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Possibilities of using polarizing microscopy for diagnosing pathological conditions of heart muscle

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ABSTRACT

Introduction: A powerful technological breakthrough in recent decades has provided instrumental solutions to important medical problems. The increased attention of many researchers was drawn to the evaluation and implementation in various medical specialties of a wide range of modern laboratory methods based on the achievements of biology, biophysics, morphology, mathematics and other sciences. This article provides information on the possibility of studying the physiological state of myocardial tissue, taking into account it optical activity and ability to be birefringent. The method of statistical Mueller-matrix analysis is analyzed, which is derived from the process of polarization visualization of the architectonic construction of normal heart muscle and influenced by pathology. The biological tissue from an optical point of view as a combination of amorphous and polycrystalline components are theoretically substantiated. The results of scientific research on the possibility of using the method of polarization microscopy for the diagnosis of pathological conditions of the heart muscle are presented.

Keywords: polarizing microscopy, biological tissues, laser, amorpho-crystalline structure, myocardial tissue.

1. INTRODUCTION

In the conditions of modern technological progress, an important task of modern biomedicine is the development of new and improvement of existing tools for instrumental diagnosis of modifications in biological tissues (BT) in cases of various diseases (Olar et al., 2019). In recent years, increased attention of many researchers has been drawn to the testing and implementation in various medical specialties of a range of modern laboratory methods based on the achievements of biology, biophysics, morphology, mathematics and other sciences, to solve many pressing problems (Bachinskyi et al., 2018; Barton et al., 2008; Patel et al., 2013). Techniques that combine the capabilities of several basic sciences receive the most attention. Thus, scientists in many countries around the world have been widely using techniques of laser polarization microscopy to study the structure of BT.

Currently, the development of laser technology has reached a fairly high level. A multitude of lasers and laser systems has been created, which by their parameters largely meet the needs of laser technology, including biotechnology (Lindon et al., 2016). They can be used for a variety of objects, among which the most interesting and complex are biological. Recently, there has been significant progress in the development of polarization methods for medical diagnostics. New directions such as optical polarization tomography and BT laser polarimetry (LP) have been developed (Furukawa, 2009). The growing number of studies in the field of LP of biological samples confirms the prospects for its use to assess structural changes and identify pathologies, including in the early stages of the disease. Determination of polarization parameters makes it facilitate to assess a number of anisotropic tissues existing in pathological and dystrophic structural changes, as well as to investigate the dynamics of these changes under the influence of various factors (Rappel & Edelstein-Keshet, 2017).

In some cases, polarization microscopy is the only method that makes the structure visible (for example, when particles cannot be stained or when their concentration is so low that they can be seen with a phase contrast or interference microscope). In many cases, polarization microscopy provides precision data on the molecular architecture in a relatively short time. When studying some species of living cells, this method is the only one, because more accurate and complex techniques require large volumes of samples or drying (which significantly affects the reliability of the results) (Koike-Tani et al., 2015). However, despite significant progress, the process of implementing laser polarimetric analysis and diagnosis in medicine is still far from complete. Existing approaches of LP are imperfect for the study of randomly inhomogeneous BT. The complexity of the processes of interaction of optical radiation with BT is due to their predominant heterogeneity. They also require in-depth study of the features of the processes of interaction of polarized radiation with human tissues during backscattering. Thus, structural complexity of biological objects, the significant diversity in the nature of how they interact with light stimulate the search for new approaches, including the creation of more advanced automated laser tools and systems, which is an important and urgent task today.

To automate and facilitate research, original experimental setups of leading Ukrainian scientific schools of polarimetry and spectropolarimetry of objects of different nature, in particular the schools of prof. S.N. Savenkova, prof. V.G. Petruka, prof. O.G. Ushenko, prof. Ya.I. Shopy and others. However, only some of these laser polarimetric devices have sufficient versatility to ensure maximum completeness of biomedical research, as they have different modes, and use an adaptive set of methods developed by the authors to process and interpret measurements of polarization characteristics of large groups of fluids and BT (connective, muscular, integumentary, bone, etc.) (Olar et al., 2019; Ushenko et al., 2014; Ushenko et al., 2019). In our study, we decided to explore the possibilities of statistical Mueller-matrix analysis in diagnosing pathological modifications in the heart muscle.

2. METHODS

The research was conducted at the Department of Forensic Medicine, Medical Law named after Professor M.S. Bocarius of the Kharkiv National Medical University (Kharkiv, Ukraine), Department of Forensic Medicine and Law, National Pirogov Memorial Medical University (Vinnytsya, Ukraine) and at the Department of Optics of the Yuriy Fedkovych Chernivtsi National University (Chernivtsi, Ukraine) during May 2021 - January 2022. In this study, we studied the potentiality of applying the method of statistical Mueller-matrix analysis to establish morphological features, pathological and postmortem changes in myocardial tissue (MT). For the study, we selected myocardial samples from the corpses who died of ischemic heart disease (IHD) ($n = 56$) and other causes of death in (heart muscle was not altered, control group $n = 30$). To obtain a histological preparation of the heart muscle, we used rapid tissue freezing and making sections using a freezing microtome. The study was performed in the traditional structure of the laser polarimeter. During the study, the microscopic sample of the heart muscle was irradiated with a He-Ne laser beam ($\lambda = 0.6328$ μm). Different states of polarization of the laser beam were formed using a polarizing illuminator. Images of histological samples of the myocardium were projected with a microlens onto a light-sensitive area of the camera. The intensities of the right and left circularly polarized components for each individual pixel were determined by rotating the transmission axis of the analyzer at an angle of ± 45º relative to the direction of the axis of a quarter of the wave plate. The obtained results were processed according to the standard algorithms of the MATLAB and Statistica software products.

3. RESULTS

Consequently, experimental researches the set of values of statistical moments (SM) of the 1st-4th order for cases of death from chronic IHD and without heart pathology is received. Figures 1 and 2 illustrate histograms of the apportionments of random values of samples of MT in normal and pathological conditions.

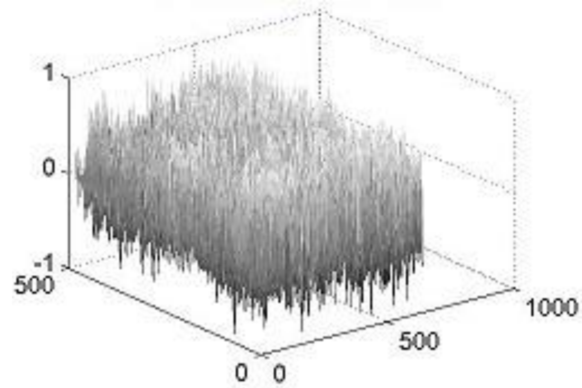

Figure 1 Histograms of distributions of random values of samples of MT of the dead from the control group.

The obtained numerical distributions of the elements of the Mueller matrix $f_{ik}(x,y)$ images of myocardial samples and the study of the dependences of experimental values revealed a significant diagnostic sensitivity to the differentiation of intact heart muscle samples from myocardium with pathological changes. Thus, in IHD we noted an increase in the extreme values of the Mueller matrix phase elements of the MT (Fig. 2). This fact indicates a significantly higher level of optical anisotropy of myosin fibrils in IHD compared to intact muscle.

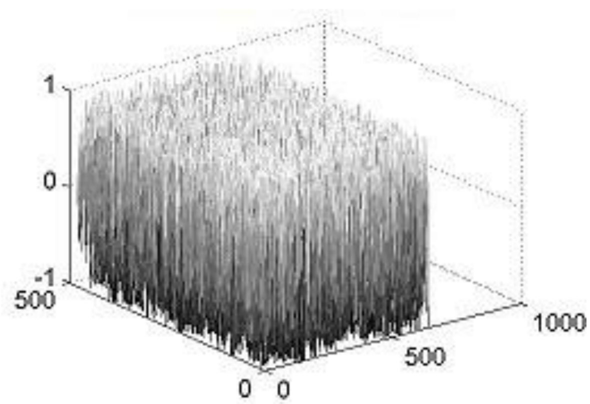

Figure 2 Histograms of distributions of random values of samples of MT at death due to IHD.

Quantitatively, the processes of change of birefringence of myosin filaments of MT at different extreme levels describes a set of SM $S_{j=1;2;3;4}$, that characterize the apportionments of the Mueller matrix elements for normal MT and in the case of the ischemic changes (table 1).

Table 1 The values of $S_{j=1;2;3;4}$, which characterize the distributions of the Mueller matrix elements for MT from the control group and in case of IHD (experimental group).

SM	Control group (n=20)	Experimental group (n=69)
Average, S_1	$0{,}63 \pm 0{,}055$	$0{,}67 \pm 0{,}061$
p_1	$>0{,}05$	
Dispersion, S_2	$0{,}28 \pm 0{,}023$	$0{,}31 \pm 0{,}025$
p_1	$>0{,}05$	
Asymmetry, S_3	$1{,}44 \pm 0{,}12$	$1{,}23 \pm 0{,}11$
p_1	$<0{,}001$	
Excess, S_4	$0{,}21 \pm 0{,}014$	$0{,}32 \pm 0{,}026$
p_1	$<0{,}001$	

According to the study results, it is determined that in the heart muscle with ischemic changes, the values of the average S_1 vary from 0.63 to 0.67, dispersion S_2 – in the range from 0.28 to 0.31, asymmetry S_3 – from 1.44 to 1.23 and excess S_4 – from 0.21 to 0.32. It was established that the SM of the 3-4th orders were the most susceptible to changes. Comparative analysis of the results showed that the standard deviation of the average value of SM $S_{j=1;2;3;4}$ does not exceed 0.05, which allows us to consider the results statistically significant. The analysis of operating parameters of the method of statistical Mueller-matrix analysis was performed (Table 2). The results show an excellent level of accuracy $Ac(S_{3;4}) \sim 90\%$, which suggests that statistical Mueller matrix analysis is effective in differentiating intact and altered MT due to IHD.

Table 2 Operational characteristics of statistical Mueller-matrix analysis in the differentiation of the MT from the control and experimental groups.

SM	$Se(S_j)\%$	$Sp(S_j)\%$	$Ac(S_j)\%$
S_1	69,8	65,2	65,6
S_2	71,4	66,7	69,0
S_3	93,6	87,3	90,4
S_4	88,9	85,7	87,3

4. DISCUSSION

It is known that at the cellular level, most BT is ordered structures that exhibit anisotropic properties (optical activity, birefringence, dichroism, etc.) during optical probing. The basis is a collagen molecule, which is a structural element of many tissues. Five molecules of collagen always form a left-handed helix due to electrostatic fields. This spiral with the initial defined direction of rotation shows a high degree of order in the collagen molecules, their molecules are shifted relative to each other at a constant angle, so they are cholesteric.

Microfibrils combine into the above-organized subfibril structures, which are right-handed supercoils. The hierarchy in connective tissues reflects the algorithm by which a BT is formed (Tuchin, 2002; Bachinskyi et al., 2018). According to this theory, the structure of many types of BT has a self-similar geometry and a common feature of their morphological formation are the processes of unlimited growth, resulting in a structured two-component system consisting of a hierarchically constructed fibrillar component (collagen proteins, myosin) and amorphous extracellular formation. The noncrystalline component is polarization isotropic, optically inactive. This approach is used for statistical modelling of optical properties of structured BT. The description of BT light scattering processes of different types is derived from the Mueller matrix. In a number of works, such an optical model of BT is theoretically and experimentally substantiated (Tuchin, 2002; Ushenko et al., 2014). These studies are built around the analytical substantiation of the processes of transfiguration of polarization of laser radiation as a result of birefringence of BT protein fibrils.

Thus, all varieties of BT are optically inhomogeneous structures, and the radiation scattered by BT becomes a carrier of information about their properties, which is contained in the photometric, spectral, polarization characteristics of light oscillations. Consequently, polarimetric studies of optical and geometric parameters of BT structure under single scattering conditions, it is established that the reason for the development of distributions of polarization states of the object field of physiologically different BT are phase shifts between polarized components of laser beams by optically uniaxial crystalline structures (Angelsky et al., 2010; Alali et al., 2012). It is established that the polarization inhomogeneity of the coherent fields of the crystal components of BT is determined by the modification of the polarization condition of the laser beam by spatially oriented birefringent fibrils (Angelsky et al., 2010). Thus, laser polarization techniques use a set of fractal, statistical, correlation relationships between the distributions of optical-anisotropic properties (linear and circular birefringence, linear and circular dichroism) of BT and two-dimensional distribution, also elements of completely polarized and depolarized components of the Mueller differential matrix of BT samples and films of biological fluids of different morphological structure and physiological state.

The greatest result obtained in the approach of LP was the relationship between a set of SM of I-IV orders, which characterize the Mueller matrix elements and the parameters of linear birefringence of fibrillar protein networks of human BT. This led to the possibility of diagnosing changes in optically inhomogeneous objects (various human BT) and the establishment of various pathological conditions (Barton et al., 2008). Today, laser polarization techniques are used in various medical fields. There are many morphological works in which the structure of various human tissues is studied, both in normal and pathological conditions, which allows to achieve significant progress in methods of laser diagnosis of the morphological construction of biological objects.

Well-known works of scientists in forensic medicine are devoted to the possibilities of applying laser polarization techniques to address topical issues that experts encounter in their daily practice (Garazdyuk et al., 2016; Bachinskyi et al., 2019). In particular, the verification of the time since death in its various types and in different pathological conditions, the time of hematoma formation, the viability of the formation of injuries, the diagnosis of acute myocardial ischemia. During the work, studies of various BT and fluid environments of the human body: skin, skeletal muscle, myocardium, brain, lungs, liver, kidneys, spleen, intestine, blood, cerebrospinal fluid, vitreous body (Garazdyuk et al., 2016; Bachinskyi et al., 2019; Bachinskyi et al., 2018).

Scientists have found that the temporal dynamics of modifications in the morphological construction of BT in the postmortem period is accompanied by dynamic regular changes in the structure of their polarization images, which is the basis for applying these techniques in determining the time since death and time of injury formation. We want to note the results, which were obtained by studying the features of postmortem changes in polarization samples of different BT. Scientists have managed to achieve the accuracy of determining the postmortem interval - 1.5 hours in the long range from 1-140 hours (Bachinskyi et al., 2018; Garazdyuk et al., 2016).

Our results confirm the diagnostic possibility of this technique in detecting pathological modifications in human MT, and correspond to the conclusions of world scientists. Based on the above, we can conclude that the proposed method are quite informative and provide important information about the structure, features and diseases of organs and systems. These techniques are easily accessible and do not require complex equipment and scarce reagents. In our opinion, in-depth study of BT and body fluids by laser polarimetric methods and compiling digital information maps for the potentiality of further use of these methods in various pathological conditions is very promising. We suppose that laser polarization techniques for reproducing the optical-anisotropic structure of polycrystalline BT networks and body fluids can be the basis for determining a set of objective criteria for screening methods for examining the population for severe pathologies of various human organ systems, which are widespread among the population of Ukraine and the world. Timely detection of such pathologies will provide a significant reduction in treatment costs, social and insurance benefits (Ushenko et al., 2010).

5. CONCLUSION

Thus, our method allows us to describe in detail the construction and characteristics of MT in normal and ischemic changes. Due to the applied statistical analysis of the distributions of the Mueller-matrix invariants of the biological layers of the MT, the magnitudes and ranges of change in the average, variance, asymmetry and excess were determined. Our results showed an excellent level of accuracy Ac (S3;4) ~ 90%, which suggests that statistical Mueller matrix analysis is effective in differentiating intact and altered MT due to IHD.

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Author Contributions

Author contributed to the research and preparation of the manuscript.

Ethical approval

The study was approved by the Medical Ethics Committee of the Kharkiv National Medical University. (Protocol No.5 dated 12.01.2022).

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Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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