http://dx.doi.org/10.35630/2199-885X/2020/10/2.1

THE USE OF PRINCIPAL COMPONENT ANALYSIS FOR EVALUATION OF MORPHOFUNCTIONAL CHANGES IN RED BLOOD CELLS UNDER THE INFLUENCE OF DIFFERENT GLUCOSE CONCENTRATIONS

Received 15 March 2020;
Received in revised form 5 May 2020;
Accepted 19 May 2020

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ABSTRACT — The article presents findings of the research investigating the effect of various glucose concentrations on the morphometric parameters of red blood cells. The principal component method was employed in order to determine the most significant indicators in the experiment. The experiment was aimed to investigate the effect of hyperglycemia on the shape, size and functional parameters of erythrocytes depending on the glucose concentration and exposure time. It was established that characteristic changes in the shape and size of erythrocytes induced by hyperglycemia are correlative with concentration of glucose and exposure time.

KEYWORDS — glucose concentration, red blood cells, morphometric parameters, principal component analysis.

INTRODUCTION

Glucose is an osmotic and chemically active substance and a long-term increase in its concentration is undesirable either in the interstitial medium or in the cell. Hyperglycemia disrupts the osmolarity of the interstitial medium and the high reactivity of glucose changes the chemical composition of structural proteins and the activity of enzymes [1].

Many authors have identified various disorders of the erythrocyte membrane resulting from the toxic effect of glucose [2]. As a result of these disorders the surface architectonics of red blood cells changes and abnormal forms of red blood cells appear [3].

The purpose of this work was to identify the possibility of using the principal component method in the assessment of morphofunctional parameters of red blood cells that were displayed under the influence of different glucose concentrations. The state of red blood cells was assessed in accordance with biophysical properties, morphofunctional parameters, indicators of poikilocytosis, anisocytosis and ovalocytosis.

Aim of study

To identify the dependence between the content of matrix metalloproteinases, their tissue inhibitors and the periodontological status in children with Type 1 DM through different endocrinopathy stages.

MATERIALS AND METHODS

During the experiment 11 control blood samples were examined, the reference values (number of er (RBC-erythrocytes or in abbreviated form — er), concentration of HB, Ht and glucose concentration) of which were determined within the normal range [4]. Blood in the amount of 4 ml was taken under standardized conditions, centrifuged at 1000 rpm, the supernatant was separated, then the er was washed in an isotonic NaCl solution three times. During the study, a series of diluted glucose solutions were prepared on buffered isotonic NaCl solution (pH 7.4) and deionized water: 1) isotonic NaCl solution — glucose concentration 0 mmol/l; 2) glucose-containing isotonic NaCl solutions — 6.5; 7.5; 10.0 and 15.0 mmol/l. Glucose concentrations corresponded to the degree of compensation of glycemia in diabetes mellitus, so the concentration of glucose in the blood up to 6.5 mmol/l corresponds to compensation, 7.5-subcompensation and higher-decompensation [5]. Each of the 5 test tubes was filled with 0.3 ml of the corresponding glucose solution on an isotonic solution with an er mass of 0.2 ml until HT — 40% was obtained. For the reliability of the results the concentration of glucose in the prepared solutions was determined using a semi-automatic stat Fax 1904 biochemical analyzer. After that, blood preparations were prepared: from the first test tube — before incubation (control) and then from all five test tubes — 10, 30, 60 and 120 minutes after incubation in a thermostat at 37° C. A total of 21 smears were obtained from a single blood sample. At each stage of incubation the preparations were previewed under a microscope at low magnification

without immersion. Preparations with all glucose concentrations incubated for 120 min were poorly visualized and were not subject to further cytomorphometric analysis. Under microscopy in these smears, normal discocytes were globulated, glued together and located in the fields of vision as aggregates and coin columns and were prone to hemolysis. Thus, in our study we worked with the morphofunctional properties of er under the influence of different glucose concentrations for 10, 30 and 60 minutes. The study of the obtained smears was carried out on the computer hardware and software complex *mekos-C-3*, the program — *Erythrocytometry*. It is known that the morphology of shaped blood elements has long been recognized as an important clinical indicator. Among the numerous hematological indicators, er morphometry is successfully used for the diagnosis of a number of different pathologies, as well as for General health assessment. The modern Arsenal of morphometric methods for analyzing peripheral blood cells is quite extensive [6]. In the study of er analysis the following characteristics were used: average diameter, area, polarization, shape factor, red blood cell formula (distribution into the main morphological types), as well as the coefficients of ovalocytosis, poikilocytosis, anisocytosis and anisochromia. The study of the nanostructure of peripheral blood erythrocytes of wounds was carried out using a scanning probe microscope NTegraPrima (NT-MDT, Russia). In the study, specially prepared microprobes were used in semi-contact mode with the use of *misalignment errors*. A preliminary selection of the area was made in an optical microscope at a magnification of 40 × 0.65, a glass cutter was used, the marked area was selected, and the sample was placed on the object table of a scanning probe microscope. The mode was used using the NT-MDT NSC 10 cantelever [7]. Two-dimensional and three-dimensional images were obtained using atomic force microscopy in 3D mode (Fig. 4).

RESULTS AND DISCUSSION

We used the principal component method to determine a small number of linear combinations of initial morphometric features of er in relation to glucose concentration which explains most of the variability of the data as a whole. Statistical data processing was performed using nonparametric analysis methods (the principal component method) and using Microsoft Office software Microsoft Exel, STATISTICA Edition and STATGRAPHICS Plus. All stages of the experiment were performed at the Department of Biomedicine and physiology of the North Caucasus Federal University. The use of the above-mentioned morphometric and statistical methods allowed us to evaluate the properties of er as an indicator of adaptation and adaptability of the body to changed conditions of the internal environment (high concentrations of glucose).

As a result of the analysis, 3 main components were identified in the control blood sample, describing 100% dispersion of morphofunctional features of er. It turned out that in the first main component, which describes 68.5% of the variance of the analyzed er features, the greatest weight among the features was

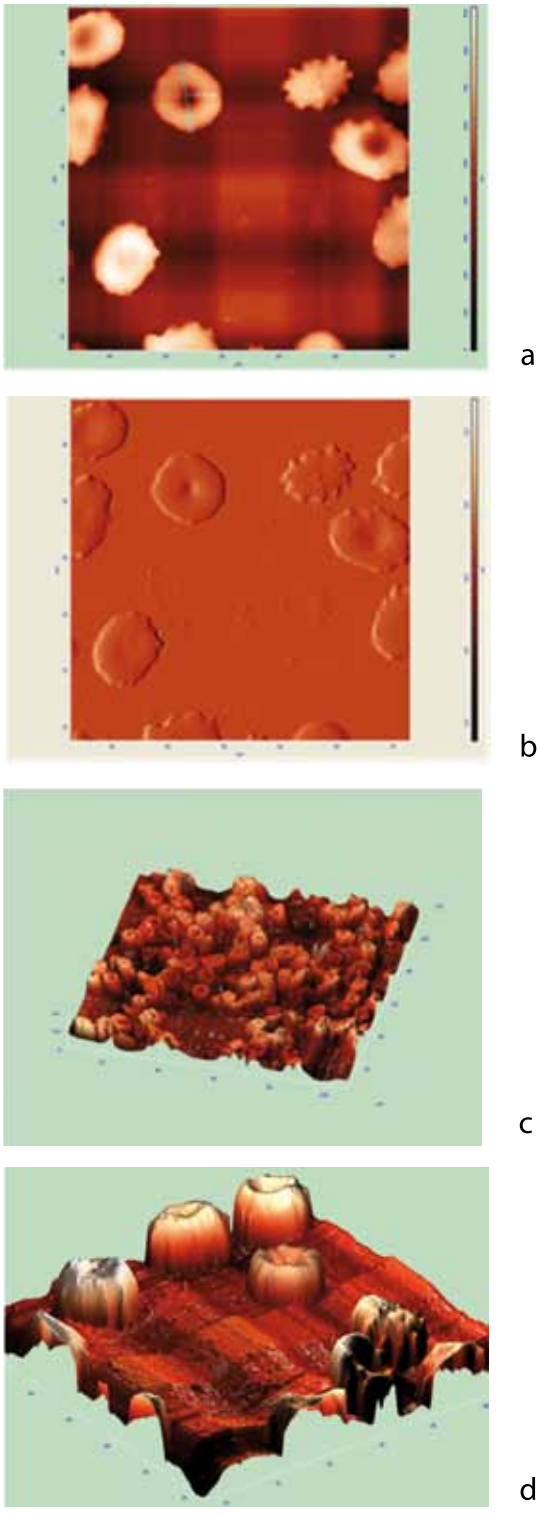

Fig. 4. *Nanostructured organization of red blood cells under the influence of high glucose concentration (10.0 mmol / l): a, b — two-dimensional image of red blood cells obtained using AFM "Integra prima"; c, d — three-dimensional image of red blood cells obtained using AFM "Integra prima"*

the number of discocytes, the ovalocytosis coefficient and the number of microcytes (Fig. 1).

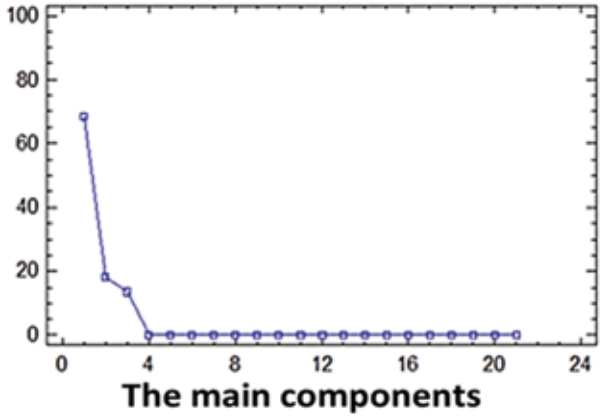

Fig. 1. *The main components highlighted in the analysis of morphometric characteristics of er in the control blood sample*

At a glucose concentration of 6.5 mmol/l, 2 main components were identified describing 100% of the variance of the analyzed features. The first main component describing and 76.4% of the variance, the largest value among the signs had a ratio of ovalocytosis, the number of discocytes and mikrocytos when the dispersion of the analyzed morphometric characters of er became predominant beshenkovichy period and the greatest weight among the signs had the number of discocytes (0,240), the coefficient of ovalocytosis (0,238) and the number of mikrocytos (0,228) (Table. 1.). In the second main component which describes 18.1% of the variance of the analyzed features, the largest changes were made by the data after 10 minutes of the experiment. Among them, the number of deformed irreversibly altered forms of er (0.327), codocytes (0.254) and microcytes (0.249) was dominant.

Analysis of a blood sample with a glucose concentration of 7.5 mmol/l also allowed us to identify 2 main components. In the first main component, which describes 58.3% of the variance of the analyzed morphofunctional parameters of er, the ovalocytosis coefficient, the number of microcytes and discocytes had the greatest informative significance among the features.

When performing the main component analysis, 2 main components were identified in a blood sample with a glucose concentration of 10.0 mmol/l. In the first main component, which describes 60.5% of the variance of the analyzed er features, the number of microcytes, spherocytes, and poikilocytosis were the most important among the features. The increase in the changes in the proportion of microcytes, in our opinion, is due to the influence of high glucose concentration which causes acceleration of the aging process of the cell and, consequently, its shrinking and reduction in size [8].

According to the method of main components at a glucose concentration of 15.0 mmol/l, 2 main components were also isolated. In the first main component, which describes 59.2% of the variance of the analyzed er features, the indicators of anisochromia and anisocytosis as well as the number of echinocytes had the highest weight among the features.

Analyzing the contribution of each of the studied indicators to the marked changes, the dominance of anisochromia, anisocytosis, echinocytes, spherocytes, ovalocytosis coefficient and discocytes was revealed. At the same time, it turned out that the greatest variability is caused by such indicators as anisochromia and the number of spherocytes. The fact that the greatest shifts are observed 10 minutes after the start of the experiment (Fig. 2) fits well into the hypothesis about the influence of high concentrations of glucose on the morphometric parameters of er and reveals a similar picture with the concentration of glucose 10.0 mmol/l (Fig. 3).

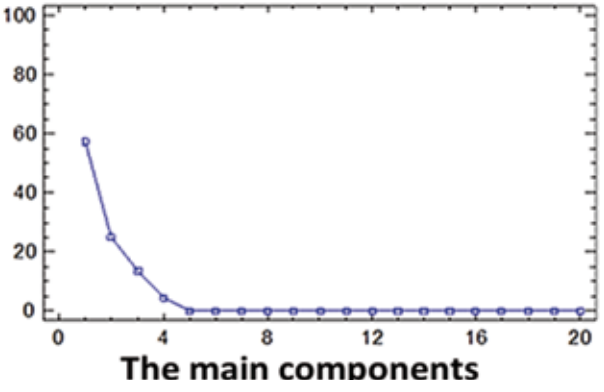

Fig. 2. *The main components identified in the analysis of morphometric features of er after 10 minutes of the experiment*

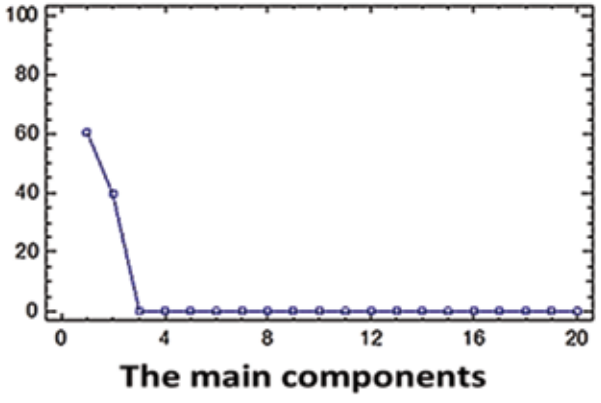

Fig. 3. *The main components identified in the analysis of morphometric features of er with a glucose concentration of 10.0 mmol/l*

Table 1. *Weight of morphometric features of er in the control blood sample*

The signs	The components		
	1	2	3
Microcytes	0,228	0,249	0,081
The normocytic	-0,224	-0,247	-0,128
Macrocytes	-0,183	-0,200	0,361
Deformed bodies	-0,200	0,327	-0,084
Discocytes	0,240	-0,204	0,063
Echinocytes	0,119	0,200	0,477
Elliptocytes	-0,249	-0,001	-0,198
Spherocytes	0,180	-0,332	0,202
Codocytes(target-shaped)	0,223	0,254	0,123
Pegmatite (bitten)	-0,110	-0,339	0,371
Dacryocytes (teardrop-shaped)	-0,221	0,202	0,227
The ratio of ovalocytosis	0,238	-0,034	0,255
Poikilocytosis	-0,250	0,157	-0,042
Anisocytosis	-0,251	0,041	0,179
Anisochromia	-0,248	0,003	0,201
Area of the object	-0,231	-0,242	0,069
Average diameter	-0,225	-0,264	0,045
Factor of the form	-0,262	-0,051	0,048
Polarization	-0,237	0,039	-0,257

This also explains the significance of the anisochromia index as one of the signs of the degree of cell aging and the course of pathological changes. The appearance of spherocytes seems to be associated with an increasing intoxication effect of glucose concentration which led to a violation of the stability of the membrane.

In addition, the combination of the appearance of spherocytes can be considered as a pre-hemolytic stage which is passed by echinocytes, stomatocytes with irreversible damage [9–18].

Analyzing the contribution of each of the studied indicators to the marked changes, the dominance of anisochromia, anisocytosis, echinocytes, spherocytes, ovalocytosis coefficient and discocytes was revealed. At the same time it turned out that the greatest variability is caused by such indicators as anisochromia and the number of spherocytes.

CONCLUSION

Thus, the erythrocyte system has a significant instability to the effects of high glucose concentrations i.e. there is no adaptive mechanisms and high hyperglycemia. The system ceases to be resistant to external effects of glucose at a concentration of 7.5 mmol/l. With a further increase in the concentration of glucose occurs as if its loosening which is shown by significant shifts in the morphometric indicators of er from normal parameters. Consequently, during the entire experiment the greatest changes occurred in blood samples with high concentrations of glucose and more stable, respectively, were blood samples with normal glucose content and glucose concentration of 6.5 mmol/l. In addition, we confirmed the hypothesis that a concentration of 7.5 mmol/l destabilizes the red blood cell system.

Apparently, it is possible that staged changes in glucose supply from the incubation medium by means of passive and facilitated diffusion with its consequent anaerobic oxidation of er, which determines the level of phosphorylation of phosphoproteins in the cell cytoskeleton, functioning of the pump and receptor apparatus of the membrane. Therefore, by changing geometry of er we can judge the initial state of the erythrocyte membrane and the level of oxidative processes of er.

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