

ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

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INFLUENCE OF ETHYL ALCOHOL ON ACCURACY OF TIME SINCE DEATH ESTIMATION BY THE METHOD OF AVTOFLUORESCENT LASER POLYARIMETRY OF POLYCRISTALLINE FILMS OF CEREBROSPINAL FLUID

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Abstract

Due to the high prevalence of death on the base of alcoholic intoxication of varying degrees, it is expedient to analyze the possibility of the influence of alcohol on the change in the structure of the polycrystalline films of the cerebrospinal fluid, and accordingly on the accuracy of the diagnosis of the time since death by the method of autofluorescent laser polarimetry.

The purpose of the work. To study the possible influence of alcohol and to develop objective forensic criteria for diagnosing its effects on the basis of analysis of changes in the polycrystalline structure of cerebrospinal fluid (CSF) films.

Materials and methods. Object of research: native films of CSF, selected in 80 corpses, in the quantity of 155 samples - 1 group; CSF from 30 healthy volunteers (group 0) - 30 samples; CSF films obtained in the deceased with the presence of ethyl alcohol in the blood, 30 samples - group 2. The methods of gas-liquid chromatography, laser-induced fluorescence and statistical processing of the results were used.

Results. The efficiency of using the methods of laser autofluorescence of polycrystalline films of the cerebrospinal fluid for the diagnosis of the time since death was proved. A statistically significant difference between cases of death from cardiovascular pathology with the presence and absence of ethyl alcohol in the organism was established by determining the magnitude of the statistical moment of the 4th order, which characterizes distribution of azimuth polarization values of microscopic images of laser-induced fluorescence. On this basis, an excellent balanced accuracy of the differentiation of the presence of ethyl alcohol in the human body ($Ac(M4) = 94\%$) was achieved.

Conclusion. Ethyl alcohol affects the structure of the polycrystalline films of the cerebrospinal fluid, which must be taken into account when estimating the time since death by autofluorescence laser polarimetric methods.

Key words: time since death, cerebrospinal fluid, ethyl alcohol, laser polarimetry, autofluorescence.

Introduction. Time since death (TSD) estimation is one of the key and for the time being still unresolved problems of forensic medicine practice. The exact setting of the time of death allows to exclude or confirm the involvement of one or another person in committing an offense, limit or expand the circle of suspects, verify the accuracy of the testimony of witnesses [1,2].

The definition of TSD is also complicated by the fact that a significant number of exogenous and endogenous factors, such as environmental conditions, the cause of death, anthropological features and the age of a person, are affected by posthumous processes occurring in the tissues of the corpse, etc. Recently, researchers began to pay attention to the impact of alcohol intoxication on the rate of flow of life processes and posthumous changes in human tissues [2].

It was observed that in the presence of alcohol in the blood of the corpses in cases of death of traumatic genesis the rosette-forming ability of lymphocytes in the posthumous period disappears earlier than in the absence of it and depends on the concentration of alcohol in the blood [3]. It was found that at concentrations of alcohol in the blood of 2,1-3,0‰ during 6-72 hours after the death rosette-forming ability of the T-general lymphocyte increases, and at a concentration of 3-5‰ it decreases. A similar nature of the change was observed for the rosette-forming ability of B-lymphocytes.

Vavilov A.Yu. [4] noticed an increase in the overall thermal conductivity of the brain and liver in death on the background of blood loss, poisoning with ethyl alcohol, mechanical asphyxia. By studying supravital zine reaction for the

diagnosis of TSD Gladkikh D.B found that its results can not be evaluated in cases where at the time of death the person was in a state of intoxication [5].

Since laser polarimetric methods are effective for the diagnosis of TSD with high accuracy [1,6,7], since they provide new information on the morphological and optic-anisotropic structure of biological objects, which makes it possible to establish a connection between their physiological state and polarization -phase parameters of the images of their architectonics [6], that is why it is advisable to assume their sensitivity and to detect alcohol in the cerebrospinal fluid (CSF) of deceased persons.

The purpose of the work. To study the possible influence of alcohol and to develop objective forensic criteria for diagnosing its effects on the basis of analysis of changes in the polycrystalline structure of CSF films.

Material and methods of research. The methods of gas-liquid chromatography, laser-induced fluorescence (measurement of coordinate distributions of intensity and azimuth polarization of microscopic images of autofluorescence of polycrystalline films (PCF) of CSF), statistical processing of results were used.

As research objects in work were used 3 groups:

group 1 - native preparations of CSF films, selected from 80 corpses of both sexes (the main group of study), who died of pathology of the circulatory system in the age of 33 to 89 years, with a previously known time of death, ranging from 1 to 26 hours - 155 specimens (some CSF samples was re-selected at different TSD intervals);

group 2 - posthumous polycrystalline planar films of CSF, obtained from persons that have died from the pathology of blood circulatory system with the presence of ethanol in the blood - 30 samples;

group 0 - 30 healthy volunteers - 30 samples.

The sampling was performed by the method of suboccipital puncture from a large occipital cistern in corpses and at spinal anesthesia in the preparation for surgical interventions in healthy volunteers [8].

Concentration of ethyl alcohol in blood was determined during the forensic medical examination of corpses in the department of forensic medical toxicology of the CMI "Regional Bureau of Forensic Medical Examination" of the Department of Healthcare of Chernivtsi Regional State Administration through gas-liquid chromatography on the device "Crystal-2000M".

PCF of CSF were formed in identical conditions by applying a drop at room temperature ($t = 220C$) to an optically homogeneous glass.

Measurement of coordinate distributions (two-dimensional arrays of values in the plane of PCF of CSF) of the azimuth values and the ellipticity of polarization at the points of the microscopic images was performed in the location of the fluorescence laser stokes polarimeter (Fig. 1), developed by the scientists of the Chernivtsi National University named after Yuriy Fedkovich [6]:

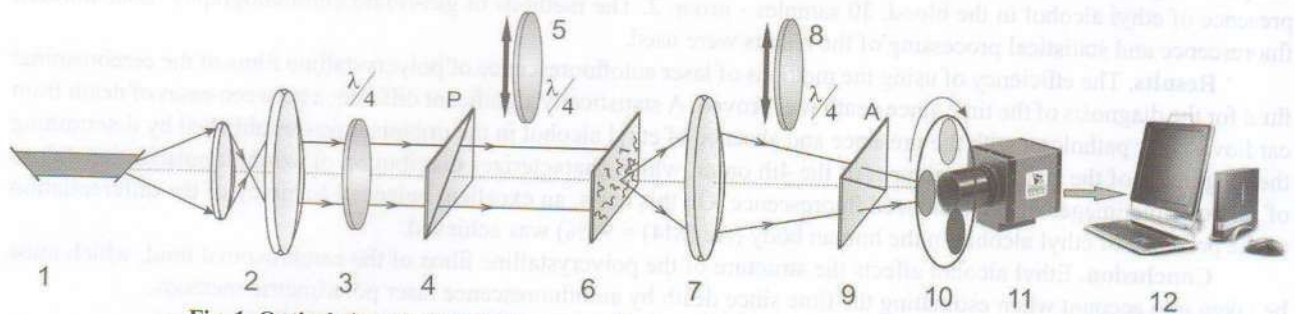


Fig. 1. Optical circuit of autofluorescence Stokes polarimeter. The explanation in the text

PCF of CSF 6 irradiations regime consisted of parallel ($\varnothing = 2 \times 10^3 \mu m$) bunch of «blue» ($\lambda_2 = 0.405 \mu m$) semiconductor laser 1. Polarization irradiator consists of two polarization elements - quarter-wave plate 3 and polarizer 4. Image 6 PFL samples using polarization object glass 7 (Nikon CFI Achromat P, the focal length - 30mm, aperture - 0.1, magnification - 4x) was projected into the light-sensitive plane 10 of CCD-camera (The Imaging Source DMI 41AU02. AS, monochrome 1 / 2 «CCD, Sony ICX205AL (progressive scan); resolution - 1280x960; photosensitive plane size - 7600x6200 μm ; sensitivity - 0.05 lx; dynamic range - 8 bit). PCF of CSF 6 samples polarization analysis was carry out by the help of quarter- wave plate 8 and polarizer 9.

To excite autofluorescence, the «blue» semiconductor laser LSR405ML-LSR-PS-II with a wavelength of 0.405 μm and a power of 50 mW was used in the position of a standard stokes polarimeter.

The main information objects for the given scheme are a set of:

- 1) directly measured distributions of the auto-fluorescence intensity of the PCF of CSF;
- 2) azimuth polarization of the laser-induced fluorescence of the PCF of CSF.

A detailed description of the calculations is given in the sources [6,7]. The main analytical tool for estimating the distributions of the intensity values and the fluorescence polarization azimuths of the PCF of CSF was the statistical

moments of the first, second, third and fourth orders, which were calculated by algorithms [6] and characterize the mean value (Z_1), dispersion (Z_2), asymmetry (Z_3) and excess (Z_4) of such distributions.

Results and discussion.

Initially, the concentration of ethyl alcohol was established according to the forensic toxicological study in the blood of the deceased 2nd group, which was $1,71 \pm 0,87$ ‰.

The next step was to study the possibilities of laser-induced autofluorescence microscopy in the developed forensic medical criteria for the study of the effect of ethyl alcohol on the time since death estimation from cardiovascular pathology using statistical analysis of intensity distributions and azimuths of polarization of microscopic fluorescence images of PCF of CSF in different spectral regions of optical radiation.

Forensic criteria for a good level of balanced accuracy were found to study the effect of ethyl alcohol on time since death by spectrally selective mapping of the intensity of the laser-induced autofluorescence of the PCF of CSF from group 1 and group 2. It is statistical moments of the 4th order characterizing the intensity distributions fluorescence in the «green-yellow» and «red» spectral regions with $Ac(M_4) = 80\% - 90\%$ (see Tab. 1).

Table 1

Statistical moments of the 1st to 4th orders characterizing the distribution of the intensity of the autofluorescence of the polycrystalline films of the cerebrospinal fluid in the «red» region of the spectrum

Statistical moments	Control group		The cause of death	
	healthy (n=30)	volunteers	pathology of the circulatory system (n=30)	alcohol poisoning (n=30)
Mean value, M_1	0,39±0,021		0,11±0,006	0,19±0,011
p_1			$p < 0,05$	$p < 0,05$
P_2			$p < 0,05$	
Dispersion, M_2	0,19±0,012		0,11±0,006	0,14±0,008
p_1			$p < 0,05$	$p < 0,05$
P_2			$p < 0,05$	
Asymmetry, M_3	0,68±0,037		0,99±0,053	1,31±0,071
P_1			$p < 0,05$	$p < 0,05$
P_2			$p < 0,05$	
Excess, M_4	0,83±0,044		1,53±0,084	1,09±0,063
p_1			$p < 0,05$	$p < 0,05$
P_2			$p < 0,05$	

For other statistical moments balanced accuracy also increases to a satisfactory level $Ac(M_{1,2,3,4}) = 75\% - 87\%$. Having investigated the statistical distributions of fluorescence azimuths, it was discovered that it is possible to achieve an excellent level of balanced accuracy on the example of the statistical moment of the 4th order, which characterizes the distributions of fluorescence azimuths in the «red» spectral region $Ac(M_4) = 95\%$. For other statistical moments, the balanced accuracy of differentiation of death due to cardiovascular pathology in the presence of ethyl alcohol in the blood also rises to a good level $Ac(M_{1,2,3,4}) \leq 90\%$

The revealed differences in the structure of PCF of CSF can be explained by the influence of ethyl alcohol on the formation of a polycrystalline film network. Probably, ethyl alcohol, penetrating through the blood-brain barrier, affects both the quantitative composition of the medium-molecular compounds, as indicated by Witer et al. [9,10], as well as on the structure of certain proteins, in particular gamma-aminobutyric acid receptors, and, thus, affects the tertiary and quaternary structure of CSF proteins [11], which is reflected in the analysis of the PCF of CSF. The obtained data prove certain influence of ethyl alcohol on the structure of PCF of CSF and allow to recommend a new methodological approach for the determination of TSD in the presence of ethyl alcohol in the blood.

Conclusions

1. The possibilities of forensic medical objective differentiation of the estimation of the influence of ethyl alcohol on the course of post-mortem processes in case of cardiovascular pathology by the method of autofluorescence microscopy of polycrystalline films of the cerebrospinal fluid were analyzed.

2. Relationships between intensity distributions and azimuths of the fluorescence polarization of the polycrystalline films of the cerebrospinal fluid of the deceased due to the pathology of the circulatory system and severe alcohol intoxication with the possible fatal outcome and the magnitude and range of changes in the statistical moments of the 1st to 4th orders characterizing such autofluorescence images were investigated.

3. A good level of balanced precision of the differentiation of the causes of death in the «red» area of the spectrum has been achieved $Ac(M_{1,2,3,4}) \leq 90\%$. At the same time, the value of the balanced accuracy of differentiation in the case of weak alcohol poisoning is significantly lower (by 10-15%) and practically does not reach the satisfactory level ($Ac < 75\%$) except for the «red» area of the spectrum ($Ac \sim 80\%$).

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ВПЛИВ ЕТИЛОВОГО СПИРТУ НА ТОЧНІСТЬ ВИЗНАЧЕННЯ ЧАСУ НАСТАННЯ СМЕРТІ МЕТОДОМ АВТОФЛУОРЕСЦЕНТНОЇ ЛАЗЕРНОЇ ПОЛЯРИМЕТРІЇ ПОЛІКРИСТАЛІЧНИХ ПЛІВОК СПИННОМОЗКОВОЇ РІДИНИ

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Анотація

У зв'язку із значною поширеністю настання смерті на фоні алкогольного сп'яніння різного ступеню, доцільним є проаналізувати можливість впливу алкоголю на зміну структури полікристалічних плівок спинномозкової рідини, а відповідно і на точність діагностики давності настання смерті методом автофлуоресцентної лазерної поляриметрії.

Мета роботи. Вивчити можливий вплив алкоголю та розробити об'єктивні судово-медичні критерії діагностики його впливу на основі аналізу зміни полікристалічної структури плівок СМР.

Матеріали і методи. Об'єкт дослідження: нативні препарати плівок СМР, відібрані у 80 трупів, у кількості 155 зразків - 1 група; СМР від 30 здорових добровольців (група 0) – 30 зразків; плівки СМР, одержані у померлих з наявністю етилового спирту в крові, 30 зразків - група 2. У роботі використовували методи газОВО-рідинної хроматографії, лазерно-індукованої флуоресценції та статистичної обробки результатів.

Результати. Було доведено ефективність використання методів лазерної автофлуоресценції полікристалічних плівок спинномозкової рідини для діагностики давності настання смерті та встановлена статистично достовірною різниця між випадками смерті від серцево-судинної патології при наявності та за відсутності етилового спирту в організмі шляхом визначення величини статистичного моменту 4-го порядку, який характеризує розподіл величини азимута поляризації мікроскопічних зображень лазерно-індукованої флуоресценції. На цій основі було досягнуто відмінної збалансованої точності диференціації наявності етилового спирту в організмі людини ($A_C(M_4)=94\%$).

Висновок. Етиловий алкоголь впливає на структуру полікристалічних плівок спинномозкової рідини, що необхідно враховувати при визначенні давності настання смерті автофлуоресцентними лазерними поляриметричними методами.

Ключові слова: давність настання смерті, спинномозкова рідина, етиловий спирт, лазерна поляриметрія, автофлуоресценція.

ВЛИЯНИЕ ЭТИЛОВОГО СПИРТА НА ТОЧНОСТЬ ОПРЕДЕЛЕНИЯ ВРЕМЕНИ НАСТУПЛЕНИЯ СМЕРТИ МЕТОДОМ АВТОФЛУОРЕСЦЕНТНОЙ ЛАЗЕРНОЙ ПОЛЯРИМЕТРИИ ПОЛИКРИСТАЛЛИЧЕСКИХ ПЛЕНОК СПИННОМОЗГОВОЙ ЖИДКОСТИ

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Аннотация

В связи с широкой распространенностью наступления смерти на фоне алкогольного опьянения различной степени, целесообразно проанализировать возможность влияния алкоголя на изменение структуры поликристаллических пленок спинномозговой жидкости, а соответственно и на точность диагностики давности наступления смерти методом автофлуоресцентной лазерной поляриметрии.

Цель работы. Изучить возможное влияние алкоголя и разработать объективные судебно-медицинские критерии диагностики его воздействия на основе анализа изменения поликристаллической структуры пленок СМР.

Материалы и методы. Объект исследования: нативные препараты пленок СМР, отобранные в 80 трупов, в количестве 155 образцов - 1 группа; СМР от 30 здоровых добровольцев (группа 0) - 30 образцов; пленки СМР, полученные у умерших с наличием этилового спирта в крови, 30 образцов - группа 2. В работе использовали методы газОВО-жидкостной хроматографии, лазерно-индуцированной флуоресценции и статистической обработки результатов.

Результаты работы. Авторами была доказана эффективность использования методов лазерной автофлуоресценции поликристаллических пленок спинномозговой жидкости для диагностики давности наступления смерти и установлена статистически достоверная разница между случаями смерти от сердечно-сосудистой патологии при наличии и при отсутствии этилового спирта в организме путем определения величины статистического момента 4-го порядка, который характеризует распределения величины азимута поляризации