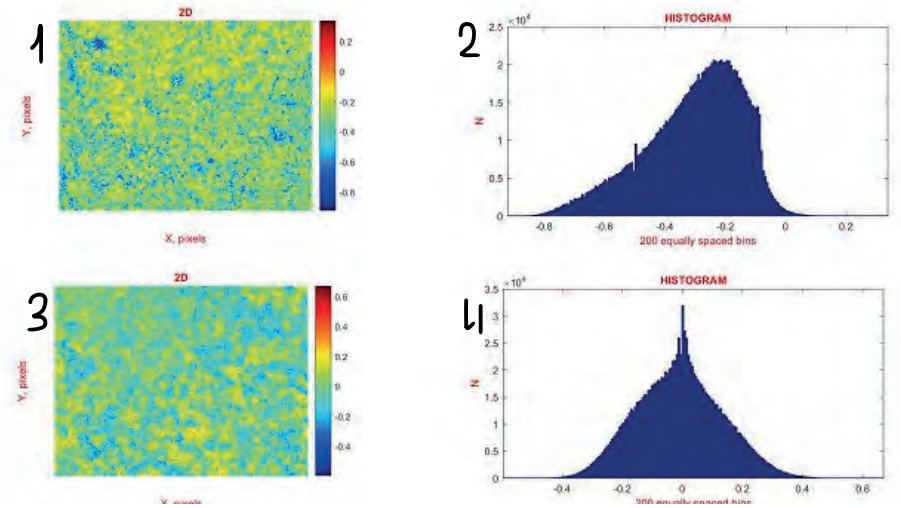

Fig. 2. Maps ((1),(3)) and histograms ((2),(4)) of the distribution of ALD value of histological brain sections of the deceased from group 2 for time of formation of a hemorrhage 6 h (1,2) and 24 h (3,4)

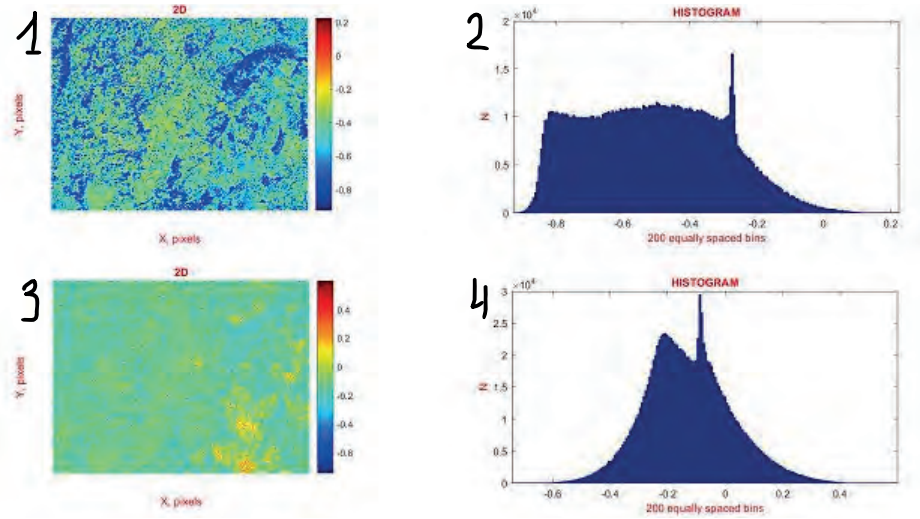

Fig. 3. Maps ((1),(3)) and histograms ((2),(4)) of the distribution of ALD value of histological brain sections of the deceased from group 4 for time of formation of a hemorrhage 6 h (1,2) and 24 h (3,4)

Table 1

Temporal dynamics of changes in statistical moments of the 1st – 4th orders, which characterize the distributions of the ALD value of histological sections of the brain of the deceased from group 2

T, год	6	12	18	24	44
SM_1	0,24±0,008	0,22±0,007	0,205±0,006	0,19±0,005	0,15±0,005
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_2	0,41±0,014	0,37±0,013	0,35±0,013	0,33±0,012	0,30±0,011
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_3	0,91±0,034	1,39±0,054	1,63±0,072	1,87±0,088	2,48±0,101
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_4	0,78±0,031	1,33±0,059	1,58±0,065	2,79±0,11	2,86±0,11
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*

Table 2

Temporal dynamics of changes in statistical moments of the 1st – 4th orders, which characterize the distributions of the ALD value of histological sections of the brain of the deceased from group 4

T, год	6	12	18	24	44
SM_1	0,21±0,006	0,192±0,005	0,18±0,005	0,17±0,004	0,15±0,004
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_2	0,36±0,009	0,33±0,008	0,31±0,007	0,285±0,006	0,253±0,006
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_3	0,69±0,021	1,09±0,029	1,31±0,033	1,52±0,055	2,12±0,097
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*
SM_4	0,83±0,026	1,36±0,034	1,63±0,043	1,89±0,049	2,68±0,099
p		*p<0,05*	*p<0,05*	*p<0,05*	*p<0,05*

When carried out analytical processing of the results of statistical processing of the topographic structure of the tomograms of ALD fibrillar networks of human brain substance samples (Figs. 2, 3) from the deceased from the experimental and control groups, a greater time range of sensitivity of this method to destructive changes in nervous tissue was revealed in comparison with polarimetric methods which were used in previous studies [18]. As a result, there is an accelerated temporal decrease in the absolute values and the range of dispersion of the ALD value with increasing time since the formation of hemorrhage, according to $SM_{1\text{-}2}$.

The following regularities of the scenario of temporal changes in the topographic structure of ALD maps have been established:

1) an increase in the value of the range of temporal linear change of SM1-4 values, which describe the distributions of the value of linear dichroism of fibrillar networks of human brain substance samples from all groups up to 24 hours, with a subsequent slowdown with an increase in time of formation of a hemorrhage up to 2 days;

2) the accuracy of determination of time of formation of a hemorrhage is 30 h ± 5 min for hemorrhages of traumatic genesis and hemorrhages of non traumatic genesis.

Conclusions

1. Taking into account the fact that it is not always possible to detect pigment in the sample, and the absence of a direct dependence of the appearance of hemosiderin on the age of the hemorrhage, it can be concluded that the histological method of examining human brain samples according to Perls does not provide accurate and objective information about the age formation of hemorrhages.

2. By temporal monitoring of changes in the magnitude of statistical moments of the 1st to 4th orders, which characterize the polarization-reproduced maps of the set of mechanisms of optical anisotropy of the polycrystalline structure of nervous tissue, the age of the formation of hemorrhages of traumatic and non-traumatic genesis was established over a time interval of up to 30 hours with an accuracy of ± 5 min.

3. The physico-optical method demonstrated a significant advantage over the classical histological method of Perls' in use in forensic medical practice to establish the age of formation of hemorrhages in the substance of the human brain.

Prospects for further research

The conducted cycle of research on the efficiency of the method of differential Müller-matrix mapping of partially depolarizing histological sections of the brain and tomographic reproduction of the parameters of optical anisotropy of their polycrystalline structure, new in forensic medical practice, revealed a high level of accuracy in differentiating the formation and determining the age of hemorrhages of traumatic origin, ischemic and hemorrhagic brain infarction.

At the same time, the impact of depolarization of laser radiation on the informativeness of this tomographic technique has been little studied. Therefore, the further development and approval of a new method of diffuse tomography for the forensic practice of studying depolarizing laser radiation of brain samples of the dead is urgently needed.

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ПОРІВНЯЛЬНИЙ АНАЛІЗ ЕФЕКТИВНОСТІ ВИКОРИСТАННЯ ГІСТОЛОГІЧНОГО ТА ФІЗИКО-ОПТИЧНОГО МЕТОДІВ ДОСЛІДЖЕННЯ ДАВНОСТІ УТВОРЕННЯ КРОВОВИЛИВІВ У РЕЧОВИНУ ГОЛОВНОГО МОЗКУ ЛЮДИНИ

М. С. Гаразджюк

Буковинський державний медичний університет
(м. Чернівці, Україна)

Резюме.

Важливе значення у судово-медичній практиці приділяють вирішенню питань, що виникають при проведенні експертизи у випадку черепно мозкової травми (ЧМТ), так як цей вид ушкоджень призводить до високої летальності та інвалідизації. При проведенні розтину тіла померлих, смерть яких настала від ЧМТ, трапляються випадки, коли травма виникає внаслідок падіння особи, у якої попередньо розвинувся інфартк головного мозку, а іноді навпаки. У такому разі важливо точно верифікувати первинну причину смерті, тому вчасне і об'єктивне встановлення часу утворення крововиливу (ЧУК) у головний мозок людини (ГМЛ) є необхідним.

Мета і завдання дослідження. Оцінити можливість встановлення ЧУК травматичного та не травматичного ґенезу у ГМЛ загальноприйнятим гістологічним методом та поляризаційно-фазовою мікроскопією, а також порівняти їх ефективність.

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Матеріали та методи дослідження. Для дослідження було відібрано зразки ГМЛ від 140 померлих осіб, смерть яких наступила від 1 до 3-х діб з моменту утворення крововиливу, відповідно до даних, узятих із медичних документів. У якості контрольної групи було відібрано зразки ГМЛ від 40 померлих у випадку смерті від ішемічної хвороби серця. Світлова мікроскопія проводилася шляхом попереднього фарбування гістологічних препаратів за методом Перлса. Фазово-поляризаційну томографію зразків проводили за допомогою поляриметра Стокса.

Результати дослідження. Було досліджено та проаналізовано пофарбовані препарати ГМЛ експериментальної та контрольної груп і виявлено, що гемосидерин був присутній не у всіх експериментальних зразках (присутній лишень у 31 зразку із 40). Не вдалося установити відповідні часові залежності давності утворення крововиливів, не залежно від ґенезу, так як на різних часових проміжках відзначається рандомна поява пігменту у дослідних зразках. Провівши аналітичне опрацювання отриманих результатів статистичної обробки топографічної структури томограм анізотропії лінійного дихроїзму (АЛД) фібрилярних мереж зразків ГМЛ від померлих з експериментальних та контрольної груп, було виявлено більший часовий діапазон чутливості даного методу щодо деструктивних змін у нервовій тканині, у порівнянні із методами, які застосовувалися у попередніх дослідженях. Як наслідок, відзначається пришвидшене часове зменшенні абсолютних значень і діапазону розкиду величини АЛД із зростанням часу з моменту утворення крововиливу.

Висновки. Поляризаційно-фазова мікроскопія продемонструвала значну перевагу у використанні, порівняно із золотим стандартом – методом фарбування за Перлсом, для ідентифікації часу утворення геморагій у головний мозок людини.

Ключові слова: черепно-мозкова травма; судова медицина; поляризаційно-фазова мікроскопія; давність утворення крововиливу

Contact information:
Martha Garazdiuk – PhD, Associate Professor of Department of Forensic Medicine and Medical Law of Bukovinian State Medical University (Chernivtsi, Ukraine).
e-mail: m.garazdiuk@gmail.com
ORCID: http://orcid.org/0000-0002-7811-3211
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Контактна інформація:
Гараздюк М. С. – к.мед.н., доцент кафедри судової медицини та медичного правознавства Буковинського державного медичного університету (м.Чернівці, Україна)
e-mail: m.garazdiuk@gmail.com
ORCID: http://orcid.org/0000-0002-7811-3211
ResearcherID: http://www.researcherid.com/rid/C-8134-2017

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