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Histopathological considerations of placenta in pregnancy with diabetes

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Abstract

The authors present the results of a study on 19 cases of pregnant women with diabetes who delivered in No. 1 Clinic of Obstetrics and Gynecology, Emergency County Hospital of Craiova, between October 1st, 2009 and September 1st, 2011. After delivery, placentas were harvested for the pathology study. The results of this study reveal: villous immaturity, villous edema, presence of basement membrane thickening, congestion of capillaries called "chorangiosis", intra- and extravillous fibrinoid and a deposit of glycogen. The authors state that although these lesions are not pathognomonic for pregnancy with diabetes, they are very suggestive and specific for this association: diabetes–pregnancy.

***Keywords*:** diabetes, pregnancy, trophoblastic, intravillous or extravillous structures.

Introduction

Placental development is characterized by three distinct periods [1]. In the early pregnancy, a series of predominantly trophoblastic critical processes of proliferation and differentiation appear, leading to the formation of intravillous or extravillous structures. The anchor of placenta in the uterus and the remodeling of the uterine spiral arteries into low resistance vessels occur later. In the second period, newly formed villi become mature, going through various stages of a multistage process. The end of gestation is associated with the expansion of placental weight by the increasing villous component.

During the first half of gestation, the trophoblast is the key tissue which is suffering the deepest changes, while extensive angiogenesis and vascularization occur in the second half of pregnancy, the endothelium becoming now the site of more important processes although there is an overlap of the two phenomena. This period is also accompanied by extensive vascular remodeling and vascular stabilization [2, 3].

Histologically, term placenta shows a large number of villi and syncytial knots. In these knots syncytiotrophoblast nuclei are arranged in clusters leaving thin areas of cytoplasm between them [4].

Although there are authors who believe that, in essence, normal microscopic morphological appearance is preserved at least in placenta of patients with diabetes with good glycemic control [3], others say, that even if a long-term balanced diabetes is maintained, or diabetes is unbalanced, placenta presents a variety of significant histological structural changes seen more frequently than in the placenta of pregnant women without diabetes [1, 5, 6].

The nature and extent of these changes depend on many variables such as the quality of glycemic control during critical periods in placental development, the methods of treatment and time of severe drift from optimum metabolic control [1].

This study tries to analyze the placental histopathological changes in the pregnancy associated with diabetes in the context of a real "epidemic" of diabetes and lack of pathognomonic information on the histological damage of the placenta in diabetes.

Materials and Methods

Materials

The database for the histopathological study was represented by a group of 19 pregnant women admitted in the Obstetrics and Gynecology Clinic of the Emergency County Hospital of Craiova who delivered and were diagnosed with diabetes.

The above-mentioned group of women with diabetes were selected and studied in between October 1st, 2009 and September 1st, 2011.

Pregnant women in the study group were aged between 25 and 35 years, 16 were at the first delivery and three at the second delivery, 17 had pre-existing diabetes (type 1 diabetes) diagnosed between 5 and 12 years before pregnancy and two were diagnosed with gestational diabetes.

The diagnosis of the disease at the time of onset was achieved by determining fasting glucose, the oral glucose tolerance test, and glycated hemoglobin.

All pregnant women in this group were treated with insulin therapy leading to optimal metabolic control. Glycated hemoglobin was also normal.

The data sources from which the material was selected for the study were the following medical documents: clinical observation sheets of pregnant women, observation sheets of the newborns, birth documents, surgery protocols, histological samples and documents with histopathological results for fetal annexes.

The study material was represented by the tissue samples and the histological slides of adnexal tissue (placenta).

Methods

The study was prospective, including cases of pregnant women diagnosed with diabetes admitted in hospital during the period of the study.

Our purpose was not to verify the existent information in the literature but only to study our group and to obtain independent information.

A computer "database" file was created where all morphological changes of placental structures for each case have been introduced and then evaluated.

Placental tissue fragments were subjected to conventional histological techniques (fixation and paraffin embedding), and then serial sections were cut from each block. The first section was stained with Hematoxylin–Eosin, usual classical method. The next two sections were stained with Alcian Blue and PAS techniques for highlighting basal membranes and extracellular matrix components of mucopolysaccharides and placental villous stroma.

Histopathological aspects were selected with an Olympus CX31 microscope, using the eyepiece with a ×4 magnification. For image acquisition we used the optical corrected objectives with a magnification of ×4, ×10, ×20 and ×40.

The most significant images were captured with a LiveViewPro digital camera directly into the computer and processed using the AnalySIS Pro software (Figure 1) and the FotoCanvas Lite v1.1 module from the ACDSee 4.0 software (Figure 2).

The preliminary processing of the data from the database was performed using the Microsoft Excel module of the Microsoft Office XP Professional software package.

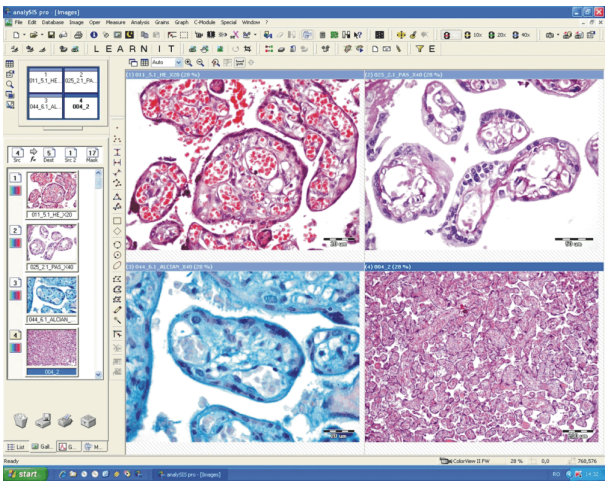


Figure 1 – ***Window of AnalySIS Pro software.***

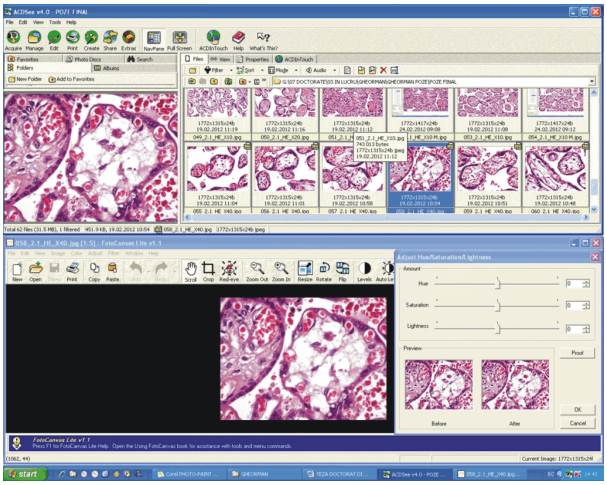


Figure 2 – ***Window of the ACDSee 4.0 software and the FotoCanvas Lite v1.1 module.***

Results

The histopathological evaluation of the 19 placentas of the women with diabetes included in our study aimed at quantifying most of the changes of placental structures reported in the literature over time.

Abnormal villous development (immaturity)

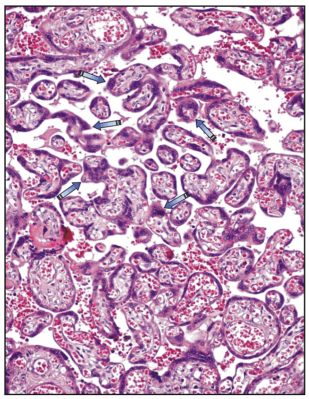
Immature intermediate villi are defined by the presence of a large stroma, loose reticular obvious channels containing Hofbauer cells. These villi predominate during the second trimester of pregnancy, persisting to term only in small nests within the center of the lobules. The most frequently reported change in the placenta of pregnant women with diabetes is the relative villous immaturity, even despite a nearly optimal metabolic control.

We also noticed the constant presence of syncytial knots, but these must be interpreted with caution because they usually are the consequence of the splitting incidence of the villi in the context of congestion caused by their increasing relative size (Figure 3).

Another morphological characteristic which allowed us to identify villous immaturity was the presence of numerous cell populations in the villous stroma, especially in the secondary and stem villi (Figures 4 and 5). In addition, regarding the villous stem, we noted the appearance of the indented edge, reducing the trophoblastic layer to extinction and its replacement by extravillous fibrinoid (Figure 5). However, nine of the cases studied showed stromal fibrosis especially in stem villi (Figure 6).

Another characteristic of immaturity was the reticular character of the extracellular matrix of villous stroma (Figure 7) with evidence of real stromal channels in which Hofbauer cells could be identified more easily (Figure 8).

Most of the time, in diabetic placentas, the presence of a continuous syncytiotrophoblast made of two cell layers can be seen, a characteristic aspect of villous development in the first quarter of pregnancy, another sign of abnormal delayed villous development (Figure 9).



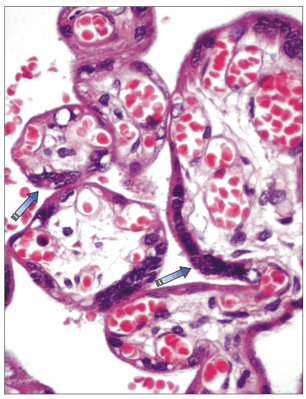
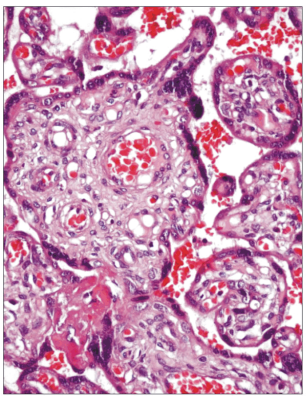


Figure 3 – ***Increased villous density, increased villous size, numerous syncytial knots (blue arrows).***



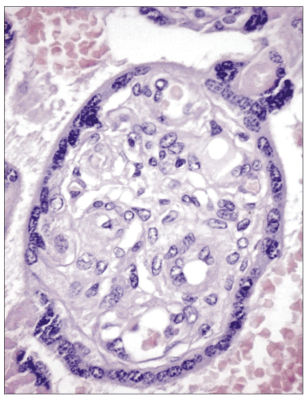
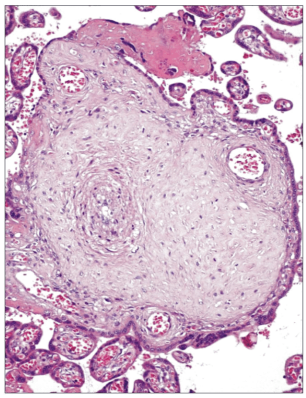


Figure 4 – ***Chorionic stroma rich in secondary cells.***



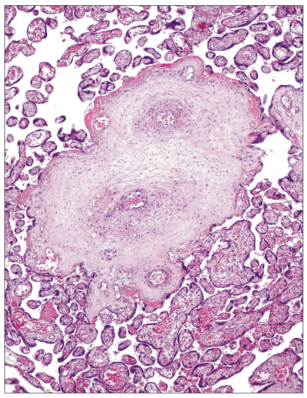


Figure 5 – ***Stem villi with indented edge, continuous trophoblastic layer and stroma with significant cell population.***

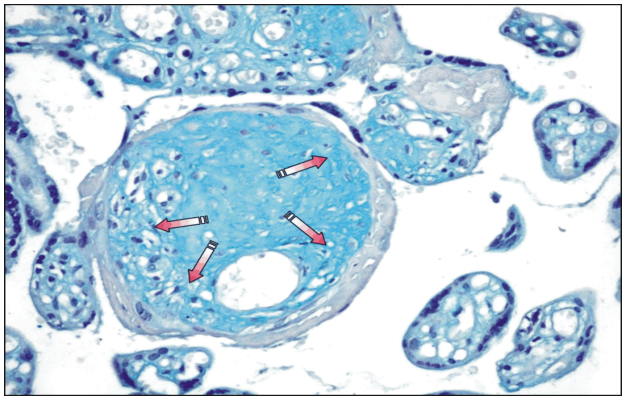
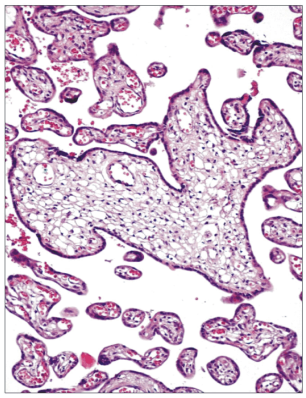


Figure 6 – ***Trend toward fibrosis in terminal stem villi.***



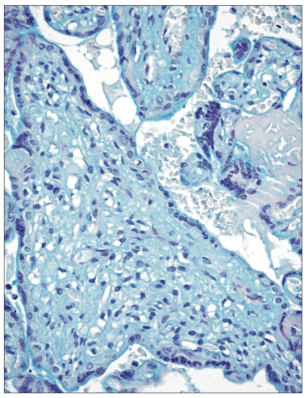
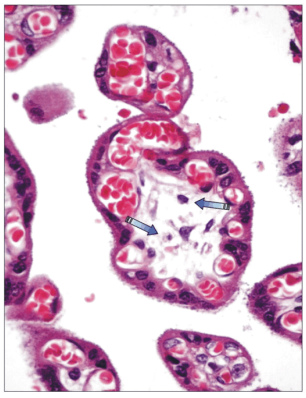


Figure 7 – ***Immature villi with loose stroma, loose reticular stroma and numerous stromal cell population.***



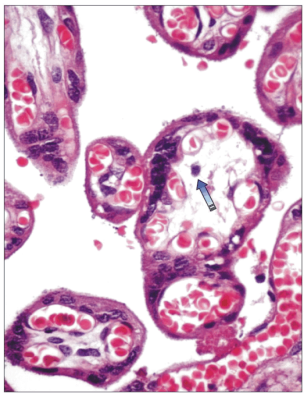
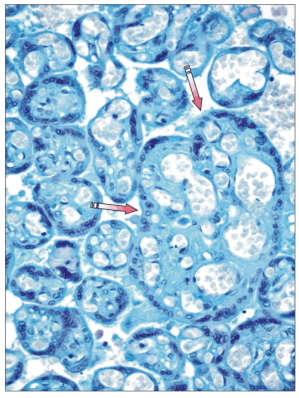


Figure 8 – ***Hofbauer cells in stromal channels of terminal villi.***



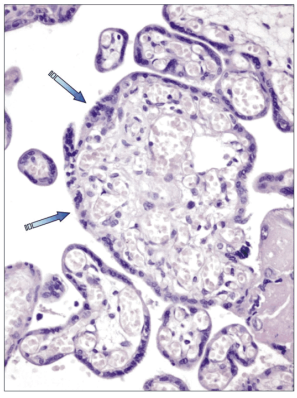


Figure 9 – ***Secondary villi with continuous trophoblastic layer.***

Villous edema

Another common abnormality found in villous parenchyma of diabetic placenta is villous edema. It is defined as the accumulation of fluid in the villous interstitium with disruption and replacement of intravillous cellular architecture. It is considered as having pathological significance, especially in premature pregnancy, and is considered one of the causes of fetal ischemia. Its etiology is still unknown. In our study, we noticed this type of change with focal distribution of distal villi in all cases studied (Figures 10 and 11).

Thickening of the basement membrane

Marked thickening of the syncytiotrophoblast

basement membrane was described in various pathological situations, one of them being maternal diabetes. All 19 cases studied showed focal thickening, but they were generally small, identified by specific staining for mucopolysaccharide components (Figure 12).

The phenomenon is significant for women with uncontrolled diabetes, has low intensity in the placenta with well-controlled diabetes and lacks in women without diabetes.

"Chorangiosis"

Another morphological aspect reported in the literature and observed in diabetic placentas studied by us is the increasing number of villous capillaries, the so-called "chorangiosis" (Figure 13) and thickening of their wall (Figure 14).

Fibrinoid

Fibrinoid means any non-cellular, eosinophilic, homogeneous material that can be identified in the placenta.

Extravillous fibrinoid has a lamellar structure and, in terms of immunohistochemistry, its surface layer is fibrinous (blood origin).

This layer may be in contact with the villous trophoblastic blade or can be followed by a homogeneous layer of matrix-type fibrinoid (secretion product that incorporates large trophoblastic cells) (Figure 15).

This type of fibrinoid either fills gaps in the trophoblastic layer, or includes the all chorionic villi or groups of villi, new aspects captured in all cases studied by us (Figure 16).

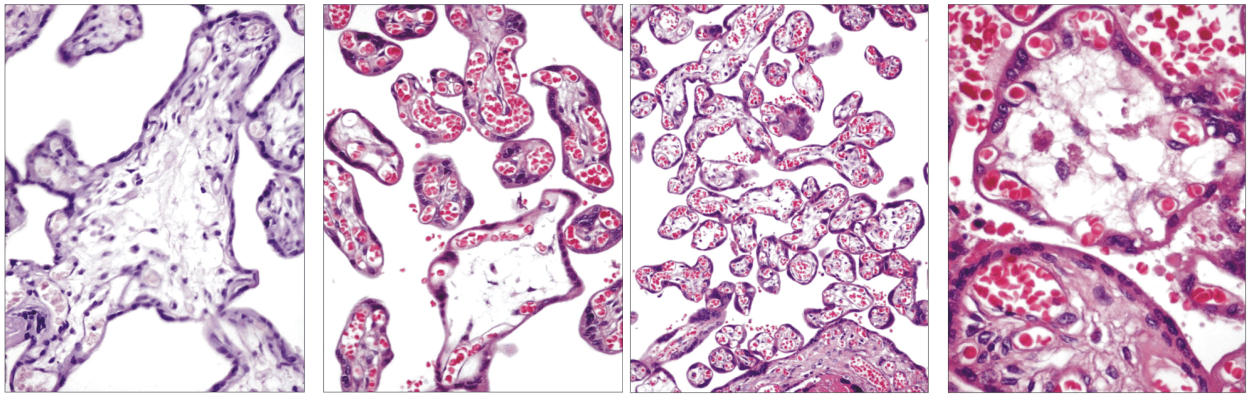


Figure 10 – ***Villous edema. Left: mature intermediate villi. Right: terminal villi.***

Figure 11 – ***Stromal edema in terminal villi.***

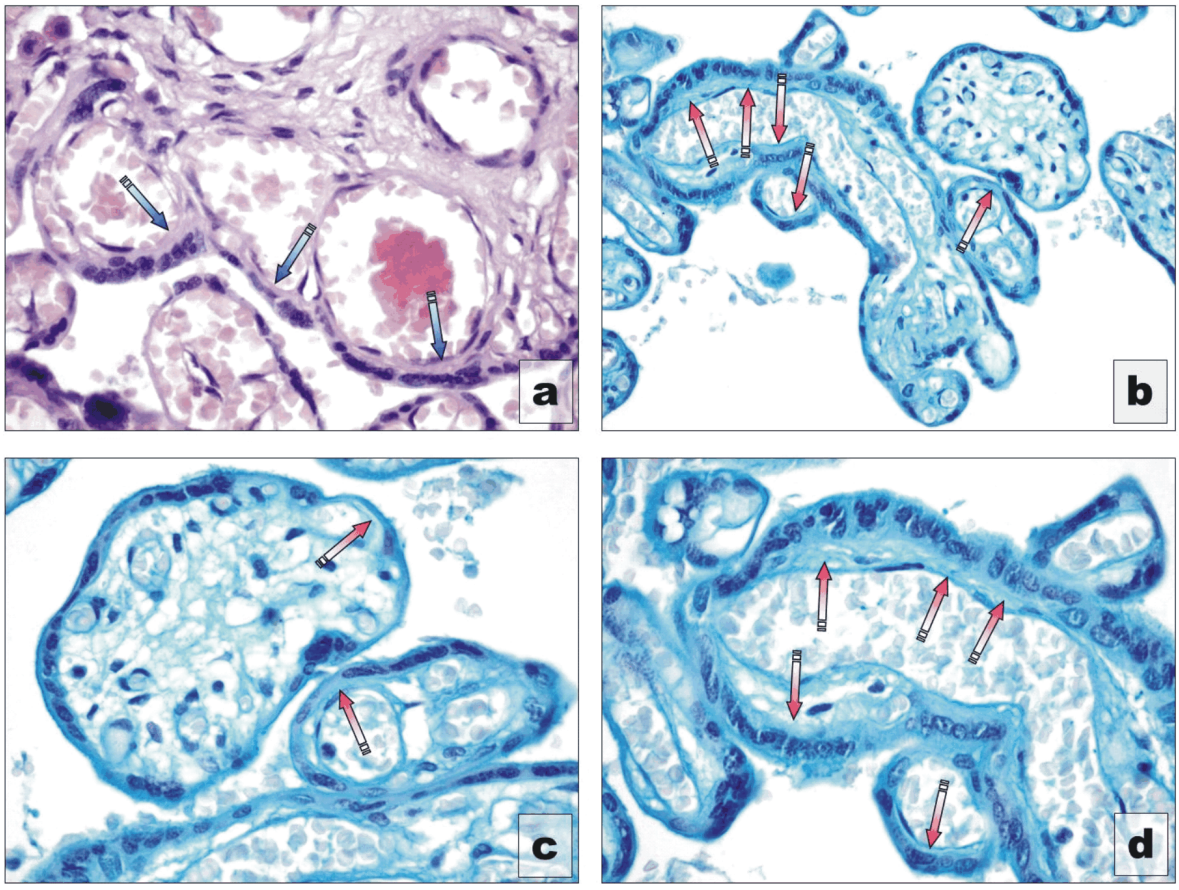


Figure 12 – ***Basement membrane thickening in villous immature trophoblast (a) and terminal/tertiary villi (b–d).***

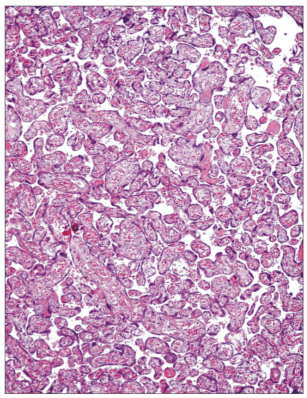
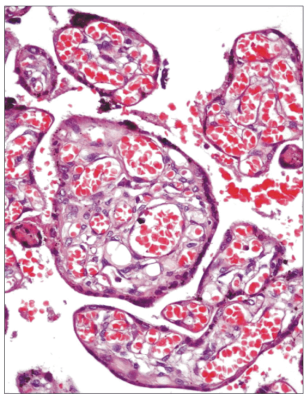


Figure 13 – *"Chorangiosis".*



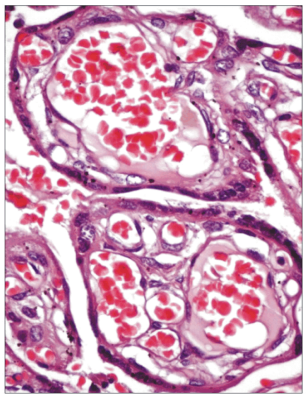
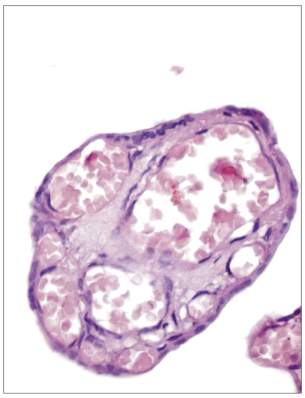


Figure 14 – *Thickening of villous capillaries.*



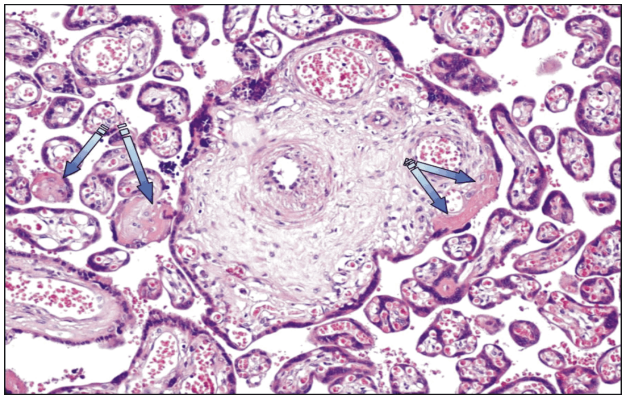


Figure 15 – *Intravillous fibrinoid in terminal stem villi (right) and the terminal/tertiary villi (left).*

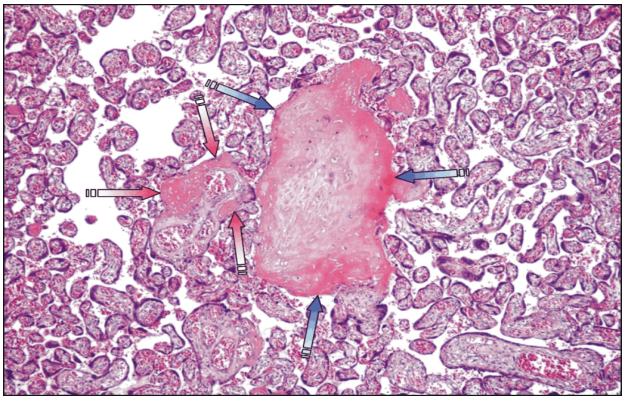


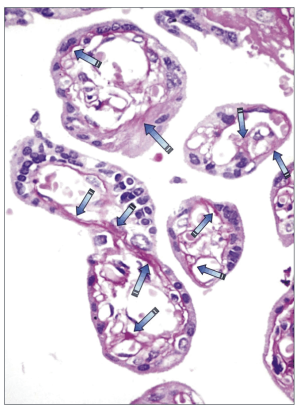
Figure 16 – *Fibrinoid necrosis of terminal stem villi (blue arrows), extravillous fibrinoid between terminal villi (red arrows).*

Intravillous fibrinoid was described as a distinct fibrinoid material appearing in the subtrophoblastic space that finally occupies the whole villous stroma. Intravillous fibrinoid deposits are increased in pathological conditions including diabetes (Figure 15).

Villous fibrinoid focal necrosis appearance, illustrated in Figure 16, was observed only in nine of the 19 placentas examined.

Glycogen deposits

In the cases studied, PAS staining allowed us to identify, on one hand, small clusters of PAS+ material with subsyncytial location, as shown above and, on the other hand, focal deposits located both in thickened basement membranes and especially in the terminal villous extracellular matrix (Figure 17).



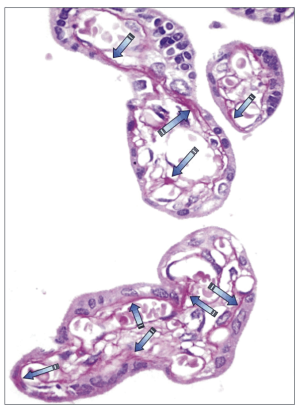


Figure 17 – *Glycogen deposits in the villous interstitium (PAS staining).*

Discussion

The aim of the histopathological evaluation of the 19 placentas from women with diabetes included in our study was to quantify as much as possible the widest range of changes in placental structures recorded over time in the literature.

Villous immaturity

Villous immaturity was defined by Altshuler G and Herman A: third quarter placentas showing enlarged villi with numerous stromal cells without syncytiotrophoblast and with syncytial knots. Benirschke K *et al.* prefer the term "abnormal villous development" (villous maldevelopment), which must be individualized (villous edema or immature hypercellular villi) rather than using the vague term "immaturity" [7].

This phenomenon was observed in villous immaturity in all cases studied, particularly in the distal branches of the villous tree. It should be emphasized that the distribution of immature appearance is focal and not diffuse and synchronous. The defining morphological features of focal abnormal villous development were increasing the size of villi especially distal ones (terminal and intermediate villi), which gives the false impression of increased villous density, although at a careful observation, the intervillous space is significant.

In normal placentas, together with villous maturation, the barrier between maternal and fetal circulation is reduced by the thinning of the syncytiotrophoblast, reduction of cytotrophoblast, decreased mean villous

diameter and fetal capillary apposition at the villous surface [8].

However, in diabetic placentas, most of the times, one can see the presence of a continuous syncytiotrophoblast consisting of two cell layers, characteristic appearance of villous development in the first quarter of pregnancy, being another sign of abnormal delayed villous development.

Villous edema

Villous edema was observed in placenta of diabetic patients, more obvious in gestational diabetes and less in well-controlled diabetes [5].

Villous edema observed in placenta from women with diabetes is less obvious in those with good metabolic control [5].

Several studies have reported the occurrence of mucopolysaccharide deposits that consist mainly in hyaluronic acid molecules in all cases of villous edema [9, 10].

Starting from the fact that hyaluronic acid molecules can retain water, it was concluded that the appearance of true villous edema in placentas of mothers with diabetes is probably the result of the appearance of abnormal deposits of mucopolysaccharides in villous stroma [11].

Thickening of the basement membrane

The marked thickening of the basement membrane of the syncytiotrophoblast, which become obvious, was described in various pathological situations, one of them being maternal diabetes [12, 13].

This thickening is the result of mucopolysaccharide storage, which may be obvious by intense Alcian Blue staining at pH 2.5 and it could be due to impaired villous trophoblastic activity such as increased production or decreased turnover of basement membrane molecules, as it is known that constituents of basement membrane components are produced by the secretion of trophoblast [7, 9].

All 19 cases studied showed focal thickening, but generally reduced, highlighted by the specific staining for mucopolysaccharide components. The phenomenon that is significant in women with unbalanced diabetes has a low intensity in the placenta of women with well-controlled diabetes and lacks in women without diabetes [5].

"Chorangiosis"

We cannot say that this is identical to diabetic microangiopathy because these injuries occur after a significant period of time, pregnancy is insufficient for creating this vascular changes but we appreciate that changes defined as "chorangiosis" may enroll in the same context. Some authors consider the increasing number of villous capillaries a sign of chronic hypoxic changes [14].

Fibrinoid

Disseminated deposits of fibrinoid material in the intervillous space and/or the villous tree are a common phenomenon in term placenta. Anatomically speaking, the nomenclature used by Fox H [15] seems to be the best, the author distinguishing two types of fibrinoid:

- perivillous fibrin (fibrinoid) surrounding more or less altered but still recognizable villi;
- intravillous fibrinoid (subsyncytial fibrinoid, villous fibrinoid necrosis) that affects the inside of villi from the beginning.

Although the two processes can interfere in time, they seem to have, at least at first, different pathogenesis. More over, the mixture of both types of fibrinoid is different [16].

Intra- or extravillous fibrinoid deposits in excess are considered pathological phenomena and they are often incompatible with normal fetal development [16].

Glycogen deposits

According to some authors, placental histological changes are mainly due to metabolic disorders that lead to the accumulation of carbohydrates and lipids in the placenta. The accumulation of carbohydrates, particularly glycogen, paradoxical since in other maternal organs the glycogen content decreases in diabetes, was identified using a histochemical stain specific for glycogen (PAS staining) [4, 17].

PAS reactivity for glycogen in diabetic placenta is stronger than normal placentas [4].

However, rigorous studies have shown the presence of more significant deposits of glycogen in placentas of diabetic mothers only around large fetal vessels [18].

Glycogen has been identified in amniotic epithelial cell cytoplasm in early pregnancy but this accumulation revealed no quantitative changes related to the existence of maternal diabetes [7, 19, 20].

Comparison with studies in the literature

The only study in the consulted literature which, in the descriptive analysis of the morphology of the placenta of diabetic mothers quantified the presence of histopathological changes observed, was that of Tewari V *et al.* in 2011 [4].

The Indian researchers' studied group consisted of 30 cases of diabetes associated with pregnancy and the morphological parameters assessed were largely the same as those evaluated in our study. As can be seen in Figure 18, observations of the two studies coincided to the some extent.

There were some differences, that is in our study we identified syncytial knots in all cases while the phenomena of fibrinoid necrosis and villous stromal fibrosis were present with a frequency lower than in the Indian study.

The general characteristics of the morphological changes found by us in the placentas studied were of low or moderate intensity, focal arrangement and for some of them such as fibrinoid necrosis and stromal fibrosis, an inconstant presence [5, 21]. This general pattern described in the literature is characteristic for properly treated and controlled diabetes [22].

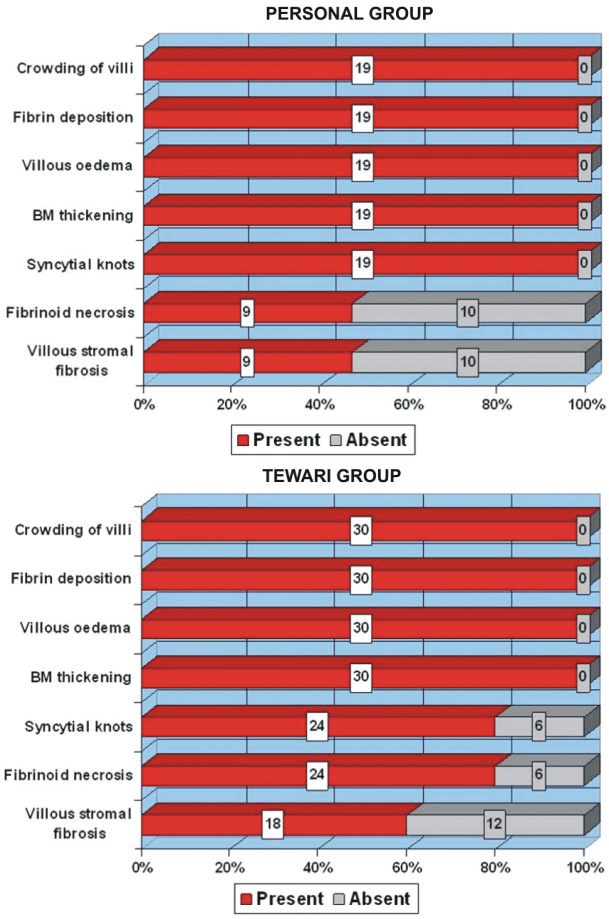


Figure 18 – ***Comparison of personal observation and the study of Tewari V et al. (2011).***

Conclusions

In summarizing the personal observations and the data reported and discussed in the consulted literature, the spectrum of the most common placental morphologic changes in structures which can be observed in placentas from mothers with diabetes are: villous immaturity, villous edema, syncytial knots, fibrinoid necrosis, and fibrin thrombi.

Villous patterns of placentas of mothers with diabetes have a high inter-individual variability that may be local and only partially explained by different degrees of disease or therapeutic success.

The pathogenesis of placental changes is still far from being fully understood, but there is a general opinion that the extent and degree depends not only on the severity and duration of maternal diabetes, but also depends on the degree of metabolic control during pregnancy.

Although the injuries presented, when analyzed individually, may occur in other diseases, when summarized they are highly suggestive for pregnancy associated with diabetes.

Acknowledgments

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