Morphological and ultrasound findings in the placenta of diabetic pregnancy

Costin Berceanu1), Adrian Victor Teteanu2,3), Anca-Maria Ofițeru1,2,4), Elvira Brătîlei5), Claudia Mehedințu5), Nicoleta Loredana Voicu1,2), Florin Adrian Szasz6), Sabina Berceanu1), Simona Vladăreanu5), Dan-Bogdan Navolan7)

1) Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania
2) PhD Student, Doctoral School, University of Medicine and Pharmacy of Craiova, Romania
3) Department of Obstetrics and Gynecology, Emergency County Hospital, Târgu Jiu, Romania
4) Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania
5) Department of Obstetrics, Gynecology and Neonatology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
6) Department of Obstetrics–Gynecology, Faculty of Medicine and Pharmacy, University of Oradea, Romania
7) Department of Obstetrics, Gynecology and Neonatology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania

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 underwent 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 placetomegaly 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.

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

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>
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<th>Table 1 – Clinical characteristics by diabetes type</th>
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<tbody>
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<td>DM associated conditions</td>
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<tr>
<td>Maternal preexisting hypertension</td>
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<tr>
<td>Preeclampsia</td>
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<tr>
<td>Diabetic neuropathy</td>
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<td>Diabetic retinopathy</td>
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<tr>
<td>Diabetic nephropathy</td>
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<tr>
<td>Urinary infections</td>
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<tr>
<td>Candida vulvovaginitis</td>
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<tr>
<td>Obesity</td>
</tr>
<tr>
<td>History of infertility</td>
</tr>
<tr>
<td>Singleton pregnancy</td>
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<tr>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>Macrosomia</td>
</tr>
<tr>
<td>IUGR</td>
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<th>Table 2 – Obstetrical US assessment</th>
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<tbody>
<tr>
<td><strong>Fetal morphology</strong></td>
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<td><strong>Fetal imaging</strong></td>
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<td><strong>Placental assessment</strong></td>
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<td><strong>Umbilical cord</strong></td>
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<td><strong>Ammniotic fluid</strong></td>
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<td><strong>Maternal–fetal Doppler profile</strong></td>
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<tr>
<td><strong>Twins</strong></td>
</tr>
</tbody>
</table>

DM: Diabetes mellitus; T1DM: Type 1 diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus; IUGR: Intrauterine growth restriction; n: No. of cases; §: 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; €: Four cases of selective IUGR and three cases of non-selective IUGR in dichorionic–diamniotic twin pregnancy; §: Selective IUGR; SD: Standard deviation.

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%,
rehydrated in distilled water, antigenic exposure in Citrate solution with pH 6, in seven cycles × 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>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Clone</th>
<th>Antigenic exposure</th>
<th>Monoclonal mouse anti-human CD34 class II</th>
<th>Dilution</th>
<th>Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CD34</td>
<td>Dako</td>
<td>QBEnd 10</td>
<td>Citrate</td>
<td>rabbit polyclonal IgG</td>
<td>1:50</td>
<td>Endothelial cells of small blood vessels</td>
</tr>
<tr>
<td>Anti-TGFβ</td>
<td>Santa Cruz Biotechnology</td>
<td>sc-398</td>
<td>Citrate</td>
<td>monoclonal mouse anti-PCNA</td>
<td>1:100</td>
<td>Cells in division in the late G1 or S phase</td>
</tr>
<tr>
<td>Anti-CD68</td>
<td>Dako</td>
<td>PC10</td>
<td>Citrate</td>
<td>monoclonal mouse anti-human CD68</td>
<td>1:100</td>
<td>Macrophages</td>
</tr>
<tr>
<td>Anti-PCNA</td>
<td>Dako</td>
<td>KP1</td>
<td>Citrate</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

CD: Cluster of differentiation; TGFβ: 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- 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 placental echogenicity 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 placent al echostucture was found in most cases (81.13%) (Table 4) (Figure 3B).

<table>
<thead>
<tr>
<th>Location, n (%)</th>
<th>Placental US findings</th>
<th>T1DM (n=37)</th>
<th>GDM (n=16)</th>
<th>T1DM + GDM (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine fundus</td>
<td>10 (27.02)</td>
<td>5 (31.25)</td>
<td>15 (28.3)</td>
<td></td>
</tr>
<tr>
<td>Anterior (± lateral)</td>
<td>12 (32.43)</td>
<td>3 (18.75)</td>
<td>15 (28.3)</td>
<td></td>
</tr>
<tr>
<td>Posterior (± lateral)</td>
<td>15 (40.54)</td>
<td>7 (43.75)</td>
<td>22 (41.5)</td>
<td></td>
</tr>
<tr>
<td>Praevia</td>
<td>1 (6.25)</td>
<td>1 (6.25)</td>
<td>1 (1.86)</td>
<td></td>
</tr>
<tr>
<td>24–28 gw &gt;40 mm</td>
<td>8 (21.62)</td>
<td>9 (56.25)</td>
<td>17 (32.07)</td>
<td></td>
</tr>
<tr>
<td>29–31 gw &gt;45 mm</td>
<td>10 (27.02)</td>
<td>12 (75)</td>
<td>22 (41.5)</td>
<td></td>
</tr>
<tr>
<td>32–34 gw &gt;50 mm</td>
<td>13 (35.13)</td>
<td>12 (75)</td>
<td>25 (47.16)</td>
<td></td>
</tr>
<tr>
<td>35–39 gw &gt;55 mm</td>
<td>16 (43.24)</td>
<td>15 (93.75)</td>
<td>31 (58.49)</td>
<td></td>
</tr>
<tr>
<td>Homogeneous</td>
<td>29 (78.37)</td>
<td>14 (87.5)</td>
<td>43 (81.13)</td>
<td></td>
</tr>
<tr>
<td>Inhomogeneous</td>
<td>8 (21.62)</td>
<td>2 (12.5)</td>
<td>10 (18.86)</td>
<td></td>
</tr>
<tr>
<td>G0 at &gt;26 gw</td>
<td>6 (17.52)</td>
<td>6 (37.5)</td>
<td>14 (26.41)</td>
<td></td>
</tr>
<tr>
<td>G1 at &gt;32 gw</td>
<td>11 (29.72)</td>
<td>7 (43.75)</td>
<td>18 (33.96)</td>
<td></td>
</tr>
<tr>
<td>G2 at &gt;35 gw</td>
<td>15 (40.54)</td>
<td>12 (75)</td>
<td>27 (50.94)</td>
<td></td>
</tr>
</tbody>
</table>

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).
placental calcifications (Figures 8 and 9) were observed in our series are the placental calcifications (26.41%) (Figure 6B). Significant overall occurrence of the basal plate fibrin deposition (26.41%) (Figure 6A) and also subchorionic fibrin deposits, with a higher susceptibility in T1DM cases compared to GDM (Figures 10 and 11) (Table 6).

Gross analysis of the placenta and umbilical cord specimens

<table>
<thead>
<tr>
<th>Gross analysis of the placenta and umbilical cord specimens</th>
<th>T1DM (n=37)</th>
<th>GDM (n=16)</th>
<th>T1DM + GDM (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight [g]*</td>
<td>621.5 ±138.9</td>
<td>658.3 ±141.5</td>
<td>639.9 ±140.2</td>
</tr>
<tr>
<td>Basal plate fibrin deposition, n (%)</td>
<td>11/72.9%</td>
<td>3/18.75%</td>
<td>14/26.41%</td>
</tr>
<tr>
<td>Subchorionic fibrin deposition, n (%)</td>
<td>19/24.32%</td>
<td>2/12.5%</td>
<td>11/20.75%</td>
</tr>
<tr>
<td>Placental calcifications, n (%)</td>
<td>6/16.21%</td>
<td>1/6%</td>
<td>7/11.32%</td>
</tr>
<tr>
<td>Placental infarction, n (%)</td>
<td>7/5.4%</td>
<td>1/6.25%</td>
<td>8/1.88%</td>
</tr>
<tr>
<td>Intervillous thrombi, n (%)</td>
<td>1/27.37%</td>
<td>1/6%</td>
<td>2/1.88%</td>
</tr>
<tr>
<td>Trivascular umbilical cord, n (%)</td>
<td>35/94.59%</td>
<td>16/100%</td>
<td>51/96.22%</td>
</tr>
<tr>
<td>SUA, n (%)</td>
<td>2/2%</td>
<td>2/2%</td>
<td>2/2%</td>
</tr>
<tr>
<td>Umbilical cord diameter [cm]</td>
<td>1.4±±1.5±±1.45±</td>
<td>0.3±±0.2±±0.25</td>
<td>1.4±±1.5±±1.45±</td>
</tr>
</tbody>
</table>
| 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 deposits, with an incidence of 20.75% (T1DM + GDM) (Figure 6B).

Phantom cells, n (%)                                        | 1/2.7%      | 1/6.25%    | 2/3.77%           |

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 thromb (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).

Placental histopathological findings

<table>
<thead>
<tr>
<th>Placental histopathological findings</th>
<th>T1DM (n=37)</th>
<th>GDM (n=16)</th>
<th>T1DM + GDM (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinoid necrosis, n (%)</td>
<td>27/72.97%</td>
<td>13/81.25%</td>
<td>40/75.47%</td>
</tr>
<tr>
<td>Intervillous fibrosis, n (%)</td>
<td>27/72.97%</td>
<td>11/68.75%</td>
<td>38/71.69%</td>
</tr>
<tr>
<td>Focal hyaline degeneration, n (%)</td>
<td>28/76.67%</td>
<td>9/56.25%</td>
<td>37/69.81%</td>
</tr>
<tr>
<td>Villous immaturity, n (%)</td>
<td>19/51.35%</td>
<td>13/81.25%</td>
<td>32/60.37%</td>
</tr>
<tr>
<td>Villous maturity, n (%)</td>
<td>18/48.64%</td>
<td>11/68.75%</td>
<td>29/54.71%</td>
</tr>
<tr>
<td>Chorangiosis, n (%)</td>
<td>21/57.65%</td>
<td>6/37.5%</td>
<td>27/59.94%</td>
</tr>
<tr>
<td>Nucleated fetal red blood cells, n%</td>
<td>13/35.13%</td>
<td>9/56.25%</td>
<td>22/41.5%</td>
</tr>
<tr>
<td>Calcifications, n (%)</td>
<td>12/32.43%</td>
<td>2/12.5%</td>
<td>14/26.41%</td>
</tr>
<tr>
<td>Lymphohistiocytic villitis, n (%)</td>
<td>4/16.12%</td>
<td>4/25%</td>
<td>8/16.12%</td>
</tr>
<tr>
<td>Placental infarctions, n (%)</td>
<td>3/8.1%</td>
<td>3/18.75%</td>
<td>6/11.32%</td>
</tr>
<tr>
<td>Syncytial nodes, n (%)</td>
<td>3/8.1%</td>
<td>3/18.75%</td>
<td>3/11.32%</td>
</tr>
<tr>
<td>Villous hypermaturity, n (%)</td>
<td>3/8.1%</td>
<td>3/18.75%</td>
<td>3/11.32%</td>
</tr>
<tr>
<td>Decidual vascuropathy, n (%)</td>
<td>1/2.7%</td>
<td>1/6.25%</td>
<td>2/3.77%</td>
</tr>
<tr>
<td>Phantom cells, n (%)</td>
<td>1/2.7%</td>
<td>1/6.25%</td>
<td>2/3.77%</td>
</tr>
</tbody>
</table>

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.

Chorangiosis 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 (56.75%) compared to T1DM (37.5%) (Figures 12 and 13).

In our study, in 9.43% of the cases. We also found decidual vasculopathy in 3.77% of the 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).
Morphological and ultrasound findings in the placenta of diabetic pregnancy

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, gyriform 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).
Morphological and ultrasound findings in the placenta of diabetic pregnancy

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

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

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

Figure 11 – Intravillous amyloid deposits (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 I diabetes mellitus (pregestational diabetes); GDM: Gestational diabetes mellitus.
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 placenta-
ciation, 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 abnor-
malities which may predominate in T1DM relative to GDM
and vice versa [20–23].

The association between diabetic pregnancy with pre-
eclampsia, 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 calci-
fications 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 calcifi-
cations 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 increasement 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 trans-
lated 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 trofoblastic
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 preconception. 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. TGFβ 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 TGFβ 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 placentaion 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.

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Corresponding author
Claudia Mehedințu, Associate Professor, MD, PhD, Department of Obstetrics, Gynecology and Neonatology, “Carol Davila” University of Medicine and Pharmacy, “Nicolae Malaxa” Clinical Hospital, 12 Vergului Highroad, 022448 Bucharest, Romania; Phone +40722–312 976, e-mail: claudiamehedintu@yahoo.com

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