

HISTOLOGICAL FETO-PLACENTAL INTERFACE CHANGES IN THE GESTATIONAL DIABETES MELLITUS

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Abstract

Introduction. Gestational diabetes mellitus (GDM) is a complication associated with pregnancy defined as any degree of glucose intolerance that occurs- or is first discovered during pregnancy, with normal values signaled before, and usually after pregnancy. **Material and Method.** This study analyzed the cellular differences that might contribute to the injuries of the feto-placental interface of insulin-controlled GDM patients. An optical microscopic analysis was performed on 26 full term placentas, of which 15 were of gestational diabetes mellitus and 11 control group. The histological observation centered upon the: *trophoblast, villous stroma and fetal capillary*. The fetal average weight was 3840 g for the studied group vs. 2760 g for the control group. **Results.** Through optical microscopy were identified varying degrees of lesions consisting of: *villous edema, proliferation and villous fibrosis of the capillaries, large number of syncytial knots, important fibrinoid necrosis, moderate fibrin thrombi, hyperplasia of the syncytiotrophoblast, chorangiomas, slightly thickened of the basement membrane of the feto-maternal interface*. **Conclusion.** The increased angiogenesis of feto-placental vessels of the terminal villi – considered to be the cause of the placental abnormalities; and the increased risk of complications for e.g. miscarriage, stillbirth, macrosomia, and congenital anomalies may be prevented by a good control over the glycemic levels.

Key words: Gestational diabetes mellitus, angiogenesis, feto-maternal interface abnormalities, trophoblast, villous stroma, fetal capillary.

Introduction

In diabetes there is an altered balance of substances such as nutrients, hormones, leptin and other cytokines which have been well documented as contributors to the potential regulation of placental function in GDM (1). The study is centered upon the analysis of histopathological modifications in placentas originating from pregnancies associated with gestational diabetes mellitus, given the lack of information on the histological pathognomonic lesions of the feto-maternal interface abnormalities.

Material and method

The samples used in this study were collected in between January 2009 – December 2011 from the 26 placentas collected immediately after delivery at „Bega” Clinic of Obstetrics, Gynecology and Neonatology, Timisoara. 15 were from patients with gestational diabetes mellitus and 11 from the control group. The placental pathological examination was performed by the same pathologist, who was blinded to the clinical data. Placental tissue samples were dissected from the central part of the placental bed.

Specimens

Two samples were collected from the middle-sections, from both – maternal and fetal – sides of the placenta. They were fixed in 4% buffered formalin, for 24 – 48 hours. Then 2 types of histological stains were used – Hematoxylin-Eosin and Masson's Trichrome. The histological examination of the slides was performed with an AmScope optical microscope in order to observe mainly the feto-maternal interface changes. The placental histopathological analyses were performed on images obtained by a digital camera. From each slide, 7 fields were randomly selected.

Hematoxylin-Eosin technique

- fixation in a 10% formalin solution
- dehydration in ethanol gradated series
- sedimentation in xylene
- sections
- paraffining
- deparaffining
- hydration and coloring with hematoxyline-eosine

Masson's Trichrome technique

- deparaffinize and hydrate to distilled water
- slides in 40 ml of Bouin's solution contained in a plastic coplin jar and microwave mix solution with beral pipet
- incubate slides in heated Bouin's solution for 15 minutes in a fume hood
- wash slides in tap water until sections are clear
- stain in working Weigert's hematoxylin 5 minutes
- wash slides thoroughly in tap water

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- 0.5% Hydrochloric acid alcohol for 5 seconds
- wash in running tap water for 30 seconds and rinse in two changes of distilled water
- stain in Trichrome solution for 15 minutes and wash slides in tap water
- rinse in 0.5% Acetic acid 10 seconds and in distilled water
- dehydrate through graded alcohols

- mount with resinous mounting media

Results

The differences between the histological changes of the placentas from the control group (of normal pregnancies) and those from the group of gestational diabetes pregnancies can be seen in Table 1.

Table 1 - Histological changes of the placentas.

Histopathological changes	Normal Pregnancy = 11		GD Pregnancy = 15	
	Number	%	Number	%
<i>Inflammatory</i>				
Villitis	0	0	0	0
Amnionitis	0	0	1	0,11
<i>Degenerative</i>				
Villous fibrosis and edema	0	0	1	0,11
Fibrinoid necrosis	1	0,11	3	27,27
Trophoblastic basic membrane thickening	0	0	4	36,36
Hyaline degeneration	2	18,18	3	27,27
Calcification	2	18,18	4	36,36
<i>Proliferative</i>				
Syncytial knots	1	0,11	3	27,27
Hofbauer hyperplasia	0	0	2	18,18
Peri- and intervillous fibrosis	0	0	4	36,36
Chorioangiosis	1	0,11	1	0,11
<i>Circulatory</i>				
Chorial and intimal edema	0	0	0	0
Interstitial hemorrhage	1	0,11	3	27,27
Nucleated red cells	0	0	4	36,36

After a general optical microscopic examination there were found varying degrees of lesions to the fetomaternal interface, trophoblast, villous stroma and fetal capillary such as:

- capillary proliferation and interstitial hemorrhage – Figure 1
- villous capillaries fibrosis – Figure 2
- syncytiotrophoblast hyperplasia – Figure 3
- trophoblastic basic membrane thickening of the fetomaternal interface – Figure 4
- peri- and intervillous fibrosis – Figure 5
- a large number of syncytial knots and Hofbauer cells hyperplasia – Figure 6

- fibrinoid necrosis – Figure 7
- nucleated red cells – Figure 8

We consider that the cause of fetal hypoxia in diabetic pregnancy remains still unknown, however the lesions at these levels are connected to the abnormalities in the structure of capillaries and the perivascular space may be an essential factor in the explanation of fetal hypoxia in diabetic pregnant women. The relationship between the vascular surface of the terminal villi to its total surface, evaluation of the endothelial structure, perivascular space and basal membrane of the trophoblast could show us the injuries constituted to the fetal-maternal interface, leaving this research area opened for further studies.

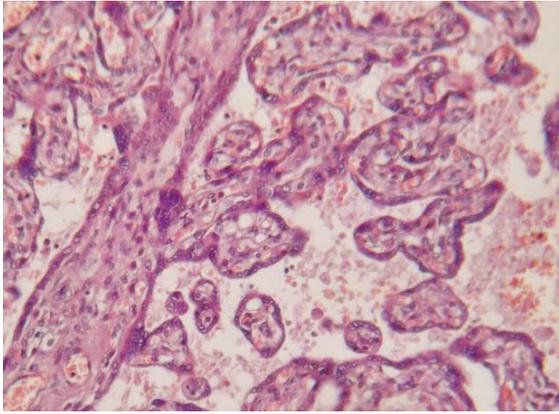


Figure 1 – capillary proliferation and interstitial hemorrhage (H.E. x 100).

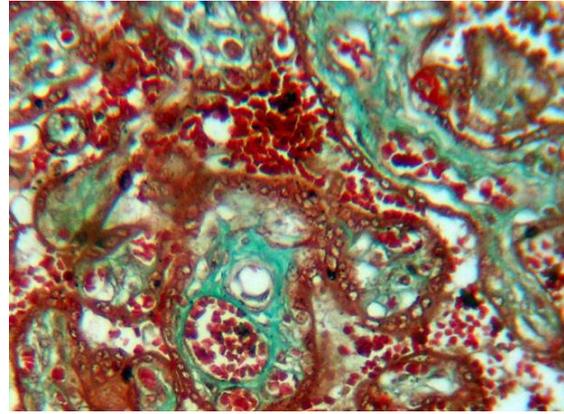


Figure 2 – villous capillary fibrosis (Masson's Trichrome x 200).

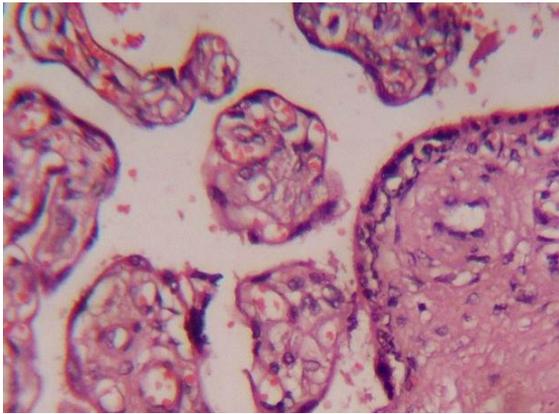


Figure 3 – syncytiotrophoblast hyperplasia (H.E. x 200).



Figure 4 – trophoblastic membrane thickening of the fetal-maternal interface (Masson's Trichrome x 400).

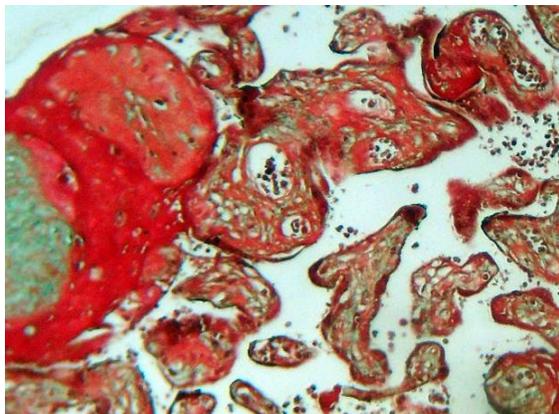


Figure 5 – peri- and intervillous fibrosis (H.E. x 100).

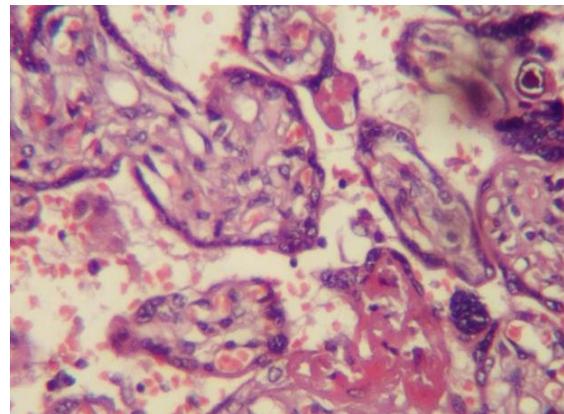


Figure 6 – a large number of syncytial knots and Hofbauer cells hyperplasia (H.E. x 200).

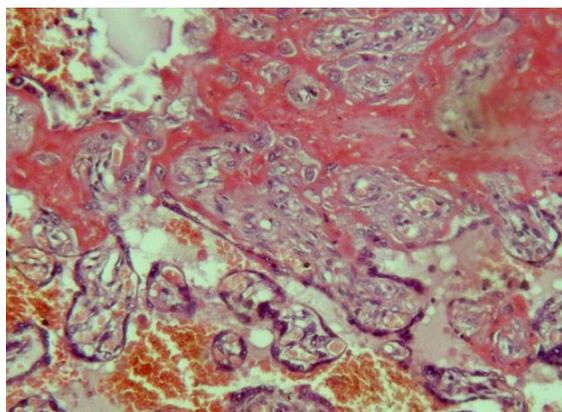


Figure 7 – fibrinoid necrosis (H.E. x 40).

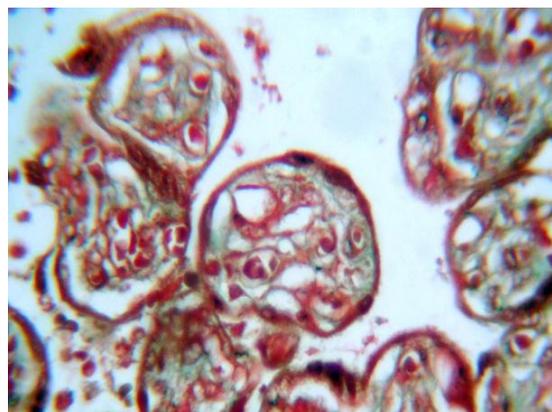


Figure 8 – nucleated red cells (Masson's Trichrome x 200).

Discussions

Optic microscopy analysis revealed various degrees of injuries consisting of numerous syncytial nuclei in the placentas of GDM, chromatin clumping, a feature of senescence, and most of them were arranged in clusters known as syncytial knots. In diabetics' placentas 25% of the villous surface is taken up by the capillary, while in normal placenta about 50% of the villous surface is taken up by the capillary bed (2). Diabetic milieu causes vascular dysfunction, increasing angiogenesis. Maternal (and fetal) hyperglycemia may impact on placental vascular permeability. The rise in blood glucose has several effects on the surrounding vasculature (3) demonstrated by the proliferation of small fetal vessels, partial or total obstruction of the vascular lumen was also seen in the vessels of the villous trunk having hemodynamic consequences (4). Fibrinoid necrosis, villous edema and villous fibrosis were observed, these being a result of hyperglycemia, as it acts as a pro-constrictor, pro-coagulator, pro-inflammatory, pro-angiogenic and pro-permeability agent (4); fibrinoid necrosis, peri- and inter-villous fibrosis, villous edema, crowding of villi and trophoblastic basement membrane were present being a real consequence of the metabolic disturbances which leads to accumulation of carbohydrate and fat in the placenta (5). These abnormalities in the fetal microcirculation are due to the microvascular changes associated with fibrinoid necrosis

and villous immaturity and can also affect oxygen exchange leading to chronic fetal hypoxia (6).

Conclusions

Histological changes in the placentas of pregnant women with gestational diabetes – disarrangements distention and proliferation of the cells of the fetal-maternal interface, or perivascular space and cells, thickening and separation of membranes of trophoblast and capillaries – are significant factors contributing to fetal anoxia with impact on placental vascular permeability. We were able to demonstrate that the diabetic milieu causes vascular dysfunction, increasing angiogenesis in pregnancy complicated with gestational diabetes mellitus. No significant relations were shown between various injuries of the fetal-maternal interface and neonatal condition, in diabetic pregnant women with fetal eutrophy.

The studies performed during the last few years brought increasing evidence to the theory that in addition to sugars, other metabolic fuels, from ketones to deranged lipid peroxidation, may be responsible for the pathomechanisms of congenital malformations. Metabolic fuels may play a crucial role and besides the classical theories about the strict glucose control, manipulations or replacements for deficient substrates, free oxygen radical scavengers and antioxidants, might become a huge promise for the near future (7).

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