Computer Morphodensitometric Analysis of Erythrocytes in the Norm and after Exposure to Small Doses of Ionizing Radiation

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Computer morphodensitometry of erythrocytes isolated from peripheral blood revealed informative parameters of three-dimensional structure of diskocytes. These parameters were used to develop a diagnostic approach to evaluate the effect of small doses of ionizing radiation on the human body.

Key Words: *erythrocytes; computer morphodensitometry; ionizing radiation*

The development of nuclear technologies and contamination of vast territories by radionuclides prompt the investigation of the effects of small doses of ionizing radiation on human organism.

Cell membranes are a target for various physical and chemical influences, including ionizing radiation. High radiochemical sensitivity of phospholipids accounts for considerable contribution of cell membranes to the radiation-induced damage. Thus, the system of biological membranes can be regarded as the second target (after nuclear chromatin) for ionizing radiation at the cellular level [1,3].

MATERIALS AND METHODS

Erythrocytes from peripheral blood of 59 children living in Moscow (norm) and 31 children in the Bryansk region (radionuclide contamination) were studied.

The cells were studied in a DiaMorf image analyzer (DiaMorf, Russia) with the use of computer morphodensitometry. This method allows one to analyze the densitometric profile of an erythrocyte. On the basis of this profile three-dimensional structure of erythrocyte can be restored for further analysis of the cell state and evaluation of conventional morphometric and photometric parameters of the erythrocyte.

The proposed modification of the method is optimized for morphodensitometry of diskocytes. In the norm, diskocyte is the predominating cytologic form of erythrocyte, and the appearance of other morphological forms is associated with advanced pathology. Therefore, changes in diskocytes are particularly interesting for the early diagnosis and diagnosis of the states with vague symptoms.

RESULTS

The method for assessing the effect of small doses of ionizing radiation on erythrocytes is based on analysis of informative morphodensitometric parameters of erythrocyte's shape.

First, we studied the distribution of erythrocytes according to morphological categories (diskocyte, stomatocytes, echinocyte, etc.) with the use of conventional methods [2]. In all the children, the erythrograms obtained were within the norm.

Based on the changes in the averaged densitometric profile, we have found that the structure of the plasma membrane of erythrocytes from children exposed to low radiation doses is changed (Fig. 1). These changes are probably associated with the damage to the skeleton of the erythrocyte plasma membrane.

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Statistical analysis of the parameters revealed changes in the erythrocytes of children from the Bryansk region in comparison with children from Moscow. Statistically significant changes were recorded predominantly for the parameters characterizing the densitometric profile of the erythrocyte (Table 1). Analysis of the dispersion diagrams for the most informative parameters (*Dx* and *Cur2*) revealed two-dimensional spaces within which the analyzed groups are separated from each other (Fig. 2).

On the basis of the results obtained we have developed a diagnostic criterion. For evaluation of this criterion we studied erythrocytes of 6 children from the contaminated region and of 3 children subjected for radiotherapy. According to this criterion, all the children can be assigned into the risk group.

Thus, with the use of classical morphological methods it was impossible to identify the children exposed to low doses of ionizing radiation, while computer morphodensitometry revealed some parameters of the three-dimensional structure of the diskocyte plasma membrane statistically ($p<0.01$) different in the group of irradiated children in comparison with the control. A diagnostic criterion has been developed on the basis of combination of informative parameters describing three-dimensional structure of diskocytes. This parameter can be used for evaluation of the effect of low radiation doses on the human body.

TABLE 1. Statistical Significance of Changes in the Mean Parameters of Erythrocytes According to Student's *t* Test

Parameter	*t* test	Significance
Morphometric		
AREA	0.98	0.66
DCIRCL	0.99	0.67
FORM P	4.72	0.999
Photometric		
OD	1.74	0.91
IOD	2.31	0.97
Morphodensitometric		
Bend	3.60	0.999
Radius	0.96	0.65
Grad1	5.46	0.999
Grad2	5.86	0.999
Grad3	4.51	0.999
Cur1	4.77	0.999
Cur2	6.90	1
Cur3	6.75	1
Dy	6.01	0.999
Dx	8.32	1
L er	5.11	0.999
Surface	5.70	0.999
Volume	3.88	0.999

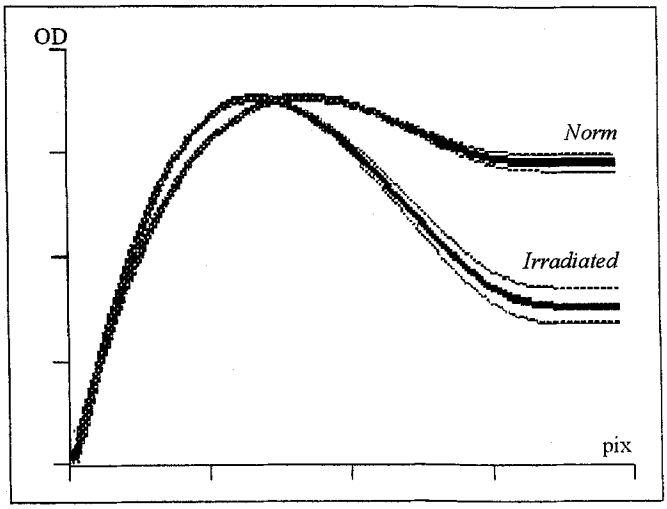

Fig. 1. Comparison of densitometric profiles of erythrocytes children exposed to low radiation doses (the Bryansk region) and children living in Moscow (control). Abscissa: space coordinate (pixel); ordinate: optical density.

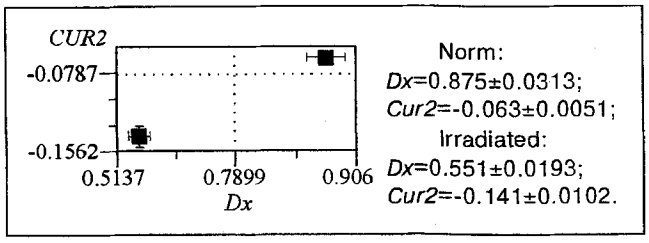

Fig. 2. Diagram of the mean parameters of morphodensitometric profile of erythrocyte. Top right: norm (Moscow); bottom left: low doses of ionizing radiation (Bryansk region).

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