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ERYTHROCYTE AT COMORBID COURSE OF CHRONIC HEART FAILURE AND ANEMIA: POSSIBILITIES OF LASER POLARIMETRY

Abstract *The article deals with the possibility of application of the methods of correlation optics and laser polarimetry for objectification of the structural changes of the erythrocyte membranes at combined course of chronic heart failure, diabetes mellitus type 2 and anemia. In patients with chronic heart failure and diabetes mellitus type 2 increasing of the anisotropic component of erythrocyte membranes was detected, which indicated conformational changes of proteins of the erythrocyte membrane structures as a result of chronic hyperglycemia. More expressed such transformations are observed during the investigation of probability and coordinate distribution of the intensity of the Fourier spectrum of laser image of the erythrocyte suspension layers at complication of the underlying disease by anemia of different degrees of severity, which serves as the basis of heterogeneity of the erythrocyte membranes structure. Quantitatively such transformation of the Fourier spectrum, caused by the change in optical anisotropy of the erythrocytes, are illustrated by the ranges of set of the statistical moments, which objectively characterize the structure of the changes of the corresponding histogram intensity. Correlation analysis revealed statistically significant direct relationship between the level of basal glycemia and the degree of anisotropy of the suspension of erythrocytes in the investigated patients, whereas the level of hemoglobin was negatively associated with the values of asymmetry and access.*
Keywords: *chronic heart failure, diabetes mellitus type 2, anemia, laser polarimetry, Fourier spectrum, erythrocyte, biological tissue.*

Introduction. Laser radiation during interaction with biological tissue (BT) can be absorbed and dissipated. Each of these processes has some information on micro- and macrostructure of the biological environment [3, 6]. Nowadays the development of the relevant methods of research of the scattered radiation by the optically active biological structures is of great topicality for obtaining new information about their structure.

The most common and sufficiently tested are spectrophotometric methods based on the analysis of the spatial changes of the intensity of the field of scattered laser radiation by the optically heterogeneous biological environments. The set of the tools and analytical methods of the polarimetric studies of the morphological structure of BT was called "laser polarimetry" [7].

Intensive development of the vector approach to the investigation of the morphological structure and physiological status of the various BT created a foundation for the development of model representations of their structure. The modeling structure of the BT [8] is based upon the idea that it is a two-component structure that consists of:

✓ optical-anisotropic component – the matrix predominantly formed by the fibrous tissue components (collagen fibers, proteins, fibrin fibers, etc.). This component is able to change the main parameters of the laser radiation during its passage through the layer of BT;

✓ amorphous component – the components of BT, which has no fibrous structure. The latter is optically neutral, i.e. one that does not change the basic characteristics of the beam of laser radiation while passing through BT.

Such spatial structure of the biological tissue is similar to the "frozen" optically-uniaxial liquid crystals.

The possibility of usage of the laser polarimetry methods for objective assessment of the erythrocytes' membranes is caused by the presence in their architecture the significant part of the specific protein structures, which, in its turn, are anisotropic from the optical point of view, that is, they are able to change the properties of the laser radiation during its passage through BT. Protein components of the erythrocyte membrane, unlike lipid, have the clear complicated hierarchical structure due to their complex level of organization. It is known that spectrin has penta- or hexagonal structure [1], which is formed by the tetramers of its molecules, that are linked to short actin microfilaments on the both ends [5]. The last acts as the connecting elements for the formation of a hexagonal mesh. The spectrin cytoskeleton of the erythrocyte maintains the definite form of the cell, therefore structural change in the ordering of the molecules can serve as a prerequisite change of the morphological structure of the erythrocyte membrane and the resulting disorder of its functional properties. Methods of the optical physics reveal and objectify the above-mentioned changes, which to our mind can expand the arsenal of the diagnostic methods of the diagnosis of the morpho-functional characteristics of the membranes of red blood cells due to various pathological conditions.

Aim of the investigation: To investigate the possible structural changes of the erythrocytes membranes in patients with chronic heart failure (CHF), diabetes mellitus (DM) type 2 and anemia of different degrees of severity by means of laser polarimetry methods.

Study design. With the help of the modern instrumental non-invasive methods of investigation a comprehensive survey of 120 patients with CHF, DM type 2 and anemia, who were hospitalized to the cardiological department of the Chernivtsi Regional Hospital for War Veterans, was conducted. The average age was 76,04±1,84 years. All examined patients according to their comorbidities were randomized into the following subgroups: I – CHF patients with comorbid DM type 2 (n=12), II – patients with CHF with comorbid anemia of different degrees of severity (n=32), III – patients with CHF, complicated by comorbid anemia and DM type 2 (n=76). The control group for comparative studies comprised 12 patients with CHF without comorbid anemic syndrome (AS) and DM type 2, whose age was not statistically significantly different from the average age of the patients of the experimental groups.

Patients of the IInd experimental group had the following distribution depending on the severity of AS: IIA subgroup – the patients with CHF with mild anemia (hemoglobin more than 91 g/L) – 16,66% of all examined patients of the experimental group) IIB subgroup – the patients with CHF with comorbid moderate anemia (hemoglobin – 71-90 g/L) – 10,00% correspondently. The patients of the III experimental group were also randomized into subgroups due to the level of hemoglobin: IIIA – 38 patients (31,67%) with CHF, DM type 2 and mild AS, IIIB – the patients with CHF, DM type 2 and moderate AS – 38 individuals (31,67%).

For the objective assessment of the functional state of erythrocytes membrane laser polarimetry of the red cell suspension smear was applied. Scheme of the optical laser polarimeter is presented on Fig. 1. Irradiation was conducted by the beam (Ø=104 mkm) of the He-Ne laser (1) with a wavelength λ=0,6328 mm. With the help of the polarizing film (quarter-wave plate and polarizer) different states of polarization of the illuminating beam were formed. Polarization images of the layers of the erythrocyte suspension (6) were formed in the plane of the light-sensitive pad (800x600) of the CCD camera (10) through the object glass (7), the resolution of which was sufficient for the measurements in the size range of structural laser images of the erythrocytes suspension (2-2000 microns).

The investigation of the optical properties of erythrocyte suspension layers was based upon the following methods:

1. Polarization imaging of the optical-anisotropic layer of the blood cells components obtained in the crossed planes of the polarizer and analyzer and statistical analysis of the received laser images.

2. Phase analysis of the polarization rendered laser image by determining the intensity of the coordinate distribution of Fourier spectrum of the red cell suspension layer.

To assess coordinate distributions of random variables their histograms were used; and we calculated set of statistical points of the 1st to the 4th grades (Fig. 2).

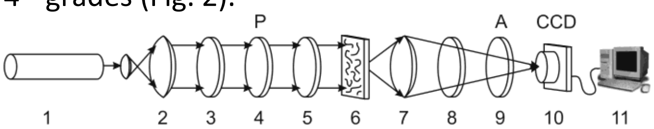

Fig. 1. Optical laser polarimeter

where: 1 – He-Ne laser; 2 – collimator; 3, 5, 8 – quarter-wave plates; 4 – polarizer; 6 – object of investigation; 7 – object glass; 9 – parser; 10 – CCD camera; 11 – personal computer

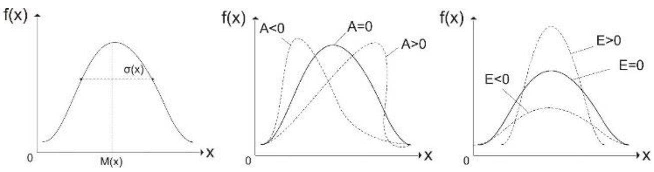

Fig. 2. Statistical points of the 1st – 4th grades (medium, variance, asymmetry, kurtosis)

Results of the investigation. Fig. 3 illustrates the coordinate (1) and probability (2) intensity distributions of Fourier spectrum of the erythrocyte suspension laser image of the patients of control group. Received results show that the intensity distribution of the histogram of Fourier spectrum has symmetrical “bell-like” distribution.

Another picture is observed during analysis of the structure of Fourier spectrum of the erythrocyte suspension laser image of the patients with CHF and DM type 2 (Fig. 4). Apparently the intensity distribution is uneven (fragment 1), and the histogram is transformed into an asymmetric dependence (fragment 2). Revealed fact indicates the growth of the anisotropic component of the red blood cells membrane, conditioned primarily by the conformational changes of the protein structure of erythrocyte membranes compared to the control group. We assume that the above-mentioned changes reflect complex disorders of the peculiarities of the erythrocyte membranes due to chronic hyperglycemia (activation of the peroxic oxidation of the biopolymers and lipids, increased production of reactive oxygen compounds, protein molecules glycolization, and, as a result, change of the conformational and spatial orientation of the protein fibrils, including integrated, of the erythrocyte membrane) [4], accompanied by worsening of the morphological features of the red blood cells membrane.

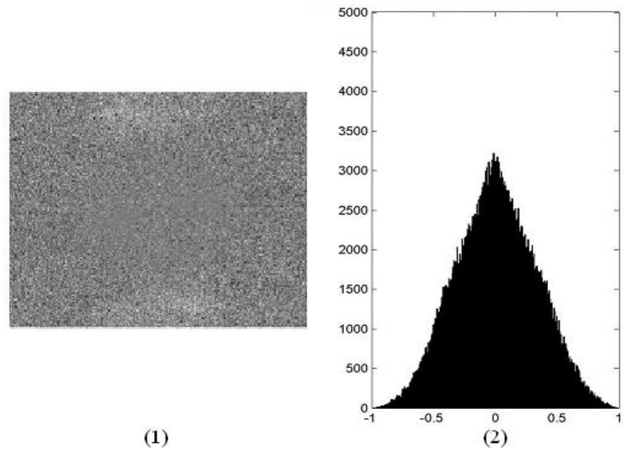

Fig. 3. Fourier-phase investigation of the erythrocyte suspension of the control group

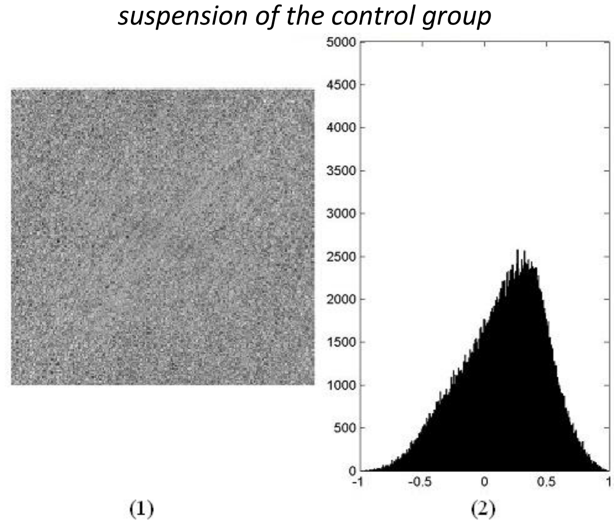

Fig. 4 Fourier-phase investigation of the erythrocyte suspension of the patients with CHF and DM type 2

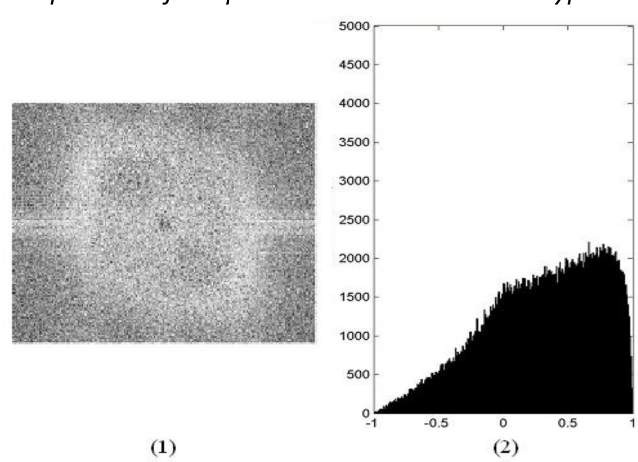

Fig. 5. Fourier-phase investigation of the erythrocyte suspension of the patients with CHF and mild anemia

More clearly such transformations are observed at the investigation of the probability and coordinate distribution of the Fourier spectrum intensity of the red cell suspension laser image of the patients of the other studied groups (Fig.5 – Fig.8). This, in our opinion, serves as the basis of heterogeneity of the erythrocyte membranes structure due to investigated comorbidity.

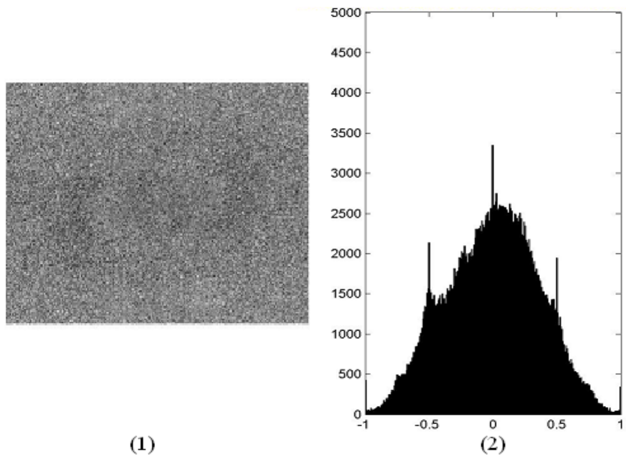

Fig. 6. Fourier-phase investigation of the erythrocyte suspension of the patients with CHF and moderate anemia

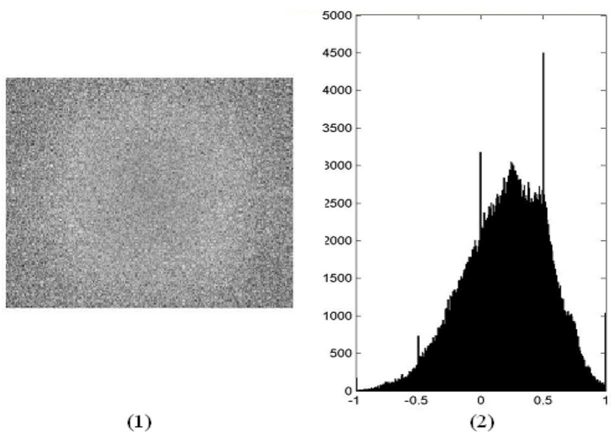

Fig. 7. Fourier-phase investigation of the erythrocyte suspension of the patients with CHF, DM and mild anemia

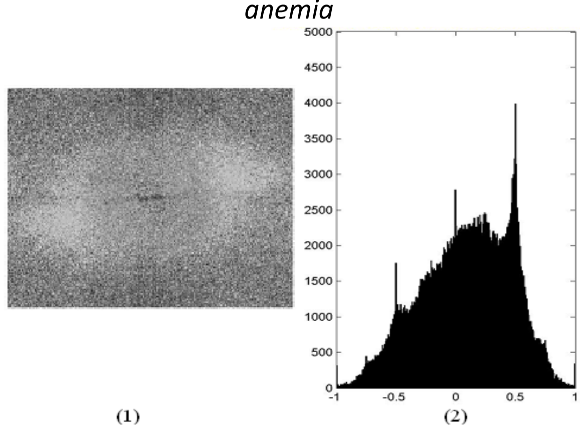

Fig. 8. Fourier-phase investigation of the erythrocyte suspension of the patients with CHF, DM and moderate anemia

The degree of anisotropy remained high in all patients of another research groups. We explained this as a progressive worsening of the morphological and functional properties of the erythrocyte membranes (primarily due to the progression of conformational changes of the protein component structure) due to comorbidity, that can not be objectified by means of traditional metods.

Additional agents that can contribute to the changes of the modification of the proteins of the red blood cells cytoskeleton and lead to the increasing of the degree of red cell suspension anisotropy is ATP and ions of Ca^{2+}, which affect the connections between the erythrocyte cytoskeleton proteins by the phosphorylation of the spectrin molecules [2]. Changes of the conformational structure of the proteins of the red blood cells cytoskeleton lead to the formation of transmembrane defects resulting in the loss of cell metabolites and ions. Destruction of only few connections in the actin-spectrin grid can cause the formation of spicules on the surface of the red blood cell. Oxidative denaturation of the protein components of the red cell membrane makes the erythrocyte sensitive to the impact of the endogenous proteases.

Quantitatively such transformations of Fourier spectrum, caused by the change of the optical anisotropy of the red blood cells, may be illustrated by the values and range changes of the set of statistical moments M; σ; A; E, which objectively characterize the structure of the changes of the corresponding intensity histograms (Table 1). Correlation analysis showed direct relationship between the level of fasting glucose and anisotropy degree of the red blood cells suspension of patients with coronary artery disease, diabetes mellitus type 2 and anemia, while the level of hemoglobin was negatively associated with the values of asymmetry and kurtosis, which, in its turn, characterize the degree of worsening of the morphological and functional properties of red blood cells of patients of this group (Table 2).

Conclusions.

1. All statistical points are sensitive to changes of the morphological and functional properties of red cell suspensions of the patients with chronic heart failure, diabetes mellitus type 2 and anemia

Table 1

Statistical points 1st - 4th degree of the coordinate intensity distribution of Fourier spectrum of the erythrocyte suspension laser image

Statistical point	Control group (n=12)	CAD + DM (n=12)	CAD + mild anemia (n=20)	CAD + moderate anemia (n=12)	CAD + DM + mild anemia (n=38)	CAD + DM + moderate anemia (n=38)
M *medium*	0,07 ± 0,009	0,11 ± 0,014	0,105 ± 0,012	0,16 ± 0,021	0,19 ± 0,032	0,31 ± 0,018 *¤ §
σ *variance*	0,16 ± 0,025	0,19 ± 0,024	0,24 ± 0,013	0,27 ± 0,016	0,29 ± 0,045 *	0,35 ± 0,031 * § #
A *asymmetry*	0,08 ± 0,100	0,63 ± 0,014 ¤	1,84 ± 0,120 * § ¤	2,62 ± 0,381 * § ¤	5,43 ± 0,290 * § #	8,13 ± 0,235 * § # ¤
E *kurtosis*	0,07 ± 0,016	0,29 ± 0,035	1,79 ± 0,295	6,18 ± 0,820 ¤	15,83 ± 1,980 * § #	27,18 ± 2,110 * § # ¤

*Note: * – difference is valid against control group, p<0,05; § – difference is valid against group CHF+DM, p<0,05; # – difference is valid against group CHF+mild anemia, p<0,05; ¤ – difference is valid against group CHF+DM+mild anemia, p<0,05*

Table 2

The correlation coefficients between the degree of red blood cells anisotropy and some laboratory parameters in patients with chronic heart failure, diabetes mellitus type 2 and anemia

Parameter	*A* asymmetry	*E* kurtosis
Hemoglobin, G/L	-0,63 *	-0,74 *
Fasting glucose, mmol/L	0,57 *	0,69 *

*Note: * – correlation coefficient is statistically valid (p<0,05)*

compared to the patients of the control group.

2. The most sensitive were changes of the statistical points of the 3rd and 4th grade (asymmetry A grew from 8 to 70 times; kurtosis E grew from 2,5 to 100 times)

3. Methods of the laser poliarimetry of the red blood cells smear with the following analysis of the statistical points of the 1st-4th grade might be used for early diagnosis of the structural and functional changes of the erythrocytes in patients with comorbid course of chronic heart failure, diabetes mellitus type 2 and anemic syndrome.

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