ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Placenta changes in pregnancy with gestational diabetes

ANTOINE EDU[1,2], CRISTINA TEODORESCU[1], CARMEN GABRIELA DOBJANSCHI[3,4], ZIŢA ZSUZSANA SOCOL[5], VALERIU TEODORESCU[6], ALEXANDRU MATEI[3,7], DINU FLORIN ALBU[2], GABRIELA RADULIAN[3,8]

[1] *Department of Obstetrics–Gynecology, "Nicolae Malaxa" Clinical Hospital, Bucharest, Romania*
[2] *Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[3] *Department of Diabetes, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[4] *Department of Diabetes, Nutrition and Metabolic Diseases, "Nicolae Malaxa" Clinical Hospital, Bucharest, Romania*
[5] *Department of Pathology, "Nicolae Malaxa" Clinical Hospital, Bucharest, Romania*
[6] *Department of Cardiology, "Prof. Dr. Theodor Burghele" Clinical Hospital, Bucharest, Romania*
[7] *Department of Obstetrics–Gynecology, "Polizu" Clinical Hospital, Bucharest, Romania*
[8] *"Prof. Dr. N. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania*

Abstract

Placental damage may be responsible for the fetal complications in pregnancies complicated by diabetes. We have analyzed the prevalence of gestational diabetes (GD) in a population of 109 pregnant women, the risk factors and the placental changes associated with gestational diabetes. Tests carried out were oral glucose tolerance test at 24–28 weeks of gestation, using the IADPSG (*International Association of Diabetes and Pregnancy Study Groups*) criteria for gestational diabetes, glycated hemoglobin, fasting insulin, total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, two-dimensional (2D) ultrasound and, also, there were analyzed macro and microscopic placental fragments from pregnant women with/without GD. It has been recorded the weight of placenta at birth and there were analyzed the possible pathological changes. The prevalence of GD was 11.9%. We have applied the direct logistic regression to determine the impact of some factors over the probability of association with gestational diabetes. The most powerful predictor was the placental maturity grade, the patients with decreased maturity grade having chances 52.6 times higher than those with an increased placental maturity grade to associate gestational diabetes. Sizes of placentas in patients with gestational diabetes mellitus were significantly increased than in patients without this diagnosis (p=0.012) from week 24–28. Pathological changes were discovered in six of the 13 placentas of women with gestational diabetes mellitus, independent of the level of glycated hemoglobin (p=0.72). The level of hyperglycemia is only partially associated with the presence of placental changes, which may be caused by other maternal factors.

Keywords: gestational diabetes, oral glucose tolerance test, placenta, microscopy, pathological changes.

Introduction

Gestational diabetes (GD) has classically been defined as any glucose intolerance identified during pregnancy [1, 2]. Generally, the pregnancy is defined as a diabetogenic event determined by the hormones produced by placenta (estrogens, progesterone, cortisol, human chorionic somatotropin, placental lactogenic hormone, prolactin), having as effect the insulin resistance, by decrease of the use of insulin-mediated glucose and the increase by 200–300% of secretion of insulin stimulated by glucose, to satisfy the metabolic needs of the fetus [3, 4].

Diagnosis of GD and the therapeutic measures, which intend to reach blood sugar targets, are important, as the values of glycemia increased over normal levels may have unfavorable consequences both for the mother and the fetus, by possible changes of fetal annexes [5, 6]. In general, the weight of placenta is higher in women with diabetes, and the precocious "aging" of placenta is more frequently found [7, 8]. Placental damage may be responsible for the high incidence of fetal complications in pregnancies complicated by diabetes.

The aim of the study was the analysis of prevalence of gestational diabetes in a population of pregnant women and the identification of the association (or not), of placental changes in pregnant women with GD.

Patients, Materials and Methods

One hundred nine pregnant women aged between 18–40 years and with pregnancy of 24–28 weeks of gestation ("Nicolae Malaxa" Clinical Hospital, Bucharest, Romania) were included in the study. There were excluded women with diabetes mellitus previously known and those with associate conditions under treatment.

For all pregnant women was carried out the oral glucose tolerance test (OGTT) with 75 g anhydride glucose being used the new criteria for diagnosing gestational diabetes [2]. Other laboratory parameters were followed: glycated hemoglobin (HbA1c), total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, fasting glucose levels. There were recorded obstetrical antecedents, prior body mass index (BMI), weight gain, perinatal events.

The diagnosis of gestational diabetes has been confirmed when any of the following plasma glucose values are exceeded: fasting plasma glucose ≥92 mg/dL (5.1 mmol/L); one hour ≥180 mg/dL (10 mmol/L); two hours ≥153 mg/dL (8.5 mmol/L), according to *International Association of Diabetes and Pregnancy Study Groups* (IADPSG) [8].

The two-dimensional (2D) ultrasound for fetal biometry and evaluation of fetal annexes (placenta and amniotic fluid) has been performed between the weeks 24–28 by

one single examiner. The placental maturity grade was appreciated and encompassed in the categories: grade 0, grade 1, grade 2 or grade 3.

At birth, it was recorded the fetal weight, the weight of placenta and there were sampled placental fragments from all pregnant women with GD included in the study, who were sent for pathological examinations. There were collected and examined 13 placentas of pregnant women with GD, and the control group comprised five placentas from women without GD.

The pathological examination was performed after prior fixation of placental fragments in formaldehyde, washing, dehydration and inclusion in paraffin block (hot paraffination), followed by cooling. The paraffin block has been cut at microtome, it has been spread on a blade, then it has been introduced in thermostat for deparaffination, followed by the staining procedure with Hematoxylin–Eosin (HE), Goldner–Szekely (GS) green light trichrome and Periodic Acid Schiff–Hematoxylin (PAS–H) stainings. The piece has been mounted on the blade with Canada balsam.

For the immunohistochemical study, there were performed 4 μm sections in the Microm HM350 rotary microtome equipped with a water system of the sections transfer (STS, microM). The histological sections were collected on poly-L-lysine covered blades and dried in a thermostat at 37^0C for 24 hours. After deparaffinization, hydration and washing of the sections, there was performed the antigen demasking, by boiling the samples in a sodium citrate, pH 6, for 21 minutes (seven cycles of three minutes) in a microwave oven. After blade cooling, they were washed in tap water and washed in distilled water for 15 minutes, followed by the blocking of endogenous peroxidase, incubating the blades in 3% hydrogen peroxide for 30 minutes at room temperature, followed by distilled water for 10 minutes and a washing in a 1% phosphate-buffered saline (PBS), for five minutes. After that, there followed the blocking of non-specific sites, using 2% skimmed milk for 30 minutes.

The prepared sections were incubated with primary antibodies, for 18 hours (over night), in a refrigerator at 4^0C. The next day, there was applied the secondary biotinylated antibody for 30 minutes at room temperature, followed by the washing in 1% PBS (three baths of five minutes), after that there was applied Streptavidin–HRP (Horseradish peroxidase) for 30 minutes, at room temperature, followed by washing the blades in 1% PBS 3×5 minutes. The signal was detected by using 3.3'-Diaminobenzidine (DAB) (Dako) and the reaction was stopped in 1% PBS. There followed the contrasting with Mayer's Hematoxylin, alcohol dehydration, xylene clarifying and blade assembling using a DPX environment (Fluka).

In our study, we used the following markers:

- anti-CD34 (clone EP373Y/ab81289, 1:100 dilution, Abcam) for highlighting placental micro-vascularization;
- anti-CD68 (clone KP1, 1:200 dilution, Dako) for highlighting the syncytiotrophoblast and the Hofbauer cells.

The pathological aspects were studied using Olympus CX31 microscope, with the ocular on ×4 magnification.

Statistical analysis

The values were expressed as mean ± SD (standard deviation) for normally distributed data. The comparisons between groups were carried out by using ANOVA for quantitative variables and χ^2 test for categorical variables. For the statistical analysis, we have used SPSS (version 18.2010) software.

Results

The anthropometrical, clinical and paraclinical characteristics of women included in the study are presented in Table 1.

Table 1 – ***Characteristics of studied pregnant women***

Group characteristics	GD+ (*n*=13)	GD– (*n*=96)	*p*
Age [years]	29.69 ± 4.83	28.08 ± 3.47	0.225
Pregnancy age [weeks]	26.31 ± 1.31	25.88 ± 1.07	0.242
Gestation number	2.23 ± 1.36	2.73 ± 1.53	0.253
Parity number	1.31 ± 0.48	1.38 ± 0.52	0.710
Weight gain [kg]	15.15 ± 9.32	7.78 ± 6.19	0.001
Initial BMI [kg/m^2]	21.85 ± 4.14	21.37 ± 3.14	0.978
Fasting blood glucose [mg/dL]	93.62 ± 28.62	82.78 ± 8.74	0.277
Insulin [microU/mL]	9.39 ± 6.91	10.47 ± 9.70	0.081
Glycated hemoglobin [%]	6.81 ± 0.48	5.38 ± 0.31	0.000
Cholesterol [mg/dL]	225.77 ± 38.22	226.26 ± 49.96	0.677
Triglycerides [mg/dL]	186.54 ± 63.39	177.33 ± 60.50	0.687
HDL-C [mg/dL]	81.00 ± 9.70	68.23 ± 14.29	0.002
LDL-C [mg/dL]	161.38 ± 39.83	143.14 ± 27.75	0.061
HOMA-IR	2.24 ± 1.92	2.21 ± 2.01	0.275
Degree of placental maturity – grade 2 [%]	61.5	64.6	0.830
Fetal weight [g]	1006.62 ± 144.81	910.54 ± 119.09	0.015
Placenta [cm]	3.35 ± 0.82	2.73 ± 0.53	0.012

GD: Gestational diabetes; Initial BMI [kg/m^2]: Pre-pregnancy body mass index; HbA1c [%]: Glycated hemoglobin; HDL-C [mg/dL]: High density lipoprotein cholesterol; LDL-C [mg/dL]: Low density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.

The global prevalence of gestational diabetes was of 11.9% (Figure 1) as compared to the results of other studies and has been preponderantly diagnosed in women ≥30 years old.

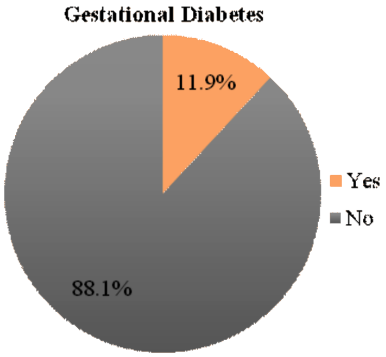

Figure 1 – ***Global prevalence of gestational diabetes, emphasized by OGTT (oral glucose tolerance test) during week 24–28 of pregnancy.***

We have applied the direct logistic regression in order to determine the impact of some factors over the likeliness of association with gestational diabetes. The model contains seven independent variables (actual BMI, initial BMI, placental maturity grade, size of placenta, level of HDL,

fetal weight, HOMA-IR). The prediction model containing these factors is statistically significant (p<0.0001), showing that the model may distinguish between patients that will associate gestational diabetes and those who will not associate this pathology. The most powerful predictor was the placental maturity grade, the patient with decreased maturity grade having 52.6 times higher than those with placental maturity grade to associate gestational diabetes, when the others factors in the model are kept constant. Another powerful predictor is the size of placenta, the patients with placentas with increased sizes having 10.9 times higher chances to associate gestational diabetes when the other factors in the model are kept constant.

The sizes of placentas measured on ultrasound between the weeks 24–28, in pregnant women with gestational diabetes mellitus, are significantly increased than in pregnant women without this diagnosis (p=0.012) (Figure 2).

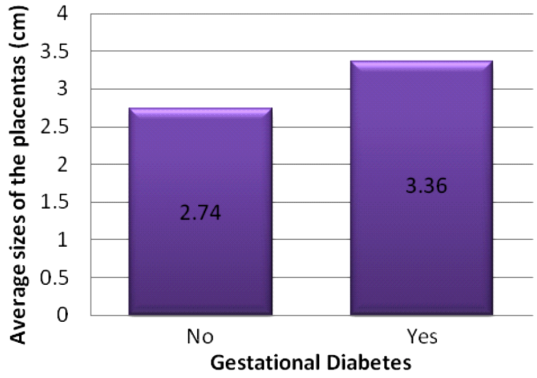

Figure 2 – ***Size of placentas of women with/without gestational diabetes. The size of placentas measured on ultrasound during the weeks 24–28, in pregnant women with gestational diabetes mellitus are significantly more increased than in pregnant women without this diagnosis.***

The placentas of six of the 13 pregnant women diagnosed with GD presented macro and microscopic changes (Table 2). Macroscopically, the most frequent pathological changes were the increase of size, volume and weight of the placenta, present only in three patients (Table 3) and fibrinoid degeneration, present in six of the 13 patients with GD (Figures 3 and 4). Microscopically, there were analyzed possible pathological changes, such as villous immaturity, villous edema, fibrinoid necrosis, syncytial nodes, fibrin thrombus.

Table 2 – ***Placental histopathological changes in pregnant women with/without GD***

Microscopic changes	No. of cases of GD– control (n=5)	No. of cases of GD+ with pathological changes (n=6)	No. of cases of GD+ (n=7)
Villous immaturity	absent	frequent	absent
Villous edema	absent	frequent	very rare
Fibrinoid necrosis	absent	absent	absent
Fibrin thrombus	present/rare	frequent	frequent

GD: Gestational diabetes.

Table 3 – ***Distribution of cases according to the placental weight in pregnant women with/without GD***

Placental weight	No. of cases of GD+ (n=13)	No. of cases of GD– (n=96)
Smaller	0	0
Normal	10	96
Larger	3	0

GD: Gestational diabetes.

The histopathological changes of placenta in patients with GD were quite varied and inconstant. The most frequent placental changes, present in 11 of 13 persons with GD, were represented by the immaturity of chorionic villus characterized by a high villus density, with the increase of villus lumenus and presence of syncytial nodules (Figure 5). Also, in five patients we identified the presence of the stromal edema in the terminal villus (Figure 6). In four patients with GD, there was observed the presence of collagen fiber densifications in the villus trunks thicker than 1mm, (Figure 7), and in one patient there were identified diffuse calcifications in the villus stroma (Figure 8).

Using the PAS–H staining, allowed us to observe the presence of certain deposits of fibrinoid extravillous and villous fibrinoid necrosis in eight patients with GD. Quite often, there was observed a moderate thickness of the basal membrane of the villous epithelium, by positive PAS deposits. Also, in the high villus trunks, there was observed the presence of high quantities of positive PAS material, microscopic aspects that show a deep alteration of the placenta structure and function (Figures 9 and 10).

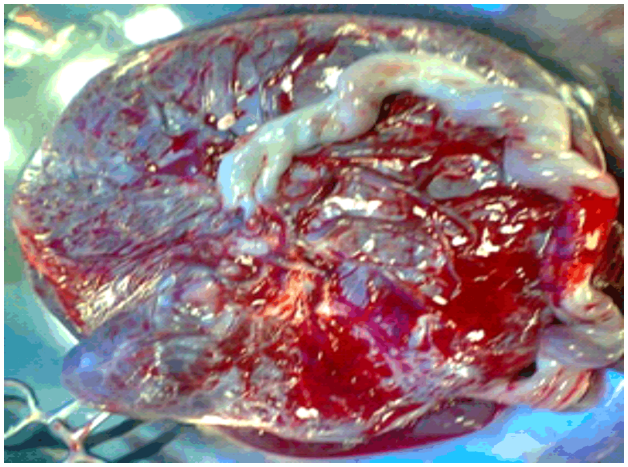

Figure 3 – ***Macroscopically normal placenta (control group).***

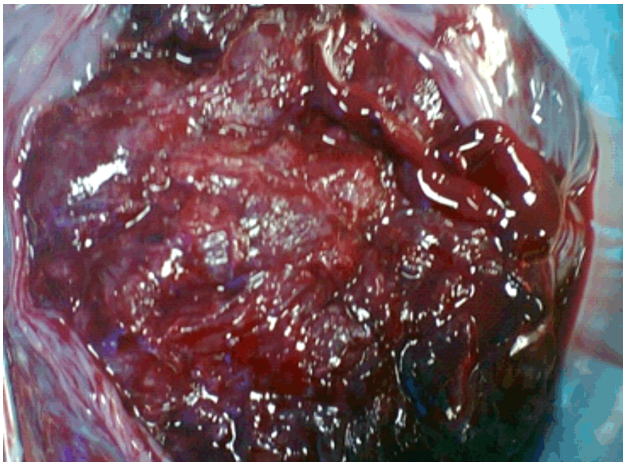

Figure 4 – ***Macroscopic image with the presence of fibrin thrombus more frequent in six of 13 pregnant women with GD.***

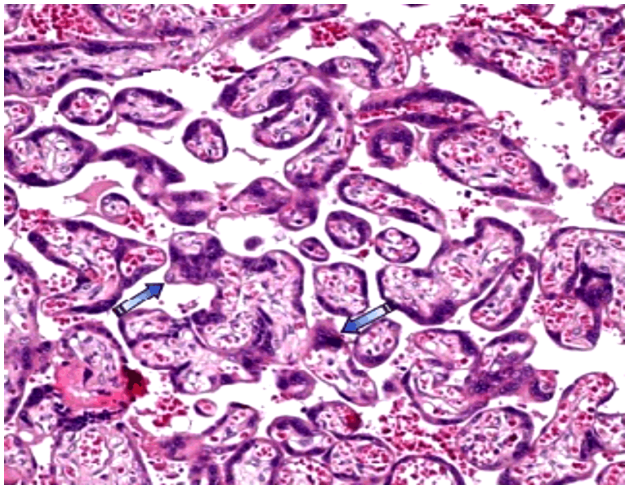

Figure 5 – ***Immaturity of chorial villosities with increased villous lumen and increased villous density; numerous syncytial nodes (blue arrows). HE staining, ×100.***

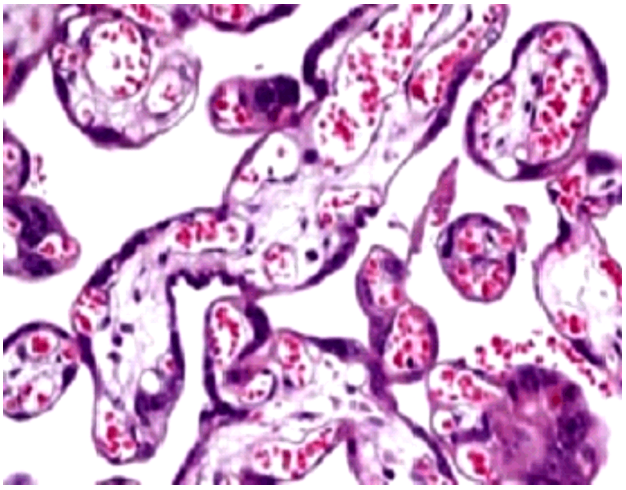

Figure 6 – ***Stromal edema at the level of terminal chorial villosities. HE staining, ×200.***

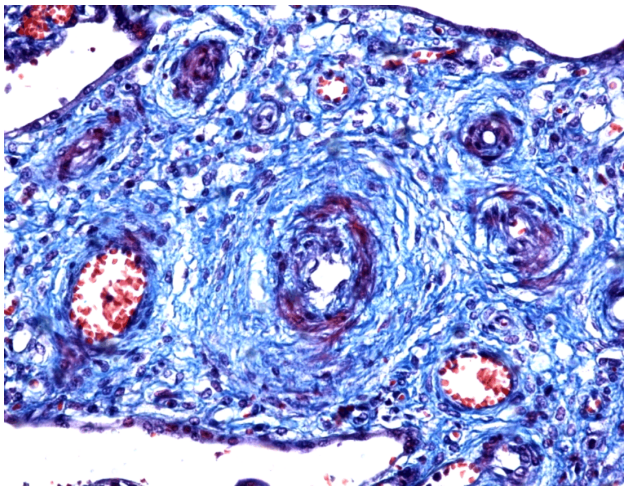

Figure 7 – ***Collagenous fibrosis in a villus trunk. GS trichrome staining, ×200.***

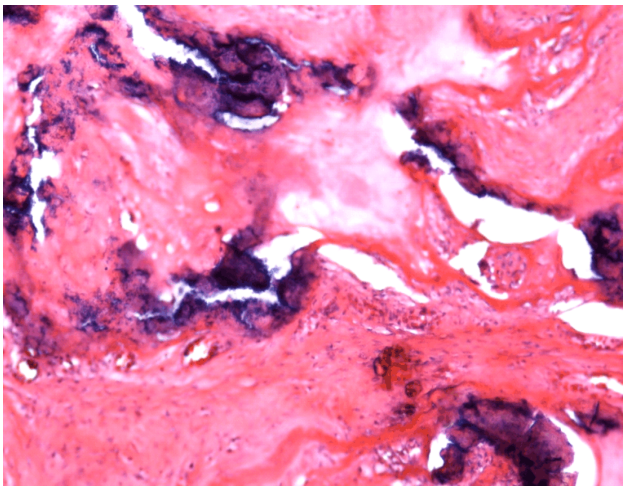

Figure 8 – ***Villous stroma with diffuse calcifications. HE staining, ×200.***

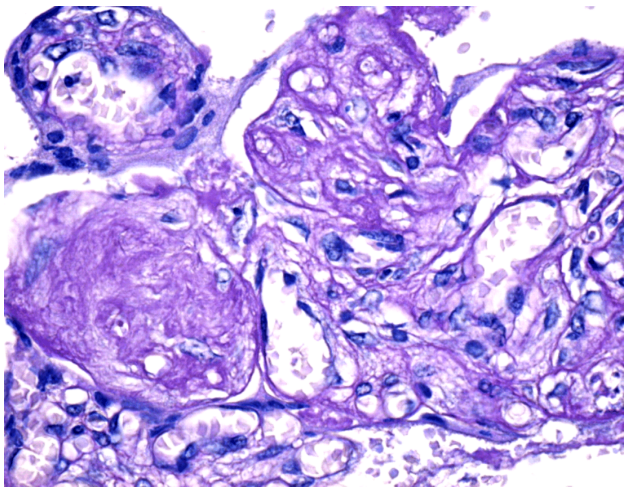

Figure 9 – ***Villous fibrinoid necrosis. PAS–H staining, ×400.***

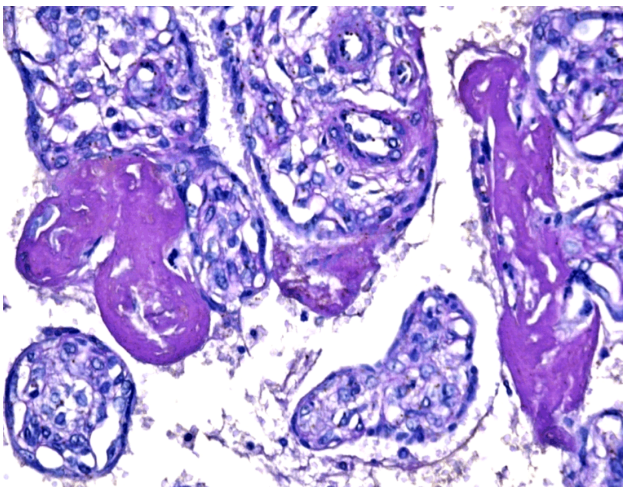

Figure 10 – ***Deposits of extravillous fibrinoid material. PAS–H staining, ×400.***

By using the anti-CD34 antibody, we could identify chorionic villus with intense phenomena of angiogenesis and a high microvascular density (Figure 11), and the anti-CD68 antibody showed a heterogeneous thickness of the syncytiotrophoblast (Figure 12).

The average value of HbA1c has not been statistically different in women with GD and histopathological changes of placenta (6.98 ± 0.39) as compared to those with GD and without such changes (6.67 ± 0.54) (p=0.72).

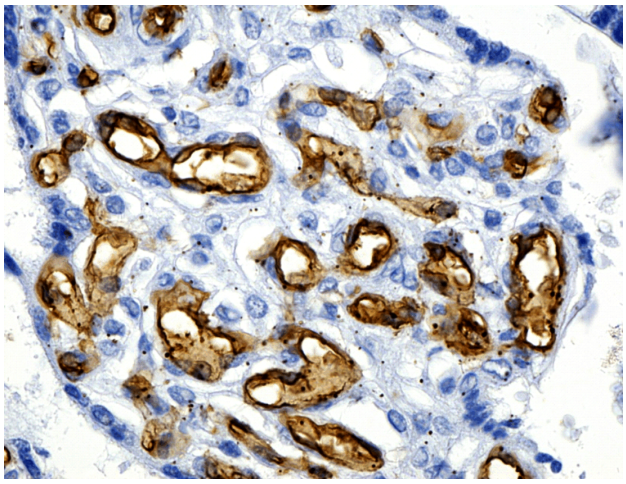

Figure 11 – ***Placental villus with an intense process of angiogenesis. Anti-CD34 antibody immunostaining, ×400.***

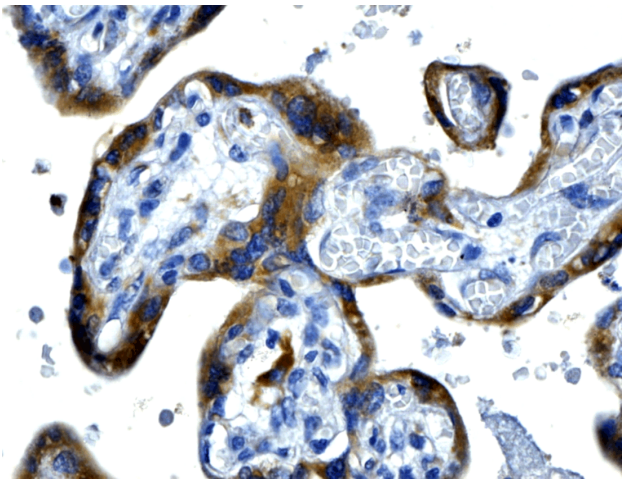

Figure 12 – ***Placental villus with variable thickness syncytiotrophoblast. Anti-CD68 antibody immunostaining, ×400.***

Discussion

The increase in size of placental mass has been noticed on ultrasound in the current study too, being directly proportional to the lack of maturation of chorial villosities by increase in diameters of villous lumen [7, 9, 10].

The increased sizes of the placenta emphasized on ultrasound even from week 24–28 may represent an independent prediction element and support the importance of screening for GD.

The determination of the placental maturity grade is necessary to understand if placenta is capable to supply to developing baby an enough quantity of nutritive substances. The terms "maturation" or "aging" of placenta are used to describe the normal changes of placenta which occur during pregnancy, estimated on ultrasound, exclusively by the presence of calcifications of placental lobes or not. Often enough, the causes of premature aging of placenta remain unknown. However, it is known the fact that in the development of these disturbances may contribute high blood pressure, diabetes and smoking [7, 9]. The obtained results support the link between diabetes and premature "aging" of placenta.

Different authors have described in women with diabetes a higher weight of placenta than the normal one and numerous structural changes that influence the normal growth and development of fetus [11]. The results obtained show that only three of 13 placentas of women with GD had a higher weight as compared to fetal weight.

Histopathological changes, described in the literature [10, 12, 13], villous immaturity (with increased villous diameter, hyper cellular stroma with reticular aspect and proliferation of Hofbauer cells, stromal fibroblast proliferation, excess of Langhans cells – cytotrophoblast, increase of thickness of basal membrane, unusual capillary densification in increased villosities, with hypercellular stroma), villous edema, syncytial nodes, fibrinoid necrosis, fibrin thrombus, have not been present in totality in each placenta [14], but they have not been completely absent in none of the six placentas from women with GD. There are evidences of no placental changes in some diabetic women or only minimal changes are found from control group. The pathogenesis of these abnormalities is still far from being full understood but probably is related not only to the degree of hyperglycemia [15, 16].

There has not been established a correlation between the presence of microscopic modifications of placenta in some women with GD and the level of HbA1c, as compared to those with GD and without such changes. These results suggest that the level of hyperglycemia is associated only partially with the placental changes, which may be due to other factors.

We consider that GD changes the placental structure and function, but many of the mechanisms that cause these changes are quite complex, due to the fact that the placenta has multiple functions and important adjusting possibilities to maternal nutrition and to its life conditions. Besides the fact that it regulates the composition and nutrient provision from mother to the fetus, the placenta is also the source of hormonal signs that affect the maternal and fetal metabolism [17, 18]. Still, most of the authors consider that hyperglycemia during GD leads to changes of the placental function, especially as far as the glucose transfer and/or use by the fetus are concerned [19]. The earlier the hyperglycemia changes appear, the higher the histopathological changes. Still, some studies showed that certain changes of the placenta continue to occur, despite the improvement of the maternal glycemia control, thus indicating the fact that hyperglycemia is not the only factor responsible for these changes [20, 21].

Conclusions

This study updates the data related to the increasing prevalence of GD and supports the importance of ultrasound monitoring, especially on placental morphology. The level of hyperglycemia is associated only partially with the occurrence of placental changes, which may be due to other maternal factors.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Metzger BE, Buchanan TA, Coustan DR, De Leiva A, Dunger DB, Hadden DR, Hod M, Kitzmiller JL, Kjos SL,

Oats JN, Pettitt DJ, Sacks DA, Zoupas C. Summary and recommendations of the Fifth International Workshop–Conference on Gestational Diabetes Mellitus. Diabetes Care, 2007, 30(Suppl 2):S251–S260.
[2] Kim C. Maternal outcomes and follow-up after gestational diabetes mellitus. Diabet Med, 2014, 31(3):292–301.
[3] Kirwan JP, Varastehpour A, Jing M, Presley L, Shao J, Friedman JE, Catalano PM. Reversal of insulin resistance postpartum is linked to enhanced skeletal muscle signaling. J Clin Endocrinol Metab, 2004, 89(9):4678–4684.
[4] American Diabetes Association. Standards of medical care in diabetes – 2013. Diabetes Care, 2013, 36(Suppl 1):S11–S66.
[5] Gibson KS, Waters TP, Catalano PM. Maternal weight gain in women who develop gestational diabetes mellitus. Obstet Gynecol, 2012, 119(3):560–565.
[6] Rudge MV, Calderon IM, Ramos MD, Abbade JF, Rugolo LM. Perinatal outcome of pregnancies complicated by diabetes and by maternal daily hyperglycemia not related to diabetes. A retrospective 10-year analysis. Gynecol Obstet Invest, 2000, 50(2):108–112.
[7] Dashe JS, McIntire DD, Twickler DM. Effect of maternal obesity on the ultrasound detection of anomalous fetuses. Obstet Gynecol, 2009, 113(5):1001–1007.
[8] Wendland EM, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, Duncan BB, Schmidt MI. Gestational diabetes and pregnancy outcomes – a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. BMC Pregnancy Childbirth, 2012, 12:23.
[9] Daskalakis G, Marinopoulos S, Krielesi 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.
[10] Rudge MV, Lima CP, Damasceno DC, Sinzato YK, Napoli G, Rudge CV, Gallego FQ, Calderon IM. Histopathological placental lesions in mild gestational hyperglycemic and diabetic women. Diabetol Metab Syndr, 2011, 3(1):19.
[11] Singer DB. The placenta in pregnancies complicated by diabetes mellitus. Perspect Pediatr Pathol, 1984, 8(3):199–212.
[12] 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.
[13] Verma R, Mishra S, Kaul JM. Cellular changes in the placenta in pregnancies complicated with diabetes. Int J Morphol, 2010, 28(1):259–264.
[14] Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic changes in gestational diabetes mellitus. Neuro Endocrinol Lett, 2015, 36(2):101–105.
[15] Barrett HL, Dekker Nitert M, McIntyre HD, Callaway LK. Normalizing metabolism in diabetic pregnancy: is it time to target lipids? Diabetes Care, 2014, 37(5):1484–1493.
[16] Roverso M, Brioschi M, Banfi C, Visentin S, Burlina S, Seraglia R, Traldi P, Lapolla A. A preliminary study on human placental tissue impaired by gestational diabetes: a comparison of gel-based *versus* gel-free proteomics approaches. Eur J Mass Spectrom (Chichester, Eng), 2016, 22(2):71–82.
[17] Hay WW Jr. The placenta. Not just a conduit for maternal fuels. Diabetes, 1991, 40(Suppl 2):44–50.
[18] Myatt L. Placental adaptive responses and fetal programming. J Physiol, 2006, 572(Pt 1):25–30.
[19] Osmond DT, Nolan CJ, King RG, Brennecke SP, Gude NM. Effects of gestational diabetes on human placental glucose uptake, transfer, and utilisation. Diabetologia, 2000, 43(5):576–582.
[20] Evers IM, de Valk HW, Mol BW, ter Braak EW, Visser GH. Macrosomia despite good glycaemic control in type I diabetic pregnancy; results of a nationwide study in The Netherlands. Diabetologia, 2002, 45(11):1484–1489.
[21] Pietryga M, Brazert J, Wender-Ozegowska E, Dubiel M, Gudmundsson S. Placental Doppler velocimetry in gestational diabetes mellitus. J Perinat Med, 2006, 34(2):108–110.

Corresponding author
Carmen Gabriela Dobjanschi, MD, PhD, Department of Diabetes, Nutrition and Metabolic Diseases, "Nicolae Malaxa" Clinical Hospital, 12 Vergului Road, Sector 2, 02441 Bucharest, Romania; Phone +40723–744 169, Fax +4021–255 35 48, e-mail: dgcarmen2004@yahoo.com

Received: December 16, 2015

Accepted: August 4, 2016