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Histomorphometric study of placental villi vascular volume in toxemia and diabetes

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Summary The quantitative changes in the vascular tree in placentas from pregnancies complicated by diabetes mellitus and preeclampsia (PE) are not well defined. The purpose of this study was to quantify placental villi cross-sectional area of capillaries assessed by a computerized morphometry system in pregnancies complicated by PE (n = 23), well-controlled pregestational diabetes mellitus (PGDM; n = 10), and healthy controls (n = 13). Our aims were to test whether villous capillarization volume was changed in PE without intrauterine growth restriction or PGDM compared with the control group and to study these effects in 3 different areas of the placenta. Examination of placentas in women with PGDM and PE revealed limited pathological changes on light microscopic examination. However, the morphometric analysis revealed a more than 5-fold decrease of villous vascular volume in PGDM compared with controls ($P = .003$) and a 1.6-fold decrease in the PE group that did not reach statistical significance. These findings show quantitative changes in the villous vascular tree in PGDM that are not detectable by conventional light microscopy and suggest that morphometric analysis of the capillary tree may have diagnostic importance in this entity. The findings differ significantly from those previously reported in pregestational diabetes and do not differ significantly from those reported in PE without intrauterine growth restriction.

1. Introduction

The placenta is a unique organ of limited life span, interposed between two separate individuals. The main functional units of the placenta are the chorionic villi; within them, fetal blood is separated from maternal blood in the surrounding intervillous space by vasculosyncytial membranes overlying dilated fetal capillaries [1,2]. In normal human pregnancy, capillary growth is biphasic, involving an initial phase of branching angiogenesis with formation of tightly looped capillaries, followed by a phase of increased nonbranching angiogenesis with formation of longer capillaries [3-5]. Vascular patterns and villous shapes vary in pregnancies complicated by fetal hypoxia; there is a shift

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toward branching angiogenesis in preeclampsia (PE) pregnancies or prevalence of nonbranching angiogenesis in pregestational diabetes mellitus (PGDM) [6-9].

Placentas from well-controlled diabetic patients are not heavier than those from healthy pregnant women but still show similar morphological abnormalities to those from patients with inadequate glycemic control [10-13].

Increased fetoplacental angiogenesis in PGDM has been described and may be associated with increased capillary villous length, endothelial proliferation influenced by relationships between pericytes and endothelial cells [6,8,9,14-17]. Proliferation of endothelial cells, significant thickening of basal membranes of trophoblast, separation of basal membranes in basal capillaries, distension and disarrangements of perivascular space, and decrease of vascular surface of terminal villi are significant factors contributing to fetal anoxia in pregnancy complicated by diabetes mellitus [14,18].

In PE, uteroplacental circulation is compromised and villi are exposed to a more focal hypoxia, which induces more heterogeneous maturation with normal or impoverished growth of capillaries [6,7]. In PE associated with intrauterine growth restriction (IUGR), capillary volumes may be reduced [6,7,19-22]. However, recent studies have suggested that the reduced capillary growth is more likely to be because of IUGR than to PE [6,7,19,23,24].

There is no absolute correlation between the severity of toxemia and the magnitude of the histological changes [2,18-20].

Taken together, this suggests a primary defect in the placental vascular tree in placentas from diabetic and preeclamptic mothers. This would be in line with the many vascular abnormalities that are prevalent in the systemic circulation in diabetic patients. The rapid growth of the placental vascular tree, which is unsurpassed in any other organ in adult life, makes it an ideal target to search for early vascular changes of diabetes. We were therefore interested to study the placental vascular tree from diabetic and preeclamptic mothers using quantitative measurements.

A systematic morphometric analysis of the placental vascular tree is required to better evaluate its functional and structural properties. Changes in the structures of placenta, using morphological criteria of villous arborization, are divisible into different topological regions. These are known as the stem, intermediate (mature and immature), and terminal villi, based on villous caliber and vessel type. Growth of villous volume during gestation is mainly confined to intermediate and terminal villi. All principal tissue compartments (trophoblast, stroma, fetal capillaries) expand during gestation. Within these villi, maturation involves differential growth of the fetal capillary bed and decline in the relative amount of stromal connective tissue [4,5,25].

This is of more than academic relevance because there are currently no completely specific histological criteria for toxemia and PGDM. The application of strict morphometric methods may help to assess subtle quantitative changes that are not apparent on regular microscopy. We therefore used a computerized morphometric detection method for analyzing capillary volume in histological cross-sectional area and compared villous capillary volume between normal placentas, placentas from mothers with well-controlled PGDM, and those from PE without IUGR subjects.

2. Materials and methods

2.1. Patient groups

Overall, 46 postdelivery placentas (38-42 weeks) from 3 different groups, which were either uncomplicated or complicated by PGDM and PE without IUGR, were examined. The relevant data were collected from a large set of patients delivered in the Misgav Ladach Hospital (Jerusalem, Israel) together with relevant clinical details, including age, weight, height, socioeconomic status, and reproductive history. Control placentas (Cos; n = 13) were from healthy women whose pregnancies were not complicated by PE, IUGR, DM, or renal disease. The PE group (n = 23) was defined by significant maternal proteinuria (>300 mg/L in 24-hour collection in the absence of urinary tract infection) and a blood pressure of higher than 140/90 mm Hg on two or more occasions in a previously normotensive women. IUGR cases were excluded by ultrasound scans and final birth weight ratio (estimated fetal weight, >10th percentile). The PGDM group (n = 10) was composed of placentas from women that were diagnosed with diabetes before 15 years, without hypertension. The mean diurnal glycemia in the PGDM group ranged between 100 and 130 mg/dL. All patients of this study were nonsmokers and were delivered vaginally in spontaneous labor.

2.2. Tissue preparation

All the placentas, including those from the control group, were treated identically in a standardized manner. Immediately after delivery, cords were clamped to ensure that placentas are not drained of blood. Fresh placental weights were determined after removing blood coagula and attached membranes. All placentas were fixed in 10% buffered formalin for 2 weeks. Each placenta was cut into 1-cm-thick choriobasally orientated tissue strips and uniformly sampled to comply with strict stereological criteria for sample selection [26,27]. Gross morphology was evaluated by a pathologist (A. M.). For histological and morphometric analyses, 3 samples at least 1 cm in diameter each were taken from constant areas of each placenta: the first section, 2 cm from the umbilical cord and 0.5 cm deeper from the subchorial region (more primary villi were measured); the second section, 4 cm from the umbilical cord and 1.5 cm deeper from the subchorial area (more secondary villi were measured); and the third section, 6 cm from the umbilical cord and 0.5 cm above the base of the placenta (more tertiary villi were measured).

Table 1 Patient characteristics

	Mothers age (decade)			Parity	
	Third	Fourth	Fifth	Primiparas	Multiparas
Control	9 (20)	4 (9)	–	1 (2)	12 (26)
Diabetes	7 (15)	3 (7)	–	2 (4)	8 (17)
Preeclamptic	14 (30)	7 (15)	2 (4)	18 (39)	5 (11)
Total	30 (65)	14 (30)	2 (4)	21 (46)	25 (54)

Values are presented as n (%).

The chosen sample was embedded with the cut face down to meet the requirements for vertical sections, which are necessary for design-based estimation of surface area [27]. Histological sections were cut at 5-μm thickness and stained with hematoxylin-eosin. Light microscopic fields of view were selected by uniform sampling [3].

2.3. Morphometric methods

The slides stained with hematoxylin-eosin were analyzed using a computerized morphometric system (WinScanArray 3; Galai, Migdal Haemek, Israel) connected to a light microscope (BH-2; Olympus, Tokyo, Japan). In every slide clean of artifacts, 0.5-cm^2 area was manually marked. Inside each area, 6 randomly selected microscopic fields (original magnification ×60) were acquired (at 1024 × 1024 resolution) using a color video camera (DXC-151AD; Sony, Tokyo, Japan). After acquisition, the images underwent automatic light analysis and noise removal procedures to ensure color and image quality standardization in all analyses. The images were given a threshold to isolate only the blood vessel lumens using red, green, blue color separation. The coverage percentage was calculated as the number of pixels covered by the threshold area/overall number of pixels in the image. The results from the 6 images were averaged to give 1 value for each specimen, describing the overall percentage of section area occupied by villous blood vessel lumens.

2.4. Statistical analysis

Statistical analyses were carried out using Excel (Microsoft Excel 2002; Microsoft Corporation, Redmond, Wash). Means and SEM were calculated for each group and for each of the 3 areas. The statistical significance of the differences was assessed using Student *t* test.

Table 2 Patient characteristics

	Gestational age (wk)	Birth weight (g)	Placental weight (g)
Co (n = 13)	38-41	3200-4000 (3527)	580-650 (606.5)
D (n = 10)	39-42	3200-4000 (3560)	620-650 (639.8)
PE (n = 23)	37-41	2900-3600 (3180)	450-620 (511)

Values are presented as range (mean).
Abbreviations. Co, control; D, diabetes; PE, preeclamptic.

3. Results

3.1. Patients

The relevant patient characteristics are given in Table 1. Of the 46 placentas evaluated, 13 were from healthy women (control group), 10 from diabetic women with type 1 diabetes mellitus, and 23 from women with proteinuric preeclamptic pregnancies without IUGR. All the diabetic mothers were well controlled in glucose and glycated hemoglobin levels. The infants birth weights ranged between 2900 and 4000 g (Table 2). No malformations were found in the neonates.

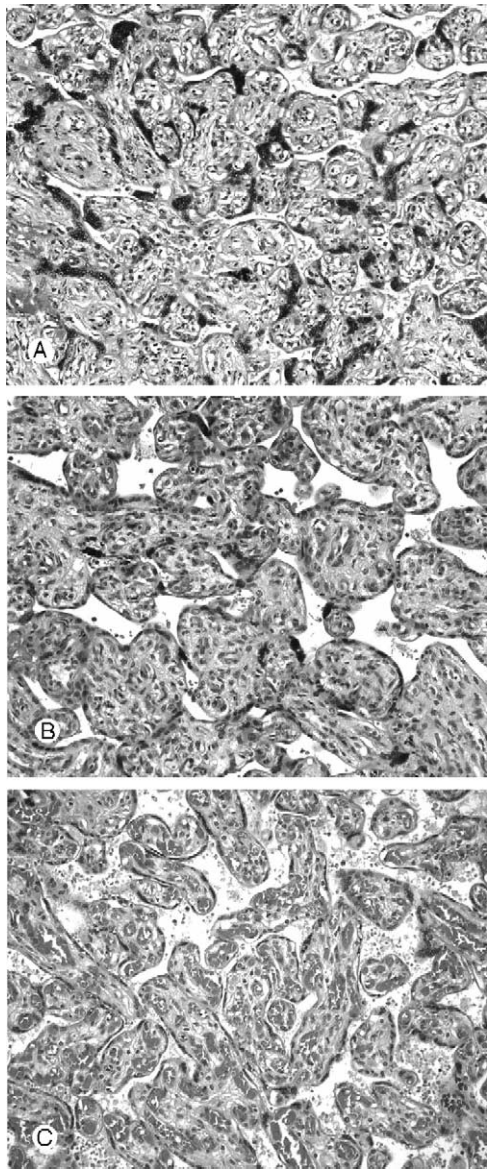

Fig. 1 Examples from the third section. A, Ischemic changes (PE), formation of prominent syncytial knots, and areas of marked branching angiogenesis (original magnification ×200). B, Presence (PGDM) of increased mature and immature intermediate villi and a relatively decreased number of terminal villi with formation of syncytial knots (original magnification ×200). C, Control group (original magnification ×200).

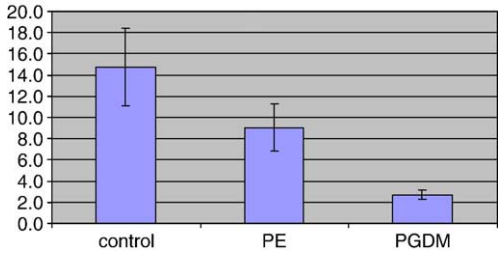

Fig. 2 Comparison of villous capillarization volume in both PGDM and PE pregnancies compared with control group.

The control and diabetic cases were delivered between 37 and 42 weeks of gestations. Most mothers were multiparous (Table 1). As expected, the mean length of gestation was 1 week longer in diabetic mothers compared with the control group (41 versus 40 weeks). Despite this, mean birth weight was similar (3527 and 3560 g) in the Co and PGDM groups, respectively (Table 2). By contrast, among the preeclamptic cases, gestational age at delivery ranged from 37 to 41 weeks, and most mothers were primiparous (Table 1). All pregnancies were delivered vaginally in spontaneous labor.

The placental weight of the 3 groups ranged from 450 to 650 g. Although the mean placental weight of the diabetic placentas was 5% heavier compared with the control group, this difference was not significant (Table 2).

The gross and microscopic examinations of placentas in preeclamptic pregnancies showed ischemic changes such as formation of prominent syncytial knots and areas of marked branching angiogenesis (Fig. 1A). Fibrinoid necrosis and intramural lipid deposition in the walls of uterine vessels (acute atherosis) were found in 15 of 23 PE placentas, and gross examination revealed infarcts in 6 PE placentas, which amounted to less than 10% of the placental volume in each case.

The villous structures of the placentas in maternal diabetes were focally edematous and dysmature with presence of increased mature and immature intermediate villi and a relatively decreased number of terminal villi with formation of syncytial knots (Fig. 1B). All Cos, including fetal membranes and umbilical cords, were normal (Fig. 1C). Light microscopic examination did not reveal any significant changes of villous capillarization among the 3 groups.

By contrast, detailed morphometric analysis showed that the relative capillary area differed significantly among the 3 groups. Although the average capillary volume was 14.8 $\pm$ 3.7 (mean $\pm$ SEM, arbitrary units) in the control group, it dropped to 9.0 $\pm$ 2.2 ($P = .099$, Student t test) and 2.7 $\pm$ 0.5 ($P = .003$) in the PE and PGDM groups, respectively (Fig. 2). The capillary volume was decreased to a similar degree in the 3 different areas of the cotyledons (Fig. 3), suggesting that this is a general phenomenon and is not restricted to specific areas of the placenta.

4. Discussion

Our morphometric study demonstrates a more than 5-fold reduction of the villous capillarization volume in PGDM placentas compared with controls. Similar degrees of reduced capillary volume were noted in all 3 areas of the cotyledon. In toxemic placentas, a 1.6-fold reduction that did not reach statistical significance was noted. When comparing the capillary volume in the peripheral zone to other zones in this group, there was a 2.0-fold reduction that was close to statistical significance ($P = .07$). Placental tissue displays a high degree of angiogenesis, a process responsible for tissue growth and placental morphology. In PE, capillarization is not altered, but capillary volume is essentially conserved because decreases in capillary length are compensated by increases in caliber [28]. Earlier findings in well-controlled PGDM showed that fetoplacental angiogenesis is enhanced exclusively by longitudinal growth (capillary villous length), but there was no evidence of altered vascular remodeling in the villi (cross-sectional shape and caliber of capillaries were preserved) [9,15-17]. Another study of placentas from patients with type I diabetes mellitus noted hypovascularization of villi [29]. Moreover, ultrastructural examination of PGDM placentas also showed a decreased vascular surface in the terminal villi [17]. Our finding of a small, not significant reduction in vascular villi volume of the placentas in the PE group is not surprising because it is in line with previous results [6,7,24,28-31]. However, the finding of a significant reduction of vascular villi volume capillarization in PGDM is in apparent contradiction with most (but not all) of the previous results. One possible explanation is that some mothers in the diabetic group also had PE. However, this is neither supported by the clinical details nor by the finding that the capillary volume in the diabetic group is actually lower than that in the PE group. An alternative explanation is the different method we have used that measures the capillary cross-sectional area assessed by computerized morphometry system. An additional explanation is the better control of blood glucose levels that is achievable today, which avoids hyperglycemic episodes at early stage of pregnancy. It is possible that increased fetoplacental angiogenesis in PGDM arises after hyperglycemic episodes before midgestation [17,32]. Although one would expect that better glycemic control would result in reduced changes, it is also possible that hyperglycemia has an opposite effect to insulin resistance and

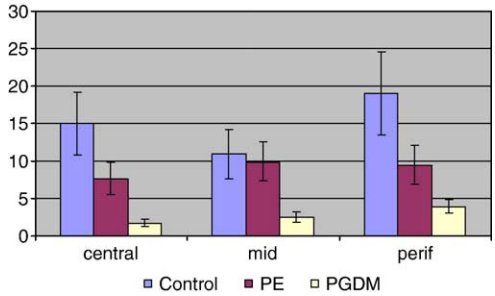

Fig. 3 Comparison of villous capillarization volume in the 3 different areas of both PGDM and PE pregnancies compared with control group.

that better glycemic control unmasks the basal effect of the diabetic process that does not depend on hyperglycemia per se. It has been shown that diabetes is associated with endothelial cell dysfunction and reduced neovascularization, and that endothelial progenitor cells are also reduced in numbers and in function in diabetes [33-35]. We suggest that the placenta shows abnormalities in angiogenesis, even in well-controlled diabetes. Because the placenta is a tissue that can be easily accessed, we suggest that it may serve as a very suitable model to study angiogenesis impairment in diabetes.

5. Summary

Our goal was to identify quantitative changes in the degree of cross-sectional vascular villi volume in the different areas of the placenta in different patient groups by morphometry. The findings of this study show that morphometry is particularly useful for assessing an organ such as the placenta where pathological abnormalities may be quantitative rather than qualitative. The present computerized measurement of villous capillarization is a quick and economical method for obtaining complex quantitative structural data that may be of diagnostic importance. We feel that the impact of our study was enhanced by adding the preeclamptic group because this another condition associated with disturbance of the maternal placental vasculature supports the validity of our methodology.

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