

# Histological Changes of the Human Placenta in Pregnancies Complicated with Diabetes

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## Abstract

Normal fetal growth and survival depends on proper development and function of the placenta. The diabetic pregnancy is characterized by numerous disturbances in fetal growth and development. This study was done to focus on the effects of gestational diabetes on the histology of the placenta to confirm the magnitude of damage caused by diabetes to human placenta. Twenty Placentas of full term pregnancy were collected from Alzawia hospital, Libya. We used histological, histochemical and CD34 immuno-histochemical stains in this study. In diabetic pregnancy, placental weight is higher in comparison to normal pregnancy. Chorionic villi showed increased number of fetal capillaries, stromal villous fibrosis, villous edema, thickness of basement membrane of syncytiotrophoblast, glycogen deposits and strong positive reaction for CD34 in the wall of blood vessels in stem villus.

**Keywords:** Placenta; Diabetes; Histological; Histochemical; Imuno-histochemical

## Introduction

The placenta is a complex organ of short life-span which is responsible for the transfer of nutrients and waste products between the fetal and maternal circulations. The metabolic and endocrine activities of the placenta are not clearly understood [1]. It is the most important and vital organ of the intrauterine life, it must integrate signals from the fetus and the mother in an effort to match fetal demand with maternal nutrient supply [2]. So, it actually plays a crucial role in fetal growth. The main functional units of the placenta are the chorionic villi, within them; fetal blood is separated from maternal blood in the surrounding inter-villous space by vasculosyncytial membranes overlying dilated fetal capillaries [3]. Histologically, a term placenta shows large number of villi and syncytial knots. In these knots, syncytiotrophoblast nuclei are aggregated together in clusters leaving zones of thin cytoplasm devoid of nuclei in between [4]. The diffusion barrier between maternal and fetal circulation comprises of five layers trophoblast, trophoblastic basement membrane, and core of supporting tissue, capillary endothelial basement membrane and endothelium [5]. Diabetes mellitus is now a major health concern in our society, according to the centers for disease control and prevention, the crude incidence of diagnosed diabetes increased 124% from 3.3 per 1000 to 7.4 per 1000 [6]. Meanwhile other Studies suggest that, the prevalence of diabetes mellitus (DM) among women of childbearing age is increasing due to more sedentary life styles, changes in diet and adolescent obesity [7]. Diabetes mellitus in pregnant women may be categorized into clinical diabetes or pregestational diabetes (women previously diagnosed with type I or type II diabetes) and gestational diabetes (GDM) which is stated as any degree of glucose intolerance with commencement or first recognition during pregnancy. Abnormal maternal glucose tolerance occurs in 3- 10% of pregnancies. Whatever, any type of diabetes mellitus during pregnancy produces variety of placental abnormalities [8]. The nature and extent of these changes depend on a number of factors, such as glucose level during the critical periods in placental development [9]. Alterations in placental function due to uncontrolled diabetes result in disturbances in fetal growth and development, macrosomia, congenital malformations and intrauterine growth retardation [10].

**Aim of the present work** is to investigate histological differences of the placenta in pregnancies complicated by gestational diabetes compared to non-diabetic pregnancies and to increase our knowledge about the histological, histochemical and immunohistochemical changes in diabetic placenta, as very few studies have been done on histochemical changes in placenta of a diabetic mother (pregestational).

## Material and Methods

### Sample

A total of twenty cases were studied, seven placentas from non-diabetic mothers and thirteen placentas from diabetic mothers. The placentas of full term pregnancy were collected from Labor Room and Gynecology operation theatre of Alzawia hospital, Libya.

### Tissue preparation

All placentas, including those from the control group were treated identically in a standardized manner. Immediately after delivery, cords were clamped to ensure that placentas are not drained of blood.

Two centimeter of tissue was taken from the center of each placenta and fixed in 10% formalin for one week. The tissue was dehydrated and followed by embedding in paraffin and 7 micron serial sections were generated with the help of rotator microtome. The tissue sections were stained with hematoxylin and eosin (H&E), Periodic -Acid Schiff (PAS), Mallory's trichrome, methyl green pyronin and Von Gieson stains [11]. CD34 immunohistochemical stain was also done.

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## Immunohistochemistry (CD 34 immunohistochemistry)

Immunostaining was carried out using the avidin-biotin-peroxidase complex method. After deparafinization and rehydration, endogenous peroxidase activity was blocked using 3% hydrogen peroxide in pure methanol for 10 minutes at room temperature. The tissues were then treated with 0.01% pepsin in 0.01M HCl at 37°C for 10 minutes. After serum blocking, using 2% bovine serum albumin, the sections were then incubated with the primary antibody for 30 minutes at room temperature.

The primary antibody was a mouse monoclonal antibody for low molecular weight cytokeratin (clone MNF, Dako A/S, Denmark), at a dilution of 1 in 50. This was then incubated using the secondary antibody CD34 for 30 minutes. Then tissue sections were examined using bright field ordinary microscope.

## Results

A total of 20 placentas were included in this study, 7 placentas were from non-diabetic mothers (control group) and 13 placentas were from diabetic mothers controlled on insulin (diabetic group).

### Gross examination

Gross observation of the placentas showed that the placentae from diabetic mothers are larger in size as compared to control group. The mean weight of the placenta in control group was 488.0 gm while in diabetic placenta, it was 950.6 gm.

### Histological results

#### Haematoxylin and eosin (H&E)

**Group 1 (Control group):** The histological findings of normal placentas group were typical of their gestational age, many villi which contain connective tissue and microvasculature, the villus connective tissue surrounded by epithelial cells of the trophoblast, including both the inner cytotrophoblast epithelium and the overlying syncytial trophoblast. In many areas nuclei of the syncytiotrophoblast layer have formed clusters or knots on the surfaces of villi (Figure 1a and 1b).

**Group 2 (Diabetic group):** On microscopic examination of placentas of diabetic mothers showed many morphological changes, which are the following:

Increased number of villous capillaries and dilated blood vessels were seen in some parts of diabetic placentas. Also congestion of many blood vessels was observed in many villi with exaggeration of blood (Figure 2a and 2b).

-Another consistent finding in villous parenchyma of diabetic placenta was villous edema, which is accumulation of fluid in the villous interstitium with disruption and replacement of intravillous cellular architecture (Figure 3a and 3b).

-Another morphological aspect reported in this group was a cellular eosinophilic, homogenous material (fibrinoid) which was seen in this group, both extravillous and intravillous. Intravillous fibrinoid appearing in the subtrophoblastic space that finally occupies the whole villous stroma. This type of fibrinoid either fills gaps in the trophoblastic layer, or includes the all chorionic villi or groups of villi (Figure 4a and 4b)

### Histochemical examination

#### Periodic acid-Schiff stain (PAS)

**Group 1 (Control group):** Chorionic villi showing a faintly

positive PAS stain in the villous core and basement membrane of the trophoblasts. Cytotrophoblast cells seen within the villous stroma. Syncytial lining is present at places. Syncytial knots are plenty (Figure 4a and 4b).

- Marked thickening of the basement membrane of syncytiotrophoblast was seen. This phenomenon was significant in placenta of diabetic women, which show strong reaction with PAS stain (Figure 5a and 5b).

- Glycogen deposits: Glycogen PAS reactivity was stronger in diabetic placentas than in normal placentas. PAS staining identify clusters of PAS positive material with subsyncytial location and in the thickened basement membranes (Figure 6a and 6b).

#### Mallory's trichrome

**Group 1 (Control group):** Cross sections of full-term placenta of this group showed normal histological architecture (Figure 6a and 6b).

**Group 2 (Diabetic group):** -The amount of interstitial collagen between the villous was significantly increased in Diabetes mellitus group compared with the control groups.

-Villous stromal fibrosis was also significantly increased (Figure 7a and 7b).

#### Van Gieson stain

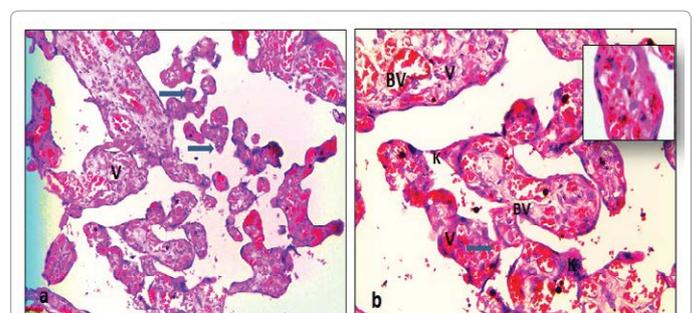
**Group 1 (Control group):** Chorionic villi showing a few collagen fibers scattered between the Villi (Figure 8a and 8b).

**Group 2 (Diabetic group):** Staining the sections of placenta from diabetic group by Van Gieson stain confirms the presence of fibrin and fibrosis in the chorionic villi that obtained by H&E, Mallory's trichrome stains respectively (Figure 9a and 9b).

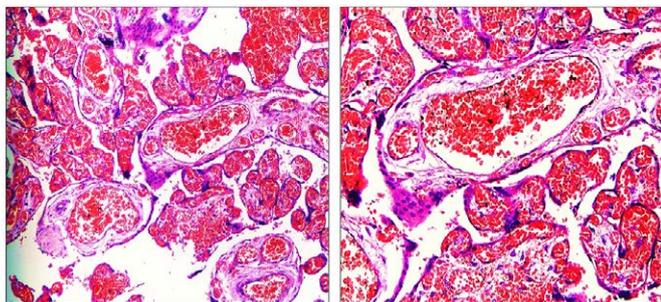
#### Methyl Green Pyronin (MGP)

**Group 1 (Control group):** The histological Sections of placentas of control group showed strong reaction for methyl green (bluish green color) and pyronin reaction (red color) in chorional epithelium as well as in the red blood cells of the fetal blood vessels (Figure 10a and 10b).

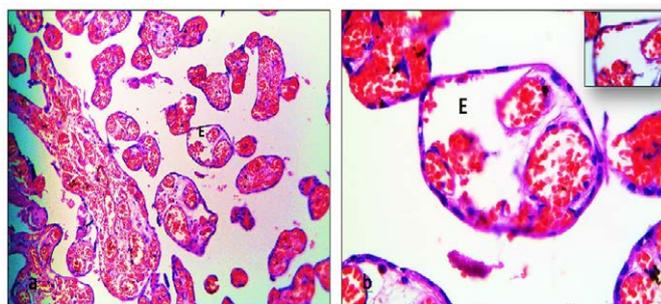
**Group 2 (Diabetic group):** Extremely weak reaction was observed in placentas of diabetic group compared with control cases (Figure 11a and 11b).



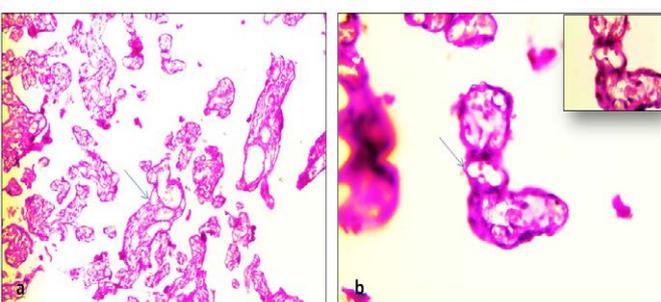
**Figure 1:** Photomicrographs of control group of human placenta showing; a) A full-term placenta includes sections of many villus stems (V), containing blood vessels (BV), smaller villus branches (arrows) which contain connective tissue and microvasculature, (H&E 10X). b) A higher magnification of previous micrograph, denoting the villus connective tissue contains blood vessels (BV), nuclei of the syncytiotrophoblast layer have formed clusters or knots (K) on the surfaces of villi, (H&E 20X). Inset



**Figure 2:** Photomicrographs of diabetic group of placenta showing; a) villi with persistence of central vessels and increased number of congested blood vessels (CBV), (H&E 10X). b) congested blood vessels (CBV) of the extraembryonic vasculature and dilated blood vessels (DBV), (H&E 20X).



**Figure 3:** Photomicrographs of diabetic group of placenta showing; a) A full-term placenta includes sections of many villus stems (V), containing edema (E), (H&E 10X). b) At higher magnification, the villus connective tissue denoting edema (E), (H&E 40X). Inset



**Figure 4:** Photomicrographs of control group of human placenta depicting a) faintly positive PAS stain in the villous core and basement membrane of the trophoblasts. Cytotrophoblast cells seen within the villous stroma. Syncytial lining is present at places. Syncytial knots are plenty (10x). b) A higher magnification of previous micrograph (40x). Inset

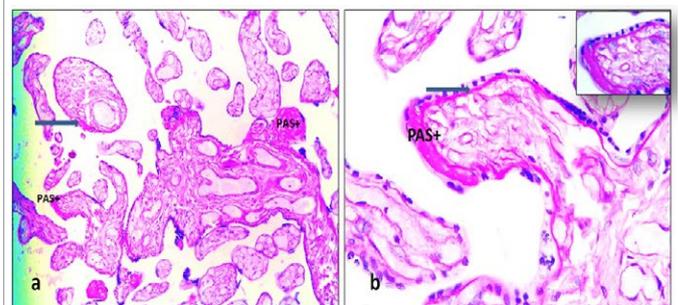
#### Immunohistochemistry (CD 34 immunohistochemistry)

**Group 1 (Control group):** In control group, the fetal blood vessels were uniformly distributed throughout the chorionic villi as shown by immunohistochemical staining of CD34 (minimum villous density), the central and peripheral parts of placentas of non-diabetic mothers, showed minimum villous vascular density (Figure 12a and 12b).

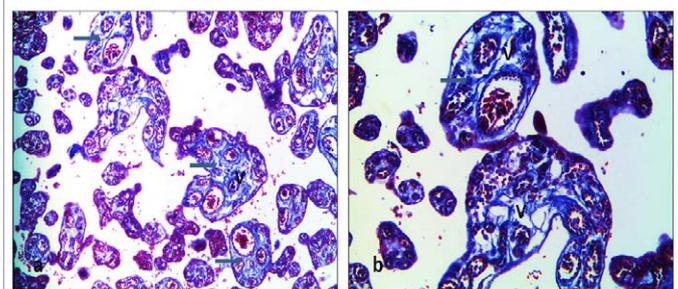
**Group 2 (Diabetic group):** In diabetic placentas, fetal blood vessels were abnormal and the chorionic villi showed excessive vascularisation i.e. CD34 immunoreactivity was strongly positive (maximum villous vascular density). Maximum villous vascular density was observed in the central and peripheral parts of the placentas of diabetic mothers (Figure 13a and 13b).

#### Discussion

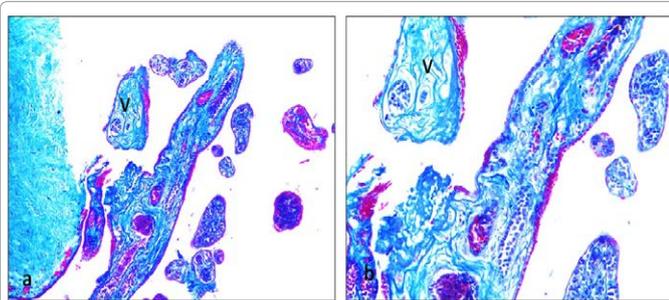
Diabetes mellitus is a common metabolic disorder, characterized by chronic hyperglycemia. It is a major cause of morbidity and mortality from long term diseases of major organ systems [7]. Apart from affecting major organ systems, D.M during pregnancy produces complications both in mother and offspring [3]. The placenta is highly specialized organ of pregnancy that supports normal growth and development of the developing fetus. The main functional unit of the placenta is the chorionic villi [2]. Gross observation in this study indicates that all placentas from pregnancies complicate by D.M, regardless age and onset of diabetes were larger in size and more in weight as compare to non-diabetic control groups. These findings are comparable to the findings of a study carried out by [8] which showed significant increase in the weight and size of placentas of diabetic mothers. But these observations are not agreed with study in which the mean weights were similar in diabetic and normal pregnancy [12]. The pathophysiology behind weight gain includes compensatory hyperplasia due to fetal macrosomia. In our study, microscopic examination of the placentas of diabetic women showed numerous pathological abnormalities such as dilated blood vessels, villous edema, fibrinoid necrosis, thickening of the basement membrane and stromal villous fibrosis. Some workers have claimed that, the placentas of diabetic women show no unusual features [12]. Furthermore studies, showed no major difference was in the microscopic changes of placentas in different groups according to White's classification of gestational diabetes mellitus [13]. While Verma in 2010 [14] has observed frequent abnormalities in such placentas but failed to agree on any consistent histopathological pattern.



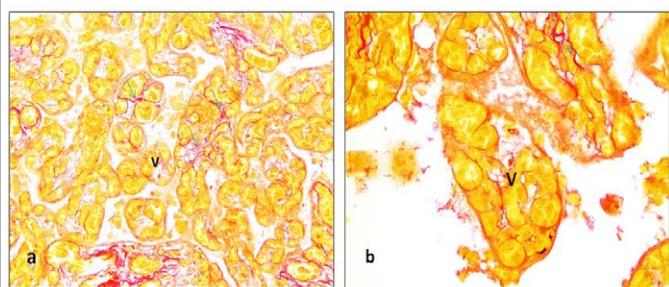
**Figure 5:** A photomicrograph of control group of human placenta denoting; a) stronger periodic acid-Schiff (PAS) reaction in diabetic placentas than in normal placentas (10x). b) PAS staining identify clusters of PAS positive (PAS+) material with subsyncytial location and in the thickened basement membranes (arrows) (40x). Inset



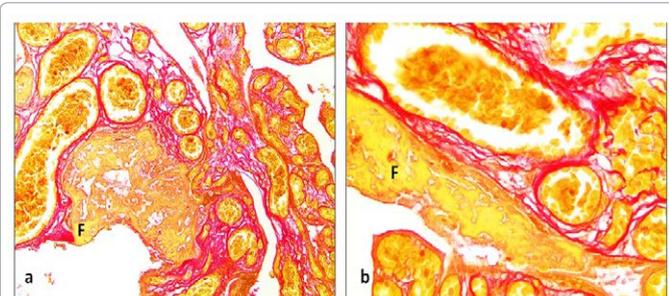
**Figure 6:** A photomicrograph of control group of human placentas showing; a) more cellular less fibrous contents of villus connective tissue (V), (10x). b) A higher magnification of previous micrograph (H&E 20X).



**Figure 7:** A photomicrograph of diabetic group of human placentas showing; a) increased amount of interstitial collagen & Villous stromal fibrosis (V), (stained blue). b) a higher magnification of Villous stroma (V) indicate increase in fibers (stained blue) (20x).



**Figure 8:** A photomicrograph of control group of human placenta showing; a) red color of collagen fibers (arrow) in interstitial tissue of villi (V), (10x). b) a higher magnification of previous A photomicrograph, (40x).



**Figure 9:** A photomicrograph of diabetic placenta showing; a) the increased amount of collagen fibers (red color) in interstitial tissue of villi in comparison with the control groups and presence of fibrin deposits (F) (10x). b) massive perivillous fibrin deposit (F), yellow in color, (40x).

### Dilated and increased number of villous capillaries

In our study, we recognize marked increase in number of villous capillaries with congestion of most of them and dilatation of the others. Some literatures have reported proliferation of small fetal vessels [15], while Geppert in 1982 [16] stated that in diabetic women, 25% of villous surface is taken up by the capillary, while the normal placenta showed 50% of the surface of the villous is taken up by the capillary surface. The increased number of villous capillaries was observed more often in the diabetic placentas compared with control placentas [17]. While Jones and Fox in 1976 [18] has shown Patchy focal syncytiotrophoblastic necrosis and indirect evidence of syncytial damage in the form of marked cytotrophoblastic hyperplasia. The syncytial necrosis appeared to be lysosomally mediated, possibly as a result of altered intracellular pH. Occasional cytotrophoblastic cells also showed degenerative changes. However Verma in 2011[19] did not notice any marked changes in the fetal capillary except that the proliferation of the fetal

capillaries in few placentas of women with gestational diabetes treated properly by insulin.

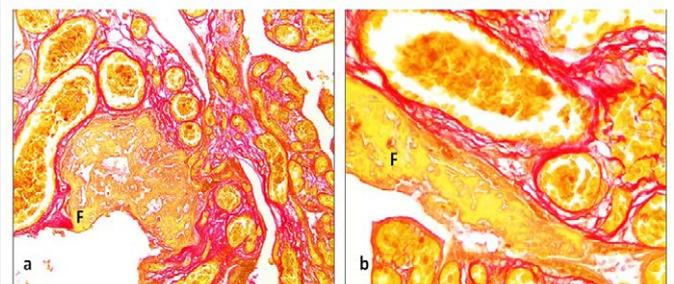
### Villous edema

The edema is accumulation of fluid in the villous interstitium with disruption and replacement of intravillous cellular architecture, it is considered to be histopathologically significant and one of the causes of fetal ischemia [20]. In our study, we noticed this type of changes with focal distribution on the distal villi in most studied cases. Starting from the fact that hyaluronic acid molecules can retain water, it was concluded that, the appearance of the true villous edema in placentas of diabetic mothers is probably the result of the appearance of abnormal deposits of mucopolysaccharides in villous stroma [21]. Verma in 2011[19] reported that, the diabetic mothers on diet control showed a mild villous edema in the villous core, in comparison with the marked villous edema that was seen in the placentas of whom controlled by insulin.

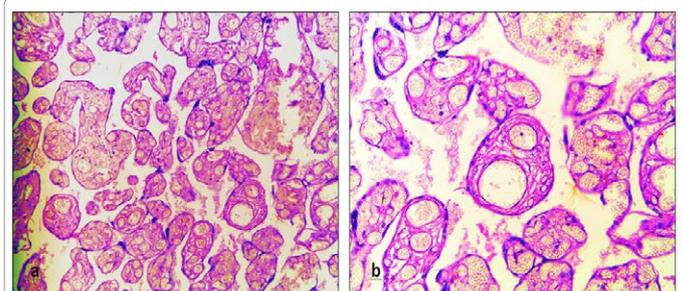
### Fibrinoid

Fibrinoid is an 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 superficial layer is a fibrinous layer (blood origin). This fibrinous layer can be in contact with the villous trophoblastic blade or followed by a homogeneous layer of matrix- type fibrinoid. Intra- villous fibrinoid deposits are increased in pathological conditions including diabetes [20].

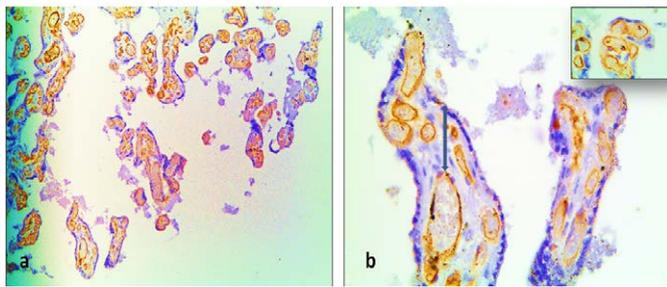
In our study we recognized both types of Fibrinoid in almost all of the cases. Disseminated deposits of fibrinoid material in the intervillous space and/or the villous tree is a common phenomenon observed in the fullterm placentas [20], which is often incompatible with normal fetal development [22].



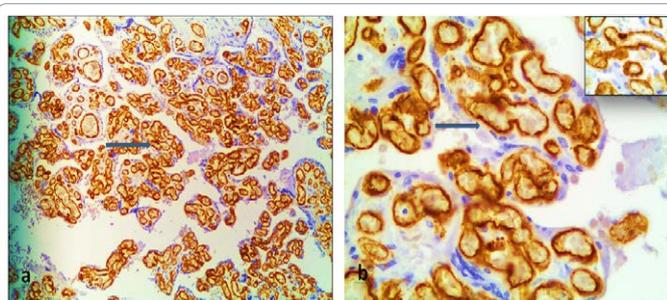
**Figure 10:** A photomicrograph of control group of placenta showing; a) strong reaction for methyl green pyronin reaction (red color) in chorionic epithelium as well as in the red blood cells of the fetal blood vessels. b) strong red reaction (arrow) in chorionic epithelium (40x).



**Figure 11:** A photomicrograph showing; a) Extremely weak reaction was observed in placentas of diabetic group compared with control cases (10x). b) higher magnification of diabetic group stained with MGP (40x).



**Figure 12:** A photomicrograph of control group of placenta staining of CD34 showing; a) minimum villous vascular density, (10x). b) the endothelium of villous vascular (arrow), (40x).



**Figure 13:** A photomicrograph of diabetic placentas showing; a) the chorionic villi showed excessive vascularization i.e. CD34 immunoreactivity was strongly positive (arrow), (10x). b) Maximum villous vascular density in the central and peripheral parts of the placentas of diabetic mothers. (40x). Inset

Fibrinoid was also reported by many authors as Younes in 1996 [23] has defined focal fibrinoid necrosis as an increase in the number of placental (Hofbauer) cells, While Daskalakis in 2008 [24] reported fibrinoid as a degenerative change in the diabetics.

### Thickening of the basement membrane

Another morphological aspect reported in our study was the marked thickening of the basement membrane of syncytiotrophoblast, which was seen in the majority of the placentas of diabetic women compared with control group. This result is in agreement with other studies where the thickening of basal membranes of trophoblast and syncytiotrophoblast was seen. Younes in 1996 [23] have measured the thickening of the basement membrane of the trophoblastic villi ( $3.2 \pm 0.35$  micrometers) and the amniotic membrane ( $1.8 \pm 0.3$  microns). Jones and Fox in 1976 [18] stated that, the focal thickening of the villous trophoblastic basement membrane was seen and this did not appear to be due to deposition of immune complexes. Khaskhelli in 2013 [25] has stated that, on histological examination of diabetic placentas, the sub-trophoblastic basement membrane thickened by a mean of 1.923 in comparison with the non-diabetic placentas which showed a mean thickening of 0.5641, and the overall difference in the mean was 1.359. He also observed a significant thickening of the basal membranes of the trophoblast. This findings were also reported by [4,19,26,27].

According to Gheorman in 2012 [20] this phenomenon is significant for women with uncontrolled diabetes, while it has a minimal affection on women with well controlled diabetes, and it is of no value in women without diabetes.

This thickening is the result of the mucopolysaccharide storage, 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 the constituents of the basement membrane are produced by the secretion of trophoblast [28,29].

### Glycogen deposits

In our study, we noted clusters of PAS positive material with subsyncytial location and in the thickened basement membranes. Glycogen accumulations were seen in placentas of all women with gestational diabetes, whether they are controlled by diet or by insulin [19].

Also, the glycogen accumulation was found within the stromal cells, corresponding to the higher glycogen content of diabetic placentas [30]. Small clusters of PAS+ material with subsyncytial location and focal deposits are located both in thickened basement membranes and especially in the terminal villous extracellular matrix [20]. PAS reactivity for glycogen in diabetic placenta is stronger than normal placentas [31].

Several studies have reported the occurrence of mucopolysaccharide deposits which consist mainly of hyaluronic acid molecules in all cases of villous edema [31,32]. However, rigorous studies have shown the presence of more significant deposits of glycogen in placentas of diabetic mothers only around the large fetal vessels [33].

Glycogen has been identified in the amniotic epithelial cells cytoplasm in early pregnancy stages, but this accumulation revealed no quantitative changes related to the existence of maternal diabetes [34-36].

According to some authors, placental histological changes in diabetics are mainly due to metabolic disorders that led to the accumulation of carbohydrates and lipids in the placenta. The accumulation of carbohydrates, particularly glycogen, is paradoxical, as in other maternal organs the glycogen content decreases in diabetes, and this was identified using a histochemical stain specific for glycogen (PAS staining) [31,37].

### Stromal villous fibrosis

The underlying cause of villous fibrosis is still unclear. One theory holds that, collagen production may be stimulated by the increased partial pressure of intravillous oxygen. As diffusion of oxygen from maternal space into the stroma, in the face of inadequate uptake by fetal capillaries, due to poor oxygen perfusion of the villous tree, might result in an increase in the oxygen content in the stroma and stimulates collagen synthesis [38]. But Verma in 2010 [14] suggested that placental infections via maternal blood stream increased in the uncontrolled diabetes. He reported that these hematogenous infections are most often associated with chronic inflammation of the villi stroma with subsequent fibrosis.

### Methyl green pyronin reaction

Methyl green pyronin reaction demonstrates two nucleic acids, namely DNA which is the main nuclear component, and RNA which presents mainly in the cytoplasm and to a less extent in the nucleus. DNA is self-replicating and determines the genetic characters, while RNA is particularly concerned with protein synthesis [39]. Methyl green pyronin stain of the chorional epithelium showed extremely weak reaction of the karyoplasm of cytotrophoblasts compared with control cases, denoting a decrease in DNA and RNA content of cytotrophoblasts. Gersell and Kraus in 2011[40] reported that the function of the trophoblasts is influenced by the utero-placental perfusion, which is reduced in diabetic pregnancy.

Madazli in 2008 [36] reported that ischemia is significantly increased in the placentas of the diabetic women and affects protein synthesis. This is not in agree with Elchalal in 2005 [41] which reported that trophoblasts and protein synthesis are stimulated by insulin. Also Alonso in 2006 [42] concluded that during gestational diabetes, the placenta is exposed to structural and functional alterations, which are indicative of the protective role of the placenta. But Pathmaperuma in 2010 [43] concluded that hyperglycemia causes intracellular glycogen accumulation with no other effects on trophoblast metabolism or protein synthesis.

### CD34 immunohistochemistry

CD34 is expressed in vascular endothelial cells of the fetal capillaries. There is an evidence for adhesion –related role for CD34 among endothelial cells [20]. Benirschke in 2006 [28] stated that high level of CD34 is typical for diabetic placenta and associated with the number of capillaries. There have been few reports, describing chorionic vascular profiles in diabetic pregnancy. Therefore, in this study, chorionic villous vascularization was investigated using CD34 immunohistochemistry. We noticed an unusual increased chorionic capillary densification in the placentas of the diabetic group. Some authors as Lewis and Benirschke in 2007 [44] considered that the increase in the density and the number of the capillaries is an indicative sign of chronic hypoxic changes associated with diabetes.

### Conclusion

This study supports the view that there is a very strong relationship between the placenta and diabetes mellitus. Clinically, the adverse effects of diabetes on the outcome of pregnancy are well established but we have seen their gross morphological and microscopical impacts on placenta. Diabetic placenta showed an increase in weight as compared to the normal placenta. Distinct histological and histochemical changes were seen in placentas of diabetic pregnant females. Microscopic examination exhibited several changes as: excessive fibrosis, increased number of chorionic fetal blood capillaries, congestion of blood vessels, villous edema, thickness of the basement membrane of the syncytiotrophoblast and increased glycogen deposits. Further researches to study these changes in vivo would be worthwhile.

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