Placenta changes in pregnancy with gestational diabetes

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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 somatomammotropin, 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 glycaemia 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 paraffinification), 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 deparaffinization, followed by the staining procedure with Hema-toxylin–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°C 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 introduced in thermostat for deparaffinization, 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% 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°C. 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 EP173Y/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 χ² 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**

<table>
<thead>
<tr>
<th>Group characteristics</th>
<th>GD+ (n=13)</th>
<th>GD– (n=96)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>29.69 ± 4.83</td>
<td>28.08 ± 3.47</td>
<td>0.225</td>
</tr>
<tr>
<td>Pregnancy age [weeks]</td>
<td>26.31 ± 1.31</td>
<td>25.88 ± 1.07</td>
<td>0.242</td>
</tr>
<tr>
<td>Gestation number</td>
<td>2.23 ± 1.36</td>
<td>2.73 ± 1.53</td>
<td>0.253</td>
</tr>
<tr>
<td>Parity number</td>
<td>1.31 ± 0.48</td>
<td>1.38 ± 0.52</td>
<td>0.710</td>
</tr>
<tr>
<td>Weight gain [kg]</td>
<td>15.15 ± 9.32</td>
<td>7.78 ± 6.19</td>
<td>0.001</td>
</tr>
<tr>
<td>Initial BMI [kg/m²]</td>
<td>21.85 ± 4.14</td>
<td>21.37 ± 3.14</td>
<td>0.978</td>
</tr>
<tr>
<td>Fasting blood glucose [mg/dL]</td>
<td>93.62 ± 28.62</td>
<td>82.78 ± 8.74</td>
<td>0.277</td>
</tr>
<tr>
<td>Insulin [mU/mL]</td>
<td>9.39 ± 6.91</td>
<td>10.47 ± 9.70</td>
<td>0.081</td>
</tr>
<tr>
<td>Glycated hemoglobin [%]</td>
<td>6.81 ± 0.48</td>
<td>5.38 ± 0.31</td>
<td>0.000</td>
</tr>
<tr>
<td>Cholesterol [mg/dL]</td>
<td>225.77 ± 38.22</td>
<td>226.26 ± 49.96</td>
<td>0.677</td>
</tr>
<tr>
<td>Triglycerides [mg/dL]</td>
<td>186.54 ± 63.39</td>
<td>177.33 ± 60.50</td>
<td>0.687</td>
</tr>
<tr>
<td>HDL-C [mg/dL]</td>
<td>81.00 ± 9.70</td>
<td>68.23 ± 14.29</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL-C [mg/dL]</td>
<td>161.38 ± 39.83</td>
<td>143.14 ± 27.75</td>
<td>0.061</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.24 ± 1.92</td>
<td>2.21 ± 2.01</td>
<td>0.275</td>
</tr>
<tr>
<td>Degree of placental maturity – grade 2 [%]</td>
<td>61.5</td>
<td>64.6</td>
<td>0.830</td>
</tr>
<tr>
<td>Fetal weight [g]</td>
<td>1006.62 ± 144.81</td>
<td>910.54 ± 119.09</td>
<td>0.015</td>
</tr>
<tr>
<td>Placenta [cm]</td>
<td>3.35 ± 0.82</td>
<td>2.73 ± 0.53</td>
<td>0.012</td>
</tr>
</tbody>
</table>

GD: Gestational diabetes; Initial BMI [kg/m²]; 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.](image-url)
Placenta changes in pregnancy with gestational diabetes (GDM). 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 other 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).

The placenta changes of the 13 pregnant women diagnosed 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 villi 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).

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

GD: Gestational diabetes.

<table>
<thead>
<tr>
<th>Placental weight</th>
<th>No. of cases of GD+ (n=13)</th>
<th>No. of cases of GD– (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>Larger</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

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 villi 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).

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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).
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


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