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Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt diabetes

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Abstract

In this study, morphometric measures of placental terminal villi and villous vessels were compared in overt, as well as gestational diabetes mellitus, and mild hyperglycemia diagnosed by oral 100 g glucose tolerance test (100 g-OGTT) and glucose profile (GP). At delivery (gestational age $\geq$ 34 weeks) a total of 207 placentas were assigned to a control group ($n = 56$) or to one of three groups complicated by mild hyperglycemia ($n = 51$), gestational diabetes ($n = 59$) and overt diabetes ($n = 41$). Placenta samples were randomly selected for blind morphometric assessment with an image analyser. Morphometric measures obtained included area and number of terminal villi and their respective villous vessels. Statistical analyses were performed using the chi-square test, ANOVA and stepwise regression ($p \leq 0.05$). Glycemic means were 86.2 mg/dL in controls, 98.9 mg/dL in mild hyperglycemia, 114.1 mg/dL in gestational diabetes and 122.1 mg/dL in overt diabetes. Our results show that abnormal maternal glycemic levels may change the placental morphometric characteristics related to materno-fetal exchanges.

Keywords: Diabetes; Hyperglycemia; Placenta; Morphometry

1. Introduction

Owing to its position between the maternal and fetal circulation, the placenta is exposed to diabetes-associated endocrine and metabolic derangements of both mother and fetus. Upon reviewing the literature, Desoye et al. [1] found no clear-cut picture of the effects of diabetes on placenta emerges, most likely because of confounding factors such as diabetes types and severity, modality of treatment, and quality of glycemic control.

According to the American Diabetes Association (ADA) [2], gestational diabetes mellitus occurs when two abnormal values are observed in the 100 g or 75 g oral glucose tolerance tests (OGTT). The glucose profile test (GP) is generally used to evaluate the quality of diabetes treatment, with fasting plasma glucose levels <90 mg/dL and/or 2-h postprandial plasma glucose <130 mg/dL [3–5]. A study in Brazil using the National Diabetes Data Group criteria [6] to assess for diabetes and Gillmer's threshold values to establish GP [3,4], unexpectedly, instead of two (normal and diabetic), found four groups of pregant women [5]: IA = normal OGTT + normal GP; IB = normal OGTT + abnormal

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GP; IIA = abnormal OGTT + normal GP; IIB = abnormal OGTT + abnormal GP.

According to ADA's criteria, groups IIA and IIB are diabetic while group IB is not because their OGTT is normal. However, Rudge et al. [7] demonstrated that in groups IB, IIA and IIB the risk for macrosomia was statically similar and perinatal mortality rate was 10-fold higher than in group IA (non-diabetic). Thus, they concluded that the adverse perinatal outcomes observed were due to hyperglycemia, which was present in the IB group, but under diagnosed by OGTT. Since then, normal OGTT and abnormal GP became the criteria used to diagnose mild hyperglycemia (IB group) and determined the treatment of these patients in the Pregnancy and Diabetes Service of Botucatu Medical School, São Paulo State University.

Several studies have described plethora, choriangiosis, edema, hypo and hyper ramification of the terminal villi, infarction, fetal-placental sclerosis, fibrotic villi and villous basement membrane changes in diabetic placentas. Diabetes has been associated with endarteritis [8]. However, its occurrence is higher in pregnant women with mild hyperglycemia [5]. Although a tight glycemic control may prevent adverse perinatal outcomes, heavier placentas have been observed in overt and gestational diabetes [9,10].

Findings on morphometric abnormalities [11–13], intervillous space (IVS) size [13–16], villous surface [15,17–20], and number of villous vessels in diabetic placentas are divergent in diabetes, villi are more vascularized, showing greater capillary length, surface area and mean vessel diameter [13]. Vascular growth is exclusively longitudinal without vascular remodeling [21].

The morphometry of placental villi and vessels is related to maternal glucose levels, and affects feto-placental function, as well as perinatal outcome. Therefore, the aim of this study was to compare the morphometric characteristics of placental terminal villi and their respective villous vessels in pregnancies complicated by either overt or gestational diabetes, or even mild hyperglycemia with non-diabetic controls. Our hypothesis is that, hyperglycemia leads to different patterns of villous and capillary growth, regardless of the test used to diagnose intrauterine hyperglycemia.

2. Materials and methods

This study was conducted in the Diabetes and Pregnancy Service of Botucatu Medical School, São Paulo State University, Brazil, and was approved by the Ethical Research Board of the institution. Placentas were obtained from consenting pregnant women.

2.1. Population

This cross-sectional study assessed the area and number of terminal villi and villous vessels, and the capillarization index in placentas, which minimum gestational age was 34 weeks ($\geq$34 weeks), with mild hyperglycemia or gestational or overt diabetes. Non-diabetic control placentas were consecutively and specifically collected for this study.

The diagnosis of gestational diabetes or mild hyperglycemia was established between 24 and 28 gestational weeks, by the 100 g-OGTT test, according to ADA's criteria [2], and the glucose profile (GP) test, according to Gillmer's threshold values [3–5,7]. At delivery (gestational age $\geq$ 34 weeks), a total of 207 placentas were assigned to a control group (normal 100 g-OGTT and normal GP, $n = 56$) or to one of three groups complicated by mild hyperglycemia (MH, $n = 51$): normal 100 g-OGTT and abnormal GP; gestational diabetes mellitus (GDM, $n = 59$): abnormal 100 g-OGTT and abnormal GP during pregnancy; or overt diabetes (OD, $n = 41$): abnormal prepregnancy 100 g-OGTT.

Aiming at a good control, a mean glucose level of 100 mg/dL on GP was sought. Gestational diabetes mellitus (GDM) and mild hyperglycemia (MH) treatment included individualized dietary advice from a qualified dietitian, or dietary advice + human insulin therapy when the GP showed fasting plasma glucose levels >90 mg/dL and/or postprandial plasma

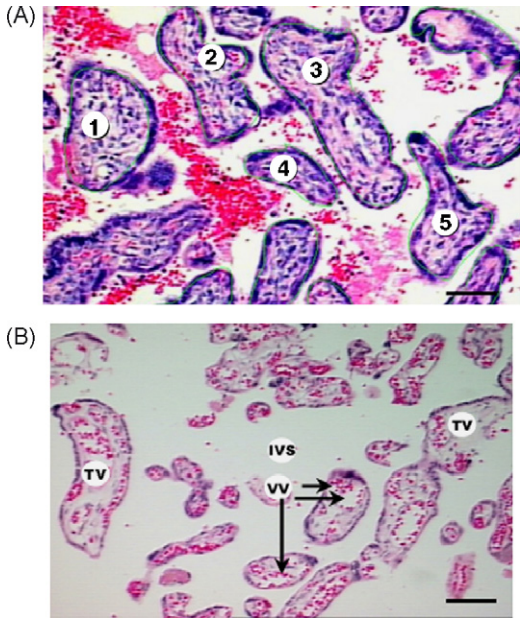

Fig. 1. Photomicrograph of terminal villi and villous vessels according to the standardization criteria in the placental morphometric analysis of the experimental groups. (A) Terminal villi totally inside the field and standardized for morphometrical analysis; terminal villous total area = 1 + 2 + 3 + 4 + 5; villi mean area = (1 + 2 + 3 + 4 + 5)/5—color HE, 200×; bar: 10 μm. (B) Terminal villi (TV) and respective villous vessels (VV, arrows) in the maternal–fetal exchange surface in contact with intervillous space (IVS)—color HE, 200×; bar: 10 μm.

glucose >130 mg/dL. All cases of overt diabetes (OD) were treated with dietary advice and human insulin therapy, regardless of prepregnancy use of hypoglycemiant drugs. Plasma glucose levels were monitored by GP (monitoring plasma glucose levels including fasting, preprandial and postprandial levels during 24 h) at least at 2-week intervals until delivery for insulin dose adjustment [2,7,22]. All glucose determinations were done using glucose oxidase method (Glucose-analyzer II Beckman, Fullerton, CA, USA).

The confounding variables studied included smoking, arterial hypertension [23] and gestational glycemic mean (GGM = mean plasma glucose in all GP performed during pregnancy), classified as adequate (GGM < 120 mg/dL) or inadequate (GGM ≥ 120 mg/dL) [7].

2.2. Placental analyses

Cords were ligatured and cut immediately after delivery. Full-depth placental samples were drawn in a systematic random manner [24] and immersed in formol saline for at least 24 h. Tissue cubes were embedded in wax, in an attempt to meet the sampling requirement for unbiased estimation of volume and area. Randomly chosen blocks from each placenta were cut at 4 μm thickness, mounted on glass microslides and stained with hematoxylin–eosine. Morphometric measures were taken from 60 blocks: 10 slides from 5 controls; 18 slides from 9 MH placentas; 16 slides from 8 GDM placentas, and 20 slides from 10 OD placentas [25].

The same pathologist performed all analyses blind. Morphological measurements were taken using a Video Image Analyser (KS-300 3.0, Zeiss®, digital camera CCD-IRIS/RGB Sony®, coupled to photomicroscope DMR, Leica®). The intermediate zone was previously examined [24] and five fields/slide counted, avoiding areas of placental infarction; areas of intervillous fibrin deposition; arterial vessels forming the primary stem and anchoring villi; and histological artifact [25]. Fifty fields of the control group, 90 of MH, 80 of GDM and 100 of OD were observed, totalizing 320 fields from 32 placentas. The terminal villi and villous vessels found totally inside the microscopic field (area = 293.9920 μm^2) were used for group comparison.

The total and mean areas (μm^2) were measured and the number of terminal villi and villous vessels were counted [25]. The mean area of villi and villous vessels was calculated by

Table 1
Background data and gestational glycemic mean (GGM)

Control variables	Control	MH	GDM	OD	*p*-Value
GGM (mg/dL)[a]	$86.2a_0$	$98.9b_0$	$114.1c_0$	$122.1d_0$	<0.05
OGTT	Normal	Normal	Altered	Altered	
GP	Normal	Altered	Altered	Altered	

	Control		MH		GDM		OD		*p*-Value
	n	%	*n*	%	*n*	%	*n*	%	
Smoking	8	14.3	6	11.8	8	13.6	5	12.2	0.980
Hypertension	22	39.3	15	29.4	24	40.7	16	39.0	0.616
Adequate GGM[a]	56	$100.0a_1$	50	$98.0a_1$	48	$81.4b_1$	22	$53.7c_1$	0.000
Pregnant women	56		51		59		41		

MH: mild hyperglycemia; GDM: gestational diabetes mellitus; OD: overt diabetes.
[a] Values followed by different letters and same index significantly differ ($p < 0.05$).

Table 2
Effect of different glycemic levels and two diagnostic criteria (OGTT and glucose profile) on placental terminal villi

Terminal villi	Control	MH	GDM	OD	*p*-Value
Total area (μm^2)[a]					
m	$68659.4a_0$	$79849.1b_0$	$68466.2a_0$	$65957.2a_0$	0.0067
S.D.	24695.0	32640.0	27326.0	28408.0	
Mean area (μm^2)[a]					
m	$15708.8a_1$	$8845.4b_1$	$12064.4a_1$	$15340.0a_1$	0.0001
S.D.	18840.0	6974.3	8890.0	14855.0	
Villi number[a]					
m	$5.9a_2$	$12.9b_2$	$7.1a_2$	$7.2a_2$	0.0001
S.D.	2.84	10.68	3.59	6.52	

MH: mild hyperglycemia; GDM: gestational diabetes mellitus; OD: overt diabetes.
[a] Values followed by different letters and same index significantly differ ($p < 0.05$).

the ratio between the total area and number of villi or vessels in the field (Fig. 1). Capillarization index (%) was defined as the total area of villous vessels and terminal villi ratio (vascular total area/villous total area × 100) [13].

2.3. Statistics

Categorical data were compared using the χ^2-test. Continuous data were compared using the unpaired Student's *t*-test or ANOVA. Stepwise regression was used to verify the gestational glycemic mean/morphometric alterations of placental villi and vessels relationship. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Maternal characteristics

Except for glycemic means, maternal characteristics did not significantly differ among groups (Table 1).

3.2. Blood glucose

Table 1 shows that gestational glycemic mean (GGM) significantly differed among groups ($p < 0.05$).

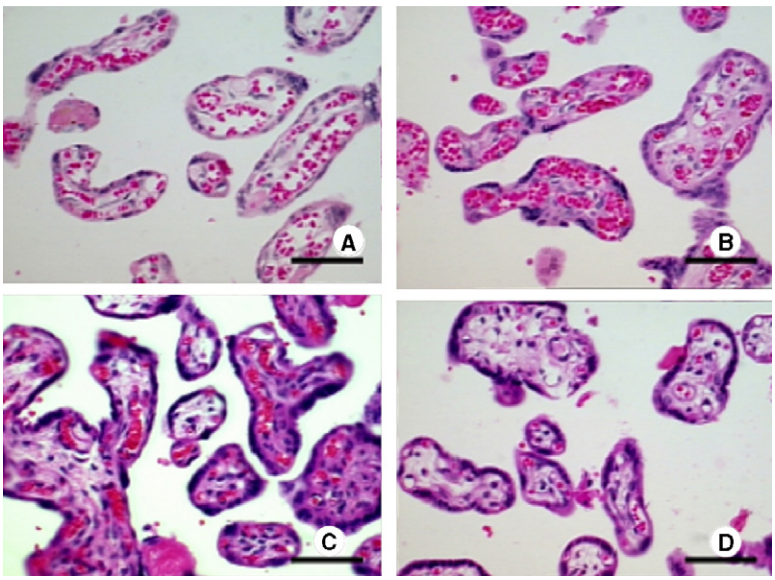

Fig. 2. Photomicrograph of placentas: control (A), mild hyperglycemia (B), gestational (GDM) (C), and overt diabetes groups (D)—color HE, 400×; bar: 5 μm.

Table 3
Effect of different glycemic levels and two diagnostic methods (OGTT and glucose profile) on peripheral villous vessels

Villous vessels	Control	MH	GDM	OD	*p*-Value
Total area (μm^2)[a]					
m	15181.6a_0	15499.6a_0	13070.8b_0	10366.9c_0	0.0001
S.D.	9017.3	6857.2	8012.8	8702.2	
Mean area (μm^2)[a]					
m	708.2a_1	399.3b_1	577.3c_1	498.5bc_1	0.0001
S.D.	556.6	259.9	387.6	548.7	
Number[a]					
m	23.4a_2	46.6b_2	23.3a_2	25.1a_2	0.0001
S.D.	8.2	21.6	8.2	21.0	
Capillarization index (%)[a]					
m	23.4a_3	23.2a_3	19.7a_3b_3	16.8b_3	0.0001
S.D.	11.9	16.4	12.2	19.0	

MH: mild hyperglycemia; GDM: gestational diabetes mellitus; OD: overt diabetes.
[a] Values followed by different letters and same index significantly differ ($p < 0.05$).

3.3. Placental morphometric parameters

3.3.1. Terminal villi

In MH pregnancies, the total area of terminal villi was higher, mean area smaller and terminal villi number greater than in normal and diabetic pregnancies (Table 2 and Fig. 2).

3.3.2. Villous vessels and capillarization index

In diabetic placentas, both the mean area and the total area of villous vessels were smaller while the number of villous vessels was similar to that in the control group. Capillarization index was smaller in OD than in the other groups. A significantly smaller mean villous vessels area and a higher number of villous vessels were found in MH pregnancies in comparison to normal and diabetic pregnancies. Consequently, the villous capillarization index was significantly lower in diabetic pregnancies (Table 3 and Fig. 2).

3.4. Coefficient value (R^2)

There was a direct relationship between GGM and total area of villous vessels ($R^2 = 0.11$; $p = 0.002$) in GDM. Inverse relationships were found between GGM and total number of villous vessels in the control group ($R^2 = 0.09$; $p = 0.034$) and DH ($R^2 = 0.23$; $p = 0.000$). There was an inverse correlation between villi number and GGM ($R^2 = 0.18$; $p = 0.000$) and a direct relationship between the total area of terminal villi and overt diabetes ($R^2 = 0.22$; $p = 0.044$) (Table 4).

Table 4
R and R^2 coefficients and p value in the significant results of multiple regression analysis between the glycemic gestational mean (GGM) and the morphometric variables of placentas from the control, mild hyperglycemia, gestational (GDM) and overt diabetes groups

	R	R^2	p-Value
Control			
Number of vessels (+)	0.30	0.09	0.034
Mild hyperglycemia			
Number of vessels (−)	0.47	0.23	0.000
GDM			
Total area villous vessels (+)	0.34	0.11	0.002
Overt diabetes			
Villi number (−)	0.43	0.18	0.000
Total area villous (+)	0.46	0.22	0.044

4. Discussion

In this study, placental morphometric characteristics was significantly increased in the mild hyperglycemia (MH) group, although GGM was adequate in 98% of the cases. In MH, the total area of placental terminal villi was higher, as well as the number of small villi and villous vessels, and capillarization index was statistically similar to control group. In both GDM and OD, the size and number of placental terminal villi as well as villi total area were similar to those in the control group. However, villous vessels total and mean areas were smaller. Thus, capillarization index was lower in OD, and intermediate in GDM, i.e., proportional to glycemic levels and diabetes type.

These data are controversial in literature. In diabetic pregnancies, the fetus may experience chronic hypoxia, with increased fetal erythrocytes and hemoglobin concentrations [26]. However, no difference in the dimensions of terminal villi and placental membranes intervillous pores have been found [13,14,27], suggesting that diabetes-related hypoxia might be due to metabolic alterations and not to placental vascularization [27]. On the other hand, feto-placental angiogenesis in well-controlled diabetes has been reported to be enhanced and to occur exclusively by longitudinal growth, no matter the severity and length of maternal diabetes mellitus [21].

Recently, the hypothetical model of the placental structural adaptative response to hypoxia, described by Desoye and Myatt [28], shows that maternal/fetal hyperglycemia leads to intrauterine hypoxia that, in turn, increases the exchange surface area to ensure adequate oxygen delivery to the fetus. Therefore, the placental morphometric characteristics observed here in MH group, specially the capillarization index similar to control, may be the initial signs of this adaptative response to hypoxia.

Maternal hyperglycemia levels are known to affect the quality and the extension of the placental exchange surface [13,29,30]: the less stringent the control, the greater the surface area [29,30]. Thus, the different placental morphologic characteristics observed here in MH, GDM and OD may be explained by the different hyperglycemia levels found. Considering the similar distribution of smoking and arterial hypertension among the groups studied, maternal hyperglycemia may be considered to be associated with intrauterine hypoxia and vascular proliferation. Moreover, multiple regression analysis showed that the higher the glycemic levels the lower the number of terminal villi and villous vessels, suggesting placenta inadequacy to

ensure maternal–fetal exchanges and fetal oxygen delivery.

In conclusion, our results show that maternal glycemic levels may change placental morphometry, suggesting that such changes are proportional to glycemic levels—low maternal hyperglycemia stimulates vascular proliferation and villous ramification in response to a lower hypoxia level, and thus assures maternal and fetal exchange. The increase in glycemic levels, proportional to the severity of the maternal clinical condition and intrauterine hypoxia, which inhibits villous angiogenesis, interferes with maternal–fetal exchanges and increases the risk of perinatal mortality, still very high in gestations complicated by maternal diabetes [31] or mild hyperglycemia [7]. This is relevant in clinical practice, as it emphasizes the importance of a strict glycemic control in pregnancies complicated by diabetes or mild hyperglycemia.

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