Morphometric Characteristics of Placental Villi in Pregnant Women with Diabetes

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We conducted a comparative morphological study of placentas from women with gestational diabetes and diabetes mellitus. Morphometry of histological preparations revealed similar type of changes in the studied parameters of placental villi in diabetes mellitus and gestational diabetes. The maximum deviation of the studied parameters from the control was noted in type 1 diabetes mellitus.

Key Words: *placental villi; diabetes; morphometry; placenta*

Diabetes mellitus (DM) in pregnant women is associated with the development of various complications for herself and her baby, including increased risk of perinatal morbidity and mortality [8]. According to the hypothesis of J. Pedersen [9], hyperglycemia in pregnancy leads to increased transplacental glucose transport inducing compensatory hyperinsulinemia in the fetus and its increased growth. Despite the fact that to date the effects of mother's hyperglycemia on the fetus are well studied, the role of the placenta in the development of these outcomes is still unclear. In particular, the question of the nature and degree of severity and prevalence of associated structural changes in the placenta at DM is not resolved yet.

Classical studies of placentae in type 1 DM describe macroscopic abnormalities of placenta, such as large weight, thickness, and plethora, combined with microscopic signs indicating disorders of the villous tree maturation [2]. These changes underlie various complications of pregnancy associated with dysfunction of the placenta [3]. However, in a series of observations, no significant differences were found [2]. Stereological study also revealed no differences in the structure of the placenta [7] or detected only individual changes, including increased surface area and volume of capillaries [6], volume of the villi, total diffusion capacity, and the volume of trophoblast [5]. These discrepancies are apparently due to different DM types grouping and different methods of controlling hyperglycemia.

Disturbances in carbohydrate metabolism that occur during pregnancy are divided into two main groups: pregestational DM and proper gestational DM.

The purpose of this study was comparative morphometric study of placental villi of pregnant women suffering from DM and gestational DM.

MATERIALS AND METHODS

The study was based on morphological analysis of 29 full-term placentas (37-40 weeks). The material from 12 pregnant women aged 26-39 years with type 1 DM was included in group 1; from 11 women aged 29-44 years with gestational DM, in group 2. All patients with type 1 DM used insulin pump therapy. Pregnant women with gestational DM were on the appropriate nutritional therapy. Control group consisted of six patients aged 23-37 years with physiological pregnancies without extragenital pathology.

After macroscopic examination, tissue fragments from the central zone of the placenta were fixed in

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10% neutral formalin. Histological examination was performed on paraffin sections stained with hematoxylin and eosin. Villous vessels were detected by immunohistochemical study using ready-to-use mouse antibodies to CD34 (clone QBEnd/10) and polymer-based detection system (Spring Bioscience). Antigen was previously retrieved by boiling the samples in citrate buffer, pH 6.0. Endogenous peroxidase activity was quenched by treating the sections with 0.3% hydrogen peroxide for 15 min. The sections were counterstained with hematoxylin.

Morphometry of the slides was performed using an image analysis system based on an Axio Imager M1 microscope using AxioVision software (Carl Zeiss). We determined the relative area of intervillous space, areas and perimeters of the terminal and mature intermediate villi, their capillaries, and syncytiotrophoblast, and the number of capillaries. Based on these morphometric parameters, we estimated the degree of vascularization of the villi calculating the ratio of the total area of capillaries to the cross-sectional area of the villi (K1), the ratio of villi perimeter to its cross-sectional area (K2), the ratio of the sum of perimeters to total capillary area in terminal villi (K3), the ratio of villi the perimeter to the sum of the perimeters of its capillaries (K4).

Statistical processing of quantitative data was performed using Statistica 6.0 software.

RESULTS

Histological examination of the placenta sections stained with hematoxylin and eosin showed that the degree of maturation of villous tree in controls corresponded to gestational age; the compensatory and adaptive as well as involution and degenerative processes were moderately expressed.

In DM and gestational DM, the intervillous space was reduced. In 8 (66.7%) placentas from women with type 1 DM and 5 (45.5%) placentas from women with

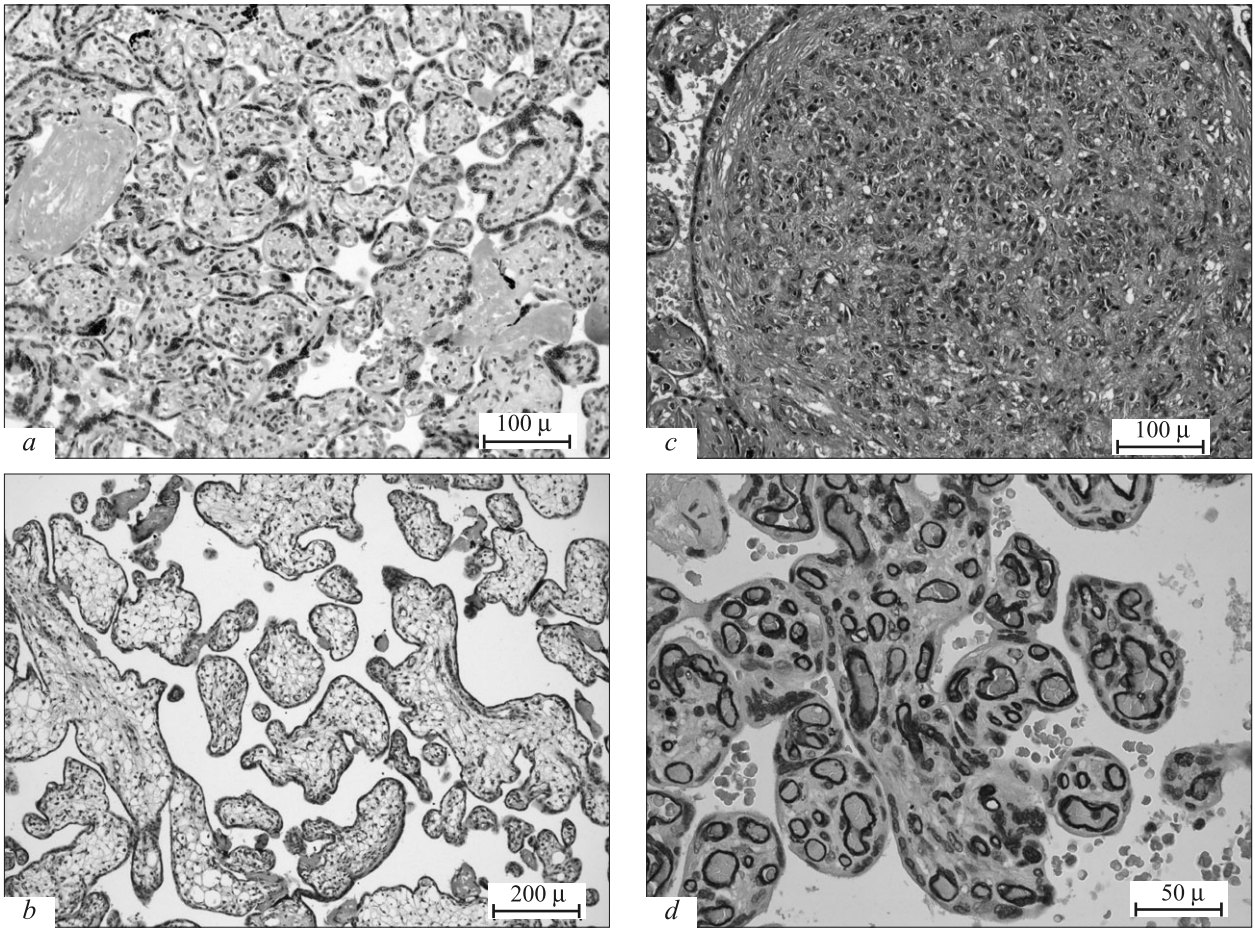

Fig. 1. Histological features of placentas from women with gestational DM (*a*) and type 1 DM (*b-d*). *a*) medium and small round villi with a large number of capillaries and syncytial nodes, intervillous space is narrow, ×200; *b*) villous edema, ×100; *c*) chorangioma in the placenta in a woman with type 1 DM, ×200; *d*) CD34 expression in villous endothelial cells, ×400. Hematoxylin and eosin staining (*a-c*), immunoperoxidase method (*d*).

TABLE 1. Morphometric Characteristics of Placental Villi in DM and Gestational DM (*M*±*m*)

Parameter	Control	DM	Gestational DM
Villus area, μ²	3090.5±364.2	2569.3±224.6	2616.6±237.1
Villus perimeter, μ	209.9±18.6	185.4±15.3	192.7±15.8
Relative area of intervillous space, %	21.8±2.3	16.9±1.3	17.2±1.3
Total capillary areas in the villus, μ²	1033.2±198.9	748.3±52.3	793.9±55.6
Mean capillary area, μ²	219.0±18.8	126.8±10.9	130.1±11.5
Total capillaty perimeters in the villus, μ	304.9±24.2	252.9±16.8	256.3±17.2
Mean capillary perimeter, μ	56.5±3.2	42.6±2.4	41.7±2.3
Number of capillaries in the villus	4.8±0.3	5.9±0.3	6.1±0.4
Degree of vascularization of the villi (K1), %	32.5±2.5	31.0±2.3	30.6±2.2
Ratio of villus perimeter to its cross-sectional area (K2)	0.07±0.01	0.07±0.01	0.07±0.01
Ratio of summary perimeter to total capillary area in terminal villi (K3)	0.30±0.02	0.34±0.02	0.32±0.02
Ratio of villus perimeter to summary perimeter of its capillaries (K4)	0.69±0.04	0.73±0.04	0.75±0.04
Ratio of villus perimeter to total cross-sectional area of capillaries	0.20±0.01	0.25±0.01	0.24±0.01

gestational DM, maturation of the villous tree lagged behind the gestational age by 2-4 weeks. In other cases, the maturity of placental villi corresponded to gestational age. In all preparations of the placenta in the main groups, the villous tree was primarily presented with round villi of small and medium caliber with a large number of capillaries and syncytial nodes (Fig. 1, *a*). Edema of some villi in placentas from women with DM was observed, it was more pronounced in type 1 DM (Fig. 1, *b*). In this group, the epithelial-capillary distance increased and syncytiocapillary membranes were not detected. In one case of type 1 DM, chorangioma of the placenta was revealed (Fig. 1, *c*). Such changes in the placentas of women with DM demonstrate predominance of the branching angiogenesis (with blood vessels branching).

Morphometry of the placenta sections from controls showed that specific area of the villous tree and area of intervillous space were 78.2±6.1 and 21.8± 2.3%, respectively (Table 1). The mean cross-sectional area and perimeter of terminal villi were 3090.5±364.2 μ² and 209.9±18.6 μ, respectively. The mean cross-sectional area and perimeter of villous capillary were 219.0±18.8 μ² and 56.5±3.2 μ, respectively, and the mean degree of vascularization of the vill (K1) was 32.5±2.5%. Of the calculated indices, the minimum coefficient was the ratio of the villus perimeter to its cross-sectional area (K2=0.07) and the maximum coefficient was the ratio of villus perimeter to the sum of capillary perimeters (K4=0.69).

Morphometry of the placenta from women with gestational DM revealed deviations of the studied parameters from the control. Thus, in both groups, the area of intervillous space was below the normal by 21.1-22.5% ($p<0.05$; Table 1). The mean size of the terminal and mature intermediate villi were also below the control values. The area and perimeter of the villi in DM were reduced by 16.9% and 11.7% and in gestational DM by 15.3% and 8.2%, respectively (Fig. 2, *a*). The calculated ratios of villus perimeter to its cross-sectional area (K2) were similar in the control and main groups.

Morphometry of placenta sections stained with antibodies to CD34 (Fig. 1, *d*) showed that the mean number of blood capillaries in the villi surpassed the normal by 23-27%. Lumen area and perimeter of a single capillary of chorionic villi in the placenta of women with DM were below the corresponding normal values by 42.1 and 24.6% ($p<0.05$) and in gestational DM by 40.6% and 26.2%, respectively ($p<0.05$; Fig. 2, *b*). At the same time, the summary area and perimeter of capillaries in a single villus were minimum in DM: they were below the normal by 27.6% and 17.1%, respectively ($p<0.05$). In gestational DM, these parameters were below the control level by 23.2% and 15.9% ($p<0.05$; Fig. 2, *c*). However, in gestational DM the calculated parameters of villous vascularization were below the control level by only 4.6% in DM and 5.8% in gestational DM (Fig. 2, *d*). In this case, the ratio of the summary perimeters of capillaries to

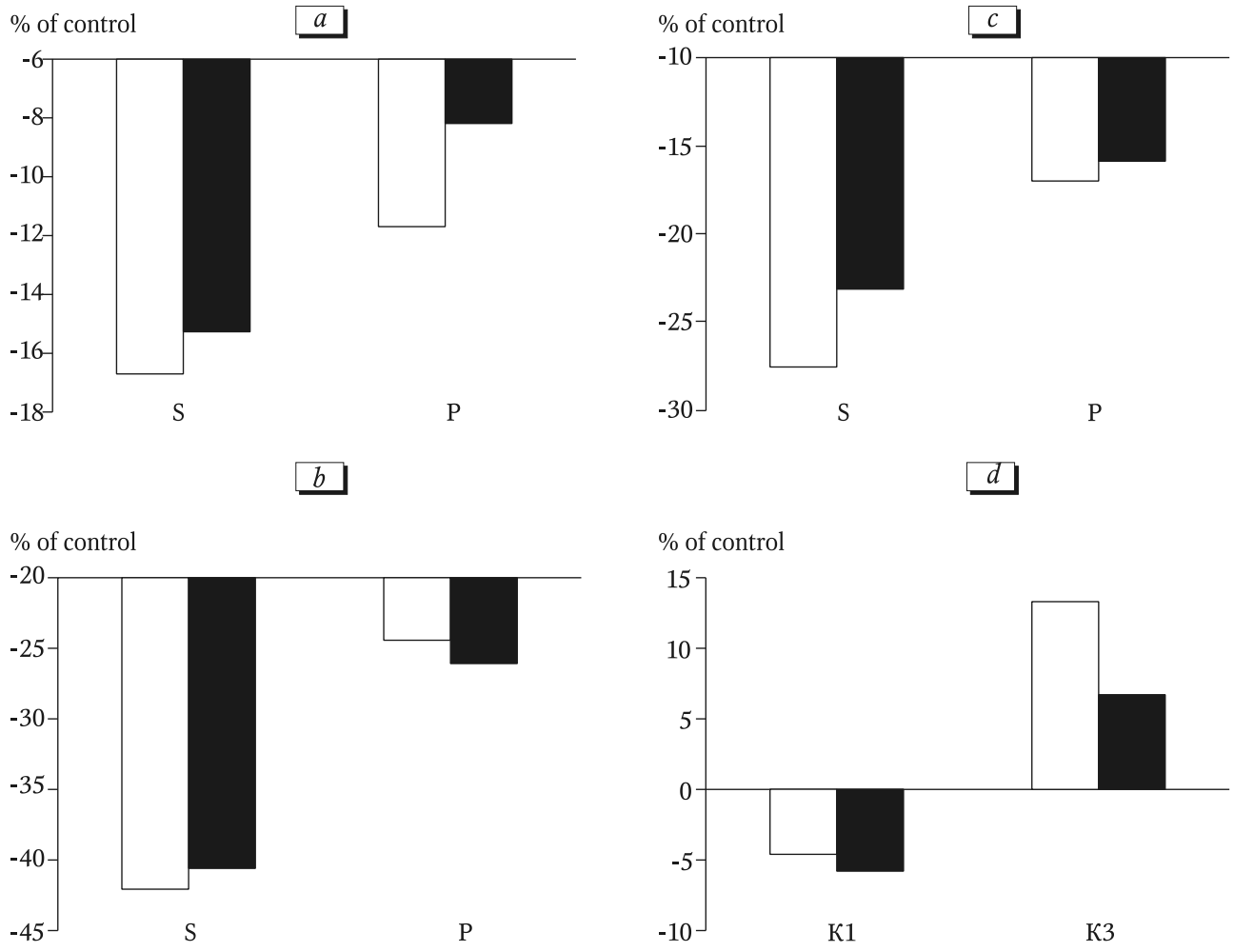

Fig. 2. Morphometric features of placental villi in women with DM type-1 (light bars*)* and gestational DM (dark bars). *a*) mean area (S) and perimeter (P) in the terminal villi; *b*) mean area (S) and perimeter (P) of terminal villi capillaries; *c*) total area (S) and perimeter (P) of terminal villi capillaries; *d*) indices of vascularization of terminal villi (K1) and ratio of summary perimeters to summary areas of terminal villi capillaries (K3).

their summary cross-sectional areas in the same villus exceeded the normal levels by 13.3 and 6.7% in DM and gestational DM, respectively (Fig. 2, *d*).

Thus, our morphometric studies revealed similar changes in the studied parameters of placental villi in type 1 DM and gestational DM. Certainly, hyperglycemia in mother has a great impact on the degree of placenta vascularization. It is known that the vascular endothelium of the placenta has some peculiarities. Blood rich in oxygen and nutrients flows through the veins of the placental villi, whereas deoxygenated blood from the fetus runs in the arteries. Placental vascular system should develop quickly enough to meet the needs of the growing fetus. In this regard, factors that are present in circulating blood should stimulate angiogenesis in the placenta. In the III trimester, placental vascular endothelium intensively expresses insulin receptors. In pregnancy associated with DM in the mother, fetal hyperinsulinemia occurs in response to hyperglycemia in the mother and, as a consequence, of the fetus, and induces growth and angiogenesis in the vascular system of the placenta. It was established that these processes start in the arterial system of placental villi [4]. Moreover, hyperglycemia increases the level of glycated hemoglobin in maternal blood; this also promotes the development of preplacental hypoxia, which, in turn, stimulates angiogenesis in the placenta [1]. The decrease in the area of intervillous space detected by us attests to rapid growth of new terminal villi after the newly formed vessels.

Deviation of the studied morphometric parameters of placental villi were most pronounced in type 1 DM. This can be explained by the fact that in women with DM the placenta develops from the very beginning in disease, manifested in hyperglycemia of varying degree and metabolic disorders. Gestational DM usually develops in the second half of pregnancy, *i.e.* when the villous tree of the placenta is already formed.

Hence, the morphometric peculiarities in the structure of placental villi in women with type 1 DM and gestational DM reflect to a certain extent the processes of compensation and dysfunction in the mother–placenta–fetus system under conditions of hyperglycemia and hypoxia.

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