Histopathological changes of placenta in pregnant women complicated with pregestational diabetes

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Abstract

The study presented the results of a study on 68 cases of pregnant women with normal and diabetes who delivered in Department of Obstetrics and Gynaecology in three Hospital at Baghdad city during the period from 1 December 2016 to 1 may 2017. After delivery, placentae were collected for the histology and histopathological studies. Numerous changes have been recorded significantly in placenta of pregestational diabetes (DM) women included: crowding of villi, increased villous number of immature intermediate villi, decreased terminal villi density, increased terminal villi size, numerous syncytial knots, basement membrane thickening in terminal villi, cytrophoblast in terminal villi, decreased vascular-syncytial membrane thickness, extravillous fibrinoid between terminal villi, fibrosis in terminal and stem villi, fibrinoid necrosis of stem and terminal villi, stem villi with indented margin, continued trophoblastic layer and stroma with respectable cell population, basement membrane thickening in immature intermediate villous, immature intermediate villi with loose reticular stroma, increased of Hofbauer cells population in immature intermediate villi, calcification intracellular as well as extracellular, chorangiosis, thickening of villi vessels, fetal vessel thrombosis, nucleated fetal RBCs, mature intermediate villi with continuous trophoblastic layer and villous edema, edema in terminal and stem villi. Although the damages presented, when analyzed individually, may incidence in other diseases, when summarized they are highly suggestive for pregnancy related with diabetes we can diagnose.

Keywords: Placenta, Pregestational diabetes, Histopathology, Pregnancy.

INTRODUCTION

Diabetes mellitus is a chronic, lifelong condition that effects on body's ability to use the energy found in food. Diabetes in pregnancy is important because of increasing rates of the disease and its effect on maternal, fetal and neonatal health, such as preeclampsia, primary caesarean delivery, macrosomia and disease and its effect on body's ability to use the energy found in food. Diabetes due to very few studies have been done on histopathological changes in placenta of pregestational diabetic mother and prospect study in this field will go a long way in discovery a solution in assessing the distractive changes in diabetic placenta in different stages of pregnancy.

MATERIALS AND METHODS

The mothers’ informed consents were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health. Fresh placentae were obtained from Department of Obstetrics and Gynaecology in three Hospital at Baghdad city during the period from 1 December 2016 to 1 may 2017. A total of 68 placentae were collected directly after delivery for the study and divided into two groups. First group, normal women and second group included women with pregnancies complicated by pregestational diabetes mellitus (DM) (cases n=34), (17 diabetes type 1, 17 diabetes type 2). Placentae were cut and sampled for histological examination. Two standard samples were taken from intact maternal surface and fetal surface and one from the central area and other from the peripheral area. Fresh placental tissue pieces were fixed with formalin (10%) [5]. Then tissue samples from placenta after delivery were prepared for histopathological studies according to routine paraffin methods [6]. Haematoxylin and Eosin staining was accomplished according to [7]. The sections were examined by compound light microscope (Meijitechno, Japan) with digital camera (Canon, Japan, 18 megapixels). Images were analysed independently with the help of expert pathologist by Multihead teaching microscope (Genex, USA). The images were captured with a Live View Pro digital camera directly into the computer in advanced embryology laboratory, Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad then adding scale bars to all images by using ImageJ software.

RESULTS

Histopathological examination of the sections from the placentae of DM mothers showed numerous changes (Figure 1) including: crowding of villi (Fig.2-A), increased villous number of immature intermediate villi and decreased terminal villi density as well as increased terminal villi size (Fig.2-B), numerous syncytial knots (Fig. 2-C), basement membrane thickening, cytrophoblast and decreased vascular-syncytial membrane thickness in terminal villi (Fig. 2-D), extravillous fibrinoid between terminal villi and fibrosis in terminal and stem villi (Fig. 2-E), fibrinoid necrosis of stem and terminal villi (Fig. 2-F), stem
villi with indented margin, continued trophoblastic layer and stroma with respectable cell population (Fig. 2-G), basement membrane thickening in immature intermediate villi (Fig. 2-H), immature intermediate villi with loose reticular stroma and increased of Hofbauer cells population in immature intermediate villi (Fig. 2-I), calcification intracellular as well as extracellular (Fig. 2-J), thickening of villi vessels and thrombosis (Fig. 3-L), nucleated fetal RBCs (Fig. 3-M), mature intermediate villi with continuous trophoblastic layer villous edema in mature intermediate (Fig. 3-N) edema in terminal (Fig. 3-O) and stem villi (Fig. 3-P) as compared to the placentae of normal mother (Figure 4, 5) which was found to be statistically significant differences with a value of P≤0.001 or P≤0.05.

Figure 1: Comparison of histopathological features of placenta in control (A) and DM (B) groups
Figure 2: A-I Section of placenta in women complicated with DM showing:

A- Crowded villi, (4X);
B- Decreased terminal villi density and increased size as well as increased immature intermediate villi (10X);
C- Numerous syncytial knots (40X);
D- Basement membrane thickness in terminal villi, more cytotrophoblast and decreased vascular-syncytial membrane thickness in terminal villi (100X);
E- Present extravillous fibrinoid between chorionic villi and fibrosis inside terminal and stem villi (10X);
F- Present fibrinoid necrosis in terminal and stem villi (40X);
G- Stem villi with indented margin, continued trophoblastic layer and stroma with respectable cell population (arrow head) (10X);
H- Thickening of syncytiotrophoblast basement membrane in immature intermediate villi (40X);
I- Immature intermediate villi with more loose reticular stroma and Hofbauer cells population (40X);

stem villous (SV) and mature intermediate villous (MV), immature intermediate villous (IV), terminal villous (TV), fibrinoid necrosis (FN), fibrosis inside (IF) chorionic villi, syncytial knots (K), basement membrane (BM), cytotrophoblast (Cy), vascular-syncytial membrane (VSM), syncytiotrophoblast (Sy), Hofbauer cell (HC), fetal blood vessel (FBV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (IMC), edema (E), loose reticular stroma (LRS); (H&E) (4X, Scale bar 500 μm); (10X, Scale bar 200 μm); (40X, Scale bar 50 μm); (100X, Scale bar 20 μm).
FIGURE 3: J-P Section of placenta in women complicated with DM showing: J- Placental calcification (40X); K- Chorangiosis (arrow head) (40X), L- Thickening of villi vessels (arrow head) and vessel thrombosis (40X), M- Present nucleated fetal RBCs (arrow head) (100X), N- Contained mature intermediate villi with continuous trophoblastic layer and edema in mature intermediate (40X), O- Edema in terminal villi (40X), P- Edema in stem villi (40X); calcification (CA), vessel thrombosis (Th), terminal villous (TV), edema (E), extravillous fibrosis, maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (TL), syncytial knot (K), fibrinoid necrosis (FN), extravillous fibrosis (EF).

DISCUSSION

In current study, we evaluate the histopathological changes of the 34 placentae from women with diabetes in comparison with normal woman was to quantify as much as possible the broadest range of changes in placental structures registered over time in the literatures. Crowding of villi was seen to be increased significantly higher in DM group in comparison with control group. [8] reported that histological anomalies such as presence crowding of villi was more frequently observed in diabetic placenta this finding agreed with our study. Previous studies have detected an increased occurrence of immature intermediate villi in placentae affected by either DM [9] compared to normal pregnancies. Due to defect in placental maturation have been related with chronic fetal hypoxia [10], a larger rate of immature intermediate villi may be revealing of a better preuterine hypoxic environment. Increasing the size of villi especially terminal and immature intermediate villi, gives the false impress of increased terminal villous density [11]. Number of studies reported an association between the frequency of immature villi and insufficient or absent terminal villi in pregestational diabetes [9]. Terminal villous size significantly increased in both diabetes groups compared to controls this result agreed with [12; 13] studies. Increased numerous of syncytial knots, bridges and sprouts are called as syncytial knotting or Tenny-Parker changes [14]. In past studies, done by [8; 11; 15], as compared with the normal placenta, placentae from pregnant
women with diabetes showed an increased incidence syncytial knots. [16; 17; 18] noticed increasing thickening trophoblastic basement membrane were present in most of the diabetic placenta in comparison of normal women. This histological change of placenta are mainly due to metabolic disturbances that leads to accumulation of carbohydrate and fat in the placenta. Whereby, this thickening is the consequence of mucopolysaccharide storage and it could be attributed to distributed villous trophoblastic activity such as increased production or decreased transformation of basement membrane molecules, as it is recognized that components of basement membrane constituents are produced by the secretion of trophoblast [14].

Figure 4: A-I Section of placenta in normal women showing: A- Normal crowded villi, (4X); B- Normal terminal villi density and size as well as normal immature intermediate villi (10X); C- Few numerous syncytial knots (40X); D- Normal basement membrane in terminal villi, few cytotrophoblast and normal vascular-syncytiotrophoblast thickness in terminal villi (100X); E- No extravillous fibrinoid between chorionic villi and fibrosis inside terminal and stem villi (10X), F- Few fibrinoid necrosis in terminal and stem villi (40X), G- Stem villi with no indented margin, no continued trophoblastic layer and stroma with normal respectable cell population (arrow head) (10X), H- Normal syncytiotrophoblast basement membrane in immature intermediate villi (40X), I- Immature intermediate villi with normal loose reticular stroma and Hofbauer cells population (40X); stem villous (SV) and mature intermediate villous (MV), immature intermediate villous (IV), terminal villous (TV), fibrinoid necrosis (FN), syncytiotrophoblast (K), basement membrane (BM), cytotrophoblast (Cy), vascular-syncytiotrophoblast (VSM), syncytiotrophoblast (Sy), Hofbauer cell (HC), fetal blood vessel (FBV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (IMC), loose reticular stroma (LRS); (H&E) (4X, Scale bar 500 μm); (10X, Scale bar 200 μm); (40X, Scale bar 50 μm); (100X, Scale bar 20 μm).
Figure 5: J-P Section of placenta in normal women showing: J- No calcification (40X); K- No chorangiosis (arrow) (40X), L- Normal thickening of villi vessels (arrow) and no vessel thrombosis (40X), M- No nucleated fetal RBCs (100X), N- Mature intermediate villi without continuous trophoblastic layer and few edema (40X), O- Few edema in terminal villi (40X), P- Few edema in stem villi (40X); terminal villous (TV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (TL).

However, in diabetic placentae, most of the times, one can observe the presence cytotrophoblast, being another sign of abnormal immature villous [19]. [20; 21] found that placental anomalies in pregestational diabetes including increased cytotrophoblastic, therefore this finding in agreement with our study. The barrier between maternal and fetal circulation is reduced by the thinning of the vascular-syncytial membrane. This can negatively affect the transplacental transport, metabolism, and oxygen distributing [22]. Decreased vasculo-syncytial formation can be due to the delayed villous maturation that might be the etiology for the improved risk for intrauterine losses in diabetic women [23].

Extravillous fibrinoid has a lamellar construction and this fibrinous layer can be in connection with the villous trophoblastic edge. This type of fibrinoid either fills spaces in the trophoblastic layer, or contains all chorionic villi or collections of villi [17]. Increased of extravillous fibrinoid deposits are reflected pathological phenomena and it was frequently inconsistent with normal fetal growth [24]. Stromal fibrosis is described abnormal when increased in the stem villi. In diabetic women, there is an increased villous stromal oxygen partial pressure, in the side of insufficient uptake by the fetal capillaries, which prompts the synthesis of collagen [25]. At placentae of diabetic women, the fibrinoid material had increased by pushing the basement membrane and pressing the complete villous stroma [14]. Histological pathologies such as the presence of fibrinoid necrosis were detected more repeatedly in DM [16; 26; 27] placentae compared with the control placenta. Regarding stem villi with
depressed margin, continued trophoblastic layer and stroma with suitable cell population, this feature permitted us to recognize villous immaturity was the presence of numerous cell populations in the villous stroma especially in stem villi. Therefore, [11; 28] found to be continued trophoblastic layer and cell population more common in DM group. [11; 29] recorded increased mature intermediate villi with continuous trophoblastic layer and as the current study this feature present more frequent with DM placentae. Noticeable thickening of the syncytiotrophoblast basement membrane was described in numerous pathological conditions; one of them is maternal diabetes. This thickening of syncytiotrophoblast basement membrane was resulted of a higher degree of nonenzymatic glycosylation or an increased quantity of the prominent type of basal lamina collagen, type IV. As well as higher substances of DNA, phospholipids, triglyceride, and of cholesterol are distinguishing features of placenta in diabetes women [14].

Immature intermediate villi are characterised by the existence of a large stroma, loose reticular noticeable channels having Hofbauer cells. These villi dominate during the second trimester of pregnancy, continuing to term only in small amount. The most often reported alteration in the placenta of diabetic women is the relative immaturity of villous, however a closely best metabolic control in these women [22]. This suggestion in agreement with [30; 31], who investigate the changes in morphology immature intermediate villi and density of Hofbauer cells in placentae from normal and diabetic pregnancies. The placental depositions are consisted of calcium phosphate [32]. These depositions are organized mostly near maternal surface in the basal plate, along the septa, perivillous space, sub choriionic space and basement membrane of placental villi, maybe due to its ability to act as placental calcium pump [33]. In many studies, calcifications observed as intracellular as well as extracellular basophilic deposits after stained with haematoxylin and eosin in the placentae of DM group [34; 35]. The increased villous chorangiosis probably a response to the relative hypoxemia due to the placentae of DM group [34; 35]. The increased villous chorangiosis probably a response to the relative hypoxemia due to an increase of VEGF expression [36] and the immaturity of the villi, which considered by centrally placed villous capillaries causing in a greater space for oxygen and nutrients to permit from maternal to fetal exchange [37]. Numerous studies described presence of chorangiosis significantly increased in placentae of pregnancies with DM [16; 21; 37].

Thickness villus vessel walls due to endothelial proliferation and thickening of the basement membrane. As well as it has been observed that increased blood glucose levels prompt oxidative stress (OS) and following variations of the placental architecture [18] principally the vascular properties, which are obvious in diabetic women. Similar results were obtained by [19] in placentae of DM women. The blood vessels in some of the terminal villi showed occluding by thrombus in DM [16] placentae, this finding in line with current study. This feature was diagnosed when a large fetal stem villous vessel was partly or completely obstructed by a thrombus [31] (Bhattacharjee et al., 2017).

Nucleated fetal RBC’s are present in the placentae vessels during the first-trimester of pregnancy, but are rare later in pregnancy and are generally absent or existing only in small numbers at term [38]. These results were associated with fetal damage and reduced fetal oxygenation in diabetic women [36; 3; 16] and reported that histological anomalies such as presence of nucleated fetal RBC’s, were more frequently observed in diabetic placenta. The accumulation of fluid in the stroma of placentae villi called edema. As hyaluronic acid molecules have the particularity to retain water, it was concluded that, the existence of abnormal deposits of mucopolysaccharides in the stroma of villi can lead to the presence of the true villous edema in placenta of diabetic pregnancies [40]. [20; 21] found that increased villous edema have been described with pregestational diabetes.

Lastly, concerning Iraqi studies, one study [41] conducted in Erbil City showed significant increase complications in diabetic pregnancy revealed immaturity of placentae villi, edema of the villi, and intervillous. As well as [42] indicated in their study at Hilla city that the terminal villi in placentae of diabetes women controlled on insulin appeared the increased number of syncytiot knots and the stroma of the villi showed villous edema, fibrinoid necrosis and fetal vessels proliferation.

**REFERENCES**


