Pathomorphological indicators of endothelial - erythrocytic dysfunction in type I diabetes: from the period of reproduction to the elderly and senile age

Tatyana V. Pavlova[1], Anton N. Kaplin[2], Irina I. Povalyaeva[3], Lyudmila O. Zemlianskaia[4], Lyubov A. Pavlova[1] , Igor Yu. Goncharov [1,5]

[1]Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia

[2]Kursk State Medical University, 3,Karl Marks St., Kursk, 305041, Russia

E-mail: drkaplin@rambler.ru

[3]Belgorod City Polyclinic, 99, Belgorodskij prospekt St., 308000, Belgorod, Russia

[4]Doctor Clinic LLC Fomina, 119, Preobrazhenskaya St., 308015, Belgorod, Russia

[5]Belgorod Shukhov State Technological University, 46 Kostyukov St., 308012, Belgorod, Russia

Abstract

Introduction: Diabetes mellitus (DM) is one of the most common diseases. According to the WHO, at present, the total number of patients with diabetes in the world has exceeded 100 million people. Endothelial dysfunction is an integrated syndrome of insulin resistance deepens it, increases vascular reactivity, and provokes cardiovascular disorders. In connection with this search for modern methods of detecting and identifying the presence of endothelial dysfunction in patients with diabetes is extremely important. Taking into account the above, the purpose of our study was to study the possibilities of using innovative pathomorphological approaches (scanning raster and atomic force microscopy) in the study of endothelial cell dysfunction in women with type I diabetes.

Materials and Methods: The research materials consisted of two groups. The first group included pregnant women with type I diabetes, the second group consisted of patients of various age groups. The study and morphometric processing were carried out in a scanning electron microscope and an atomic force microscope. The geometric parameters of erythrocytes were calculated as area, volume, max Z, perimeter.

Results and discussion: When studying erythrocytes, it was shown that the sizes of different groups of cells did not differ significantly. Pathological forms of erythrocytes were represented by: transitional forms (capable of reverse transformation): ellipses, discocytes with a crest, flat disks, discocytes with outgrowth, discocytes with multiple outgrowths, erythrocytes in the form of a "mulberry berry"; prehemolytic forms (with changes that are irreversible): domed, spherical, in the form of a "deflated ball"; degenerative. There was a clear tendency to an increase in their content with increasing age of the patients, which, apparently, is associated with the duration of the course of the disease. In the older age groups of patients with diabetes mellitus I, the erythrocyte cavity depth indicators varied significantly, therefore, there was no significant increase in this indicator compared to the biological norm group for this age; on the contrary, it was decreased. In the samples of the uterus of pregnant women with type I DM, obtained during the caesarean section, the vessels had a greater tortuosity than in the control group. Moreover, their diameter varied significantly. Foci of destruction were identified in the endothelium. The distance between the folds of endothelial cells increased

In the lumen of a part of the vessels, fibrin was determined in the form of separate filaments and layers, platelets, erythrocytes with stasis and sludge. The structure and shape of erythrocytes was often disrupted.

Conclusions: Even with a short duration of the disease, deviations of the main hemorheological properties and indicators are observed, including such as changes in the basic geometric parameters of erythrocytes, their ability to aggregate and deform. Diagnosis of hemorheological disorders in diabetes can serve as an early marker of target organ damage. The study proved that changes in carbohydrate metabolism in diabetes mellitus significantly affect the properties of the endothelial-erythrocytic system, and they can be diagnosed using various modern microscopy methods at an early stage.

Key words: diabetes mellitus, pregnant, erythrocytes, electron, atomic force microscope

doi.org/10.31838/hiv22.02.16 Received: 20.07.21, Revised: 10.08.21, Accepted: 25.09.21

Introduction

Diabetes mellitus (DM) is one of the most common diseases. According to the WHO, at present, the total number of patients with diabetes in the world has exceeded 100 million people (3% of the world's population) (Babik et al., 2018; Giraldo-Grueso & Echeverri, 2020). It increases by 57% annually and doubles every 12-15 years. Carbohydrate metabolism, disturbed in diabetes mellitus, can cause vascular lesions, micro- and macroangiopathies (Lawrence, 2011; Sehyun, Yunhee, Narayanan, & Megha, 2007; Shi & Vanhoutte, 2017), which is largely due to dysfunctions of the endothelial cells themselves. Endothelial dysfunction is an integrated syndrome of insulin resistance (Cho, Mooney, & Cho, 2008; Pavlova et al., 2020; Popova, Berezikova, & Mayanskaya, 2010), deepens it, increases vascular reactivity, and provokes cardiovascular disorders (Sattar & Preiss, 2018; Vecchie, Montecucco, Carbone, Dallegri, & Bonaventura, 2019). It should be noted that the appearance of this syndrome is one of the earliest signs of vascular lesions in patients with diabetes and can be detected at the initial stages of the disease, even before the appearance of atherosclerotic plaques, and in the later stages it can provoke spasm (Lunder, Janić, & Šabovič, 2019; Sosunkevič, Rapalis, Marozas, Čeponis, & Lukoševičius, 2019), the formation and growth of plaques (Kuznik et al., 2012), followed by its rupture. In connection with this search for modern methods of detecting and identifying the presence of endothelial dysfunction in patients with diabetes is extremely important (Avramenko, Litvinov, & Malinina, 2019; P Hoffman, 2014; Pavlova et al., 2020). Taking into account the above, the purpose of our study was to study the possibilities of using innovative pathomorphological approaches (scanning raster and atomic force microscopy) in the study of endothelial cell dysfunction in women with type I diabetes.

Materials and Methods

The research materials consisted of two groups. The first group included pregnant women with type I diabetes with a compensated course (20) aged 25-35 years (average age 29.5 ± 4.5 years), and 10 women without endocrinopathies and obstetric pathology (26.5 ± 2.5), located on the basis of the Perinatal Center of the Belgorod regional Clinic Hospital of St. Joasaph in 2018-19. To clarify the diagnosis, general clinical, radioimmunological, instrumental research methods were used. Clinical blood and urine tests were performed, and biochemical parameters were studied. Childbirth in all surveyed women was carried out by performing an abdominal cesarean section for a period of 38-40 weeks.

The second group consisted of patients of various age groups, in whom the study was carried out at the clinical base of the City Polyclinic of Belgorod 6 in the period from 2019-2020. These are 52 women with type 1 diabetes. Among them were: 1) 15 women in the 25-44 age group (average age 33.5 ± 3.2 years); 2) 18 - 45-59 years old (average age 52.5 ± 2.2 years); 3) 12 - 60-75 years old (average age 72.8 ± 4.4 years); 4) 10 women - 76-80 years old (average age 77.5 ± 2.5 years). The control group consisted of 20 women in whom the disease was not diagnosed (10 women aged 25-36 and 10 women in the age group 60-75).

Erythrocytes of the venous blood of women were examined. To study native cells, the erythrocyte mass was washed and blood smears were formed. To determine the tissue changes in the vessels of the uterus, its fragments were taken during a cesarean section. The samples were examined under an electron microscope without additional processing. The study and morphometric processing were carried out in a scanning electron microscope (SEM) "FE1 Quanta 600 FEG" and an atomic force microscope (AFM) "Ntegra-Aura". SPM images were obtained in the semicontact AFM mode using DCP11 series cantilevers with a radius of curvature of 70 nm. Statistical processing of AFM images was carried out using the "Image Analysis P9" (NT-MDT) software package. The geometric parameters of erythrocytes were calculated as Area - the cross-sectional area of the particle at the level of half the height of the erythrocyte, Volume - the volume of the particle, Max Z - the value of the local maximum, the height of the erythrocyte measured from the general zero level, Perimeter - the cross-sectional perimeter, as the diameter of a circle whose area is equal to the sectional area Diameter = $2\sqrt{(Area / \pi)}$.

Statistical analysis was not applied because there was no need to compare values. The task of the authors was to a greater extent to study the possibility of using modern microscopic methods for assessing quantitative characteristics and identifying patterns.

Results and Discussion

When studying the state of erythrocytes, thrombohemorrhagic changes were revealed in all patients with grade I diabetes. This was manifested in the presence of erythrocyte stasis and sludge phenomenon, which in the early stages of the disease may be the initial sign of disseminated intravascular coagulopathy, and later become the basis for the formation of blood clots. In some cases, we have already observed them in patients aged 45-59 years. Another manifestation of thrombohemorrhagic changes was poikilocytosis, an increase in the number of acanthocytes and echinocytes, as well as the

formation of individual spines on the surface of erythrocytes.

When studying erythrocytes, it was shown that the sizes of different groups of cells did not differ significantly. The microcytes were 6.20 ± 0.15 μm, normocytes - 7.50 ± 1.05 μm (from 6.50 μm to 8.90 μm), macrocytes 9.50 ± 0.50 μm (from 9.0 μm to 10.90 μm), megalocytes - 11.50 ± 1.0 μm (11.0 μm and more). At the same time, the content of microcytes in pregnant women (25-35 years old) and non-pregnant women (25-44) with type 1 diabetes did not differ significantly (14.50 ± 1.50% 13.00 ± 1.60%), but it was higher than in the control groups (7.50 ± 1.50% and 6.30 ± 1.40%). The content of macrocytes was, respectively: 15.50 ± 1.10% and 14.30 ± 1.80% (14.30 ± 1.10% and 13.20% ± 2.05%). Individual megalocytes appeared in the blood of patients with type I diabetes, both pregnant and not. With age, the content of microcytes progressively decreased, reaching 10.30 ± 1.30% in the 76-80 age group, while the number of macrocytes (18.50 ± 1.20%) and megalocytes (2.50 ± 0.25 %) increased (10.50 ± 1.25%). These changes are characteristic of the moderate stage of anisocytosis.

Pathological forms of erythrocytes were represented by: transitional forms (capable of reverse transformation): ellipses, discocytes with a crest, flat disks, discocytes with outgrowth, discocytes with multiple outgrowths, erythrocytes in the form of a "mulberry berry"; prehemolytic forms (with changes that are irreversible): domed, spherical, in the form of a "deflated ball"; degenerative. There was a clear tendency to an increase in their content with increasing age of the patients, which, apparently, is associated with the duration of the course of the disease. The data obtained by us with atomic force microscopy confirm the fact that disorders of carbohydrate metabolism specific for diabetes mellitus (even at the stage of latent changes) lead to an increase in the proportion of macrocytes and polymorphism in the size of erythrocytes, sphericity of the shape, and the appearance of a significant number of deformed and hemolyzed cells.

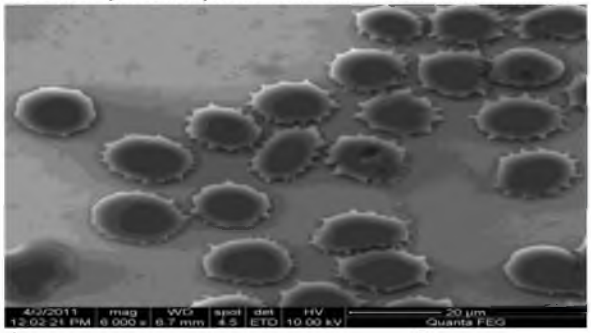
Table 1. The state of erythrocytes in the venous bed of women with type I diabetes mellitus.

Age groups	Preg.	Number of patients	Area, μm ^{2}	Volume μm ^{3}	MaxZ, μm	Perimeter, μm	Diameter, μm
Norma (25-32)	yes	n=5	44.50±1.50	4.70±0.90	0,55±0.20	33.40±1.7	7.30±0.80
DM I (25-35)	yes	n=5	30.5±1.20*	4.51±0.50	0.72±0.21*	41.80±1,6*	6.40±0.70
Norma (25-36)	no	n=5	44.30±2.50	4.60±0.40	0.53±0.07	33.20±1.40	7.40±1.10
DM I (25-44)	no	n=5	31.10±1.10*	4.51±0.50	0.72±0.21*	41.80±1.6*	6.40±0.70*
DM I (45-59)	no	n=5	42.0±3.10	4.32±0.54	0.73±0.05*	42.05±2.10*	6.10±0.55*
Norma (60-75)	no	n=5	44.90±3.5	4.80±1.40	0.56±0.08	33.20±1.40	7.50±1.80
DM I (60-75)	no	n=5	50.10±3.20*	3.52±0.45*	0.28±0.05*	32.80±2.25	8.20±1.70*
DM I (76-80)	no	n=5	43.25±2.30	5.52±1.20*	0.57±0.06	29.30±1.41*	7.35±1.05

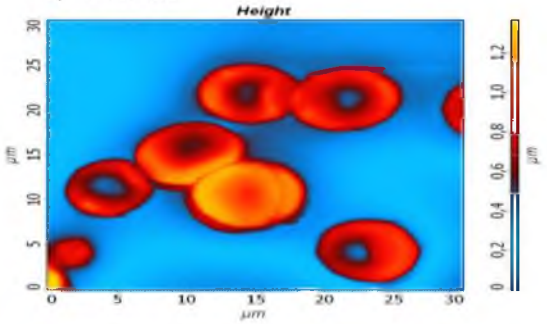
**p <0.05 - in comparison with the control group.*

When studying erythrocytes using atomic force microscopy (Table 1), it was noted that the average erythrocyte area in the group of pregnant women with diabetes mellitus (30.5 ± 1.20 μm) was significantly reduced compared to that in the group of pregnant women without this endocrinopathies (44.50 ± 1.50 μm) This tendency to a decrease in the area of venous blood erythrocytes persisted when comparing other age groups of patients with type I diabetes with groups of biological norm.

The fundamental property of the erythrocyte is its volume (Volume). As can be seen from Table 1, this indicator, however, was significantly reduced only in the group of patients with DM I 60-75 (3.52±0.45 μm^3) compared with the control group for this age category (4.80±1.40 μm^3). In the blood of pregnant women with concomitant diabetes mellitus I of the compensated course, an increase in the number of pathologically altered forms of erythrocytes was clearly noticeable (Fig. 1), while the index of the depth of their depression, compared with that in the group of healthy pregnant women, was significantly increased (0.72±0.21 μm and 0.55±0.20 μm, respectively).



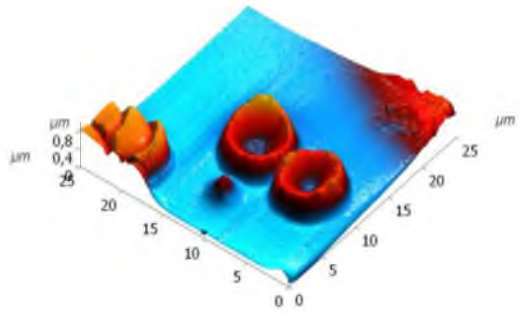
A



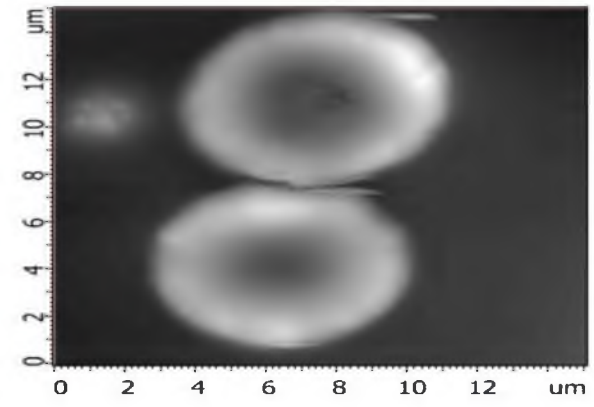
B

Figure 1. Blood of a pregnant woman with diabetes 1. Stasis and sludge of erythrocytes. Part of cells with an elongated shape. The number of microcytes is increased. On the surface of most cells there are spines (A, B). The indicator of the depth of the erythrocyte cavity is increased (B). Figure: A. REM (x8000). Figure: B. AFM. 2D image

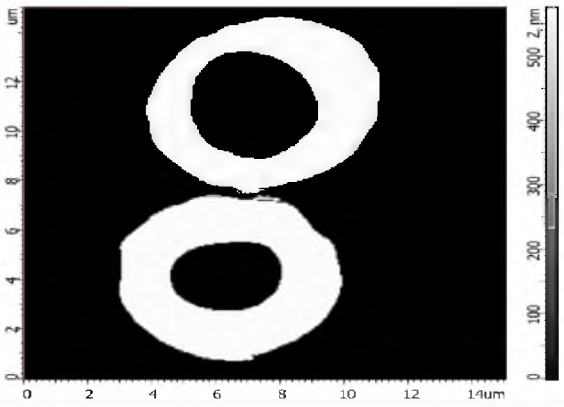
It should be noted that the index of the depth of the erythrocyte cavity in the groups of younger patients with diabetes I (25-44 years old and 45-59 years old) was also significantly higher than in the control group for this age (0.72 ± 0.21; 0, 73 ± 0.05 and 0.53 ± 0.07 μm, respectively). On the images of venous erythrocytes obtained by atomic force microscopy of one of the patients with diabetes mellitus I, 27 years old, we recorded the onset of thrombus formation (Fig. 2).



A



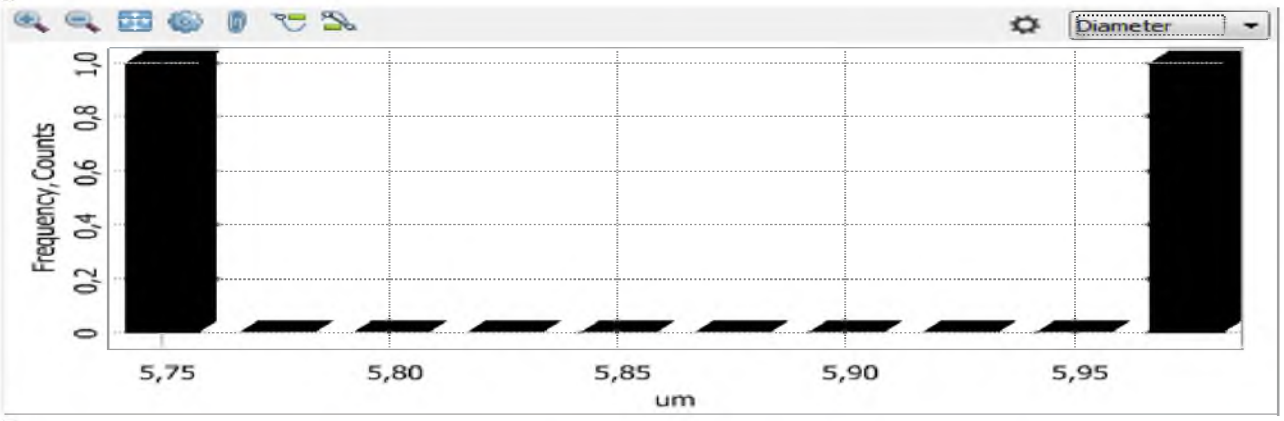
B



C

	Area	Volume	MaxZ	Perimeter	Diameter
Unit	um*um	um*um*nm	nm	um	um
Average	27.119	3317.843	779.419	43.441	5.874
SD	1.150	100.609	55.244	2.382	0.124

D



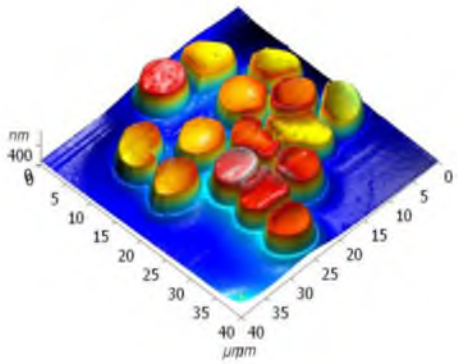
E

Figure 2. The blood of patients with type I DM (27 years). Stasis and sludge of erythrocytes. The

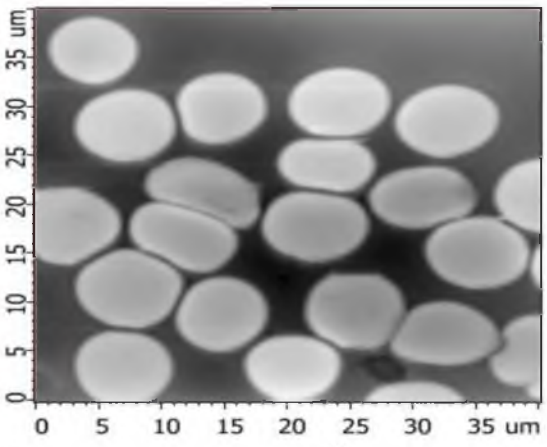
onset of thrombus formation (A). The indicator of the depth of the erythrocyte cavity has been increased (A, B, C). AFM.

A - three-dimensional, B - two-dimensional image, C - cell outlines, D - digital indicators, D - graphic image.

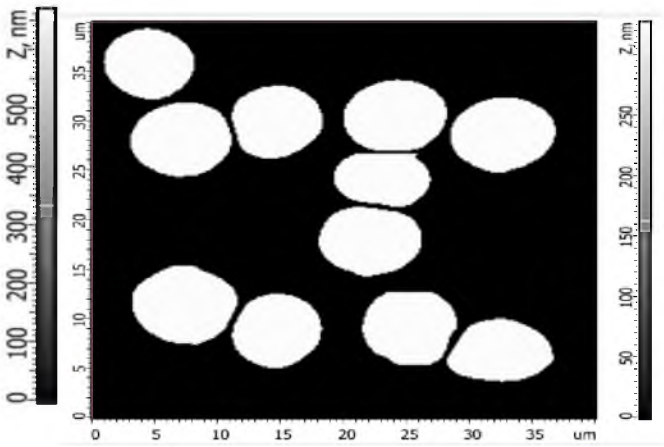
In the older age groups of patients with diabetes mellitus I, the erythrocyte cavity depth indicators varied significantly (Fig. 3), therefore, there was no significant increase in this indicator compared to the biological norm group for this age; on the contrary, it was decreased. So, its value in the group of patients with diabetes I, 76-80 years old, was 0.28 ± 0.05 μm, and in the control group for this age - 0.56 ± 0.08 μm. As well as in the blood of younger patients with this endocrinopathy, the phenomena of erythrocyte stasis and sludge, the initial stages of blood clots were observed.



A



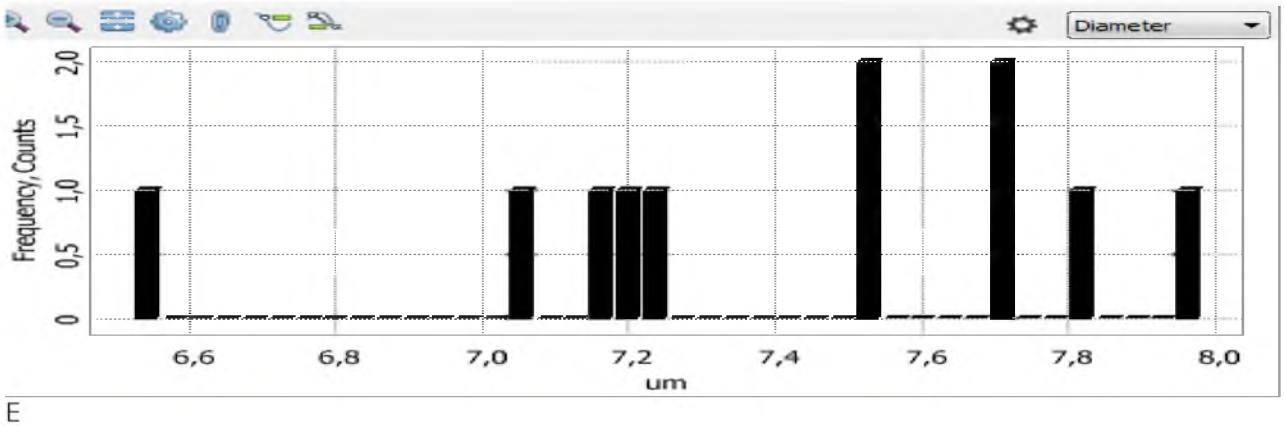
B



C

	Area	Volume	MaxZ	Perimeter	Diameter
Unit	um*um	um*um*nm	nm	um	um
Average	43.411	6045.982	552.562	28.705	7.423
SD	4.577	2399.156	48.296	1.650	0.399

D



E

Figure 3. Blood of a patient with type I DM (76 years old). Stasis and sludge of erythrocytes. The onset of thrombus formation (A). Changing the shape of cells. Various indicators of the depth of the cavity of erythrocytes (A, B, C). AFM. A - three-dimensional, B - two-dimensional image, C - cell outlines, D - digital indicators, D - graphic image.

In the samples of the uterus of pregnant women with type I DM, obtained during the caesarean section, the vessels had a greater tortuosity than in the control group. Moreover, their diameter varied significantly. The length of the vessels on the histograms (up to significant bending) was 1.4 ± 0.3 μm (3.1 ± 0.7 μm in the control group). Foci of destruction were identified in the endothelium. The distance between the folds of endothelial cells increased to 4.6 ± 1.4 μm (2.0 ± 0.5 μm) ($p < 0.05$). In addition, there were thickenings, partially, sometimes up to 60%, covering the lumen of the vessels, where thrombus formation was more often observed, which is clearly seen with AFM (Fig. 4).

The height of endothelial cells, respectively, was: 2.5 ± 0.3 μm (3.45 ± 0.7 μm) ($p < 0.05$). In the lumen of a part of the vessels, fibrin was determined in the form of separate filaments and layers, platelets, erythrocytes with stasis and sludge. The structure and shape of erythrocytes was often disrupted. The size varied from 3.50 μm to 8.30 μm. An increase in the content of microcytes was observed. In the endometrium and myometrium, significant changes in the shape and structure of erythrocytes, their diapedesis were noted. Some of them (11.5 ± 1.5%) - with impaired shape (Fig. 4).

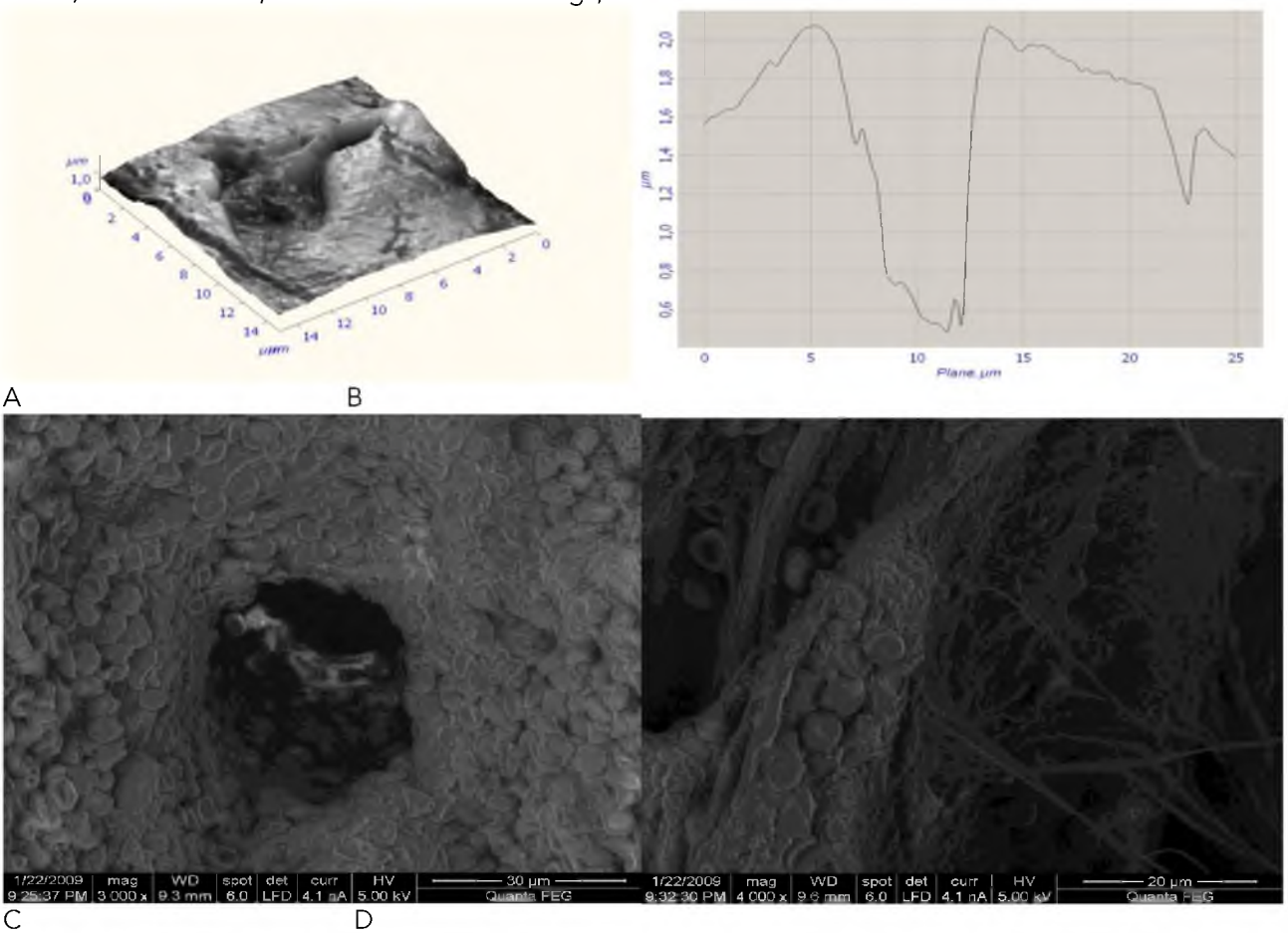


Figure 4. A fragment of the uterus in type 1 diabetes. Myometrium with vessels with a slightly altered shape (Fig A), uneven, thickened endothelium (A, D), congested vessels (C, D). The lumen is narrowed due to the endothelium protruding in separate fragments (A).

Figure B – histogram. Figure A – 3D image, AFM. Figure C – SEM, x3000. Figure D – SEM, x4000).

Conclusion

Even with a short duration of the disease, deviations of the main hemorheological properties and indicators are observed, including such as changes in the basic geometric parameters of erythrocytes, their ability to aggregate and deform. Diagnosis of hemorheological disorders in diabetes can serve as an early marker of target organ damage. The study proved that changes in carbohydrate metabolism in diabetes mellitus significantly affect the properties of the endothelial-erythrocytic system, and they can be diagnosed using various modern microscopy methods at an early stage.

References

Avramenko, T. V., Litvinov, S. K., & Malinina, O. B. (2019). Indicators of Endothelial Dysfunction in Pregnant Women with Gestational Diabetes Mellitus.

International Academy Journal Web of Scholar(4 (34)), 16-18. doi:https://doi.org/10.31435/rsglobal_wos/30042019/6443

Babik, B., Peták, F., Agócs, S., Blaskovics, I., Alács, E., Bodó, K., & Südy, R. (2018). Diabetes mellitus: endothelial dysfunction and changes in hemostasis= Diabetes mellitus: endothelial dysfunction and changes in hemostasis. *Orvosi hetilap, 159*(33), 1335-1345. doi:https://doi.org/10.1556/650.2018.31130

Cho, Y. I., Mooney, M. P., & Cho, D. J. (2008). Hemorheological disorders in diabetes mellitus. *Journal of diabetes science and technology, 2*(6), 1130-1138. doi:https://doi.org/10.1177/193229680800200622

Giraldo-Grueso, M., & Echeverri, D. (2020). From endothelial dysfunction to arterial stiffness in diabetes mellitus. *Current diabetes reviews, 16*(3), 230-237. doi:https://doi.org/10.2174/1573399814666181017120415

Kuznik, B. I. i., Vitkovskiy, Y. A., Zakharova, M. Y. e., Klyuchereva, N. N., Rodnina, O. S., & Solpov, A. V. (2012). Aggregation activity of blood formed elements in patients with type 1 and type 2 diabetes mellitus. *Diabetes mellitus, 15*(2), 49-53. doi:https://doi.org/10.14341/2072-0351-5518

Lawrence, J. M. (2011). Women with diabetes in pregnancy: different perceptions and expectations. *Best Practice & Research Clinical Obstetrics & Gynaecology, 25*(1), 15-24. doi:https://doi.org/10.1016/j.bpobgyn.2010.10.003

Lunder, M., Janić, M., & Šabovič, M. (2019). Prevention of vascular complications in diabetes mellitus patients: focus on the arterial wall. *Current vascular pharmacology, 17*(1), 6-15. doi:https://doi.org/10.2174/1570161116666180206113755

P Hoffman, R. (2014). Vascular endothelial dysfunction and nutritional compounds in early type 1 diabetes. *Current diabetes reviews, 10*(3), 201-207. doi:https://doi.org/10.2174/1573399810666140613124326

Pavlova, T. V., Petrukhin, V. A., Kaplin, A. N., Malyutina, E. S., Selivanova, A. V., & Zemlianskaia, L. O. (2020). New Approaches in Assessing the Clinical and Pathomorphological Aspects of Obstetric Pathology in the Structure of the Mother-Placenta-Fetus using Atomic Force Research. *Systematic Reviews in Pharmacy, 11*(6).

Popova, A. A., Berezikova, E. N., & Mayanskaya, S. D. (2010). Mechanism of endothelial dysfunction development., *64*(4), 7-11.

Sattar, N., & Preiss, D. (2018). Research digest: updates on diabetes in pregnancy. *The Lancet Diabetes & Endocrinology, 6*(4), 272. doi:https://doi.org/10.1016/S2213-8587(18)30076-7

Sehyun, S., Yunhee, K., Narayanan, B., & Megha, S. (2007). Erythrocyte deformability and its variation in diabetes mellitus. Indian Journal Exp. Biol., *45*(1), 121-128.

Shi, Y., & Vanhoutte, P. M. (2017). Macro- and microvascular endothelial dysfunction in diabetes. Diabetes., *9*(5), 434-449. doi:https://doi.org/10.1111/1753-0407.12521

Sosunkevič, S., Rapalis, A., Marozas, M., Čeponis, J., & Lukoševičius, A. (2019). Diabetic vascular damage: review of pathogenesis and possible evaluation technologies. *IEEE Access, 7*, 148511-148521. doi:https://doi.org/10.1109/ACCESS.2019.2946689

Vecchie, A., Montecucco, F., Carbone, F., Dallegri, F., & Bonaventura, A. (2019). Diabetes and vascular disease: is it all about glycemia? *Current pharmaceutical design, 25*(29), 3112-3127. doi:https://doi.org/10.2174/1381612825666190830181944