

Morphological and ultrasound findings in the placenta of diabetic pregnancy

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Abstract

The purpose of this study is to analyze the morphological, histological, immunohistochemical and ultrasound findings in the placenta of maternal type 1 and gestational diabetes, to compare the pathological changes of the placental structure in the two types of metabolic disruptions, but also to establish correlations with the expression of these findings, influenced by different associated conditions. This multicenter study includes 53 pregnancies, of which 37 with pregestational and 16 with gestational diabetes. All cases undergone specific obstetric ultrasound assessment and detailed placental scan. There were assessed 49 singleton and four twin pregnancies, all of which having live births as fetal outcome. Maternal preexisting hypertension, preeclampsia and obesity were the main associated conditions. Placental ultrasound scan revealed increased placental thickness even from the second trimester, with significant increases in the first half, and placentomegaly at the end of the third trimester. Macroscopic analysis of the placentas and umbilical cords has shown that the placentas of women with diabetes are heavier, and abnormal cord insertion has been also found. Gross analysis of maternal and fetal surfaces of the placentas revealed certain changes in both metabolic conditions. We observed 14 types of placental pathological findings in pregestational and 11 in gestational diabetes. In diabetic placenta, it is not appropriate to discuss about specific changes, but rather about a pathological diabetic pattern, influenced by associated conditions. Preconceptional and first trimester glycemic control is the key element, and euglycemia throughout pregnancy is a purpose whose accomplishment depends the maternal–fetal outcome.

Keywords: gestation, diabetes, sonography, pathology, diabetic pattern, glycemic control.

Introduction

The placenta is a morpho-functional complex with a central metabolic role during the gestation period. Is the critical organ responsible for the facilitation of nutrient uptake, waste elimination, and gas exchange between mother and fetus [1]. Also, placenta is synthesizing various hormones, regulates the transport of maternal fuels to the fetus and facilitates maternal metabolic adaptations to different stages of pregnancy [2].

During a pregnancy complicated by diabetes mellitus (DM), the placenta undergoes a number of functional and structural pathological changes [3].

Maternal DM complicating pregnancy may result in fetal morbidities, including fetal abnormalities, stillbirth or fetal growth dysfunctions, which have been significantly associated with placental vascular abnormalities [3–6].

DM complicates pregnancy with combinations of growth promoting and growth restricting forces, which may alter the normal growth patterns of both the fetus and placenta [7].

In the early pregnancy, a series of predominantly

trophoblastic essential processes of proliferation and differentiation occur, leading to the development of intra-villous or extravillous structures [8].

Due to its role as maternal–fetal interface, the placenta is exposed to metabolic endocrine disorders in both the maternal and fetal circulations, on one hand the maternal diabetic environment is in contact with the syncytiotrophoblast, and on the other hand, by the fetal side, with the endothelium, because of the presence of receptors, transporters, ion channels or other molecules on both placental surfaces [2].

DM may affect the maternal intrauterine environment by altering uteroplacental vascular function either by the mediators of oxidative stress or by inflammation [7, 9].

None of the abnormalities found in the placenta of the diabetic women are in any way specific to the diabetic state, though when taken together the spectrum of abnormalities forms a very characteristic pattern [10].

Placental development is otherwise a complex process, mostly completed at the end of the second trimester, thus any insult of the maternal diabetic environment during the

essential period of placental differentiation in the first and second trimester will likely result in placental changes that may have subsequent effects on the fetus [2, 11–13].

Various metabolic or biological maternal dysfunctions may have implications in terms of the dimensions of the placental structure, among which is also the DM.

Placentomegaly is diagnosed by ultrasound (US), when the placental thickness is greater than 40 mm in the second trimester, or 60 mm in the third trimester of pregnancy [14].

Based on sonographic morphology, in DM the thickening of the placental structure is of homogeneous type, with a uniform echotexture [14, 15].

The aim of this study is to establish morphological, histological, immunohistochemical (IHC) and US correlations in the placenta of maternal type 1 diabetes mellitus (T1DM), or pregestational, but also in cases of gestational diabetes mellitus (GDM).

Patients, Materials and Methods

This multicenter study (see affiliations of the authors) has been conducted on a group of 53 selected cases diagnosed with T1DM or GDM. The above-mentioned group of pregnant women with DM were selected and studied over a two-year period (January 2016–December 2017).

The main characteristics of the patients in the study group are represented by the average age of 31 years old (20–42 years old) and DM associated with hypertension, preeclampsia, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, urinary infections, *Candida* vulvovaginitis, obesity or history of infertility. All the patients in the study group are Caucasian.

In our study group, there were 37 (69.81%) cases diagnosed with T1DM and 16 (30.19%) cases with GDM. Clinical data were abstracted from medical records.

Among the obstetric characteristics of the patients in the study are included 49 (92.45%) singleton pregnancies and four (7.54%) twin pregnancies, all of them having live births as fetal outcome (Table 1).

Fetal growth abnormalities have been identified in 25 (47.16%) cases, of which 17 (32.07%) cases of fetal macrosomia and eight (15.09%) cases of intrauterine growth restriction (IUGR).

US assessment of the cases in the study group has been performed both *via* two-dimensional (2D) conventional technique and three-dimensional (3D) or tomographic US imaging, as well as spectral, color or power Doppler (Voluson 730 Pro, Voluson E6, Voluson E8 Expert, US machines, equipped with RAB4-8L, RAB4-8D, and RIC5-9-D US probes, GE Healthcare and Samsung H60 ultrasound system equipped with CV1-8AD transducer, Samsung Medison).

Obstetrical US assessment included fetal morphology and biometry, as well as placental, umbilical cord and amniotic fluid evaluation, maternal–fetal Doppler profile and in the case of multiple pregnancies, the diagnosis of chorionicity and amnionicity (Table 2).

The macroscopic analysis of the specimens included the placental weight and the number of umbilical cord blood vessels, also looking for perivillous or subchorionic fibrin depositions, maternal floor infarction/massive basal plate fibrin deposition, placental infarction, subchorial thrombosis, intervillous thrombi or placental calcifications.

Table 1 – Clinical characteristics by diabetes type

DM associated conditions	n (%)		
	T1DM	GDM	T1DM + GDM
Maternal preexisting hypertension	7 (18.91)	2 (12.5)	9 (16.98)
Preeclampsia ^{1#}	9 (24.32)	3 (18.75)	12 (22.64)
Diabetic neuropathy	3 (5.66)	–	3 (5.66)
Diabetic retinopathy	5 (13.51)	–	5 (9.43)
Diabetic nephropathy	1 (2.7)	–	1 (1.88)
Urinary infections	16 (43.24)	6 (37.5)	22 (41.5)
<i>Candida</i> vulvovaginitis	19 (51.35)	7 (43.75)	26 (49.05)
Obesity	7 (18.91)	2 (12.5)	9 (16.98)
History of infertility	11 (29.72)	4 (25)	15 (28.3)
Singleton pregnancy	34 (91.89)	15 (93.75)	49 (92.45)
Twin pregnancy	3 (8.1)	1 (6.25)	4 (7.54)
Macrosomia	15 (40.54)	2 (12.5)	17 (32.07)
IUGR	7 ^{2#} (18.91)	1 ^{3#} (6.25)	8 (15.09)

DM associated conditions	Mean ± SD		
	T1DM	GDM	T1DM + GDM
Birth weight [g]	3735±995	3920±1045	3827.5±1020
Gestational age at birth [weeks]	37.4±1.9	38.2±1.3	37.8±1.6

DM: Diabetes mellitus; T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus; IUGR: Intrauterine growth restriction; n: No. of cases; ^{1#}: Blood pressure <140/90 mmHg at the first prenatal visit (1st trimester) – hypertension and proteinuria (proteins ≥0.3 g/24 h) after 20 gestational weeks; ^{2#}: Four cases of selective IUGR and three cases of non-selective IUGR in dichorionic–diamniotic twin pregnancy; ^{3#}: Selective IUGR; SD: Standard deviation.

Table 2 – Obstetrical US assessment

Fetal morphology	▪ Fetal head, CNS;
	▪ Fetal face;
Fetal biometry	▪ Thorax, cardio-vascular and respiratory systems;
	▪ Abdomen and pelvis, digestive system, kidneys, urinary tract and genital organs;
Placental assessment	▪ Spine and fetal skeleton.
	▪ BPD, OFD, HC, AC, FL, HL, TL, EFW (BPD-HC-AC-FL).
Umbilical cord	▪ location, thickness, echotexture, volume, immature appearance.
	▪ number of vessels, insertion, coiling, diameter.
Amniotic fluid	▪ amniotic fluid index.
Maternal–fetal Doppler profile	▪ UA, MCA, uterine artery.
Twins	▪ placental location, T sign, lambda sign, interfetal membrane, biometry or morphology discordance.

US: Ultrasound; CNS: Central nervous system; BPD: Biparietal diameter; OFD: Occipitofrontal diameter; HC: Head circumference; AC: Abdominal circumference; FL: Femoral length; HL: Humeral length; TL: Tibial length; EFW: Estimated fetal weight; UA: Umbilical artery; MCA: Middle cerebral artery.

The placenta specimens resulting after birth were fixed in 10% buffered neutral formalin, processed by paraffin embedding and Hematoxylin–Eosin (HE), Masson's trichrome and Periodic Acid–Schiff (PAS)–Hematoxylin stainings. We sectioned the biological material using the HM350 rotary microtome, equipped with a water bath section transfer system (STS, microM). In order to perform the IHC study, the histological sections were applied to poly-L-Lysine slides and kept to thermostat at 37°C for 24 hours. IHC technique has been used for immunostaining: dewaxing, dehydration in alcohols with decreasing concentrations: 100%, 96%, 90%, and 70%,

rehydration in distilled water, antigenic exposure in Citrate solution with pH 6, in seven cycles \times 3 minutes, inhibition of endogenous peroxidase with 2% hydrogen peroxide for 30 minutes, washing in distilled water and phosphate-buffered saline (PBS) 15 minutes, uncovering of specific antigenic sites in 3% milk powder solution for 30 minutes.

Subsequently, the primary antibody was applied (Table 3) and left overnight (18 hours) at 4°C. The next

day, the slides were left at ambient temperature for 30 minutes, washed in PBS, the second antibody (mouse/rabbit IgG antibody, VC002-025, R&D Systems, VisUCyte HRP Polymer, one hour), developed with 3,3'-Diaminobenzidine (DAB) (Dako), Hematoxylin nuclei labeling, dehydrated in 70%, 90%, 96% and 100% alcohol, clarified in xylene for 30–45 minutes and mounted with balsam of Canada.

Table 3 – Immunohistochemical panel of antibodies

Antibody	Manufacturer	Clone	Antigenic exposure	Secondary antibody	Dilution	Labeling
Anti-CD34	Dako	QBEnd 10	Citrate	Monoclonal mouse anti-human CD34 class II	1:50	Endothelial cells of small blood vessels
Anti-TGF β	Santa Cruz Biotechnology	sc-398	Citrate	rabbit polyclonal IgG	1:50	Induction factor for cellular transformation
Anti-PCNA	Dako	PC10	Citrate	monoclonal mouse anti-PCNA	1:100	Cells in division in the late G1 or S phase
Anti-CD68	Dako	KP1	Citrate	monoclonal mouse anti-human CD68	1:100	Macrophages

CD: Cluster of differentiation; TGF β : Transforming growth factor beta; PCNA: Proliferating cell nuclear antigen; IgG: Immunoglobulin G.

The research meets the conditions of the ethical guidelines and legal requirements, and was approved by each Ethical Committee of the Universities of Medicine and Pharmacy (see authors' affiliations). Informed consent was obtained from every patient included in the study.

Results

The prevalence of maternal preexisting hypertension and preeclampsia in the study group have been 16.98% and 22.64%, higher in patients with T1DM (18.91% and 24.32%, respectively) compared to those with GDM (12.5% and 18.75%, respectively). Diabetic neuropathy, retinopathy and nephropathy have been diagnosed only in T1DM cases, with poor glycemic control both pre-gestational and during pregnancy. Urinary infections and *Candida* vulvovaginitis had an increased incidence (>40%) in both T1DM patients and those with GDM, due to the favorable diabetic environment. Macrosomia had an incidence of 32.07%, with a higher frequency (>40%) in fetuses from mothers with T1DM. On the other side, IUGR had an incidence of 15.09%, higher in cases with T1DM, taking into account also multiple pregnancies.

The US examination of placental characteristics in our series revealed increased placental thickness even from the second trimester, especially in GDM cases (56.25%), with significant increases in placental thickness in the first half of the third trimester both in T1DM and GDM cases, with more than half of the cases (58.49%) presenting placentomegaly at the end of the third trimester (Table 4) (Figure 1).

Immature appearance of placenta has been also observed since the second trimester, predominantly in GDM cases (37.5%), such that during the third trimester, this finding increased progressively, exceeding 40% towards the end of the last trimester, both for patients with T1DM and those with GDM (Figure 2).

Placental location by US has been predominantly at the uterine fundus and anterior or anterolateral (56.6%), followed by posterior or posterolateral localization (41.5%). One case of placenta praevia was in the GDM group

(Figure 3A). Also, the homogeneous placental echotexture was found in most cases (81.13%) (Table 4) (Figure 3B).

Table 4 – US assessment of the placenta in diabetic pregnancy

Placental US findings	T1DM (n=37)	GDM (n=16)	T1DM + GDM (n=53)
Location, n (%)			
	Uterine fundus	10 (27.02)	5 (31.25) (28.3)
	Anterior (\pm lateral)	12 (32.43)	3 (18.75) (28.3)
	Posterior (\pm lateral)	15 (40.54)	7 (43.75) (41.5)
Thickness*, n (%)			
	Praevia	–	1 (6.25) (1.88)
	24–28 gw >40 mm	8 (21.62)	9 (56.25) (32.07)
	29–31 gw >45 mm	10 (27.02)	12 (75) (41.5)
Echotexture, n (%)			
	32–34 gw >50 mm	13 (35.13)	12 (75) (47.16)
	35–39 gw >55 mm	16 (43.24)	15 (93.75) (58.49)
	Homogeneous	29 (78.37)	14 (87.5) (81.13)
Immature appearance, n (%)			
	Inhomogeneous	8 (21.62)	2 (12.5) (18.86)
	G0 at >26 gw	8 (21.62)	6 (37.5) (26.41)
	G1 at >32 gw	11 (29.72)	7 (43.75) (33.96)
	G2 at >35 gw	15 (40.54)	12 (75) (50.94)

US: Ultrasound; T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus; n: No. of cases; gw: Gestational weeks; *: Measured at the widest diameter in the sagittal plane; G: Grannum score.

Macroscopic analysis of the placentas and umbilical cords, has shown that the placentas of women with diabetes are heavier, compared to standard medians in unaffected pregnancies, in our study with an average of 640 g and a mean weight increased in GDM cases compared to those with T1DM (658 g vs. 621 g). In our series, we identified two bivascular umbilical cord singleton pregnancies in the T1DM group, this being an isolated anomaly (Table 5).

Table 5 – Macroscopic analysis of the placenta and umbilical cord

Gross analysis of the placenta and umbilical cord specimens		T1DM (n=37)	GDM (n=16)	T1DM + GDM (n=53)
Placental weight [g]*		621.5 ±138.9	658.3 ±141.5	639.9 ±140.2
Basal plate fibrin deposition, n (%)		11 (29.72)	3 (18.75)	14 (26.41)
Subchorionic fibrin depositions, n (%)		9 (24.32)	2 (12.5)	11 (20.75)
Placental calcifications, n (%)		6 (16.21)	–	6 (11.32)
Placental infarction, n (%)		2 (5.4)	1 (6.25)	3 (5.66)
Intervillous thrombi, n (%)		1 (2.7)	–	1 (1.88)
Trivascular umbilical cord, n (%)		35 (94.59)	16 (100)	51 (96.22)
SUA, n (%)		2 (5.4)	–	2 (3.77)
Umbilical cord diameter [cm]		1.4± 0.3	1.5± 0.2	1.45± 0.25
Twist direction, n (%)	Left	26 (70.27)	12 (75)	38 (71.69)
	Right	11 (29.72)	4 (25)	15 (28.3)
	Normal	29 (78.37)	12 (75)	41 (77.35)
Twisting, n (%)	Excessive	3 (8.1)	1 (6.25)	4 (7.54)
	Lack	5 (13.51)	3 (18.75)	8 (15.09)
	Central/pericentral	33 (89.18)	14 (87.5)	47 (88.67)
Cord insertion, n (%)	Marginal	3 (8.1)	2 (12.5)	5 (9.43)
	Velamentous	1 (2.7)	–	1 (1.88)
Meconium staining		–	1 (6.25)	1 (1.88)

T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus; *: Without attached umbilical cord or membranes; n: No. of cases; SUA: Single umbilical artery.

According to these findings, we noticed an increased diameter in the umbilical cord compared to standard medians, but also an incidence of 7.54% of excessive twisting, or a lack of twisting in 15.09% of cases (Figure 4, A and B).

Abnormal cord insertion has been found in six cases, including two multiple pregnancies, in 9.43% of the cases marginal and in 1.88% velamentous insertion, these findings might be correlated with IUGR (Figure 5, A and B).

Gross analysis of maternal and fetal surfaces of the placentas revealed in both T1DM and GDM groups a significant overall occurrence of the basal plate fibrin deposition (26.41%) (Figure 6A) and also subchorionic fibrin depositions, with an incidence of 20.75% (T1DM + GDM) (Figure 6B).

Other macroscopic findings that have also been observed in our series are the placental calcifications (16.21% – T1DM only), placental infarction (5.66%) (Figure 7A), and intervillous thrombi (1.88%) (Table 4) (Figure 7B).

The most common pathological finding in our series was fibrinoid necrosis (Figures 8 and 9), in a 75.47% overall ratio, with no statistically significant difference between T1DM (72.97%) and GDM (81.25%). Intervillous fibrosis is another placental observation in our study, in

a 71.69% overall ratio, more common in the T1DM group (72.97%), compared to GDM (68.75%). Focal hyaline degeneration was recorded in 69.81% of the analyzed placentas, with a higher susceptibility in T1DM cases compared to GDM (Figures 10 and 11) (Table 6).

Table 6 – Placental pathology in T1DM and GDM

Placental histopathological findings	T1DM (n=37)	GDM (n=16)	T1DM + GDM (n=53)
Fibrinoid necrosis, n (%)	27 (72.97)	13 (81.25)	40 (75.47)
Intervillous fibrosis, n (%)	27 (72.97)	11 (68.75)	38 (71.69)
Focal hyaline degeneration, n (%)	28 (75.67)	9 (56.25)	37 (69.81)
Villous immaturity, n (%)	19 (51.35)	13 (81.25)	32 (60.37)
Villous maturity, n (%)	18 (48.64)	11 (68.75)	29 (54.71)
Chorangioma, n (%)	21 (56.75)	6 (37.5)	27 (50.94)
Nucleated fetal red blood cells, n (%)	13 (35.13)	9 (56.25)	22 (41.5)
Calcifications, n (%)	12 (32.43)	2 (12.5)	14 (26.41)
Lymphohistiocytic villitis, n (%)	6 (16.21)	4 (25)	10 (18.86)
Placental infarctions, n (%)	3 (8.1)	3 (18.75)	6 (11.32)
Syncytial nodes, n (%)	3 (8.1)	–	3 (5.66)
Villous hypermaturity, n (%)	3 (8.1)	–	3 (5.66)
Decidual vasculopathy, n (%)	1 (2.7)	1 (6.25)	2 (3.77)
Phantom cells, n (%)	2 (5.4)	–	2 (3.77)

T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus; n: No. of cases.

Concerning the placental maturation, villous immaturity was found in 81.25% of GDM cases, whereas in T1DM placentas only occurred in 51.35%, with an overall placental maturation deficiency of 60.37%.

On the other hand, villous maturity has been recorded in 54.71% of the placentas in the study.

Chorangioma has been found in over 50% of cases overall, with a significant difference in T1DM cases (56.75%) compared to GDM (37.5%) (Figures 12 and 13).

The presence of nucleated fetal red blood cells was found predominantly in GDM cases (>50%), compared with T1DM (35.13%) (Table 6).

Placental calcifications (Figures 8 and 9) were observed predominantly in T1DM cases (32.43%), while in GDMs only 12.5%, with a global ratio of 26.41% on our series.

Another finding noticed especially in the GDM group (25%) is lymphohistiocytic villitis, compared to T1DM (16.21%).

Placental infarctions were detected in over 11% of cases (Table 6).

Syncytial nodes and villous hypermaturity (both 8.1%) and phantom cells (5.4%) were found only in T1DM cases. We also found decidual vasculopathy in 3.77% of the studied placentas.

Peripheral villous cells are positive for immunostaining with the anti-transforming growth factor beta (TGFβ) antibody (Figure 14, A and B), multiple cells being in the division (Figure 15), and macrophages are also present in the periphery of placental villi (Figure 16).



Figure 1 – US at 34(+1) gestational weeks in a GDM pregnancy demonstrating a 55.8 mm thick placenta – placentomegaly. US: Ultrasound; GDM: Gestational diabetes mellitus.

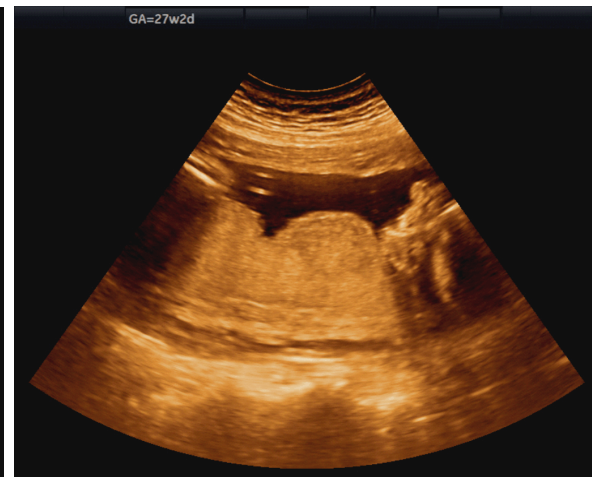


Figure 2 – US at 27(+2) gestational weeks in a T1DM pregnancy demonstrating thick and immature appearance of placenta (G0). US: Ultrasound; T1DM: Type 1 diabetes mellitus (pregestational diabetes).

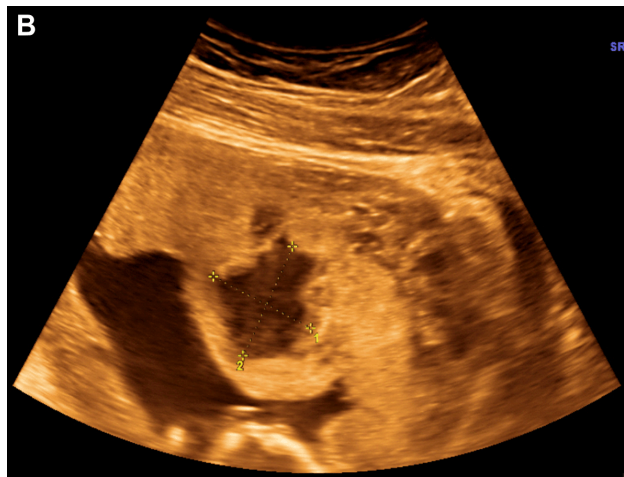
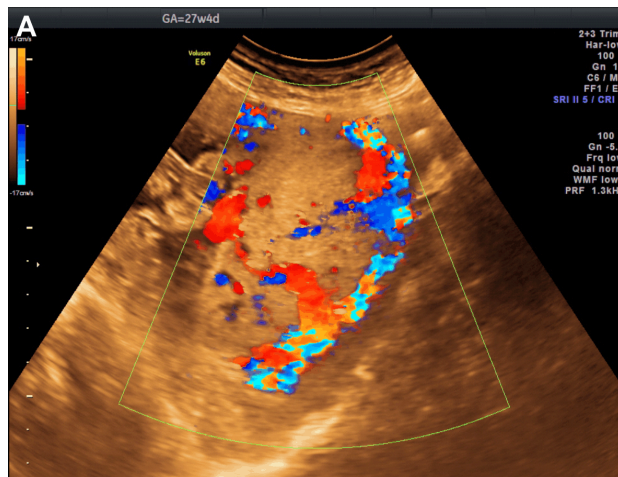


Figure 3 – (A) Second trimester US in a GDM pregnancy demonstrating placenta praevia with suggestive 2D and color Doppler characteristics, including increased vascularity and lacunae; (B) 2D US image demonstrating inhomogeneous appearance due to placental chorangioma in a T1DM pregnancy. US: Ultrasound; 2D: Two-dimensional; GDM: Gestational diabetes mellitus; T1DM: Type 1 diabetes mellitus (pregestational diabetes).

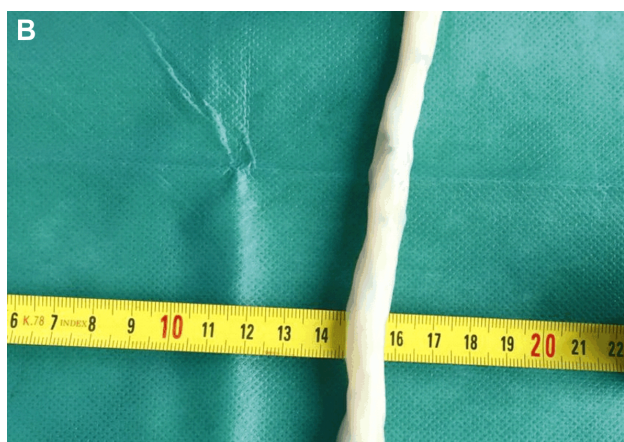


Figure 4 – (A) Excessive cord twisting in a T1DM pregnancy. Note the umbilical cord diameter of 1.6 cm; (B) Lack of cord twisting and thick umbilical cord in a GDM pregnancy. T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus.

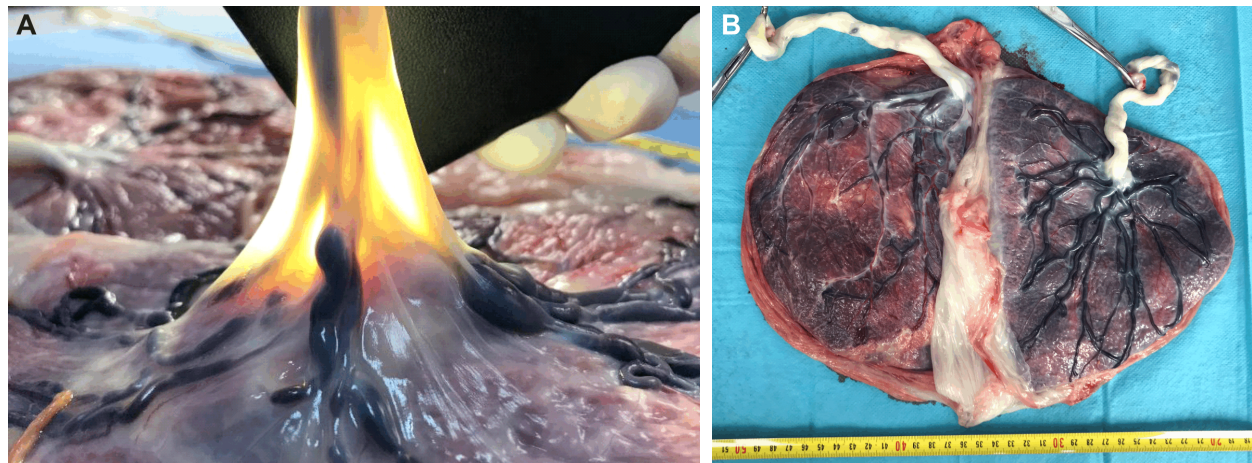


Figure 5 – (A) Gross analysis of the placenta and umbilical cord in a T1DM dichorionic–diamniotic twin pregnancy demonstrating velamentous cord insertion, with the fetal vessels running through the membranes, unprotected by Wharton's jelly in the emerging segment of the placental disk; (B) Macroscopic analysis of the placentas and umbilical cords in a GDM dichorionic–diamniotic twin pregnancy demonstrating marginal and eccentric cord insertions. T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus.

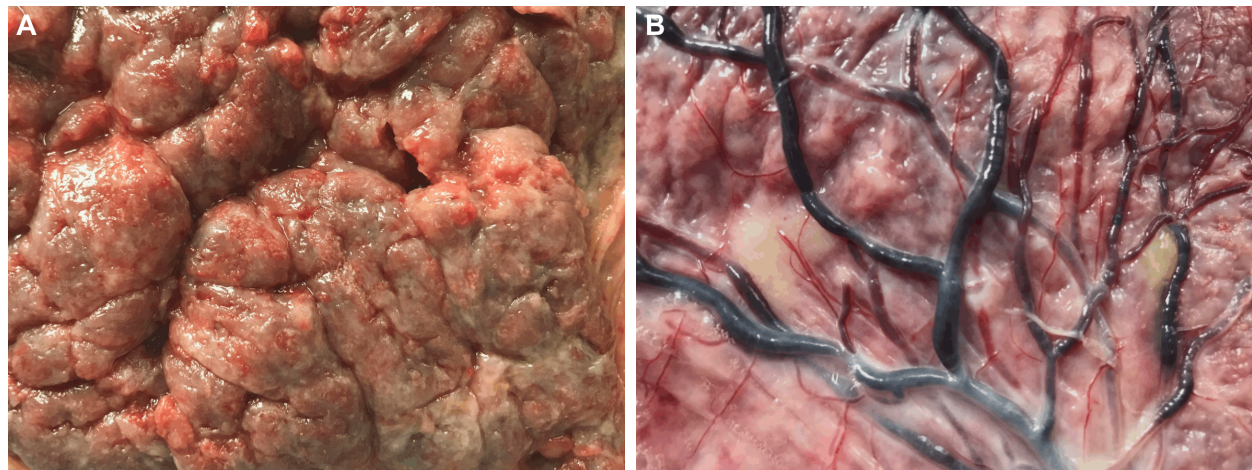


Figure 6 – (A) Maternal surface of the placenta from a T1DM pregnancy demonstrating basal plate fibrin deposition, with a greyish-yellow, gyriiform appearance; (B) Placenta from a GDM pregnancy demonstrating subchorionic fibrin deposition, which is apparent as a laminated white plaque at the fetal surface. T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus.

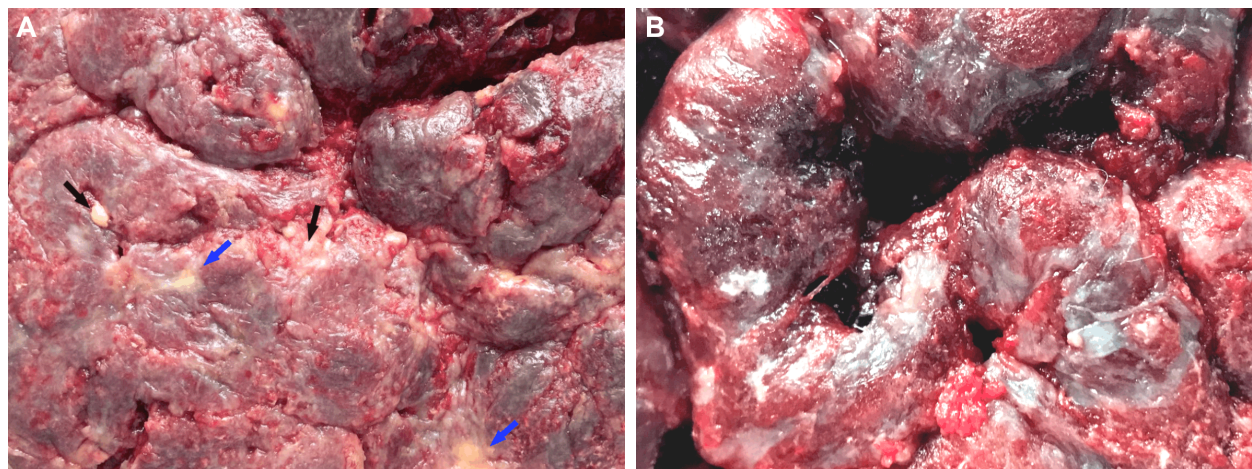


Figure 7 – (A) Maternal surface of the placenta from a T1DM pregnancy demonstrating placental infarction with a roughly triangular shape and a white-yellowish color (blue arrows). Note also the small and scattered flecks demonstrating placental calcifications (black arrows); (B) Maternal surface of the placenta from a T1DM pregnancy demonstrating an approximately oval intervillous thrombus with a soft, dark red appearance. T1DM: Type 1 diabetes mellitus (pregestational diabetes).

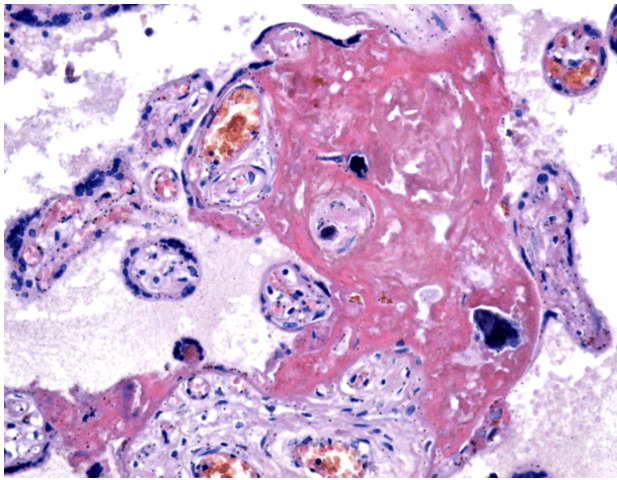


Figure 8 – Calcium depositions (blue areas) and perivillous fibrinoid (pink areas). Placental calcifications were observed predominantly in T1DM cases (HE staining, 200×). T1DM: Type 1 diabetes mellitus (pregestational diabetes).

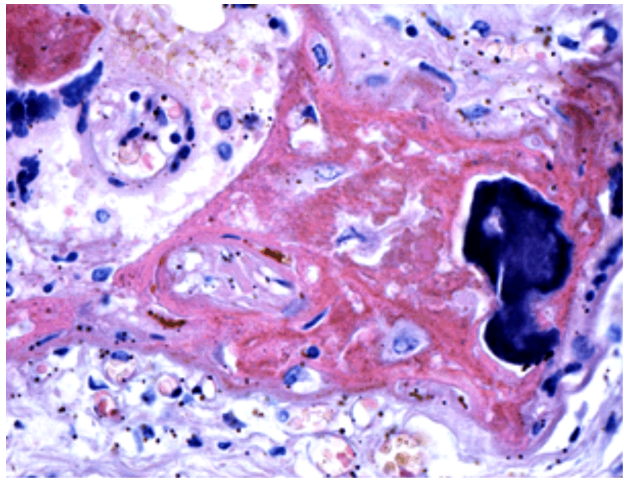


Figure 9 – Calcium depositions (blue areas) and perivillous fibrinoid (pink areas) (HE staining, 400×).

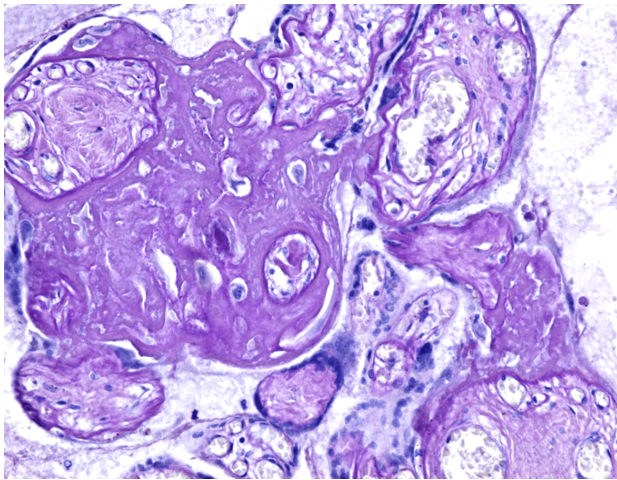


Figure 10 – Perivillous amyloid depositions (PAS-Hematoxylin staining, 400×).

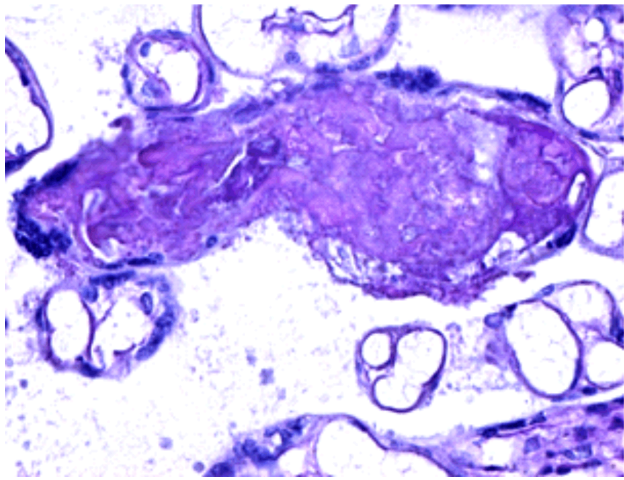


Figure 11 – Intravillous amyloid depositions (PAS-Hematoxylin staining, 400×).

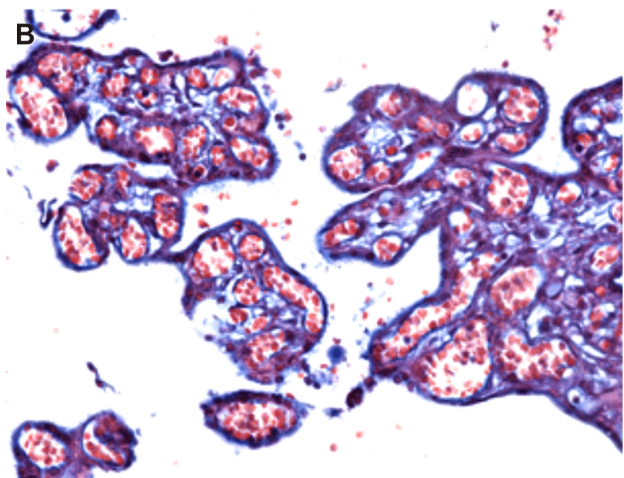
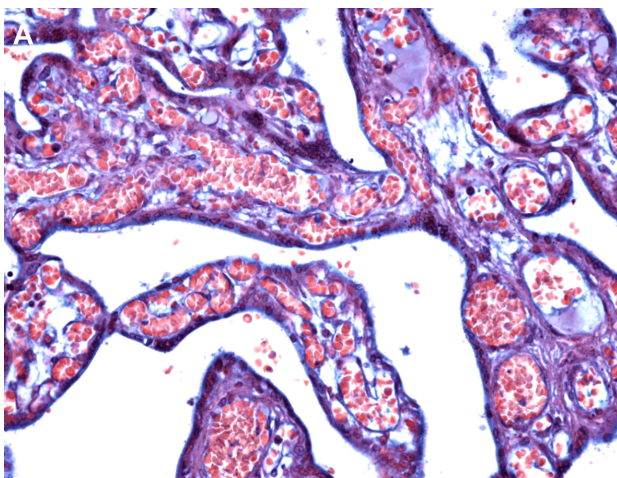


Figure 12 – (A and B) Chorangiosis – an increased number of intravillous capillary vessels (>10/villi). Chorangiosis has been found in over 50% of cases overall, with a significant difference in T1DM cases (56.75%) compared to GDM (37.5%) (Masson's trichrome staining, 400×). T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus.

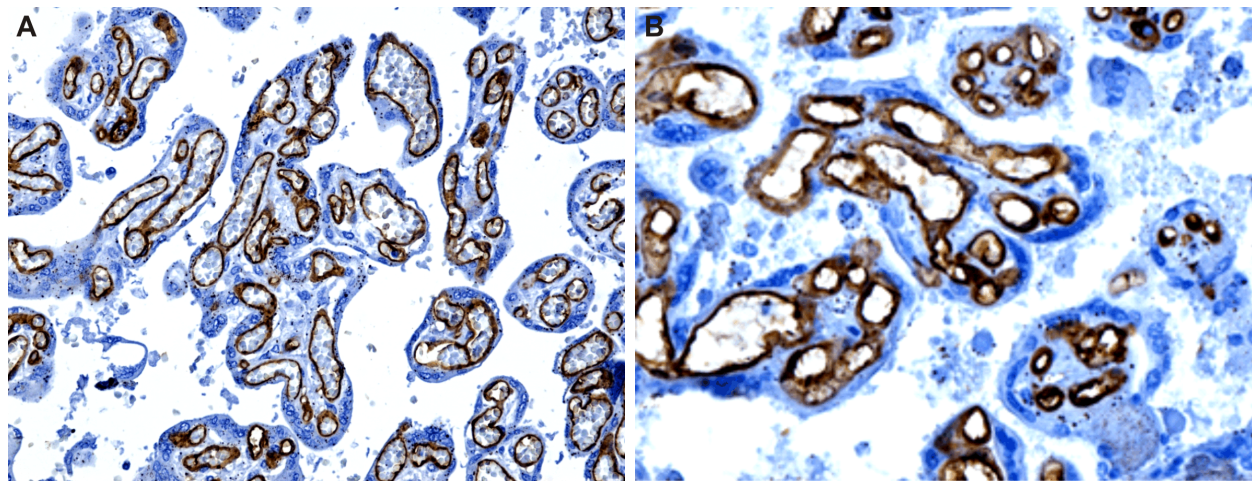


Figure 13 – (A and B) Chorangiosis – an increased number of intravillous capillary vessels (>10/villi). Immunostaining with anti-CD34 antibody: (A) 200 \times ; (B) 400 \times .

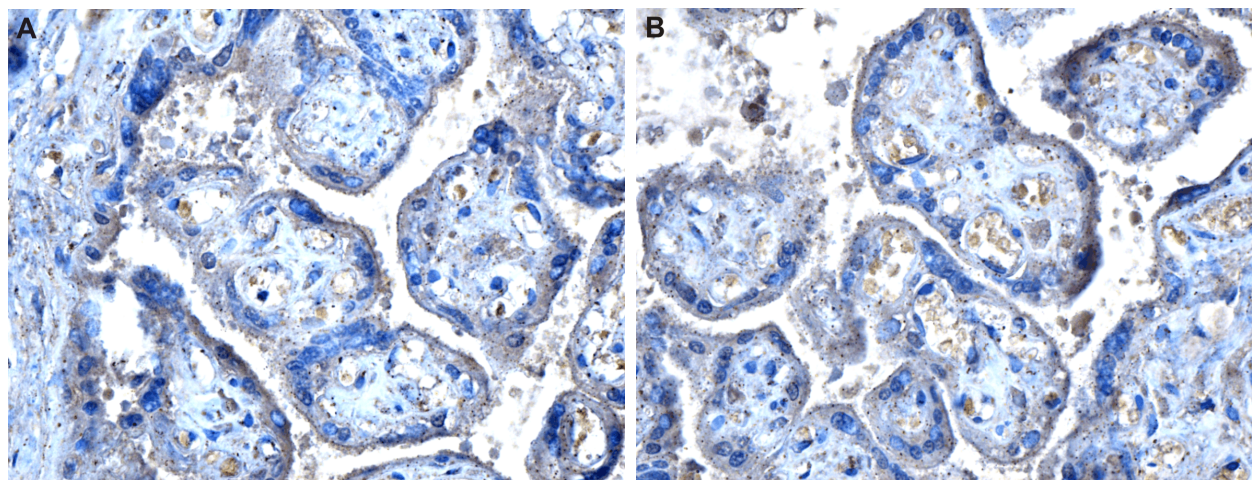


Figure 14 – (A and B) Positive reaction of tumor growth factor beta (TGF β) in peripheral villous cells (Immunostaining with anti-TGF β antibody, 400 \times).

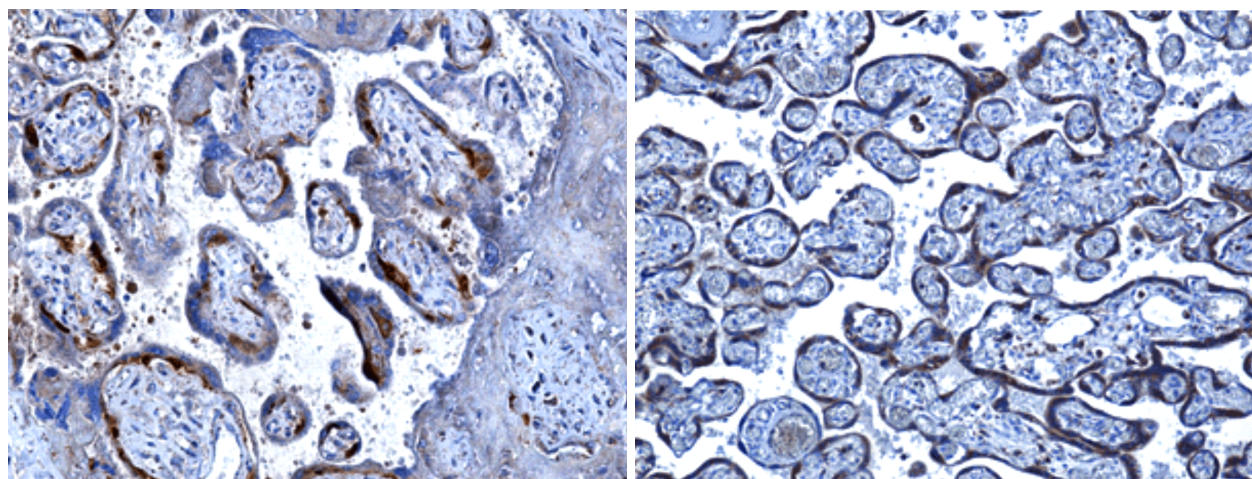


Figure 15 – Cells in the division (Immunostaining with anti-PCNA antibody, $\times 200$).

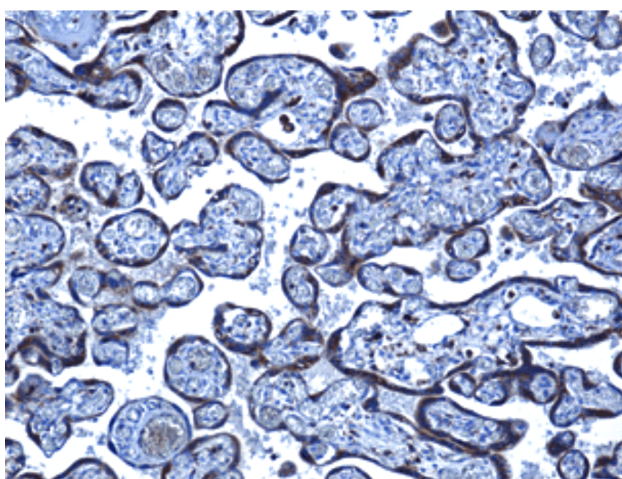


Figure 16 – Macrophages in the periphery of placental villi (Immunostaining with anti-CD68 antibody, $\times 100$).

✉ Discussions

It is widely accepted that pregestational DM has a significant impact on pregnancy outcome, compared with GDM [16].

During pregnancy, the trophoblast and placenta undergo structural changes, which might be influenced by various

mechanisms, including the oxidative stress occurring particularly in T1DM [2].

In diabetic pregnancies, the increased oxidative stress to the cell may create the basis for early damage in vessels formation of the placenta [7, 17–19].

In this study, we included cases with T1DM and GDM, analyzing the US and pathological changes occurring in

the placenta, comparing them with each other and also with the standard medians of some parameters, where appropriate. Different pathophysiology of these two diabetic conditions may differentially impact placentation, despite similar medical care and glycemic control [1].

Preeclampsia or maternal preexisting hypertension more frequently associated with T1DM than GDM, are associated with distinct placental pathological abnormalities which may predominate in T1DM relative to GDM and vice versa [20–23].

The association between diabetic pregnancy with preeclampsia, maternal hypertension or obesity, are widely accepted, therefore changes in placenta could reflect the influence of these abnormalities on placental function and development.

Daskalakis *et al.* [24] have found a 40% chorangiosis ratio in a series of 40 GDM placentas, while Huynh *et al.* found this modification in 38.1% out of 126 GDM pregnancies, including women with preeclampsia [3].

In our GDM group, we found a 37.5% chorangiosis ratio, including women with preeclampsia, which is comparable to the data presented above, but our research is limited by the lower number of cases.

Regarding the chorangiosis in T1DM, Evers *et al.* found this abnormality in 41.4% of the placentas ($n=58$) [25], while in our research we observed it in 56.75% of the cases ($n=53$).

This difference in a similar number of cases could be based on the more effective glycemic control of cases in the above authors' study, given that 33% of their patients used the insulin pump during pregnancy. In our series, we had four (10.81%) patients with insulin pump during pregnancy in the T1DM group.

Rudge *et al.* [26] found a 50.6% of placental calcifications ratio in the T1DM placentas and a 12.5% in GDM pregnancies. The same authors observed also a 85.5% of focal hyaline degeneration ratio in the T1DM placentas and a 75% in the GDM group.

On our data, we observed a 32.43% placental calcifications ratio in the T1DM placentas and a 12.5% in GDM pregnancies. In the same manner, we found 75.67% of focal hyaline degeneration ratio in the T1DM pregnancies and a 56.25% in the GDM placentas.

As we can see from these data, some are comparable and some are divergent, but these issues can also be attributed to glycemic control, which in our series was not obtained in all cases due to patient-dependent poor surveillance. We can also discuss other variables, such as medical or metabolic associated conditions.

The human placenta is supplied on one hand by maternal circulation, but it contains instead, in this interface unit a vascular complex that is entirely of fetal origin and continuous with the vasculature of the developing fetus [27].

There are so far many studies on angiogenesis and vascular remodeling in diabetic placenta, but there is not possible to discuss about specific abnormalities, but rather about a placental pathological diabetic pattern.

Although it has long been thought that chorangiosis may be a specific feature of diabetic placenta, recent studies provide data on multiple changes that may occur at this level, depending on effective glycemic control

and factors associated with this condition, the most common being preeclampsia and preexisting maternal hypertension.

However, the most studied and documented pathological changes in diabetic placenta appear to be chorangiosis [7] and placental villous immaturity [7, 28–34].

DM has been linked to accelerated microangiopathy and this may be associated with capillary hypertension and, as a consequence, with changes in capillary permeability [27].

The increased villous vascularity may be a response to the relative hypoxemia due to the immaturity of the villi, this being characterized by centrally placed villous capillaries, resulting in a greater distance for oxygen and nutrients to pass from maternal to fetal circulation [35].

Also, hypoxia, increased vascular endothelial growth factor, free oxygen radicals, cytokines, inflammatory mediators and, last but not least, hyperglycemia, are known to impair endothelial barrier function or to have angiogenic effects, affecting the structure and functional capacity of placenta and fetal vascular background [27, 36–40].

Desoye *et al.* [41] consider that the oxidative stress arising from increased placental mitochondrial activity and production of reactive oxygen species, nitric oxide, carbon monoxide, and peroxynitrite is a general underlying pattern of altered placental function and vascular reactivity [42], all of this being mechanisms that are likely to contribute to general fetal endothelial dysfunction in diabetes [41–43].

In our study, placentas from pregnancies complicated with GDM were observed to be heavier compared to those from T1DM. This finding is correlated with the US perception of placentomegaly, which in general terms refers to a placental thickness of more than 40 mm in the second trimester of pregnancy [14, 44, 45].

Regarding placentomegaly we observed on our series, a constantly increasing incidence in the 24–39 gestational weeks range from 32% to 58%.

Maternal DM is associated with enlargement of the capillary surface area, the villous stroma is slightly edematous [41], and there is a capillary proliferation and insertion of small newly formed vessels, penetrating the trophoblast [41, 46–48]. Therefore, this leads to hypervascularization and surface increase of the maternal–fetal exchange interface, facilitating oxygen diffusion at the placental level, as a compensatory mechanism for the impaired maternal–fetal transfer of diffusion-limited substances [41, 49, 50].

Placentomegaly occurs as a result of the increase in parenchymal tissue cellularity [41], and this is translated on US by placental thickness and echotexture, and is also correlated with fetal macrosomia, confirming the close correlation of placental weight and thickness with that of the offspring [51, 52].

In pregnancy associated with DM, the quality of maternal metabolic control, both preconceptional and during the gestation period, influences the rate of pregnancy complications, especially those related to inappropriate placentation, correlated with the trophoblastic invasion process in the decidua [2].

Both placentomegaly and fetal macrosomia tend to ameliorate through an effective glycemic control during pregnancy [41].

In our series, we noticed both pregnant women with T1DM and GDM who did not have constant glycemic control during gestation, even more, in the T1DM group there were cases with poor glycemic control in pre-conception. These aspects, correlated with associated pathology, especially preeclampsia, preexisting or pregnancy induced hypertension or obesity, could explain the significant number of placental pathological findings (14 in T1DM, 11 in GDM) in our study.

Boileau *et al.* consider that lower fetal insulin concentrations, which results from better maternal glucose control, may potentially limit the mitogenic effect of insulin in placental cells [53].

Moreover, Huynh *et al.* found that women with T1DM, despite insulin therapy, had less well-controlled glycemia compared to women with GDM, these differences probably reflecting the earlier onset and longer duration of diabetes in pregnancies of women with T1DM compared to those with GDM [3].

In this study, the involvement of macrophages in placental inflammatory processes is also observed, being present on the periphery of placental villi and having a role in the pro-inflammatory factors [interleukin (IL)-6] production. TGF β is increased, but not significantly. These factors probably indicate placental inflammation in women with gestational diabetes [54–56]. These data demonstrate the infiltration and accumulation of monocytes and macrophages in the placenta and it is important to note the occurrence of placental lesions. Moreover, excess macrophages in the placenta, contributes to the occurrence of a chronic inflammatory status [57]. In inflammation, T-cells are capable of secreting regulatory cytokines, such as TGF β to counterbalance the destructive potential of the inflammation [58]. One of the proteins synthesized at the end of the G1 and in the S phase of the cell cycle is proliferating cell nuclear antigen (PCNA) [59]. PCNA plays an important role in DNA synthesis, repair and regulation of the cell cycle and is commonly used as a proliferation marker [60]. In human placenta, the most intense expression of PCNA has been identified in the villi and invasive cytotrophoblasts. Furthermore, PCNA expression has been identified in the syncytiotrophoblast, stromal villous cells, decidual cells and decidual glandular cells [61]. PCNA expression was noticeably observed in the first trimester of pregnancy and was reduced to the term pregnancy [62]. In term placentas of mothers with gestational diabetes, an intense positive reaction is observed only in the periphery of the villi. Immunoreactivity to PCNA increases in placental cytotrophoblast in women with preeclampsia, indicating increased proliferative activity compared to normal [63–67].

These data and our data patterns come to highlight the importance of both maternal–fetal US assessment throughout the diabetic pregnancy, especially in the second and third trimesters of gestation, and on the other hand, to confirm the purpose of obtaining and maintaining an effective and constant glycemic control throughout pregnancy.

Conclusions

In T1DM and GDM, the maternal–fetal interface bear morphological changes related in particular to placental immaturity and chorangiosis. In diabetic placenta, it is not appropriate to discuss about specific changes, but rather about different associations that can establish a pathological diabetic pattern, influenced by the associated conditions, especially preeclampsia. The spectrum of morphological changes depends on glycemic control, metabolic control and associated condition management. US is essential both for the assessment of possible fetal complications or abnormal placentation and their management. US findings are related to the morphological changes of the placental structure. Preconceptional and first trimester glycemic control is the key element in diabetic pregnancy. Equally, euglycemia throughout pregnancy is a purpose whose accomplishment depends the maternal–fetal outcome.

Conflict of interests

The authors declare that they have no conflict of interests.

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Author contribution

Costin Berceanu and Adrian Victor Tetileanu equally contributed to this article.

References

- [1] Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta*, 2015, 36(2):101–114.
- [2] Desoye G, Kaufmann P. The human placenta in diabetes. In: Djelmiš J, Desoye G, Ivanišević M (eds). *Diabetology of pregnancy*. Series "Frontiers in Diabetes", vol. 17 (Editors: Porta M, Matschinsky FM), Karger, Basel, 2005, 94–105.
- [3] Huynh J, Yamada J, Beauharnais C, Wenger JB, Thadhani RI, Wexler D, Roberts DJ, Bentley-Lewis R. Type 1, type 2 and gestational diabetes mellitus differentially impact placental pathologic characteristics of uteroplacental malperfusion. *Placenta*, 2015, 36(10):1161–1166.
- [4] Kovo M, Schreiber L, Bar J. Placental vascular pathology as a mechanism of disease in pregnancy complications. *Thromb Res*, 2013, 131(Suppl 1):S18–S21.
- [5] Starikov R, Inman K, Chen K, Lopes V, Coviello E, Pinar H, He M. Comparison of placental findings in type 1 and type 2 diabetic pregnancies. *Placenta*, 2014, 35(12):1001–1006.
- [6] Lewis RM, Demmelmair H, Gaillard R, Godfrey KM, Hauguel-de Mouzon S, Huppertz B, Larque E, Saffery R, Symonds ME, Desoye G. The placental exposome: of fetal adiposity and postnatal body composition. *Ann Nutr Metab*, 2013, 63(3): 208–215.
- [7] Roberts DJ, Raspollini MR. Histopathology of placenta. In: Hod M, Jovanovic L, Di Renzo GC, de Leiva A, Langer O (eds). *Textbook of diabetes and pregnancy*. 2nd edition, Informa Healthcare, UK, 2008, 41–46.
- [8] Gheorman L, Pleșea IE, Gheorman V. Histopathological considerations of placenta in pregnancy with diabetes. *Rom J Morphol Embryol*, 2012, 53(2):329–336.
- [9] Roberts DJ, Oliva E. Clinical significance of placental examination in perinatal medicine. *J Matern Fetal Neonatal Med*, 2006, 19(5):255–264.
- [10] Fox H, Sebire NJ. *Pathology of the placenta*. 3rd edition, Saunders–Elsevier, 2007, 203–234.
- [11] Ringholm L, Juul A, Pedersen-Bjergaard U, Thorsteinsson B, Damm P, Mathiesen ER. Lower levels of placental growth

- hormone in early pregnancy in women with type 1 diabetes and large for gestational age infants. *Growth Horm IGF Res*, 2015, 25(6):312–315.
- [12] Segura MT, Demmelmaier H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC, Rueda R, Koletzko B, Campoy C. Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. *Placenta*, 2017, 57: 144–151.
 - [13] Desoye G, van Poppel M. The feto-placental dialogue and diabetes. *Best Pract Res Clin Obstet Gynaecol*, 2015, 29(1):15–23.
 - [14] Dashe JS, Hoffman BL. Ultrasound evaluation of the placenta, membranes and umbilical cord. In: Norton ME, Scoutt LM, Feldstein VA (eds). *Callen's ultrasonography in obstetrics and gynecology*. 6th edition, Elsevier, Philadelphia, 2017, 674–703.
 - [15] Porat S, Fitzgerald B, Wright E, Keating S, Kingdom JC. Placental hyperinflation and the risk of adverse perinatal outcome. *Ultrasound Obstet Gynecol*, 2013, 42(3):315–321.
 - [16] Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY (eds). *Williams obstetrics*. 23rd edition, McGraw-Hill Medical, New York, 2010, 1104–1125.
 - [17] Myatt L, Kossenjans W, Sahay R, Eis A, Brockman D. Oxidative stress causes vascular dysfunction in the placenta. *J Matern Fetal Med*, 2000, 9(1):79–82.
 - [18] Li H, Yin Q, Li N, Ouyang Z, Zhong M. Plasma markers of oxidative stress in patients with gestational diabetes mellitus in the second and third trimester. *Obstet Gynecol Int*, 2016, 2016:3865454.
 - [19] Gheorman V, Gheorman L, Ivănuș C, Pană RC, Gogăna AM, Pătrașcu A. Comparative study of placenta acute fetal distress and diabetes associated with pregnancy. *Rom J Morphol Embryol*, 2013, 54(3):505–511.
 - [20] Shams F, Rafique M, Samoo NA, Irfan R. Fibrinoid necrosis and hyalinization observed in normal, diabetic and hypertensive placentae. *J Coll Physicians Surg Pak*, 2012, 22(12):769–772.
 - [21] Gutaj P, Zawiejska A, Mantaj U, Wender-Ożegowska E. Determinants of preeclampsia in women with type 1 diabetes. *Acta Diabetol*, 2017, 54(12):1115–1121.
 - [22] Kelly CB, Hookham MB, Yu JY, Jenkins AJ, Nankervis AJ, Hanssen KF, Garg SK, Scardo JA, Hammad SM, Menard MK, Aston CE, Lyons TJ. Subclinical first trimester renal abnormalities are associated with preeclampsia in normoalbuminuric women with type 1 diabetes. *Diabetes Care*, 2018, 41(1): 120–127.
 - [23] Wotherspoon AC, Young IS, McCance DR, Holmes VA. Evaluation of biomarkers for the prediction of pre-eclampsia in women with type 1 diabetes mellitus: a systematic review. *J Diabetes Complications*, 2016, 30(5):958–966.
 - [24] Daskalakis G, Marinopoulos S, Kriesi V, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Placental pathology in women with gestational diabetes. *Acta Obstet Gynecol Scand*, 2008, 87(4):403–407.
 - [25] Evers IM, Nikkels PG, Sikkema JM, Visser GH. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. *Placenta*, 2003, 24(8–9):819–825.
 - [26] Rudge MVC, Lima CP, Damasceno DC, Sinzato YK, Napoli G, Rudge CVC, Gallego FQ, Calderon IMP. Histopathological placental lesions in mild gestational hyperglycemic and diabetic women. *Diabetol Metab Syndr*, 2011, 3(1):19.
 - [27] Leach L, Mayhew TM. Vasculogenesis and angiogenesis in the diabetic placenta. In: Djelmiš J, Desoye G, Ivanišević M (eds). *Diabetology of pregnancy*. Series "Frontiers in Diabetes", vol. 17 (Editors: Porta M, Matschinsky FM), Karger, Basel, 2005, 110–126.
 - [28] Stanek J. Chorangiogenesis of chorionic villi: what does it really mean? *Arch Pathol Lab Med*, 2016, 140(6):588–593.
 - [29] Ogino S, Redline RW. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and chorangiogenesis. *Hum Pathol*, 2000, 31(8):945–954.
 - [30] Soma H, Murai N, Tanaka K, Oguro T, Kokuba H, Fujita K, Mineo S. Angiogenesis in villous chorangiogenesis observed by ultrastructural studies. *Med Mol Morphol*, 2013, 46(2):77–85.
 - [31] Schwartz DA. Chorangiogenesis and its precursors: underdiagnosed placental indicators of chronic fetal hypoxia. *Obstet Gynecol Surv*, 2001, 56(9):523–525.
 - [32] Gupta R, Nigam S, Arora P, Khurana N, Batra S, Mandal AK. Clinico-pathological profile of 12 cases of chorangiogenesis. *Arch Gynecol Obstet*, 2006, 274(1):50–53.
 - [33] Rossi R, Scillitani G, Vimercati A, Fiore MG, Mastrodonato M, Resta L. Diabetic placenta: ultrastructure and morphometry of the term villi. *Anal Quant Cytopathol Histopathol*, 2012, 34(5):239–247.
 - [34] De La Ossa MM, Cabello-Inchausti B, Robinson MJ. Placental chorangiogenesis. *Arch Pathol Lab Med*, 2001, 125(9):1258.
 - [35] Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol*, 2000, 92(1): 35–43.
 - [36] Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. *Placenta*, 2004, 25(2–3):103–113.
 - [37] Zygmunt M. Placental circulation: clinical significance. *Early Pregnancy*, 2001, 5(1):72–73.
 - [38] Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta*, 2004, 25(2–3): 114–126.
 - [39] Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. *Placenta*, 2004, 25(2–3): 127–139.
 - [40] Helske S, Vuorela P, Carpén O, Hornig C, Weich H, Halmesmaki E. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. *Mol Hum Reprod*, 2001, 7(2):205–210.
 - [41] Desoye G, Shafir E, Hauguel-de Mouzon S. The placenta in diabetic pregnancy: placental transfer of nutrients. In: Hod M, Jovanovic L, Di Renzo GC, de Leiva A, Langer O (eds). *Textbook of diabetes and pregnancy*. 2nd edition, Informa Healthcare, UK, 2008, 47–56.
 - [42] Myatt L. Placental adaptive responses and fetal programming. *J Physiol*, 2006, 572(Pt 1):25–30.
 - [43] Farias M, San Martín R, Puebla C, Pearson JD, Casado JF, Pastor-Anglada M, Casanella P, Sobrevia L. Nitric oxide reduces adenosine transporter ENT1 gene (SLC29A1) promoter activity in human fetal endothelium from gestational diabetes. *J Cell Physiol*, 2006, 208(2):451–460.
 - [44] Wan Masliza WD, Bajuri MY, Hassan MR, Naim NM, Shuhaila A, Das S. Sonographically abnormal placenta: an association with an increased risk poor pregnancy outcomes. *Clin Ter*, 2017, 168(5):e283–e289.
 - [45] Perović M, Garalejić E, Gojnić M, Arsić B, Pantić I, Bojović DJ, Fazlagić A, Gardiner H. Sensitivity and specificity of ultrasonography as a screening tool for gestational diabetes mellitus. *J Matern Fetal Neonatal Med*, 2012, 25(8):1348–1353.
 - [46] Lash GE, Pitman H, Morgan HL, Innes BA, Agwu CN, Bulmer JN. Decidual macrophages: key regulators of vascular remodeling in human pregnancy. *J Leukoc Biol*, 2016, 100(2):315–325.
 - [47] He S, Gleason J, Fik-Rymarkiewicz E, DiFiglia A, Bharathan M, Morschauser A, Djuretic I, Xu Y, Krakovsky M, Jankovic V, Buensuceso C, Edinger J, Herzberg U, Hofgartner W, Hariri R. Human placenta-derived mesenchymal stromal-like cells enhance angiogenesis via T cell-dependent reprogramming of macrophage differentiation. *Stem Cells*, 2017, 35(6):1603–1613.
 - [48] Yu J, Zhou Y, Gui J, Li AZ, Su XL, Feng L. Assessment of the number and function of macrophages in the placenta of gestational diabetes mellitus patients. *J Huazhong Univ Sci Technolog Med Sci*, 2013, 33(5):725–729.
 - [49] Leach L, Lammiman MJ, Babawale MO, Hobson SA, Bromilou B, Lovat S, Simmonds MJ. Molecular organization of tight and adherens junctions in the human placental vascular tree. *Placenta*, 2000, 21(5–6):547–557.
 - [50] Babawale MO, Lovat S, Mayhew TM, Lammiman MJ, James DK, Leach L. Effects of gestational diabetes on junctional adhesion molecules in human term placental vasculature. *Diabetologia*, 2000, 43(9):1185–1196.
 - [51] Kc K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. *Ann Nutr Metab*, 2015, 66(Suppl 2):14–20.

- [52] Taricco E, Radaelli T, Nobile de Santis MS, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. *Placenta*, 2003, 24(4):343–347.
- [53] Boileau P, Caüzac M, Pereira MA, Girard J, Hauguel-De Mouzon S. Dissociation between insulin-mediated signaling pathways and biological effects in placental cells: role of protein kinase B and MAPK phosphorylation. *Endocrinology*, 2001, 142(9):3974–3979.
- [54] Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N; West of Scotland Coronary Prevention Study. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes*, 2002, 51(5):1596–1600.
- [55] Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA*, 2003, 289(14):1799–1804.
- [56] Atègbo JM, Grissa O, Yessoufou A, Hichami A, Dramane KL, Moutairou K, Miled A, Grissa A, Jerbi M, Tabka Z, Khan NA. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab*, 2006, 91(10):4137–4143.
- [57] Garcia Lloret MI, Winkler-Lowen B, Guilbert LJ. Monocytes adhering by LFA-1 to placental syncytiotrophoblasts induce local apoptosis *via* release of TNF- α . A model for haematogenous initiation of placental inflammations. *J Leukoc Biol*, 2008, 68(6):903–908.
- [58] Cavaillon JM. [Cytokines in inflammation]. *C R Seances Soc Biol Fil*, 1995, 189(4):531–544.
- [59] Takahashi T, Caviness VS Jr. PCNA-binding to DNA at the G1/S transition in proliferating cells of the developing cerebral wall. *J Neurocytol*, 1993, 22(12):1096–1102.
- [60] Start RD, Cross SS, Clelland C, Silcocks PB, Rogers K, Smith JH. Delay in fixation does not affect the immunoreactivity of proliferating cell nuclear antigen (PCNA). *J Pathol*, 1992, 168(2):197–199.
- [61] Korgun ET, Celik-Ozenci C, Acar N, Cayli S, Desoye G, Demir R. Location of cell cycle regulators cyclin B1, cyclin A, PCNA, Ki67 and cell cycle inhibitors p21, p27 and p57 in human first trimester placenta and deciduas. *Histochem Cell Biol*, 2006, 125(6):615–624.
- [62] Danihel L, Gomolcák P, Korbél M, Pruzinec J, Vojtassák J, Janík P, Babál P. Expression of proliferation and apoptotic markers in human placenta during pregnancy. *Acta Histochem*, 2002, 104(4):335–338.
- [63] Arnholdt H, Meisel F, Fandrey K, Löhrs U. Proliferation of villous trophoblast of the human placenta in normal and abnormal pregnancies. *Virchows Arch B Cell Pathol Incl Mol Pathol*, 1991, 60(6):365–372.
- [64] Hustin J, Foidart JM, Lambotte R. Cellular proliferation in villi of normal and pathological pregnancies. *Gynecol Obstet Invest*, 1984, 17(1):1–9.
- [65] Jones CJ, Fox H. An ultrastructural and ultrahistochemical study of the human placenta in maternal pre-eclampsia. *Placenta*, 1980, 1(1):61–76.
- [66] Lyall F, Myatt L. The role of the placenta in pre-eclampsia – a workshop report. *Placenta*, 2002, 23(Suppl A):S142–S145.
- [67] Redline RW, Patterson P. Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. *Hum Pathol*, 1995, 26(6):594–600.

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