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The branching pattern of villous capillaries and structural changes of placental terminal villi in type 1 diabetes mellitus

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ABSTRACT

Maternal diabetes is associated with changes of the placental structure. These changes include great variability of vascularity manifested by strikingly hypovascular as well as hypervascular terminal villi. In this paper, normal placental terminal villi and pathological villi of type 1 diabetic placentas were compared concerning the structure of villous stroma, spatial arrangement of villous capillary bed and quantitative assessment of capillary branching pattern.

Formalin fixed and paraffin embedded specimens of 14 normal and 17 Type 1 diabetic term placentas were used for picrosirius staining, vimentin and desmin immunohistochemistry and confocal microscopy. 3D models of villi and villous capillaries were constructed from stacks of confocal optical sections.

Hypervascular as well as hypovascular villi of diabetic placenta displayed changed structure of villous stroma, i.e. the collagen envelope around capillaries looked thinner and the network of collagen fibers seemed less dense. The desmin immunocytochemistry has shown that stromal cells of hypervascular as well as hypovascular villi appeared nearly or completely void of desmin filaments. In comparison with normal villi, capillaries of hypovascular villi had a smaller diameter and displayed a markedly wavy course whereas in hypervascular villi numerous capillaries occurred in reduced stroma and often had a large diameter. The quantitative assessment of capillary branching has shown that villous capillaries are more branched in diabetic placentas.

It is concluded that type 1 maternal diabetes enhances the surface area of the capillary wall by elongation, enlargement of diameter and higher branching of villous capillaries and disrupts the stromal structure of terminal villi.

1. Introduction

It is obvious that the function of organs depends on the parameters of capillary bed, i.e. its volume, structure of the capillary wall, rate of blood flow, spatial arrangement and relationship to the neighboring structural components of the organ (e.g. epithelium). The knowledge of the normal structure, quantity and arrangement of capillaries is necessary for the complete understanding of their functions and pathologies.

Maternal diabetes mellitus represents a serious complication of pregnancy associated with increased maternal and fetal risks, and manifests itself also in the structure and function of placenta. Placenta is interposed between mother and fetus, and mediates the influence of maternal milieu on fetus. Placental vascular bed is interconnected with fetal circulatory system, and placental capillaries play a key role in the transfer of oxygen, nutrients and metabolites between maternal and fetal blood. As the fetal development is fully dependent on their functional efficiency, any disorder of them may impair fetal well-being.

Metabolic disturbances of diabetic mother have an impact on placental structure. Although there are no typical structural features, the great variability of the vascular and stromal component of terminal placental villi is often conspicuous. As shown in studies based on transmission and scanning electron microscopy, in normal terminal villi the villous stroma occupies the central part. It consists above all of stromal cells and collagen fibrils. Stromal cells display long cytoplasmic processes extending toward the wall of peripherally located capillaries and into the central core. Collagen fibrils are mainly concentrated around the capillary wall forming a mantle-like structure, whereas their bundles organized into a network in the central core together with

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projections of stromal cells are delimiting so called stromal channels [1,2]. Variable number of capillary profiles are located peripherally, i.e. under the trophoblast, and often form vasculosyncytial membranes.

Besides villi displaying normal vascularity and structure of stroma similar to normal placenta, the microscopic picture shows more or less frequent pathologically changed villi. Those villi are usually of larger diameter and display remarkable variability in the number and diameter of capillary profiles. One form of pathological villi appears markedly hypovascular, i.e. containing sparse narrow capillaries usually embedded in high amount of loose stroma. Due to their structure resembling villi of immature placenta, i.e. larger diameter, voluminous loose stroma and sparse small vessels distant from trophoblast and thus not forming vasculosyncytial membranes, those villi are often characterized as villi displaying immaturity (hypomaturity, maturation defects, maturation disorders) [3–7]. In some diabetic placentas, villi with marked stromal fibrosis (higher amount of collagen) can also be found. The other form of pathological villi is characterized by numerous, and frequently large and congested capillary profiles. Due to their hypervascularity, the amount of stroma is usually decreased [3–7] and a decreased amount of collagen is also described [8]. The excessive villous hypervascularity defined as the occurrence of 10 or more capillary profiles in at least 10 villi in 10 or more foci in at least 3 noninfarcted placental areas, which were found under 10× objective magnification, is called chorangiosis. This phenomenon is associated with increased adverse perinatal outcomes [9].

Quantitative estimations of villous capillaries gave ambiguous results, however the majority of those studies found increased angiogenesis in the diabetic placenta [10–16]. Although the knowledge of the arrangement of microvascular bed is necessary for understanding its function in all organs, there is no study giving information whether type 1 maternal diabetes (DM 1) influences three-dimensional organization of villous capillaries. Despite previously published papers studying angioarchitecture of peripheral placental villi we have shown that our approach using confocal microscopy and adequate software meets necessary requirements of such studies [17]. In our previous papers we demonstrated that the villous capillary bed of term placenta is commonly branched, and signs of both the longitudinal and sprouting capillary growth are present till the end of pregnancy [18]. Using methods of confocal microscopy, topological schemes and 3D reconstruction we compared the spatial arrangement of villous capillary bed in normal placentas and placentas in gestational diabetes mellitus (GDM), and have found that in placentas from GDM the villous capillary bed is more complicated due to an enhanced capillary branching [19]. These results are in contrast to the previously accepted opinion that the placental capillary growth in the second half of pregnancy is exclusively longitudinal [14].

As mentioned above, structural differences between normal and diabetic placentas were described repeatedly, but the data regarding density of capillary branching and spatial organization of villous capillary bed in type 1 diabetes are not available. Similarly, only weak attention was paid to the structural changes of villous stroma in otherwise exhaustive papers dealing with the morphology of diabetic placenta (e.g. Ref. [20]), and surprisingly, we have found no contemporary paper characterizing an influence of maternal diabetes on the organization of villous stroma.

As placental capillaries represent a key component of villous membrane and play a very important role in maternofetal transport, we would like to contribute to better elucidation of the impact of maternal diabetes on their structure and function. In this study we quantified villous capillary branching and examined differences in spatial organization of villous capillaries and structure of villous stroma in normal placentas and placentas in DM 1 using methods of confocal microscopy, 3D reconstruction and immunohistochemistry.

2. Methods

Human placentas were obtained with the written informed consent of mothers and the collection of placental tissue was approved by the Ethics Committee of the First Faculty of Medicine and General University Hospital of the Charles University in Prague.

All mothers were white European women. They declared that they were non-smokers. In the control group the mothers were examined for the flow velocity waveforms in umbilical vessels at 36th or 37th week of pregnancy, the mothers in the diabetic group were examined 3–4 times during the last trimester. Recordings of flow velocity waveform in umbilical arteries and vein were performed with 5–7 MHz Acuson-Antares (Siemens) real-time convex scanner with pulsed and color Doppler options. A 125 Hz high pass filter was used to eliminate signals from slow moving tissues. Output energy of the units did not exceed 50 mW/cm^2 spatial peak temporal average intensity. The umbilical artery blood velocity signals were obtained from free floating central part of the cord. All recordings were obtained during periods of fetal apnea and without movements. The maximum blood flow velocity waveform was characterized by measuring pulsatility index (PI). The average values of three consecutive blood velocity waveforms analyses were taken as the final result. PI was correlated to normal reference values [21]. Patients with preeclampsia, chronic hypertension, IUGR as well as abnormal results of Doppler velocimetry, which can signalize subsequent development of preeclampsia or higher risk for fetal IUGR [22,23], were excluded.

The results achieved in normal and diabetic group were compared using two-sampled Student's *t*-test. We found no statistical differences of PI between normal and diabetic group and no patient with abnormal flow velocity waveforms was included into this study.

2.1. Collection of placentas

Placental specimens were collected at the end of gestation from 14 healthy mothers and from 17 mothers suffering from DM 1. Data regarding mothers, newborns and placentas were recorded in Table 1 and Table 2. The comparison of birth weights (3470 ± 343 vs. 3441 ± 679), placental weights (607 ± 120 vs. 574 ± 110) and fetoplacental ratios (F/P) (5.49 ± 0.76 vs. 6.02 ± 0.63) did not show significant differences of these parameters between control and diabetic group respectively.

2.2. Tissue processing

Placental full-thickness tissue pieces were taken by the systematic uniform random sampling [19]. Each specimen was cut transversally into three blocks corresponding to the parabasal, middle and parachorial placental zone. Each of them was cut into two pieces. One half of pieces was fixed for histology and immunohistochemistry in 4% formaldehyde solution and the other half was fixed for examination by confocal microscopy in 4% formaldehyde containing 0.5% eosin. Following fixation and randomizing of tissue orientation the specimens were embedded in the paraffin wax.

2.3. Histology and immunohistochemistry

For histology and immunohistochemistry the tissue blocks were cut at the thickness of 7 μm. In order to show histological structure of normal and diabetic placenta, selected sections were stained with hematoxylin-eosin method. For demonstration of the degree of villous fibrosis, the picrosirius staining was used [24].

Table 1
Characteristics of the control group. F/P = birth weight/placental weight.

Patient No.	Age (years)	Gestational week/Mode of delivery	Newborn (g/cm)	Placenta (g)	F/P
1	30	39/S.C.	M 3700/50	900	4.11
2	20	41/S.C.	F 3030/48	555	5.46
3	30	40/S.C.	M 3870/52	635	6.09
4	24	41/S.C.	M 3730/53	620	6.02
5	26	39/S.C.	F 2781/45	435	6.39
6	33	39/S.C.	M 3470/50	710	4.89
7	37	40/S.C.	M 4110/53	740	5.55
8	26	39/spont.	F 2890/50	390	7.41
9	31	40/spont.	F 3380/49	600	5.63
10	38	39/spont.	M 3490/50	535	6.52
11	31	39/S.C.	M 3560/50	605	5.88
12	23	40/spont.	F 3520/52	580	6.07
13	35	38/S.C.	F 3175/50	590	5.38
14	31	40/S.C.	F 3880/50	603	6.43