

New Aspects in the Study of Clinical and Morphological Features of Uterine Blood Flow in Type 1 Diabetes Mellitus in the Mother

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ABSTRACT

The aim of the study was to develop new approaches to the study of modern aspects of the clinical and morphological features of uterine blood flow in type I diabetes mellitus (DM) in the mother. Under our supervision, there were 40 pregnant women with type I DM. Clinical examination methods generally accepted in obstetric practice were used to assess the condition of the fetus, placenta, and blood flow in the fetal vessels and uterine arteries. Light microscopy, atomic force microscopy (AFM), and scanning electron microscopy (SEM) of 2 uterine fragments obtained during cesarean section were performed. Samples were examined using a FE1 Quanta 200 3D scanning microscope, analyzed, and morphometric and imaging were performed. Processing AFM images was formulated using the NOVA software (NT-MDT, Russian Federation). Results obtained allow us to judge the adverse effect of diabetes on the mother's body during pregnancy and childbirth. Among the complications of pregnancy, it should be noted: the state of preeclampsia (8%), fetoplacental insufficiency (88%), the threat of abortion (52%), polyhydramnios (25%). Morphologically, we revealed plethora of the endometrium. A change in the shape of the myometrium with the presence of fragmentary destructive changes in the myometrium, including focal necrosis and damage to the vascular system of the pregnant uterus. The paper substantiates the possibility of using scanning electron and atomic force microscopy as express methods for morphofunctional diagnosis of a woman's condition in pregnancy pathology.

Keywords: diabetes mellitus, uterus, atomic force microscopy, scanning electron microscopy

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INTRODUCTION

Based on the data presented in the World Health Organization (WHO) Global Report on Diabetes, diabetes mellitus (DM) can be seen as an epidemic of no communicable etiology (Weinert 2010). WHO predicts a further increase in the number of cases to 380 million people by 2025, of which patients with type I DM will make up 10-20% (Pavlova et al. 2016). Pregnancy during hyperglycemia is accompanied by impaired homeostasis and changes in the placenta and the fetus during all periods of their formation (Burenkova et al. 2011), which increases the risk of fetal death, presenting a serious medical, social and economic problem (Evsyukova & Kosheleva 2009).

The formation and functioning of the placenta in combination with increased activity of the endocrine glands during pregnancy leads to a hormonal surge, which in turn is accompanied by activity of contrinsular hormones (Pavlova et al. 2017), which contributes to the development of insulin resistance (IR). With IR, there is a violation of insulin-mediated vasodilation, which is accompanied by a decrease in NO production, followed by a consistent development of systemic endothelial dysfunction (Burenkova et al. 2011). Along with this, the production of endogenous glucose and the reorientation of carbohydrates with activation of hyper lipogenesis, which are involved in increasing the level of free fatty acids and enhancing

ketogenesis, increase in the liver (American Diabetes Association 2009).

Thus, the course of pregnancy with its characteristic changes in the hormonal background in DM is of particular interest (Pavlova et al. 2012). The development of new approaches to the study of the clinical and morphological features of uterine blood flow in type I DM in the mother can solve the problem of increasing the frequency of adverse pregnancy outcomes (Pavlova et al. 2019).

Diabetic angiopathy, especially with a long course of this process, leaves its mark on the structure and function of the uterus during pregnancy. So, in the spiral arteries with type I DM, fibrinoid transformation of the muscle membrane and narrowing of the lumen of the vessel are observed (Pavlova et al. 2015). Severe obliterating angiopathy occurs even outside the area of cytotrophoblast invasion. In the vascular system of the pregnant uterus, lesions similar to diabetic angiopathy of other organs and systems of a pregnant woman are observed. Also, with type I DM, in all biopsy samples of the placental uterine bed, a pronounced pathology of the uteroplacental arteries with a violation of the components of the muscular wall and narrowing of the lumen is revealed. Cytotrophoblast cells and the surrounding fibrinoid don't penetrate deep into the walls of the uterine arteries, but spread only within the adventitia of their endothelial segments. The same changes are observed in the myometrical segments of the uterine arteries.

Diabetic angiopathy, pronounced mainly in the spiral arteries in the area of the placental uterus, reduces the lysing potential of the cytotrophoblast and thereby helps to slow down the invasion, which subsequently reduces the increase in the volume of uteroplacental blood flow at the end of II and in the third trimester of pregnancy. In the endometrium, insufficient cytotrophoblastinvasion, as well as gestational rearrangement of the uteroplacental arteries in diabetic angiopathy, lead to a reduction in uteroplacental circulation, which limits the release of arterial blood into the intervillous space and contributes to the development of chronic fetoplacental insufficiency (Fedorova et al. 2001; Ershova et al. 2018).

MATERIAL AND METHODS

The study was conducted on the basis of the Perinatal Center of the Belgorod Regional Clinical Hospital. St. Joasaph (Belgorod), Kursk City Clinical Maternity Hospital (Kursk). The morphological study of placentas was carried out on the basis of the Department of Pathology of the Medical Institute of Belgorod State University, the Department of Pathological Anatomy of the Kursk Medical State University, as well as at the Scientific-Educational and Innovation Center "Nanostructured Materials and Technologies" of Belgorod State University.

Under our supervision, there were 40 pregnant women with compensated course of type I DM. The control group consisted of 10 women whose pregnancy proceeded without concomitant obstetric and extragenital pathology. All observed women underwent the necessary set of generally accepted clinical and laboratory studies, and were also examined by related specialists (therapist, endocrinologist, neurologist, ophthalmologist) (Order of the Ministry of Health of the Russian Federation of November 1, 2012), followed by a comparison of the obtained data with the morphological signs of the placenta. Anamnestic data are obtained from pregnant cards and birth histories. Ultrasound investigation was carried out on ultrasonic devices SSD-650 ("AlokaKo., Ltd", Japan). Using ultrasound scanning, the position and presentation of the fetus were determined, fetometry was performed, the placenta (thickness, location, correspondence of the "maturity" of the placenta to the pregnancy period, pathological structural changes), and the amount of amniotic fluid were evaluated, and an echographic study of each pregnant woman was carried out from 1 to 7 times. Assessment of the state of blood flow (dopplerometry) was performed for women from the second half of pregnancy (from 20 weeks), while were examined not only the main vessels of the fetus (umbilical cord, thoracic descending aorta of the fetus, middle cerebral artery), but also the spiral arteries of the uterus.

All women delivery was performed by planned C-section on 38-39 weeks, and during the operation were taken 2 fragments of the uterus with a size of 0.6 ± 0.2 mm (in the future, the blocks were used for atomic force microscopy (AFM) and scanning electron microscopy (SEM) (Baro & Reifenberger 2012). For scanning electron microscopy (SEM) were used untreated or fixed in a standard glutaraldehyde fixative samples, which were then examined

with a scanning microscope «FE1 Quanta 200 3D». Were also carried out tissue analysis, morphometry and imaging, both after short-term fixation in a formalin solution, and on paraffin blocks in the «Ntegra-Aura» probe laboratory. AFM-images were processed using the «NOVA» software (NT-MDT, Russian Federation).

In the course of statistical processing, all data were checked for normality by the Shapiro-Wilk and Kolmogorov-Smirnov tests, as a result of which it was established that the data are subject to a normal distribution, in connection with which the Student's t-test as parametric method was used in further statistical analysis. The result was considered reliable at $p < 0.05$.

RESULTS AND DISCUSSION

We have shown that changes in the mother's organism with type I DM lead to an unfavourable course of pregnancy and child birth. So, in the structure of extragenital pathology in patients with type I DM, the most common are: anemia and obesity - in 16 (40%) and 14 (35%) cases, respectively, then urinary tract infections 10 (25%) and thyroid diseases, especially goiter with an euthyroid state, in 10 (25%) cases. Hypertension occurs in 4 (10%) cases, and myocardial dystrophy in 3 (7.5%) cases. Among the complications of type I DM, diabetic angiopathy was observed in 16 (40%), retinopathy in 7 (17.5%), polyneuropathy in 10 (25%) cases.

Among the complications of pregnancy, it should be noted: preeclampsia - 3 (7.5%), chronic fetoplacental insufficiency - 35 (87.5%), the threat of abortion - 20 (50%), polyhydramnios - 10 (25%) cases. A frequent feature of the outcome of the course of pregnancy in women with type I DM is premature birth - in 14 (35%) cases. It is also necessary to note the untimely discharge of amniotic fluid observed in 5 (12.5%) women with type I DM. Ketoacidosis, a complication of DM, was observed in 19 (47.5%) pregnancies. In most cases, fetal hypoxia was noted. As a result of the study, we found that the most frequent pathology detected by ultrasound screening is polyhydramnios and pathological structural changes, also in 14 (35%) cases of preeclampsia were detected hemodynamic disturbances from I to III degrees.

Morphologically we revealed plethora of the endometrium (Fig. 1). Its thickness varied over a fairly wide range from 300 to 1000 μm . Moreover, the greatest changes in the relief were observed with type I DM complicated by preeclampsia. Fragments of a destructive tissue lesion were revealed in the endothelium. Endometrial tissue contained erythrocytes, the value of which was in a fairly wide range from 3.31 to 5.94 μm and averaged $4.35 \pm 0.84 \mu\text{m}$, with values of 4.95 ± 0.32 in the control group and the absence of such a spread in cell sizes ($p > 0.05$) (Fig. 1a). The shape of individual cells was changed: microcytes, spherocytes, dermocytes, bubble cells, individual poikilocytes were observed. Due to the disturbed structure of plasma membranes and changes in the architectonics of surface plasmatic erythrocyte outgrowths, their surface irregularities were recorded. By studying the profile of the cell, a decrease in the cavity of discocytes was established, on average, to $0.27 \pm 0.03 \mu\text{m}$, at $0.38 \pm 0.04 \mu\text{m}$ in the control group ($p < 0.05$). The ratio of the diameter of

the erythrocyte to the diameter of its hollow was 25.1 units (Fig. 1b). There is hemolysis of individual erythrocytes, which does not always capture the cell completely, as confirmed by AFM. In addition to changes in red blood cells, marked disturbances in capillary blood flow are noted: stasis, sludge and thrombosis.

Examination of arterioles and venules revealed the presence of a complex network of capillaries associated with them. Venules often had excessive expansion up to the formation

of thin-walled lacuniform “venous lakes” with a diameter of more than $105 \pm 29 \mu\text{m}$. The clearance of the spiral arteries was often curved and narrowed due to the thickening of the vessel wall (Fig. 1). In the endothelium of the arteries are observed folding disorders (Fig. 1c) and partial destruction sites with the adhesion of red blood cells, which are interconnected by plasmatic outgrowths and fibrin (Fig. 1c). The length of the vessels in the histograms was $1.5 \pm 0.3 \mu\text{m}$ ($2.9 \pm 0.4 \mu\text{m}$ in the control group) ($p < 0.05$).

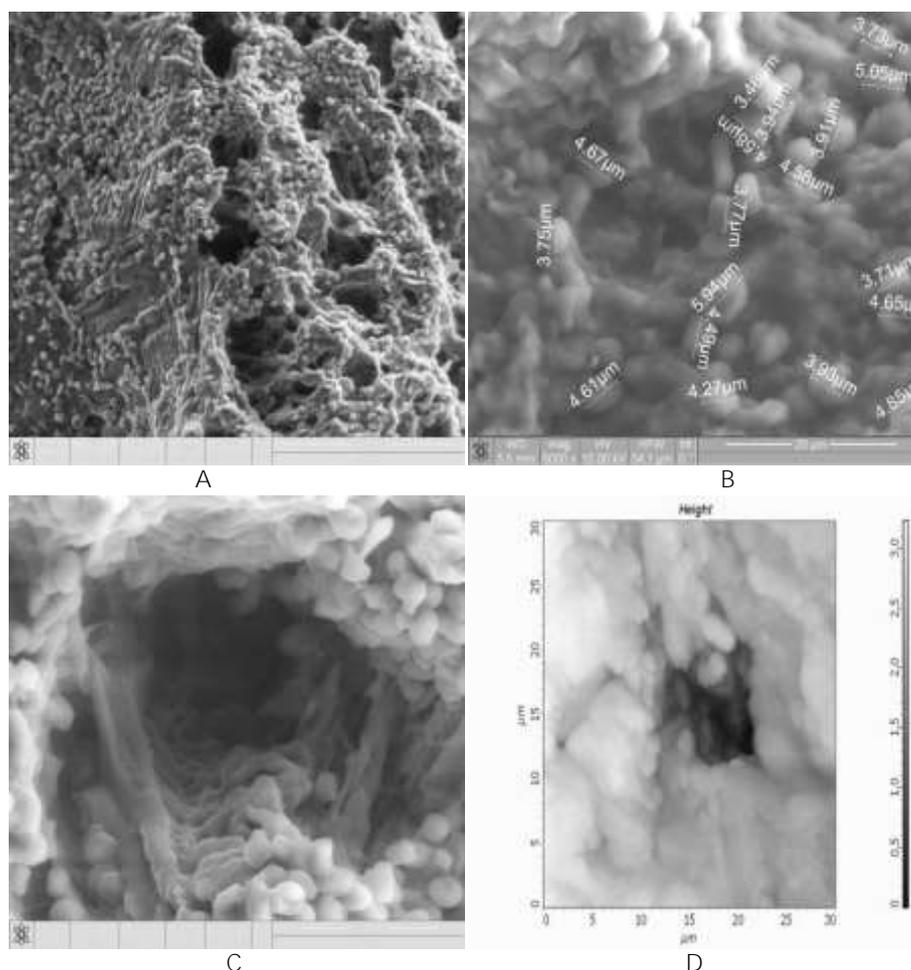


Figure 1A: A fragment of the uterine endometrium in type I DM. The endometrium is full-blooded. The vascular wall is changed (thickened, with impaired folding of the endothelium and fragmentary destruction). Red blood cells vary in size and shape. Their adhesion, sludge and fusion with fibrin are observed

Figure 1B – The ratio of the diameter of the red blood cell and the diameter of the cavity. Fig. B fragment Fig. A.

Figure 1C – Folding of the endothelium. Fig. C fragment Fig. A.

Figure 1D– Adhesion of red blood cells, their connection with each other, by means of plasma processes and fibrin – atomic force microscopy, three-dimensional image. Fig. D fragment Fig. A.

SEM A (x1000), B (x5000), C (x4000)

In the study of myometrium using scanning 3D microscopy, a change in its shape, a clearly pronounced three-layer structure were recorded (Fig. 2). The outer layer of the myometrium is represented by layers of muscle cells in dense connective tissue, which have fragments of dystrophic changes in type I DM, sometimes up to the formation of focal necrosis, the presence of which is very important to consider when choosing tactics for pregnancy and childbirth in women with type I DM. The next thick middle muscle layer penetrated by

blood vessels is characterized by the presence of signs of alternative changes. A muscle in its composition contains hypertrophied myocytes, which are separated by collagen fibers. Along the periphery, the bundle is surrounded by layers of connective tissue penetrated by blood vessels. In some cases, especially when type I DM combining with preeclampsia, an impregnation of a bundle of muscle fibers with red blood cells can be detected.

The arterial network of the uterus begins with arcuate

arteries, from which then radial arteries go deep into the myometrium and pass into the final branches - spiral arteries. The vein system is represented by many cavities coated with a thin muscle membrane endothelium. In type I DM in the walls of the vessels revealed: thickenings, in some places foci of alterative and sclerotic changes, violation of folding and the shape of the spiral arteries, in some cases in combination with narrowing of the lumen of the vessel. In the lumen of the vessels were found stasis and sludge of erythrocytes, especially along the luminary edge, the presence of fibrin and parietal

thrombosis. In addition to impaired blood flow and changes in arteries, a violation in the structure (mainly spherocytes) and in the shape (violation of the shape of 10-12%) of erythrocytes (Fig. 2a) was often detected. Blood clots were observed mainly in the venous network. In the endothelium, there is a violation of folding, foci of destructive changes, individual layers and fibers of fibrin directed into the lumen of the vessel, as well as diapedesis of individual cells through the vascular wall. The total length of the vessels in the histograms is $1.4 \pm 0.4 \mu\text{m}$ (Fig. B).

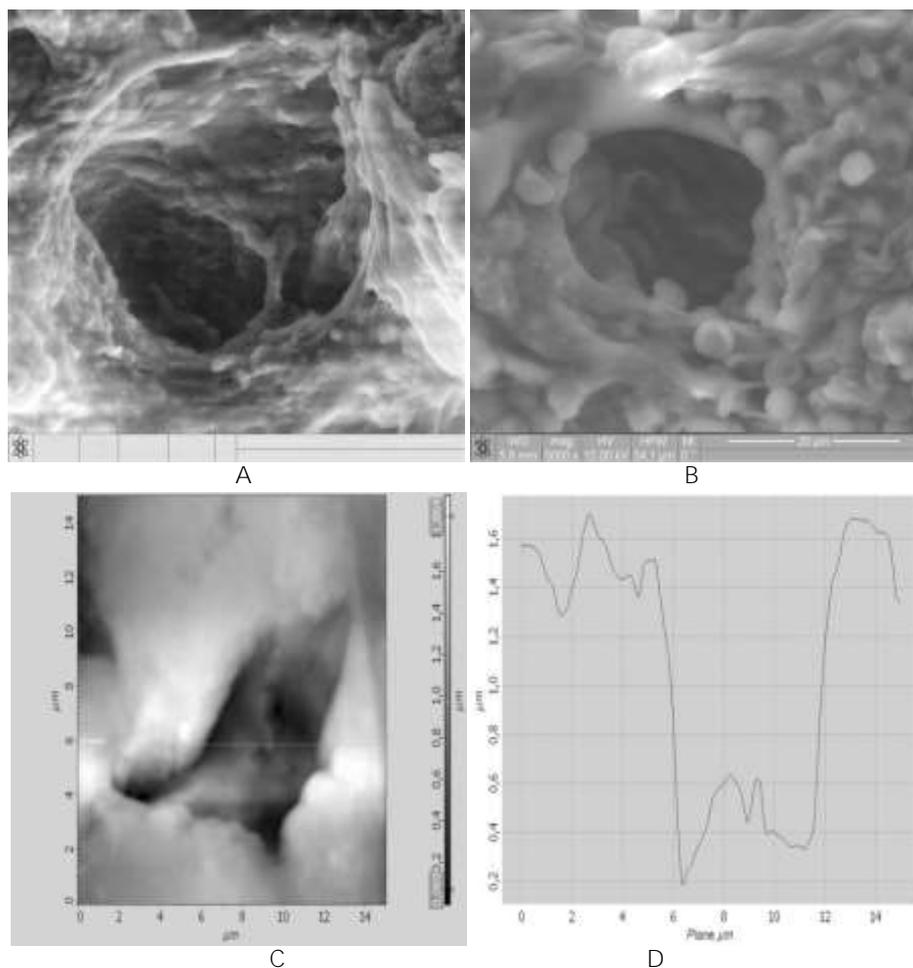


Figure 2: A fragment of the uterine myometrium in type I DM. The vascular wall is altered (thickened, with impaired folding of the endothelium and fragmentary destruction of the wall). Red blood cells vary in size and shape. Their adhesion, sludge and fusion with fibrin are observed, as well as diapedetic hemorrhages.

Figure 2A – Violation of the folding of the vessel wall, the shape and structure of red blood cells.

Figure 2 B – Fragment Fig. A. SEM **A**(x3000), **B**(x5000)

Figure 2C – Length of blood vessels (three-dimensional histogram).

Figure 2D – Graphical representation of the depth of vessels in the uterine myometrium fragment in type I DM shown in Fig **C**.

CONCLUSION

Thus, the structural changes that we identified in the vascular system of a pregnant uterus with type I DM are an integral part of diabetic angiopathy in other organs and systems of a pregnant woman. The presence of fragmentary destructive changes in the myometrium, including focal necrosis, must be taken into account during pregnancy and childbirth. The possibilities of scanning microscopy, both

electronic and atomic force, can be successfully used as express methods for morphofunctional diagnosis of a woman's condition in pregnancy pathology.

CONFLICT OF INTEREST

None

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