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SPECTRAL-SELECTIVE LASER AUTOFLUORESCENT POLARIMETRY OF OPTICALLY ANISOTROPIC STRUCTURES OF THE MYOCARDIUM IN POSTMORTEM DIAGNOSIS OF ACUTE CORONARY INSUFFICIENCY

Key words: *acute coronary insufficiency, postmortem diagnostics, autofluorescence*

Abstract. *Investigations of 69 samples of the myocardium following acute coronary insufficiency (ACI), 69 with chronic ischemic heart disease and 20 specimens of the control group were carried out. Operative characteristics of spectral-selective laser autofluorescent polarimetry of optically anisotropic structures of myocardium in comparison with the traditional method of ACI verification have been stated. The investigated method demonstrated a high level of the balanced accuracy for ACI diagnostics*

Introduction

Diagnostics of acute coronary insufficiency (ACI) in the period till 6 hours from its beginning is a complicated task of forensic medical practice, and taking into consideration suddenness of the event and frequent absence of witnesses, the question about the violent character of death arises [2]. Therefore, to verify ACI the necessity concerning the addition of existing and development of new methods for objective identification of the indicated pathological state could not be put off.

Combination of autofluorescent analysis with Muller-matrix one has been shown to be efficient method for ACI diagnostics. However, it is possible to carry out selective registration of autofluorescent radiation in a certain range in order to increase accuracy of the method that simultaneously will become the representation of biochemical and structural changes in cardiac hystiocytes in case of acute ischemia [1,5,6].

Aim of the research - to investigate possibilities of using spectral-selective laser autofluorescent polarimetry of optically anisotropic structures of the myocardium for postmortem ACI diagnostics.

Materials and methods

Taking of the material was carried out during the period of 2010-2015 years in the premises of the municipal service "Regional bureau of forensic medical examination" at compounded light, air temperature 18-22°C and relative humidity 60-75%. In all cases sampling was conducted in different anatomical regions. 69 samples of myocardium after ACI and chronic ischemic heart disease (CIHD) each in all and 20 samples of myocardium of the cadavers died due to the violent death with a short agonal period have been examined. Blocks in 1cm3 of volume, cut on freezing microtome with sections of thickness 30±5 mkm were formed. Sections were dried. Dried native sections were delivered to the laboratory of the department of correlative optics and spectroscopy of Yu. Fedkovych National University, Ukraine, Chernivtsi. Sampling for forensic-histological study that consisted of staining with hematoxylene, principle fuchsine, picric acid according to Lie method was carried out in parallel.

Experimental measurement was carried out in standard disposition of stocks-polarimeter, modified for autofluorescence investigations.

Measurements of coordinate distributions of autofluorescence I intensity was conducted in the plane of photosensitive ground of digital camera, and on the basis of the obtained data file (p x k) of Muller matrix invariants was calculated, the values of which were determining optic activity of myosin molecules r_{14} and crystallization degree of the myocardium r_{41} on three conditionally singled out spheres: "short-wave" ($\Delta\lambda_{max}$=0.63mkm ч0.46mkm); "average-wave" ($\Delta\lambda_{max}$=0.5mkm ч0.55mkm) and "long-wave" ($\Delta\lambda_{max}$=0.63mkm ч0.65mkm). Afterwards the totality of their statistical moments of the first-fourth orders was calculated. Sensitivity, specificity and balanced accuracy were calculated according to the standards of demonstrated medicine [4].

Discussion of the results

Series of spectral-selective on short-waves ($\lambda^{(1)}_{max}$) =0.45mkm, average ($\lambda^{(2)}_{max}$) =0.55mkm, and long-wave ($\lambda^{(3)}_{max}$)=0.63mkm areas of autofluorescent images of the sections of the groups under study is presented in Fig. 1-Fig. 3.

Altogether, the obtained data illustrate the presence of laser autofluorescence of the native sections in all spectral ranges. The highest intensity of autofluorescent images (Fig. 1) is implemented for short-wave part ($\lambda^{(1)}_{max}$=0.45mkm) of the spectrum, and in long-wave area ($\lambda^{(3)}_{max}$ =0.63mkm) of the spectrum

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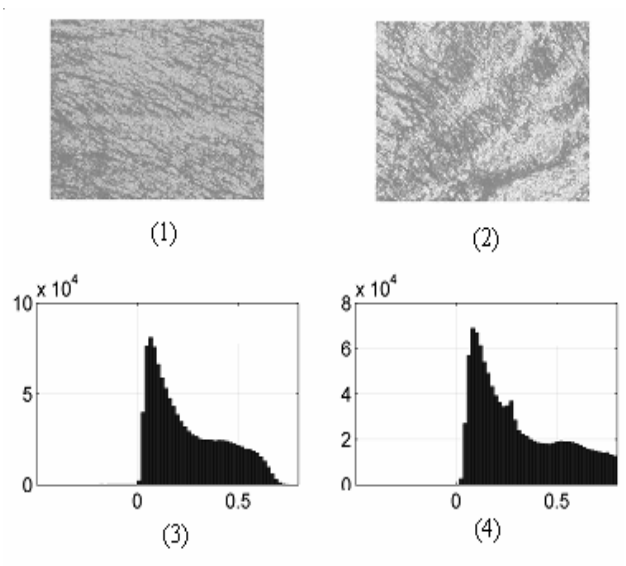

Fig.1. Autofluorescent images of the native sections of the myocardium of both groups at ($\lambda^{(1)}_{max}$) =0.45mkm: 1-autofluorescent map of CIHD; 2-autofluorescent map of ACI; 3- histogram of values at CIHD; 4- histogram of values at ACI

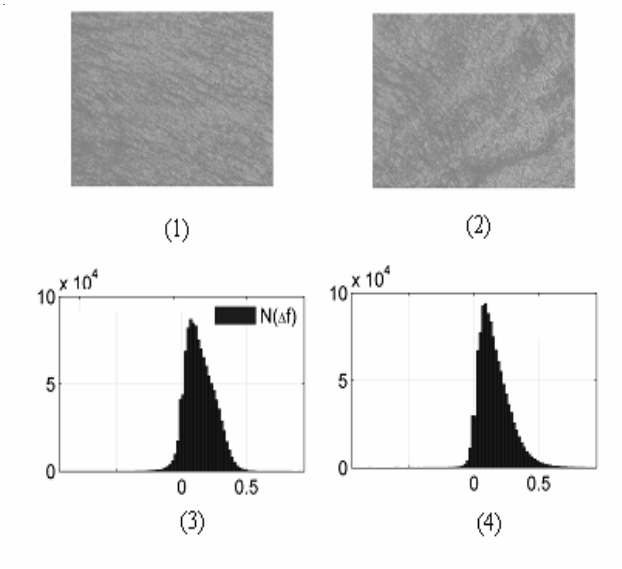

Fig.2. Autofluorescent images of myocardial sections of both groups at ($\lambda^{(2)}_{max}$) =0.55mkm: 1-autofluorescent map of CIHD; 2- autofluorescent map of ACI; 3- histogram of values at CIHD; 4- histogram of values at ACI

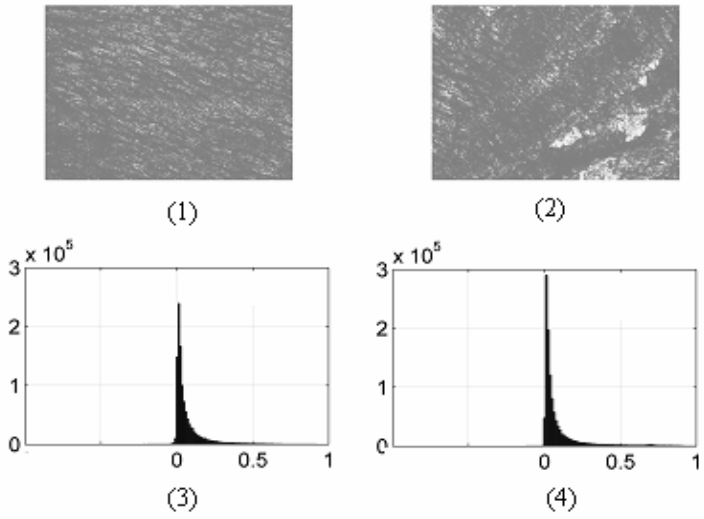

Fig.3. Autofluorescent images of myocardial sections of both groups at ($\lambda^{(3)}_{max}$)=0.63mkm: 1-autofluorescent map of CIHD; 2- autofluorescent map of ACI; 3- histogram of values at CIHD; 4- histogram of values at ACI.

such images are the least intensive (Fig. 3).

The data obtained may be connected with "short-wave" spectral localization of autofluorescence maxima of birefracted protein chains formed by optically active myosin, collagen, elastin and HAD.

In that way, lowering of intensity of autofluorescence of the native myocardium sections both ACI and CIHD was observed that, respectively, impeded direct AI verification of the myocardium on the basis of the presence of autofluorescence intensity decrease depending upon the registration area.

Statistically indicated changes of the structures of autofluorescent images between groups are accompanied by an increase of the average and ($M_1 = \frac{1}{N}\sum_{j=1}^{N} I_j$) dispersion ($M_2 = \frac{1}{N}\sum_{j=1}^{N} (I)_j^2$) of distribution I at ($\lambda^{(1)}_{max} = 0{,}45$mkm) (Table 1).

The analysis of the data, given in Table 1, showed that for short-wave potion the most perspective indices of the spectral-selective laser autofluorescent polarimetry for the establishment of ACI were the average and dispersion, which constituted M_1 0,67 ± 0,051 and M_2 0,27 ± 0,023.

Statistical moments $M_{i=1;2;3;4}[(I(p \times k))]$ for $\lambda^{(2)}_{max} = 0{,}55$mkm have been established (Table 2). The most sensitive statistical parameters for differentiation AI of the myocardium on spectral-selective autofluorescents polarimetric images of the sections have been detected. The average M_1 and dispersion M_2 are such statistical moments.

Table 1

Statistical moments $M_{i=1;2;3;4}[(I(p\times k))]$ for the method of spectral-selective laser autofluorescent polarimetry at $\lambda^{(1)}_{max}$ = 0,45 mkm

Statistical moments	Cause of death		
	Control (n=20)	CHID (n=69)	ACI (n=69)
Average, M_1	0,36 ± 0,025	0,51 ± 0,037	0,67 ± 0,051
Dispersion, M_2	0,12 ± 0,009	0,19 ± 0,018	0,27 ± 0,023
Asymmetry, M_3	0,44 ± 0,032	0,36 ± 0,033	0,41 ± 0,031
Excess, M_4	0,41 ± 0,03	0,39 ± 0,034	0,35 ± 0,028

Table 2

Statistical moments $M_{i=1;2;3;4}[(I(p\times k))]$ for the methods of experimentally-selective laser autofluorescent polarimetry at $\lambda^{(2)}_{max}$ = 0,55 mkm

Statistical moments	Cause of death		
	Control (n=20)	CHID (n=69)	ACI (n=69)
Average, M_1	0,56 ± 0,045	0,33 ± 0,024	0,42 ± 0,036
Dispersion, M_2	0,21 ± 0,015	0,12 ± 0,011	0,16 ± 0,014
Asymmetry, M_3	0,25 ± 0,022	0,21 ± 0,017	0,24 ± 0,021
Excess, M_4	0,21 ± 0,019	0,25 ± 0,019	0,22 ± 0,017

Statisticalmoments $M_{i=1;2;3;4}[(I(p\times k))]$ for $\lambda^{(3)}_{max}$ = 0,63 mkm have been established (Table 3). The average and dispersion were found to be the most sensitive statistical parameters of differentiation of spectral-selective autofluorescent polarimetric images of the sections in the group under study in spectral scope $\lambda^{(3)}_{max}$=0.63mkm.

Data of the balanced accuracy of the method of statistical analysis of laser spectral-selective autofluorescent polarimetric images of the native myocardial sections are presented in Table 4. Only balanced accuracy as one of the most significant integral indices that simultaneously shows sensitiveness and specificity of the method of statistical analysis of laser spectral-selective autofluorescent images of myocardial sections has been analyzed. The analysis was carried out for indices, which were the most perspective for ACI verification on all parts of visible spectrum that is, for the average and dispersion.

The analysis of the data, cited in Table 4, has

Table 3

Statistical moments $M_{i=1;2;3;4}[(I(p\times k))]$ for the method of spectral-selective laser autofluorescent polarimetry at $\lambda^{(3)}_{max}$=0.63mkm

Statistical moments	Cause of death		
	Control (n=20)	CHID (n=69)	ACI (n=69)
Average, M_1	0,43 ± 0,034	0,21 ± 0,016	0,31 ± 0,025
Dispersion, M_2	0,25 ± 0,019	0,13 ± 0,012	0,19 ± 0,014
Asymmetry, M_3	0,88 ± 0,074	0,72 ± 0,061	0,84 ± 0,072
Excess, M_4	0,51 ± 0,042	0,39 ± 0,024	0,45 ± 0,039

Table 4

Accuracy of the method of statistical analysis of the spectral-selective autofluorescent images of myocardial sections

Parameters	Ac($\lambda^{(1)}_{max}$),%	Ac($\lambda^{(2)}_{max}$),%	Ac($\lambda^{(3)}_{max}$),%
M_1	*76* *a*=53;*b*=16 *c*=49; *d*=20	62 *a*=44;*b*=25 *c*=41; *d*=28	66 *a*=48;*b*=21 *c*=45; *d*=24
M_2	80 *a*=56;*b*=13 *c*=52; *d*=17	64 *a*=46;*b*=23 *c*=45; *d*=24	69 *a*=49;*b*=20 *c*=46; *d*=23

shown that the usage of statistical analysis of laser spectral-selective autofluorescent polarimetric images of the native sections of the myocardium with determination of the average and dispersion is the most perspective on short-wave part of visible spectrum ($Ac(\lambda^{(I)}_{max})$ =76% - 80% portion.

Conclusion

Thus, using the method of laser spectral-selective autofluorescent polarizing microscopy of myocardial sections with well balanced accuracy ($Ac(\lambda^{(I)}_{max})$ =76% - 80% enabled to realize verificiation of ACI and differentiation of the indicated pathological condition with CIHD ,all in all, the possibilities of the diagnostic test in case of high quality of the balanced accuracy and successful reproduction of the data.

The results obtained enable to assert that Muller-matrix mapping of optic anisotropic molecular endogenic fluorophores shows a sufficiently good level of the balanced accuracy for ACI diagnostics.

Perspectives of further investigations

Methods of spectral-selective laser Muller-matrix polarimetry, in particular, for autofluorescence of nicotineamideadeninnucleotide in postmortem diagnostics of acute ischemia of the myocardium require further development.

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СПЕКТРАЛЬНО-СЕЛЕКТИВНА ЛАЗЕРНА АВТОФЛУОРЕСЦЕНТНА ПОЛЯРИМЕТРІЯ ОПТИЧНО АНІЗОТРОПНИХ СТРУКТУР МІОКАРДА У ПОСМЕРТНІЙ ДІАГНОСТИЦІ ГОСТРОЇ КОРОНАРНОЇ НЕДОСТАТНОСТІ

О.Я. Ванчуляк

Резюме. Проведено дослідження 69 міокардів з гострою коронарною недостатністю (ГКН), 69 з хронічною ішемічною хворобою серця та 20 контрольної групи. Встановлено операційні характеристики спектрально-селективної лазерної автофлуоресцентної поляриметрії оптично анізотропних структур міокарда порівняно із традиційним методом верифікації ГКН. Досліджуваний метод продемонстрував високий рівень збалансованої точності для діагностики ГКН.

Ключові слова: гостра коронарна недостатність, посмертна діагностика, автофлуоресценція.

СПЕКТРАЛЬНО-СЕЛЕКТИВНАЯ ЛАЗЕРНАЯ АВТОФЛУОРЕСЦЕНТНАЯ ПОЛЯРИМЕТРИЯ ОПТИЧЕСКИ АНИЗОТРОПНЫХ СТРУКТУР МИОКАРДА В ПОСМЕРТНОЙ ДИАГНОСТИКЕ ОСТРОЙ КОРОНАРНОЙ НЕДОСТАТОЧНОСТИ

О.Я. Ванчуляк

Резюме. Проведено исследование 69 препаратов миокарда после острой коронарной недостаточностью (ОКН), 69 после хронической ишемической болезни сердца и 20 контрольной группы. Установлено операционные характеристики спектрально-селективной лазерной автофлуоресцентной поляриметрии оптически анизотропных структур миокарда по сравнению с традиционным методом верификации ОКН. Исследуемый метод продемонстрировал высокий уровень сбалансированной точности для диагностики ОКН.

Ключевые слова: острая коронарная недостаточность, посмертная диагностика, автофлуоресценция.

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