Morphology of Placental Villi and Development of Hemorrhages in Very Small Preterm Newborns

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We performed a comparative morphometric and immunohistochemical studies of the placentas collected in preterm labor after 27-33-week gestation. Predominating branched angiogenesis processes and more pronounced vascularization of the placental villi were found in the group of newborns with hemorrhagic complications. These changes were paralleled by high expression of VEGF in all villous structures.

Key Words: *villus; hemorrhage; morphometry; preterm newborn; placenta*

Morphological and functional immaturity of organs and systems in preterm newborns is a factor essential for prenatal morbidity and mortality. Hemorrhages and hematomas are frequent complications. For example, the incidence of intracerebroventricular gemorrhagies in very small (1000-1499 g) preterm newborns can reach 20% [5,8]. In extremely small (500-999 g) newborns these gemorrhagies develop in 45% observations [10]. The incidence of pulmonary hemorrhages in very small babies varies from 2 to 12%, half of these cases with lethal outcomes [9].

These complications in preterm newborns are attributed to be due to immaturity of the hemostasis system components and unfavorable factors of the intrauterine and postnatal periods. One more factor contributing to high incidence of hemorrhagic complications is immature vascular wall in the newborns. The development of the vascular system depends on the expression of vascular endothelial growth factor (VEGF). In disease this factor stimulates the vascular wall permeability and acts as a vasodilator. In addition, VEGF is a chemoattractant stimulating monocyte activation and migration [4,11]. The placenta, in turn, produces numerous growth factors, including VEGF [1].

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We studied the relationship between morphometric characteristics of the placenta and development of hemorrhagic episodes in very small preterm babies.

MATERIALS AND METHODS

Morphological analysis was carried out in 16 placentas collected in preterm deliveries after 27-33-week pregnancies (mean term 30.5 weeks). All newborns (8 girls and 8 boys) were very small (1000-1499 g, mean weight 1297.1 g). In 6 newborns (group 1) the early neonatal period was aggravated by intracerebroventricular (4 patients), pulmonary (1 patient), and gastrointestinal (1 patient) hemorrhages. No hemorrhagic complications developed during the neonatal period in 10 patients (group 2).

After macroscopic examination of the placentas, tissue fragments were cut out from the central zones and fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections stained with hematoxylin and eosin.

Immunohistochemical studies were carried out by the standard method with ready to use mouse antibodies to CD34 (clone QBEnd/10) for detection of villous vessels and with rabbit polyclonal antibodies to VEGF, using polymeric system for detection (Spring Bioscience). Preliminary antigen unmasking was carried out by boiling the specimens in citrate buffer (pH

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6.0). Endogenous peroxidase was blocked by treating the sections in 0.3% hydrogen peroxide (15 min). Hematoxylin was used as the basal stain. The expression of VEGF was evaluated in syncytiotrophoblast (SCT), vascular endotheliocytes, and terminal and mature intermediate villous mesenchyma by a semiquantitative score (0 points: no reaction; 1 point: weak reaction; 2 points: moderate reaction; 3 points: manifest reaction).

Morphometric analysis of preparations was carried out under a microscope with image analysis system AxioImager M1 and AxioVision software (Carl Zeiss). The areas and perimeters of mature intermediate and terminal villi, their capillaries, and number of these capillaries were evaluated. Based on these morphometric parameters, the indexes of villous vascularization were calculated as proportions of total area of villous capillaries to its section area (K1), villous perimeter to section area (K2), sum of perimeters to sum of capillary area in a villus (K3), and villous perimeter to sum of its capillaries' perimeters (K4). Numerical data were statistically processed by Statistica 6.0 software.

RESULTS

The weights of placentas from women with preterm deliveries varied from 185 to 420 g (mean weight 237.1 g), which was generally somewhat lower than the normal gestation value. The placental/fetal index varied from 0.13 to 0.33 (mean value 0.19). Newborn body weights, placental weights, and placental/fetal indexes virtually did not differ in the groups.

The structure of the villous tree in histological preparations stained with hematoxylin and eosin generally corresponded to that for gestation term. The bulk of the villous tree were mature intermediate villi; there were also groups of immature intermediate and terminal villi (Fig. 1, *a*). Rapid maturation of the villous tree with predominating terminal villi and predominance of branched angiogenesis in the forma-

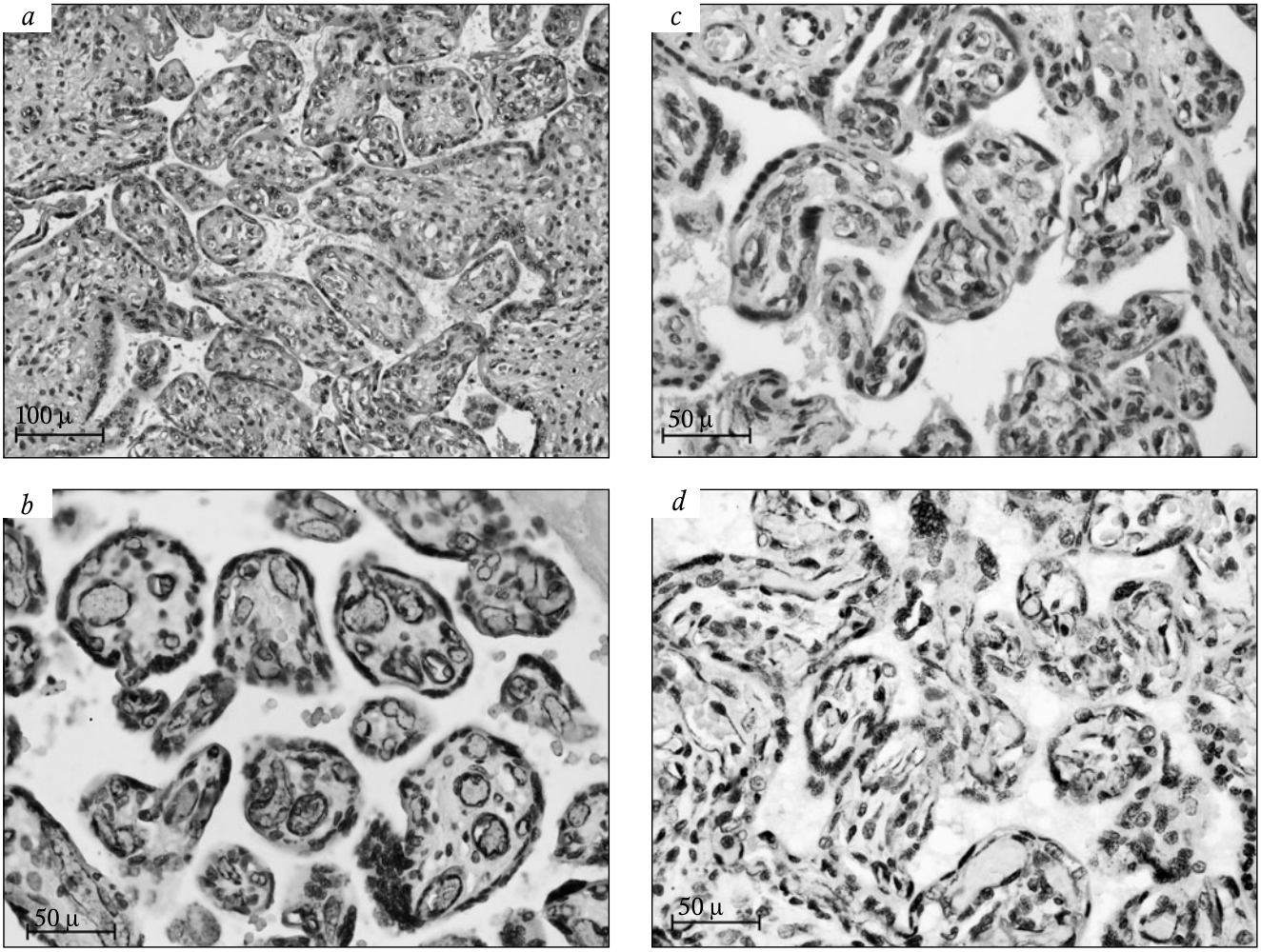

Fig. 1. Morphological characteristics of placentas in groups 1 (*a*, *d*) and 2 (*b*, *c*). *a*) mature intermediate villi predominate; *b*) expression of CD34 in placental villous endotheliocytes; *c*, *d*) VEGF expression in placental villous structures. Hematoxylin and eosin staining (*a*, ×200), immunoperoxidase method (*b-d*, ×400).

TABLE 1. Morphometric Characteristics of Placental Villi in Preterm Delivery of Very Small Newborns (*M*±*m*)

Parameter	Group 1	Group 2
Area of villus, μ²	2741.9±211.8	2558.7±206.3
Perimeter of villus, μ	189.5±16.1	183.1±15.6
SCT area, μ²	697.6±43.9	647.7±42.3
Total capillary area in a villus, μ²	633.0±24.5	568.0±19.7
Mean capillary area, μ²	109.4±4.3	101.2±4.1
Sum of capillary perimeters in a villus, μ	184.7±10.2	206.2±11.6
Mean capillary perimeter, μ	36.2±1.9	37.0±2.1
Number of capillaries in a villus	5.1±0.3	5.5±0.3
Villous vascularization (K1), %	23.1±1.7	22.2±1.6
Villous perimeter/cross-section area (K2)	0.07±0.01	0.07±0.01
Sum of capillary perimeters/sum of capillary cross-section areas in a villus (K3)	0.35±0.02	0.38±0.02
Villous perimeter/sum of its capillary perimeters (K4)	1.06±0.06	0.91±0.06
Villous perimeter/sum of its capillary cross-section section areas	0.39±0.02	0.36±0.02

tion of the placental villi were seen in the placentas of women whose children developed hemorrhagic complications.

Morphometric analysis of histological preparations showed differences in the numerical values of the placentas in the two groups (Table 1, Fig. 2). The mean cross-section area and perimeter of a terminal villus in the group with hemorrhagic complications were 7.2 and 3.5% higher ($p>0.05$) than the values in the group without complications. On the other hand, the proportion of villous perimeter to its section area (K2) was the same in both groups. The area of villous SCT reflecting to a certain measure the maternal blood—fetus exchange was 7.8% higher in group 1 ($p>0.05$).

Analysis of villous vascularization showed rather interesting facts (Fig. 1, *b*). The mean cross-section area of capillaries in a villus and the summary cross-sections area of capillaries in a villus were 8.1 and 11.4% greater, respectively, in the group with hemorrhagic complications. On the other hand, the placental villi in this group had lesser mean numbers of capillaries (by 7.3%) and lower total and mean perimeters of these villous capillaries per villus (by 10.4 and 2.2%, respectively). The villous vascularization index (K1) in this group was 4.1% higher. In addition, the ratio of villous perimeter to the sum of its capillary perimeters was by 16.5% higher and the ratio of villous perimeter to the sum of cross-section areas of its capillaries was 8.3% higher.

Immunohistochemical studies of mature intermediate and terminal villi in all the examined placentas showed positive expression of VEGF in the cytoplasms of all cells. Moderate reaction was seen in villous capillary endotheliocytes and mainly weak reaction in the mesenchymal cells and cytotrophoblast in group 2 (Fig. 1, *c*). Higher intensity of VEGF reaction was found in group 1 placentas (Fig. 1, *d*). The highest values surpassing the parameters in group 2 by 14.3%, were found in endotheliocytes. The level of its expression in SCT cells was 65.1% higher ($p<0.05$), in mesenchymal cells 55.8% higher ($p<0.05$).

Hence, the placentas of women, who gave birth to very small premature children with hemorrhagic complications, were characterized by predominating branched angiogenesis processes and more intense

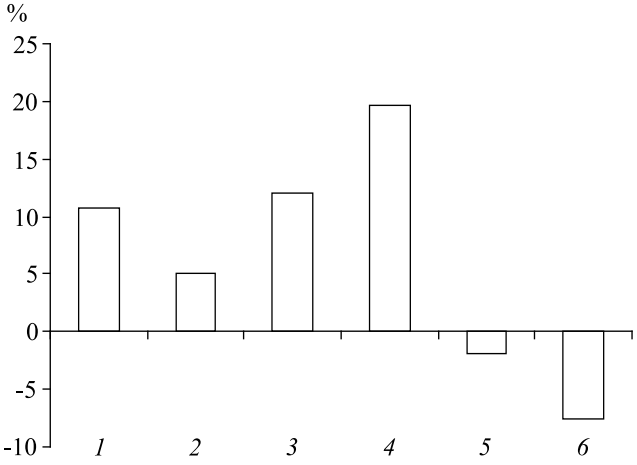

Fig. 2. Changes in morphometric values of placental villi in group 1: *1*) mean area of a villus; *2*) mean perimeter of a villus; *3*) summary capillary cross-section area in a villus; *4*) mean capillary cross-section area in a villus; *5*) summary perimeter of capillaries in a villus; *6*) mean perimeter of a capillary in a villus. Ordinate: increase of the parameters in group 2.

vascularization of the villi. These changes were paralleled by high expression of VEGF in all villous structures. This increase of VEGF expression in endotheliocytes of small and large vessels of the villous tree indicated its high transport to the fetal blood system.

Normally the local concentrations of VEGF in tissues are strictly regulated. In disease, even a slight elevation of VEGF expression leads to an increase of vascular permeability. Experiments on newborn transgenic mice showed that elevated VEGF content in the lung tissue led to the development of pulmonary hemorrhages [6].

The main source of intracerebroventricular hemorrhages is the germinative matrix, abundantly vascularized accumulation of the neuroglia precursor cells in the developing brain. High fragility of the germinative matrix vessels is explained by the so-called "rapid" angiogenesis, as a result of which vessels with insufficient content of pericytes, immature basal membrane, and glial fibrillar acidic protein deficiency form in the astrocyte adjacent nerve terminals [2]. High concentrations of VEGF and angiopoietin II can be responsible for rapid angiogenesis in the germinative matrix [3,7].

Hence, changes in the placenta detected in our study can be the factors modulating the angiogenesis in the germinative matrix and hence, causing the development of intraventricular hemorrhages in the brain and promoting increase of vascular permeability and development of hemorrhages in other organs of preterm newborns.

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