New Aspects in the Study of Clinical and Morphological New Aspects in the Study of Blood in Type II Diabetes Mellitus

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ABSTRACT

The aim of the research was to study red blood cells in type II diabetes mellitus (DM), both an independently occurring disease, and in combination with hypertension, using innovative research methods (atomic force and scanning electron microscopy with elemental analysis). In the course of the study, we observed 20 patients aged 40 to 50 years. All of them were divided into groups of 5 people: without somatic and endocrine pathology and infectious diseases; with moderate type II DM; stage II hypertension; with a combination of type II DM and hypertension. Each has passed the necessary set of generally accepted clinical and laboratory examination methods together with related specialists (therapist, endocrinologist, neurologist, ophthalmologist). Blood was taken from each patient for subsequent light, atomic force and scanning electron microscopy. A morphometric analysis of the tissues in the Ntegra-Aura instrument was also performed. We observed the most pronounced changes in the structure of erythrocytes in people with a combination of type II DM and hypertension: the number of degenerative forms increases sharply, and the number of discocytes decreases. In this regard, such altered red blood cells can't perform their functions in full. The article substantiates the possibility and future prospects of using atomic force and scanning electron microscopy with elemental analysis for independent type II DM, and in combination with arterial hypertension.

Keywords: diabetes mellitus, atomic force microscopy, scanning electron microscopy, red blood cells.

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INTRODUCTION

Currently, more than 422 million people worldwide have various forms of diabetes mellitus (DM).By 2030, this will be the seventh cause of death in the world. In its first Global Diabetes Report, WHO emphasizes the sheer magnitude of diabetes and the potential for changing the current situation (Pavlova et al. 2016).

A political basis for taking concerted action to combat this disease has already been formed. It is also identified as sustainable development goal of the United Nations Political Declaration and the WHO Global Plan of Action for Non-communicable Diseases. In this report, WHO indicated the need to scale up the prevention and treatment of diabetes (American Diabetes Association 2014).

A complex of metabolic changes detected in diabetes (Chang et al. 2017), as a rule, leads to a violation of the rheological properties of blood (Chang et al. 2017). Even with a short duration of the disease, deviations of the main hemorheological parameters, such as blood viscosity, the ability of red blood cells to aggregate and deform, are observed (Bashuk et al. 2014).

Increased blood viscosity, hyperaggregation, violation of the deformability of red blood cells lead to a decrease in capillary blood flow, ischemia, hypoxia of organs and tissues in DM (Jaman et al. 2018). Hemorheological disorders lead to the development of arterial hypertension. A connection was established between impaired myocardial perfusion in diabetes and the presence of red blood cell hyperaggregation (Neamtu et al. 2015). The complex of microrheological disorders (Szablewski & Sulima 2017) that occur in DM is involved in the pathogenesis of the development of its late vascular complications (Loyola-Leyva et al. 2018).

Diagnosis of hemorheological disorders in diabetes can serve as an early marker of target organ damage (Zhang et al. 2015) and various system changes (Blaslov et al. 2019). Also, further study of hemorheological features in diabetes is important for a more complete understanding of the pathogenesis of the disease (Vilahur 2018), in the long term - to search for effective ways to correct violations (Pavlova 2013; Bedel et al. 2018; Pourmousa et al. 2018).

We carried out a number of works indicating the need for innovative research methods (atomic force and scanning electron microscopy with elemental analysis) in studying this problem (Pavlova 2012), which requires further study.

MATERIAL AND METHODS

The study was performed on the basis of the Belgorod City Polyclinic.
Patients (5 in each group) aged 40 to 50 years were taken for examination.

Each has passed the necessary set of generally accepted clinical and laboratory examination methods together with related specialists (therapist, endocrinologist, neurologist, ophthalmologist).The recipients were presented as follows: a group of people without somatic and endocrine pathology and infectious diseases; with type II DM of moderate severity (fasting glycemia increased to 14 mmol/l, daily glucosuria did not exceed 40 g/l, ketosis or ketoacidosis occasionally developed, were revealed diabetic angioneuropathies of various localization and functional stages); stage II hypertension (established on the basis of blood pressure and the presence of changes in one or more target organs); group of patients with a combination of type II DM and hypertension.

Blood was taken for atomic force and scanning electron microscopy. After that, samples were prepared consisting of a suspension of red blood cells, previously washed from plasma. To perform atomic force microscopy, they were placed on a defatted glass slide for immunohistochemical analysis and then in a wet Ntegra-Aura chamber for scanning. Using this method allows, in addition to reducing the time of the study itself, to obtain scans of cells with high resolution, without disrupting their vital functions, shape and native size (Baro & Reifenberger 2012).

A morphometric analysis was performed at Ntegra-Aura device. In addition, the samples were viewed in scanning electron microscopes: «FE1 Quanta 200 3D» and «FE1 Quanta 600 FEG» without further processing.

Elemental analysis (oxygen, carbon, phosphorus, calcium, magnesium, iron, nitrogen, aluminum, sodium, and sulfur) was performed using a detector for recording characteristic x-ray spectra «EPAX» company that was integrated with a scanning electron microscope «Quanta 600 FEG». It was based on the occurrence of continuous fluorescence radiation during the bombardment of the studied samples by a beam of primary x-rays. The sensitivity of the method was 0.1-0.3%, depending on the type of atoms.

In the statistical analysis of the material, the calculation of intensive and extensive indicators of average values was carried out, the reliability of the differences between the average and relative values was determined by the Student criterion. The result was considered reliable at $p < 0.05$.

RESULTS AND DISCUSSION

Analyzing the data of scanning electron microscopy in a group of practically healthy people, we obtained data on the state of red blood cells. So, the average indicators of their sizes amounted to 7.72 ± 0.48 μm. The largest percentage of the red blood cell population was represented by normocytes (68.40 ± 1.43%), microcytes amounted to 14.30 ± 1.32%, macrocytes - 16.40 ± 0.13% (Table 1).

Table 1: The ratio of the types of red blood cells (in size)

Redbloodcells	Proportional cell content (% of total)			
	Healthy (n=5)	DM II (n=5)	Hypertension (n=5)	DM II +hypertension (n=5)
Microcytes	14.30±1.32	19.40±3.20* **	15.10±2.28	12.30±3.28*
Normocytes	68.40±1.43	55.50±1.45*	54.90±3.21*	57.60±1.23*
Macrocytes	16.40±0.13	23.30±1.08* **	27.20±0.88*	26.10±0.27*
Megalocytes	1.20±0.28	3.20±0.08* **	5.20±0.12*	5.40±0.08*

Note. * - p <0.05when compared with healthy people
** - p <0.05 when compared DM II with patients with GB

The diameter of the cells was: microcytes - 6.1 ± 0.09μm, normocytes - 7.3 ± 0.10μm, macrocytes - 9.5 ± 0.09μm (Fig. 1).The prevalence of discocytes was shown - 88.67 ± 2.44%. When studying the surface of red blood cells using atomic force microscopy, it was found that the depth of their cavity, calculated by studying the profile of the cell, was 0.25 ± 0.06 μm. When calculating the ratio of the diameter of the red blood cell to the size of the cavity, this value was 21.5 ± 2.3 units.

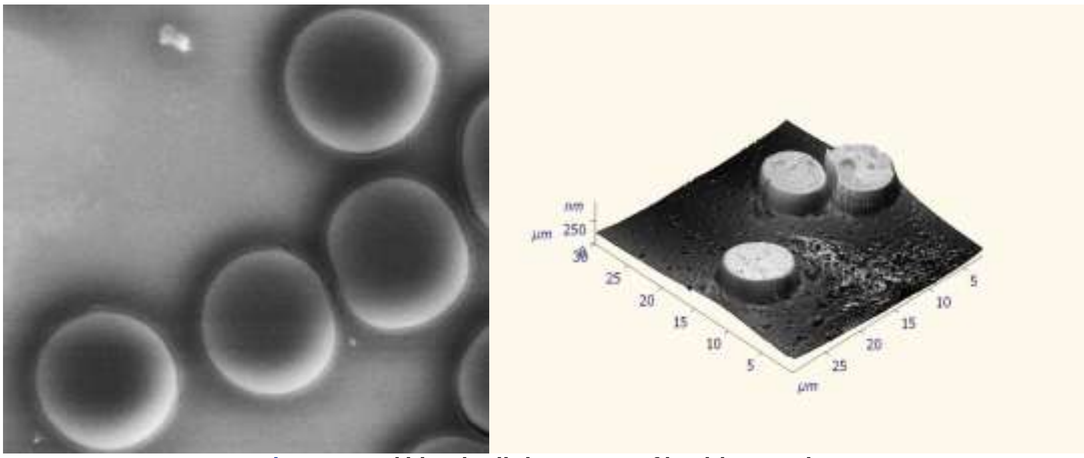

Figure 1: Red blood cells in a group of healthy people.
Fig. A - Normocytes are mainly observed (SEM, x15000).
Fig. B - The structure of the pores is not broken (3D image, atomic force microscopy).

The results of hemoscanning showed that in individuals with type II DM and hypertension the following changes were observed, most pronounced when these two pathologies were combined.Anisocytosis was expressed, which manifests itself in the direction microcytosis and macrocytosis. The number of discocytes significantly decreases, while the number of reversibly altered transient and especially degenerative forms, on the contrary, increased (Table 2).

Table 2: The ratio of the types of red blood cells

Red blood cells	Proportional cell content (% of total)			
	Healthy (n=5)	DM II (n=5)	Hypertension (n=5)	DM II + hypertension (n=5)
Discocytes	88.67±2.44	70.30±0.92*	73.30±2.92*	72.03±0.62*
Reversiblymodified (transient)	11.00±2.39	18.52±1.06*	20.02±1.14*	23.91±1.05*
Irreversiblymodified (prehemolytic)	0.17±0.17	8.18±0.48$^{*\,**}$	5.18±0.51*	9.11±0.42*
Degenerativeforms	0.33±0.21	4.00±0.35$^{*\,**}$	2.50±0.30*	4.08±0.31*

Note. * - $p < 0.05$ when compared with healthy people
** - $p < 0.05$ when compared DM II with patients with GB

Secondary absolute erythrocytosis was detected. Poikilocytosis with a change in the shape of red blood cells was observed periodically. Ovalocytes appear. The number of echinocytes increased. The number of prehemolytic forms is increasing. The cells were closely connected with each other with the help of spines, which made it possible to slow down the blood flow with the subsequent formation of a blood clot, which is especially typical for DM (Fig. 2).

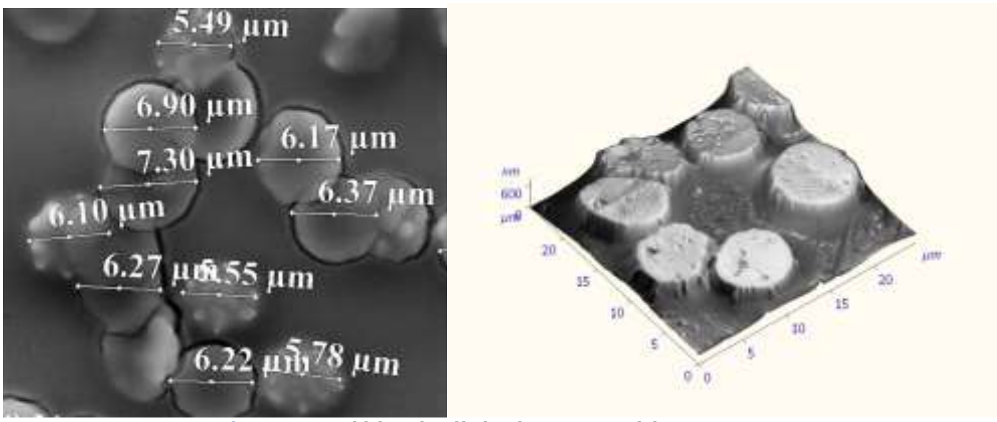

Figure 2: Red blood cells in the group with type II DM.
Fig. A -Stasis and sludge cells. Normocytes are mainly observed. A large number of microcytes. Outgrowths on the cytolemma(SEM, x6000).
Fig. B. - Violation of the pore structure (3D image, atomic force microscopy).

The erythrocytes of patients with hypertension, according to hemoscanning data, were also represented mainly by the size and shape of erythrocytes. The number of macrocytes and megalocytes increased (compared with the group of practically healthy individuals), pronounced poikilocytosis was observed with the appearance of prehemolytic and degenerative forms.On the cytoplasmic membrane of erythrocytes, outgrowths of various sizes and pore structure disturbances were revealed (Fig. 3).

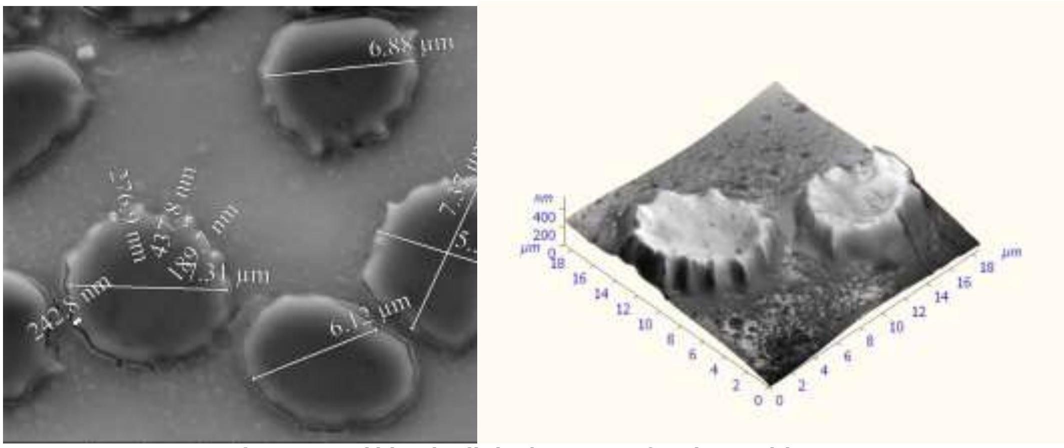

Figure 3: Red blood cells in the group of patients with GB.
Fig. A - Normocytes are mainly observed. Cells increase in diameter. A change in their forms appears. The depth of the discocyte cavity is significantly increased. Outgrowths on the cytolemma of various sizes (SEM, x12000).
Fig. B. - The structure of the pores is broken (3D image, atomic force microscopy).

When studying the surface of erythrocytes, it was revealed that the depth of the discocyte cavity in type II DM was 0.29 ± 0.08 µm, hypertension - 0.16 ± 0.07 µm, and in the combined version - 0.20 ± 0.04 µm. When studying the ratio of the diameter of the red blood cell to the size of the cavity, it was shown that this indicator increased with hypertension and a combination of type II DM and hypertension (Fig. 4), due to an increase in the number of macrocytes and the depth of the cavity.

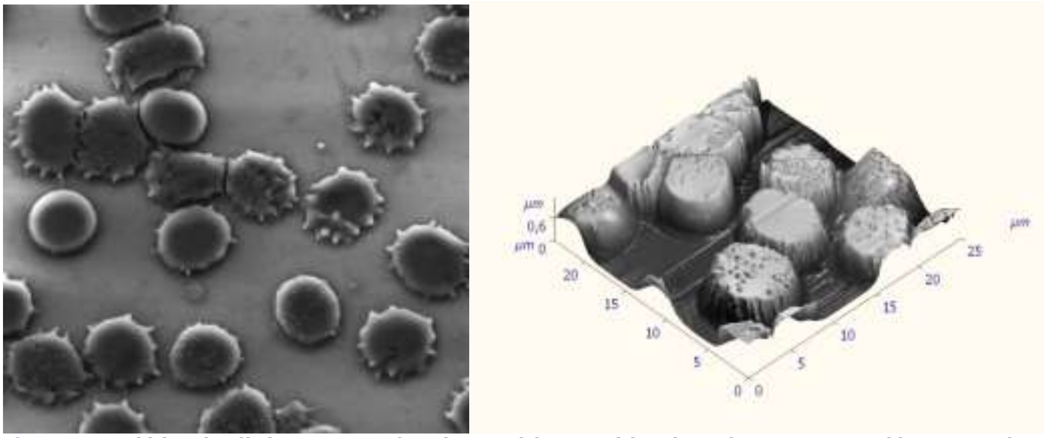

Figure 4: Red blood cells in a group of patients with a combination of type II DM and hypertension.
Fig. A - Stasis and sludge cells. The shape of part of the cells is changed. Degenerative forms appear. Outgrowths on the cytolemma of various sizes. Their number is increasing (SEM, x12000).
Fig. B. - The structure of the pores is broken (3D image, atomic force microscopy).

According to the results of atomic force and scanning electron microscopy with elemental analysis, we were able to establish the proportional content of atoms of various chemical elements in the erythrocytes of patients of each of the studied groups (Table 3).

Table 3: **Proportional atomic composition of red blood cells**

Element	Proportional atomic content of an element (in% of the total number of atomic contents in an erythrocyte)			
	Healthy (n=5)	DM II (n=5)	Hypertension (n=5)	DM II + hypertension(n=5)
C	46.88±0.10	49.43±0.08	47.64±0.12	50.11±0.25
N	20.42±0.48	18.66±1.56	18.50±2.98	20.23±3.12
O	18.37±0.03	14.34±0.03*	15.23±0.25	13.37±0.08
Na	3.11±0.02	2.69±0.02**	4.85±0.04	4.56±0.05
S	0.79±0.07	1.38±0.02	1.23±0.02	0.37±0.03
Cl	5.33±1.01	5.76±0.20**	7.44±0.07	6.65±0.02
K	2.88±0.03	2.67±0.06	1.87±0.05	1.69±0.05
Others	2.11±0.44	3.00±0.04	2.32±0.20	2.44±0.29

Note. * - p <0.05 when DM II compared with healthy people
** - p <0.05 when compared DM II with patients with GB

The results of the elemental composition of erythrocytes showed that the differences in the content of carbon, sodium, sulfur, and potassium in the erythrocytes of patients of different groups were insignificant and varied within rather narrow limits. A significant decrease in the content of oxygen atoms compared with a group of people without somatic and endocrine pathology and infectious diseases (18.37 + 0.03) was recorded in the group with type II DM (14.34 + 0.03). Such a change can lead to a decrease in the amount of oxygen given to tissues and, consequently, to their ischemia, hypoxia of organs.

It should also be noted a decrease in the content of sodium atoms and an increase in the content of chlorine atoms in red blood cells with type II DM (in comparison with the red blood cells of patients with hypertension).The cause of this phenomenon may be acidosis developing in diabetes, as well as a violation of the activity of sodium-potassium ATPase. In this case, there is a transition of chlorine to red blood cells from plasma and the leveling of the content of this anion between them, and the amount of intracellular sodium decreases due to a change in the work of the above enzyme.

CONCLUSION

Thus, the metabolic changes detected in diabetes do lead to a violation of the rheological properties of bloodin particular due to changes in the size, shape and elemental composition of red blood cells.All this can lead to ischemia and hypoxia of tissues and organs.
The use of light, electron and atomic force microscopy, as well as the study of the elemental composition of red blood cells in various pathologies, in particular diabetes and hypertension, in the futuremay change the scientists' views on their pathogenesis, as well as on the diagnosis and correction of changes.

CONFLICT OF INTEREST

None

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