

Effect of Gestational Diabetes on Gross Morphology, Histology and Histochemistry of Human Placenta

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Abstract

Gestational diabetes is the glucose intolerance of varying severity and complicates about 2-4% of pregnancies. While there is a surfeit of associative data that demonstrate the placental adaptive responses to gestational diabetes, the mechanisms at placental level remain elusive.

One objective of this study was to investigate various anatomical, histological and some histochemical changes in placenta of gestational diabetes patients with re-evaluation of some mechanisms of placental adaptive responses to gestational diabetes. A second objective was to find whether the placenta adapts to diabetes and ultimately protects the fetus or whether it contributes to the adverse fetal outcome with diabetic pregnancies despite good care of these gestations.

Two groups each of 30 placentas were collected at term and post Caesarian Section (CS) deliveries as one group was the control group (control) and the other group was collected from patients with gestational diabetes and were treated with zinc insulin. After morphological data assay, central and peripheral biopsies were processed for histological and histochemical assay.

The diabetic placentas showed mild increase in diameter, central thickness and weight. This study confirmed that the villous portion with its corresponding intervillous space is the structural and functional unit of the placenta. Syncytial clumps among peripheral placenta were bigger than those of central placenta of the diabetic group and best examined by Hematoxylin and Eosin stain and to a lower extent by Van Gieson stain for light microscopy. The diabetic placentas showed marked increase of the chorionic villi which appeared more crowded centrally while the villous vasculature was higher peripherally. The increased young, immature and unspecialized villi among the diabetic placentas explained the enhanced fetal hypoxia with subsequent increased neonatal morbidity and mortality. These anatomical, histological and histochemical findings put diabetes in the moderate-high risk factors of vascular placental pathology. Also, placenta was not the primary cause to markedly affect the perinatal morbidity as the placenta showed a good degree of potentiality to adapt with derangements of gestational diabetes. So, the elevated rates of perinatal morbidity and mortality among diabetic deliveries were most probably due to metabolic abnormalities occurred in mother and fetus because of whatever kind of diabetes.

Conclusion: Placenta itself is always perfect, innocent and helpful in managing and preventing complications via its endogenous mechanisms. It was necessary histologically to examine several preparations with different and specific measures to obtain detailed picture of the totality of the placenta structure. Lastly, the premium key in gestational diabetes is to apply scientific exogenous measures in harmony and accordance with early diagnosed and strictly controlled endogenous placental measures.

Keywords: Gestational diabetes; Human placenta; Morphology; Histology

Introduction

Gestational Diabetes Mellitus (GDM) is described as glucose intolerance of varying severity with the onset of first recognition during pregnancy and disappears with delivery [1]. Gestational diabetes complicates approximately 2-4% of pregnancies and it is the major cause of macrosomia and perinatal mortality and usually associated by clinical hyperglycemia, hyperlipidemia, hyper-insulinemia and placental endothelial dysfunction [2,3]. Classical morphological investigations of placental structure have shown a varying degree of changes in the syncytiotrophoblast, cytotrophoblast, trophoblastic basement membrane, and fetal vessels [4,5].

Overall, since 1950s [6-8] most authors reported a relative placental immaturity due probably to a high proportion of villi with stromal edema and focal fibrinoid necrosis [9-11]. While there is a surfeit of associative human and animal data that demonstrate the placental adaptive responses to gestational diabetes, the mechanisms at the placental level remain elusive. One objective of this study was to investigate various anatomical, histological and some histochemical changes in placenta of gestational diabetes patients with re-evaluation of some mechanisms of placental adaptive responses to gestational diabetes. A second objective was to find whether the placenta adapts to

diabetes and ultimately protects the fetus or whether it contributes to the adverse fetal outcome with diabetic pregnancies despite good care of these gestations.

Methods

Placentas

Thirty placentas from women with non-complicated (well controlled) Gestational DM and all were treated with zinc insulin and were referred to as the diabetic group. All were chosen randomly of variable ages, parities, races, weights, heights and socio-economic states. The control group included 30 placentas were collected from

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normal healthy women. All the placentas were collected at term and post Caesarian Section (CS) delivery in both groups with a consent from chosen women. Mothers were assessed for number of gravida, para, and abortions or stillbirth while babies were assessed for their weight and condition (alive, distressed or dead). The placentas immediately in fresh state post-delivery were examined for shape, color, diameter, thickness, weight, attachment of umbilical cord (cord centrality) and number of cotyledons and then provided to the Histology Department where biopsies were taken from both central and peripheral areas for further details.

Histological and Histochemical Methods

Placental biopsies - central and peripheral - were fixed in 10% formal saline followed by dehydration in ascending grades of alcohols (50-100%). Clearance was held with xylene then impregnation in three successive changes of soft paraffin at 50°C and embedding in paraffin wax. Five micrometer-thick serial sections were cut, mounted and stained by Harris' Hematoxylin and Eosin (H&E.), Masson's Trichrome (Aniline Blue or Light Green), Van Gieson's method, Mc Farlane's modification of the Picro-Mallory method and histochemical Periodic Acid-Schiff (PAS) reaction.

All chemicals utilized in the present study were products of Sigma Chemical Company (St. Louis, MO, USA).

Data analysis

Analysis of statistical data was conducted for mothers' parameters, babies' weights and placentas morphology. The critical value for significance was $P \leq 0.05$.

Results

Gross anatomical results and statistics: Figure 1 (Histogram) represented the placentas of control group with normal discoid (45%) or oval (55%) flat-cake shape, dark bluish maroon color, eccentric-attached cord (70%) or central (30%), mean diameter of 18.15 cm, mean central thickness of 2.43 cm, mean number of cotyledons of 19.8 and mean weight of 643.5 g.

The placentas of the diabetic group showed a flat-cake shape tending to be oval (60%) more than the normal control (55%) as the oval shape was suitable with the larger placentas often present with diabetic pregnancies. Diabetic placentas had dark bluish-maroon color and the eccentric cord-attachment was more prevalent (80%) than that of the control placentas (70%). The eccentric cord could be due to the more prevalent oval shape of the enlarged diabetic placentas. No considerable infarctions, hematomas or calcifications were noticed among the maternal placental surface of the diabetic group.

Figure 2 (Histogram) showed the diabetic placentas with a mild increase in mean weight (678.08 g), higher than controls (643.5 g) by a mean difference of 34.58 g.

As regard the mean baby weight, there was a highly significant (*) increase ($P < 0.0005$) among Infants of Diabetic Mother (IDM) weighed 3.898 Kg, higher than controls (3.310 Kg.) with a mean difference of 588 g.

Table 1 showed a mild increase of the mean diameter (18.25 cm) among diabetic placentas higher than controls (18.15 cm) with a mean difference of 1.0 mm. Also, there was a mild increase of the mean central thickness (2.67 cm), higher than controls (2.43 cm) with a mean difference of 2.4 mm. Cotyledons' mean number appeared almost the same (19.86) as normal controls (19.8). There was no significant

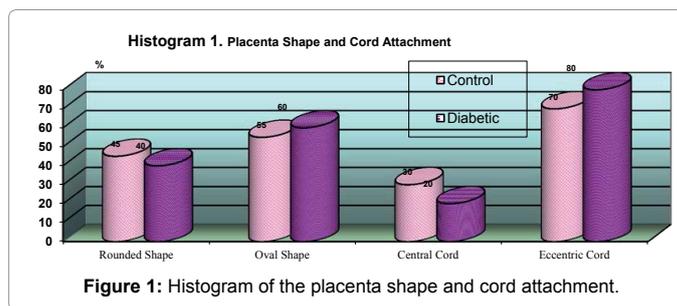


Figure 1: Histogram of the placenta shape and cord attachment.

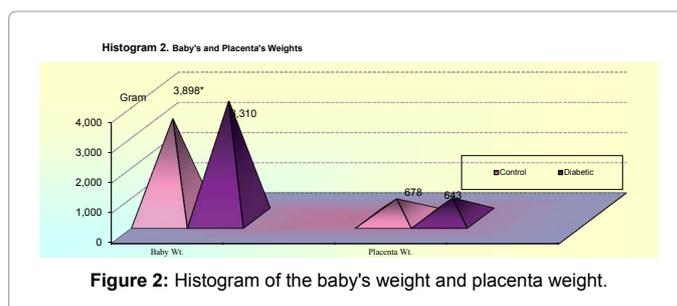


Figure 2: Histogram of the baby's weight and placenta weight.

difference between the diabetic and control groups as regard maternal history for gravidity (gravida) and parity (para).

Histological Results

By Hematoxylin and Eosin stain, the central section of gestational diabetic placenta (Figures 3-7) showed the capsule with normal cellular and fibrous content while the chorionic villi appeared crowded and markedly increased with no marked increase in their capillaries. Vertically, the intervillous sinuses (spaces) at the maternal side were congested with maternal blood but they were less filled with blood at the fetal (basal) side where the main feeding (fetal) blood vessels appeared congested and dilated. The peripheral section of gestational diabetic placenta (Figures 4, 6 and 8) showed the capsule slightly thickened and increased number of chorionic villi but less frequent than the central section. Markedly increased capillaries were noticed in villous cores and markedly increased size and number of syncytial clumps on the villous surface. The capillary endothelial cells were more flattened while their basement membrane was intact and thickened. The villous stroma was not increased and stromal edema spaces were moderate while stromal lipid content appeared as vacuoles and obviously decreased.

By Masson's Trichrome stain (Figures 9-12), Van Gieson stain (Figures 13-16) and Mallory stain (Figures 17-20) confirmed the same results of H&E, but explored mature RBCs, basement membranes and fibrinoid material in a better way.

Histochemical Results

By PAS stain, the gestational diabetic placenta showed strong positive PAS reaction at the central capsule especially the deep areas while the peripheral capsule appeared thickened and showed strong reaction at both deep and circumferential areas due to the increased peripheral fibrinoid content (increased fibrinoid necrosis) (Figures 21 and 22). The central trabeculae also showed strong reaction while the villous cores showed moderately high reaction at central sections whereas the reaction appeared moderate at peripheral sections. Little fibrinoid material was noticed around both central and peripheral villi.

Peripheral villi showed more frequent blood capillaries than

Independent samples Test					
t test for Equality of Means					
		t	df	Sig. (2-tailed)	Mean Difference
GRAVDA	Equal Variances Assumed	-1.484	48	0.144	-0.83333
PARA	Equal Variances Assumed	-0.138	47	0.891	-8.62E-02
BABY_WT	Equal Variances Assumed	-4.494	48	0	-0.58848
PL_WT	Equal Variances Assumed	-0.67	48	0.506	-34.58
Central Thic	Equal Variances Assumed	-1.765	48	0.084	-0.2467
DIAMETER	Equal Variances Assumed	-0.234	48	0.816	-0.1
No of Cotyledons	Equal Variances Assumed	-0.176	48	0.861	-6.67E-02

Group Statistics					
	Status	N	Mean	Std. Deviation	Std. Error Mean
GRAVDA	1	20	3.90000	1.74416	0.39001
	2	30	4.73333	2.06670	0.37733
PARA	1	20	2.50000	2.50000	3.4412
	2	29	2.58621	2.58621	0.45895
BABY_WT	1	20	3.30975	3.30975	0.11579
	2	30	3.89823	3.89823	7.41E-02
PL_WT	1	20	643.50000	643.50000	15.78265
	2	30	678.08000	678.08000	40.69551
Central Thic	1	20	2.42500	2.425000	0.11047
	2	30	2.66667	2.66667	8.42E-02
Diameter	1	20	18.15000	18.15000	0.43695
	2	30	18.25000	18.25000	1.93E-01
No of Cotyledons	1	20	19.80000	19.80000	0.30435
	2	30	19.86667	19.8667	0.23358

*Significance at P<0.05 level. *t=t-value *d.f.=degree of freedom
 **Baby weight was significantly increased; 3.898-3.310=0.588 Kg=588 g
 **Placental weight was insignificantly increased; 678.08-643.5=34.58 g
 **Central thickness was insignificantly increased 2.667- 2.425=0.238 cm=2.38 mm.
 **Diameter was insignificantly increased; 18.25-18.15=0.1 cm=1.0 mm
 **Cotyledons number was insignificantly increased; 19.86-19.80=0.06.

Table 1: Group Statistics for T-Test and Significance (2-tailed). Status 1=Control Group. Status 2=Diabetic Group.

central villi but central capillaries were more congested. The basement membranes of all capillaries were thickened and manifested a strong PAS reaction. The main feeding blood vessels were congested and dilated especially at central placenta. Note the condensation of reticular fibers interior to the syncytial cell layer which had no basement membrane particularly at peripheral villi (Figures 23 and 24).

Discussion

As stated by Dubova et al. [12] and Huynh et al. [13] that placental vasculopathic abnormalities differ by maternal diabetes type, potentially reflecting underlying pathophysiologic mechanisms, we secluded the present study to women gained GDM and excluded any woman who already had any type of DM before pregnancy. The placentas of the control group appeared discoid or flat-cake, dark bluish brown with more centrally-attached cord than diabetic placentas and having a mean placental diameter of 18.15 cm, a mean central thickness of 2.43 cm, a mean number of cotyledons of 19.8 and a mean weight of 643.5 g. These measures were within the same range of the results of some previous studies [14,15].

No considerable infarctions, thrombosis, hematomas or calcifications were noticed among the maternal placental surface among the diabetic group which was contradictory to Salge et al. [16] who recorded those findings among diabetic groups, which may be attributed to poorly controlled diabetes of their studied patients, or might be attributed to placental hypoxic overlap lesions (acute-on-chronic) being associated with clinical complications of pregnancy and

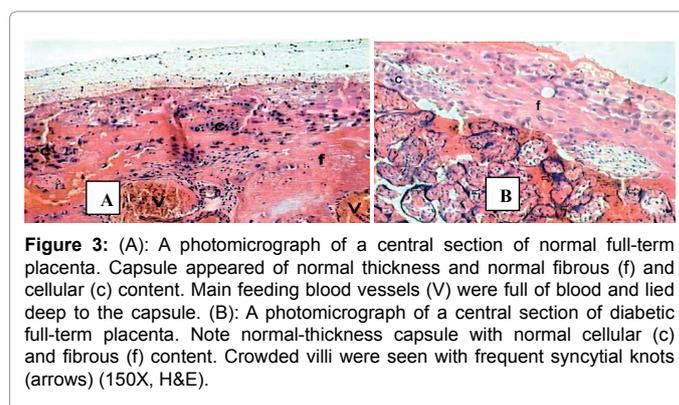


Figure 3: (A): A photomicrograph of a central section of normal full-term placenta. Capsule appeared of normal thickness and normal fibrous (f) and cellular (c) content. Main feeding blood vessels (V) were full of blood and lied deep to the capsule. (B): A photomicrograph of a central section of diabetic full-term placenta. Note normal-thickness capsule with normal cellular (c) and fibrous (f) content. Crowded villi were seen with frequent syncytial knots (arrows) (150X, H&E).

predispose to thrombotic lesions as stated by Stanek [17].

The placentas of the diabetic group showed a mild increase in diameter, central thickness and weight when compared with the controls, which had been similarly found by many previous researchers [18,19]. This could be attributed to placental hyperplasia in response to diabetes and appeared in the form of a moderate increase in parenchymatous (syncytio-vascular) tissue and the significant accumulation of non-parenchymatous tissue (stroma, glycogen, lipids, tissue fluid edema) according to the results of this study and many previous ones [19,20].

The increased mean diameter (18.25 cm) and mean central thickness (2.67 cm), although insignificant, but denoted parallel to the increased

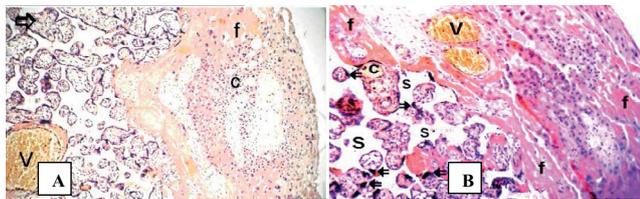


Figure 4: (A): A photomicrograph of a peripheral section of normal full-term placenta. The capsule appeared of normal thickness with normal fibrous (f) and cellular (c) content. Lower amount of chorionic villi appeared containing more capillaries (arrow). Main feeding blood vessel (V) was full of blood. (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed thickened capsule with increased fibrinoid material (f). Less crowded villi were seen with increased villous capillaries (C) while the syncytial knots (arrows) were increased in size. The subcapsular feeding vessels (V) were congested and dilated while less amount of maternal blood was seen in the intervillous sinuses (S) (150X, H&E).

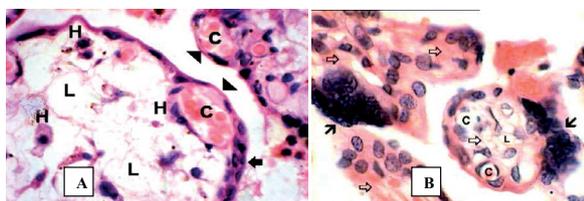


Figure 5: (A): A photomicrograph of a central section of normal full-term placenta. A syncytial clump (arrow) was thin and kept apart from the villous capillary (C). The syncytial covering (arrowhead) was thin and attenuated at the villous capillaries. Macrophages Hofbauer cells (H) were acidophilic and seen around capillaries and in villous core. Intercellular lipid (L) seen as vacuoles were frequent in the villous core. (B): A photomicrograph of a central section of diabetic full-term placenta showed numerous and big syncytial clumps (dense arrows). The villous stroma was increased (lucent arrow) and edema spaces were mild. Lipid seen as vacuoles (L) was decreased. Villous capillaries (C) were not increased (1000X, H&E).

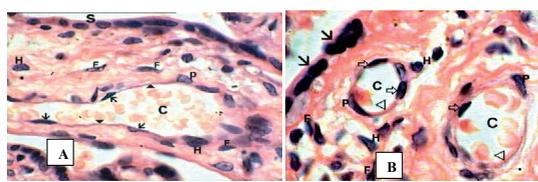


Figure 6: (A): A photomicrograph of a peripheral section of normal full-term placenta. A villous capillary (C) cut longitudinally and showed intact endothelial cells (arrows) with intact basement membrane (arrowheads), Pericytes (P), Hofbauer phagocytes (H) near the blood vessel, Fibroblasts (F) and syncytial cells (S) with dark nuclei and no clear cell boundaries. (B): A photomicrograph of a peripheral section of diabetic full-term placenta. Many capillaries (C) were seen in the villous core with intact endothelial cells (lucent arrows) and pericytes (P) while the basement was thickened (arrowheads). Hofbauer phagocytes (H) were seen near the villous capillaries while fibroblasts (F) were seen in a normal stroma. Dark nucleated syncytial cells (dense arrows) were seen on the villous margin (1000X, H&E).

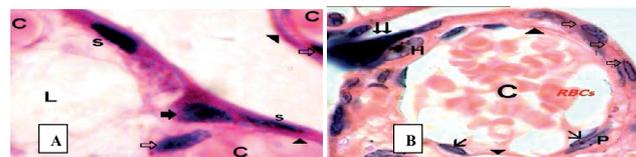


Figure 7: (A): A photomicrograph of a central section of normal full-term placenta. Flattened endothelial cells (lucent arrows) of villous capillary (C) rest on a basement membrane (arrowheads) with normal thickness and surrounded by a pericyte cell (dense arrow). Note the dark nucleus of a syncytial cell (s) with basophilic dark cytoplasm. The syncytial layer was attenuated at the capillary side to the surface. Intercellular lipid (L) seen as vacuoles in the villous core. (B): A photomicrograph of a central section of diabetic full-term placenta showed a big syncytial clump (double arrows) while the villous capillary (C) was congested with thickened basement membrane (arrowheads) under intact endothelial cells (dense arrows). Pericytes (P) appeared normal surrounding the endothelial cells. Hofbauer phagocyte (H) was located near the villous capillary. The syncytial layer appeared attenuated over the capillary surface (lucent arrows) (2000X, H&E).

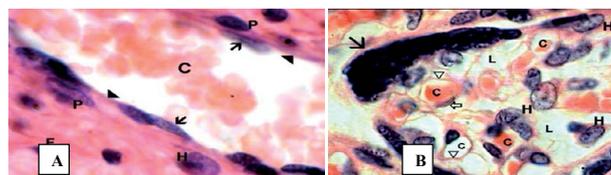


Figure 8: (A): A photomicrograph of a peripheral section of normal full-term placenta. A villous capillary (C) showed intact flattened endothelial cells (arrows) with normal thickness intact basement membrane (arrowheads), pericytes (P), Hofbauer phagocytes (H) near the blood vessel, and fibroblasts (F). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed a big syncytial clump (dense arrow). Many capillaries (C) were seen in the villous core with intact and thickened basement membrane (arrowheads) basal to intact endothelial cells (lucent arrow). Hofbauer phagocytes (H) were seen near the villous capillaries. Decreased vacuoles were seen represented stromal lipid (L) while stromal edema spaces were moderate (2000X, H&E).

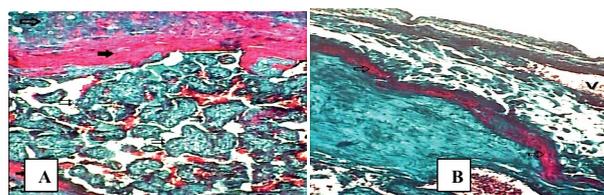


Figure 9: (A): A photomicrograph of a central section of normal full-term placenta showed the capsule (up) and the trabeculae (down) with normal collagen (lucent arrows) and fibrinoid (dense arrows) content. The chorionic villi (double arrows) were of normal amount and a normal fair amount of maternal blood in the intervillous spaces. (B): A photomicrograph of a central section of diabetic full-term placenta showed normal-thickness capsule with its collagen (blue) while the fibrinoid (arrows) content (pinkish red) appeared moderately decreased. The subcapsular blood vessels (V) appeared congested and dilated (150X, Masson's Trichrome).

mean placental weight and hyperplasia among the diabetic group. Increased placental diameter represented an increase in the area of endometrial attachment i.e. placental exchange area, whereas increased central thickness represented an increase of trophoblastic angiogenesis and density of blood vessels i.e. placental efficiency. This adaptive response of the placenta to gestational diabetes was explained by some

previous studies [18] where the role of increased trophoblast (both amount and function) was compensatory to the changes in placental transport activity, hormone production and substrate metabolism due to diabetes. This correlation may appear contradictory to the study of Pathak et al. [21] who concluded that the macroscopic morphological features of the placenta cannot predict the presence or absence of the

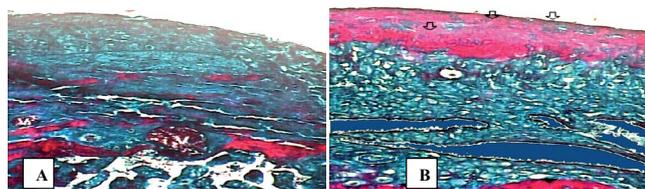


Figure 10: (A): A photomicrograph of a peripheral section of normal full-term placenta showed normal-thickness capsule with its collagen (blue) content. The fibrinoid (pinkish red) content appeared mainly at the deep area of the capsule. The subcapsular blood vessels (V) were seen of normal size. (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed slightly thickened capsule with its collagen (blue) of normal amount. The fibrinoid (pinkish red) content was more increased at the peripheral circumferential area (arrows) but appeared normal at the deep area (arrowheads). (150X, Masson's Trichrome).

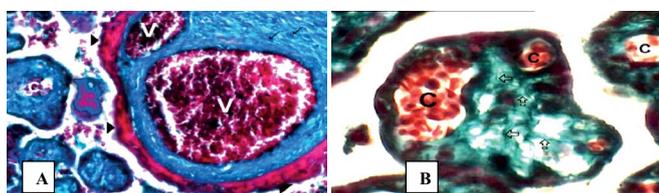


Figure 11: (A): A photomicrograph of a central section of normal full-term placenta showed normal amount of chorionic villi with normal villous capillaries (C). The villous stroma contains normal amount of collagen (blue) and fibroblasts (arrows) while the fibrinoid (arrowheads) material appeared circumferential around the trabeculae containing the main feeding blood vessels (V) which appeared normal and full of blood mostly RBCs (400X, Masson's Trichrome). (B): A photomicrograph of a central section of diabetic full-term placenta. The chorionic villi acquired more stromal collagen fibers (arrows) with mild stromal edema spaces. Villous capillaries (C) were less frequent but congested with fetal blood mostly of mature RBCs. No fibrinoid material being noticed around the villi (1000X, Masson's Trichrome).

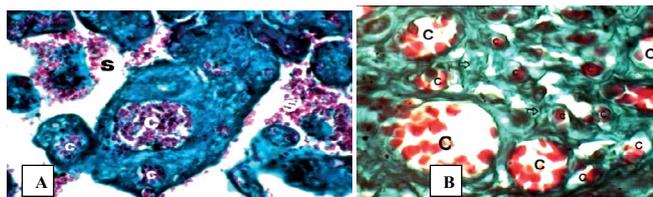


Figure 12: (A): A photomicrograph of a peripheral section of normal full-term placenta showed normal amount of chorionic villi with normal villous capillaries (C) and stroma which appeared mostly formed of collagen (blue). Syncytial clumps are less frequent. No marked layer of fibrinoid material around the villi. The intervillous sinuses (S) contained less amount of maternal blood (400X, Masson's Trichrome). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed young villi with less stroma (arrows) and more villous capillaries (C) which appeared congested and dilated while stromal edema spaces were moderate (1000X, Masson's Trichrome).

histological placental lesions, nor are these lesions in general associated with differences in cord centrality, placental eccentricity or cord coiling. Despite expected to be significant, the increased central thickness among the diabetic placentas was insignificant because of the small sample size utilized in the present study (30 placentas) which probably could reach the significant level in case of bigger sample size as recorded in some previous studies [18,19,22,23].

On the other hand, human data showed that placentas of IUGR

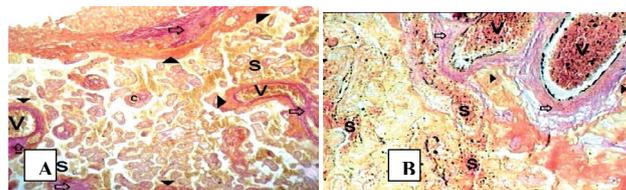


Figure 13: (A): A photomicrograph of a central section of normal full-term placenta showed normal thickness capsule and trabeculae with their content of collagen (arrows, pink) and fibrinoid material (arrowheads, orange). Note the normal amount of chorionic villi with normal villous capillaries (C). Note the fair amount of maternal blood (yellow-brown) in the intervillous sinuses (S) while fetal blood filling the main feeding vessels (V). The stroma of the trabeculae and villi got normal amount of collagen (pink) whereas fibrinoid coat (orange) being seen at the trabeculae. (B): A photomicrograph of a central section of diabetic full-term placenta showed crowded chorionic villi and congested intervillous sinuses (S) full of maternal blood (RBCs were red because of congestion). Congested and dilated main feeding blood vessels (V) full of fetal blood and surrounded by collagen fibers (arrows) while the fibrinoid material (arrowheads) was seen at the trabeculae near the capsule. The nuclei of the syncytial and endothelial cells appeared brown (150X, Van Gieson).

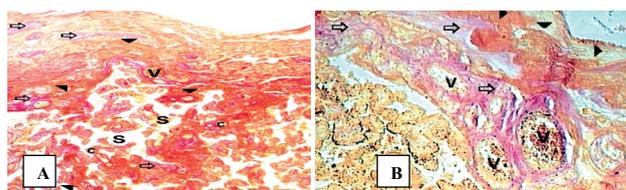


Figure 14: (A): A photomicrograph of a peripheral section of normal full-term placenta showed normal thickness capsule with its content of collagen (arrows) and fibrinoid material (arrowheads). Normal amount of chorionic villi with normal villous capillaries (C) and fair amount of maternal blood (deep yellow) in the intervillous sinuses (S) and feeding vessels (V). The stroma of the trabeculae and villi contained normal amount of collagen (pink) while the fibrinoid around the villi was little. (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed slightly thickened capsule with increased collagen (arrows) content while the fibrinoid material (arrowheads) was increased at the circumferential areas of the capsule. Note moderately increased amount of the chorionic villi (150X, Van Gieson).

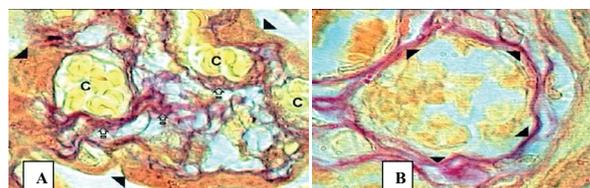


Figure 15: (A): A photomicrograph of a central section of normal full-term placenta showed a normal chorionic villous with normal capillaries (C) full of fetal blood where mature RBCs colored yellow. The stroma of the villous being contained normal amount of collagen (arrows) and surrounded by fibrinoid material (arrowheads). (B): A photomicrograph of a central section of diabetic full-term placenta showed the villous capillaries dilated and surrounded by thickened basal collagenous layer of the basement membrane (arrowheads) (1000X, Van Gieson).

(Intra - Uterine Growth Restriction) were not simply smaller versions of a term placenta, but they display alterations in placental vasculogenesis, in trophoblastic transporters [22] and trophoblast hormone production

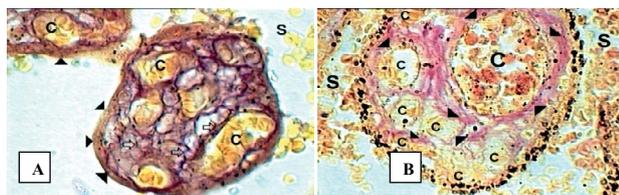


Figure 16: (A): A photomicrograph of a peripheral section of normal full-term placenta showed normal chorionic villi with capillaries (C) full of fetal blood (mostly of mature RBCs). The stroma of the villous being contained normal amount of collagen (arrows) while a thin fibrinoid layer was seen around the villi (arrowheads). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed a chorionic villous with increased villous capillaries (C) appeared congested while the villous stroma (arrowheads) was increased only around the capillary base. The intervillous sinuses (S) were congested with maternal blood. The nuclei of the syncytial cells appeared dark brown (400X, Van Gieson).

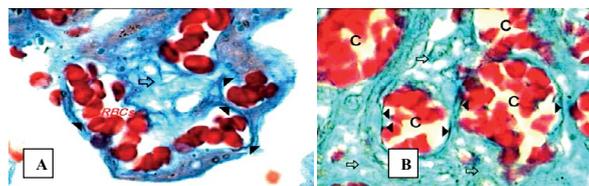


Figure 19: (A): A photomicrograph of a central section of normal full-term placenta showed a chorionic villous with normal content of collagen (arrow) stroma and capillaries with normal thickness basement membrane (arrowheads) and full of fetal blood mostly of mature RBCs (fetal polycythemia). (B): A photomicrograph of a central section of diabetic full-term placenta showed a chorionic villous with increased content of collagen (arrows) stroma with mild stroma edema spaces. The capillaries (C) were congested and dilated with thickened basement membrane (arrowheads) (1000X, Mallory).

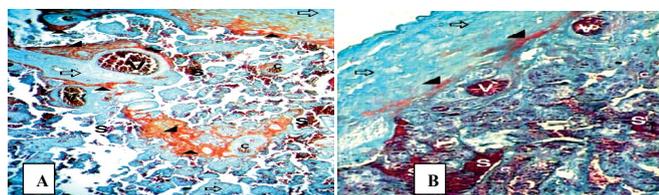


Figure 17: (A): A photomicrograph of a central section of normal full-term placenta showed normal amount of the chorionic villi with their content of collagen (blue). The fibrinoid material (red) appeared normal in the trabeculae, around big anchoring villi and at deep areas of the capsule (arrowheads) which showed a normal amount of collagen (arrows). The main feeding vessels (V), the villous capillaries (C) and the intervillous spaces (S) were full of a fair amount of blood. (B): A photomicrograph of a central section of diabetic full-term placenta showed increased and crowded chorionic villi with their content of collagen (arrows). The fibrinoid material (red) appeared few at deep areas of the capsule (arrowheads) which showed a normal amount of collagen. The main feeding vessels (V), the villous capillaries (C) and the intervillous spaces or sinuses (S) were congested with blood (150X, Mallory).

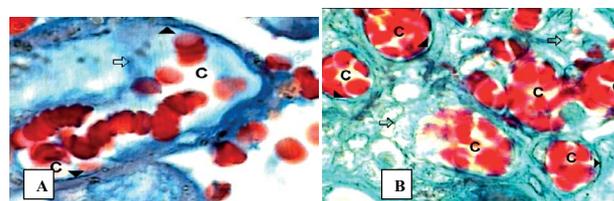


Figure 20: (A): A photomicrograph of a peripheral section of normal full-term placenta showed a normal peripheral chorionic villous with less amount of collagenous stroma (arrows) while its capillaries were full of fetal blood and their endothelium was intact with its basement membrane (arrowheads). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed a chorionic villous with increased and congested capillaries (1000X, Mallory).

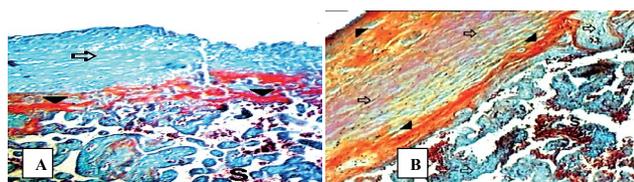


Figure 18: (A): A photomicrograph of a peripheral section of normal full-term placenta showed normal amount of the chorionic villi with their content of collagen (arrows) which filled most of the capsule. The fibrinoid material (arrowheads) appeared at deep areas of the capsule. Note normal amount of maternal blood being seen in the intervillous spaces and sinuses (S). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed moderately increased amount of the chorionic villi and moderately congested intervillous sinuses (S). The capsule was thickened and the fibrinoid material (arrowheads) appeared at peripheral and deep areas of the capsule. Collagen stroma (arrows) was slightly increased in the capsule but appeared normal at the villi (150X, Mallory).

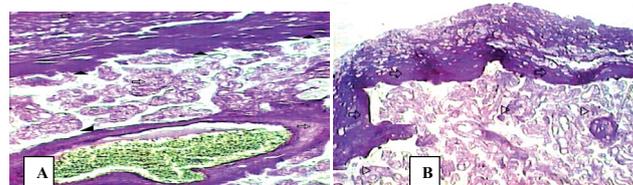


Figure 21: (A): A photomicrograph of a central section of normal full-term placenta showed a moderate PAS positive reaction at the capsule, chorionic villi and in the villous stroma around a main feeding blood vessel (arrows). The reaction was more intense at the capsule and around big vessels due to the more abundant reticular fibers and the fibrinoid material (arrowheads) therein. (B): A photomicrograph of a central section of diabetic full-term placenta showed a moderately high PAS positive reaction at the crowded villi (arrowheads). The capsule showed stronger reaction at its deep area (arrows) due to the more abundant reticular fibers in addition to the fibrinoid material. (150X, PAS).

and enzyme activity [24]. The placenta of the over-nourished adult ewe - a model of IUGR - showed less proliferation of fetal trophoblast and reduced expression of angiogenic factors, which lead to reduction in placental mass (diameter and thickness), blood flow, fetal glucose, amino acids and O₂ concentration [25]. This explains the opposite outcome results due to gestational diabetes where the maternal condition is relatively under-nourished despite hyperglycemia of both mother and fetus which causes also a reduction in maternal plasma

insulin level and decreased maternal weight gain.

Among the diabetic group, there was a significant increase of the mean weight of IDM (infant of diabetic mother) with 588 g difference higher than the control group. This macrosomia or Large for Gestational Age (LGA) infant among diabetics was recorded before by numerous studies [26-28].

Macrosomia among IDM could be attributed to fetal hyperglycemia due to maternal hyperglycemia which would produce fetal hyperinsulinemia with inability of the fetus to fully down-regulate insulin receptors. This condition proceeds to elevate insulin action with a

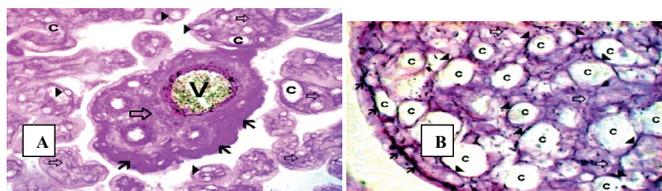


Figure 22: (A): A photomicrograph of a peripheral section of normal full-term placenta showed a moderate PAS positive reaction at the chorionic villous stroma (lucent arrows) and the basement membrane (arrowheads) of the capillaries (C). Reticular fibers and fibrinoid material (dense arrows) were more condensed with big villi carrying big feeding blood vessel (V) rather than in the stroma of small floating villi (400X, PAS). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed a moderate PAS positive reaction at the villous core (lucent arrows). The villous capillaries were much increased and showed stronger PAS reaction at their thickened basement membranes (arrowheads). Note the condensation of reticular fibers (dense arrows) interior to the syncytial cell layer which had no basement membrane (400X, PAS).

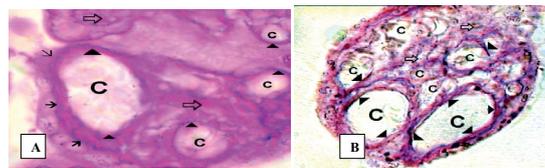


Figure 24: (A): A photomicrograph of a peripheral section of normal full-term placenta showed a moderate PAS positive reaction at the chorionic villous core stroma (arrows) and the basement membrane (arrowheads) of the capillaries (C). Note the condensation of reticular fibers (dense arrows) interior to the syncytial cell layer which had no basement membrane. No fibrinoid coat seen around the villous (2000X, PAS). (B): A photomicrograph of a peripheral section of diabetic full-term placenta showed frequent villous capillaries with stronger PAS reaction at their thickened basement membranes (arrowheads). Note a moderate PAS reaction at the villous core (arrows) (1000X, PAS).

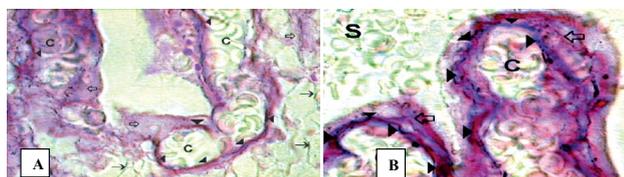


Figure 23: (A): A photomicrograph of a central section of normal full-term placenta showed a moderate PAS positive reaction at the chorionic villous stroma (lucent arrows) and the basement membrane (arrowheads) of the capillaries (C). Minimal reticular fibers at the villous core showed PAS positive reaction (dense arrows) (1000X, PAS). (B): A photomicrograph of a central section of diabetic full-term placenta showed a moderately high PAS positive reaction at the chorionic villous core stroma (arrows). The basement membrane (arrowheads) of the capillaries was thickened and showed intense PAS positive reaction (dark magenta red). Both the villous capillaries (C) and the intervillous spaces (S) were dilated and congested with fetal and maternal blood respectively (2000X, PAS).

kept high affinity of insulin receptors yielding hyper-insulinism. This in turn produces exaggerated insulin anabolic action resulting in increased glycogenesis and visceromegaly (organomegaly) especially in the liver, heart, muscles and viscera but not the brain which lacks glycogenesis. Also, the combination of hyperglycemia, hyperinsulinism and increased free fatty acids availability from the mother leads to acceleration of triglyceride synthesis in fetal adipocytes and consequent obesity (adiposity) of IDM, as glucose offered as a source of glycerophosphate needed for the esterification of free fatty acids. Some previous researchers had concluded the same explanations for macrosomia of IDM [29].

Usually, there is a direct proportion between the new-born infant weight and placental weight as claimed by previous studies [30-32]. In the present study, placental weight was insignificantly increased while IDM weight was significantly increased, as most infants were born Appropriate for Gestational Age (AGA) e.g. < 4 Kg, and their placentas weighed almost normal (up to 800 g) while fewer infants were born Large for Gestational Age (LGA) with macrosomia e.g. >4.5 Kg, and had placentas weighed higher, up to 1.2 kg. These results were similar to mild diabetes type-2 infants (IDM) through the findings of some previous studies [33,34] who recorded insignificant increase of placental weight with IDM + AGA while the more affected (moderate or severe diabetes) IDM + LGA showed significant placental weight increase.

Therefore, gestational diabetes behaves similar to type-2 diabetes

and follows it in all conditions of either mild or moderate or severe. Mild gestational and type-2 diabetes shared the presence of some endogenous pancreatic insulin which resulted in mild maternal hyperglycemia, mild hyperlipidemia and mild fetal hyperinsulinism with mild consequences ending by an infant AGA with mild adiposity. However, the placenta in this condition would grow normally in the first trimester (<13th week) before the onset of gestational diabetes - around 24th week of gestation when insulin resistance usually begins - (with mild affection in case of mild type-2 diabetes) and develop fair vasculogenesis stimulated by hypoxia within physiological level, whereas oxygenation of villi reduces trophoblastic proliferation as claimed by Benirschke and Kaufmann [35]. In the second and third trimesters; the time of onset of gestational diabetes, hypoxia could kept in a mild pathological state and would not much stimulate trophoblast proliferation and angiogenesis, so, no expectation of significant increase in placental weight.

In moderate or severe cases of gestational or type-2 diabetes, the increased hyperglycemia, elevated hyperlipidemia and the resulted fetal severe hyperinsulinism would end by LGA infants while their placentas would be exposed to exacerbated hypoxia, oxidative and nitrate stresses which might highly stimulate trophoblast proliferation ending in a significant increase of placental weight. This was also claimed by some previous studies [30]. Furthermore, this could explain why 30-40% of gestational diabetic women will get type-2 diabetes within a decade (10 years) later, as stated before by the United States National Center for Health Statistics [36].

There was no marked or significant difference between the diabetic and control groups as regard maternal history for gravida or para. This offered an advantage to explore seldom the placental and fetal changes due to diabetes without being affected by changes in the aforementioned parameters.

There was a significant increase of the rate of previous abortions (past history) among diabetic mothers (53.3%) when compared with controls (20%). This report was in agreement with some previous studies [37,38], whereas Crane et al. [39] claimed no effect of gestational diabetes on spontaneous abortion.

The villous portion with its corresponding IVS can be considered as the structural and functional unit of the human placenta through which gas exchange, nutrient supply and waste disposal occur in addition to formation and release of many hormones into the maternal blood [40].

In the present study, the normal control placentas showed their chorionic villi lined only by external syncytiotrophoblast layer while

the cytotrophoblast Langhans cells were absent as stated before by some authors for placenta at term [41,42]. The absence of Langhans cytotrophoblast layer could be attributed to its manifest mitotic division at the 16th week of gestation to form the syncytial trophoblast and become confluent together as a syncytial layer. This confluence or incorporation in one homogeneous layer without basement membranes will potentate the transport efficiency through it to meet the increased metabolic requirements of the growing fetus particularly during the second half of gestation (19th-38th week).

However, some authors claimed that remnants of cytotrophoblast cells persist until term in the form of cells having characters intermediate between cyto- and syncytio-trophoblast cells [43]. Their findings were not contradictory to our claim of confluence of both layers together, as the syncytial trophoblast was derived only from the cytotrophoblast cells throughout gestation which had been proved by mitotic activity and DNA synthesis occurring only in the cytotrophoblast. Also, the syncytiotrophoblast secretion of HCG was initially due to chorionic GnRH secreted by the cytotrophoblast [44].

The gestational diabetic placentas of the present study showed no differences regarding the confluence of the cytotrophoblast with the syncytiotrophoblast and showed absence of the cytotrophoblast layer at term, as was found by other previous studies [45,46]. This was logically accepted, as the onset of gestational diabetes usually started around the 24th week of gestation when insulin resistance began which was later to the time of confluence of both cyto- and syncytio-trophoblast at 16th week. These were the same findings in previous studies on type-1 diabetes [19] and type-2 diabetes [33] in spite of presence of maternal hyperglycemia before 16th week of gestation - the time of confluence of both cyto- and syncytio-trophoblast layers.

The syncytio-trophoblast layer appeared as strong-basophilic cells lacking intercellular boundaries with darkly-stained and irregularly-dispersed nuclei often aggregated or clustered at the villous surface to form syncytial clumps or knots. Syncytial basophilia could be attributed to the abundance of free ribosomes, RER, lysosomes, phagosomes and secretory vesicles or granules of hormones formed by syncytial cells [40].

In the present study, the syncytiotrophoblast and syncytial clumps or knots were best examined and explored for light microscopy by Hematoxylin and Eosin stain and to a lesser extent by Van Gieson's stain and least explored by Masson's Trichrome or Mallory stain.

The syncytial knots or clumps appeared as localized clusters with close aggregation of nuclei that undergo marked degenerative changes probably to remove senescent nuclear materials away from the adjacent functional and metabolically active syncytial areas. This was also explained before by some authors [47] who postulated that in case if these senescent cells did not pushed aside and rather kept in place, the villous surface would be covered by a very thick layer of these aged cells and blood feto-maternal exchange function would be progressively impeded. Different were the syncytial sprouts which being continually formed throughout gestation, some of which were the initial stages for the development of new branch of villi while many others project into the maternal blood and detached forming syncytial emboli that may pass to the mother's lungs. It had been estimated that about 100,000 syncytial sprouts pass into maternal circulation. At the lungs, they undergo lysis, but occasionally they may become a neoplastic focus [40].

The diabetic placentas of the present study showed marked increase of syncytial clumps and knots which become more frequent at central sections but appeared bigger in size among peripheral areas of placenta.

Increased number and frequency of syncytial clumps or knots centrally more than peripherally could be attributed to the more increase of villi themselves due to hypoxia caused by diabetic insult to placenta. However, these young villi were immature and unspecialized, therefore the rate of cellular senescence and/or degeneration would be also increased where those non-functional aged and/ or necrotic cells should be dragged apart towards the syncytial clumps (the recycle bin). These findings were in agreement with many previous studies [19,33,35,48].

The syncytial clumps among peripheral placenta were bigger in size than those of central placenta of the diabetic group which could be due to lower oxygen tension (more hypoxia) existed at peripheral regions which would fasten cellular senescence and/or necrosis and the more consequent accumulation of bigger syncytial knots. This comes in agreement with some former study [48].

The chorionic villi showed marked increase among the diabetic group when compared with the normal control group. Horizontally, those villi were more crowded with higher stromal content centrally than peripherally while the villous vasculature was more increased peripherally than centrally. Although, the greater increase was among the terminal and intermediate villi, they were young, immature and unspecialized with relatively lower parenchymal syncytiovascular membranes. However, the greater number of villi represented an increase in villous volume, villous syncytial surface area and total trophoblast volume. These finding were in harmony with many other former studies [19,49-52].

Villous changes among the diabetic placenta could be attributed to exacerbation and conversion of physiological hypoxia (which is normally needed for organogenesis, vasculogenesis, angiogenesis and trophoblast development) to a pathological hypoxia lead to more oxidative and nitrate stresses. These explanations were also suggested by some previous study [53,54]. However, Soma et al. [55] presumed that trophoblast cells may play an important role for gas transfer mechanism under hypoxic state at high altitude. So, pathological hypoxia could enhanced expression of the Placental Growth Factor (PLGF), angiogenic Vascular Endothelial Growth Factor (VEGF) and angiopoietins, causing increase in the amount and ramification of villi and hypercapillarization (branching angiogenesis) of their vasculature. This was also concluded by Srinivasan et al. [56] who observed more instances of chorangiomas in placentae that have suffered significant hypoxic insults due to maternal diseases complicating pregnancy.

The effect of hypoxia was explained by some studies [56] that it might be mediated through the transcription of hypoxia-inducible factor HIF-1 α which activates gene transcription in response to varying oxygen concentration in an inversely proportional manner. Fetoplacental angiogenesis could be processed through mobilization of bone marrow angioblasts i.e. Endothelial Precursor Cells (EPCs) or via capillary sprouts from the pre-existing vessels by the existing endothelial cell or the surrounding pericytes. This could explain one adaptive response of the diabetic placenta to increase its efficiency as regard hormone production, substrate metabolism and transporter activity among both uteroplacental and fetoplacental circulation. The later adaptive response was also similarly explained by other authors [30,56-60].

Peripheral regions of diabetic placenta gained more villous vasculature (angiogenesis and hyper-capillarization) than central regions which could be due to lower oxygen tension (more hypoxia) existed at the peripheral regions that would be more exacerbated by diabetes. Also, the increased vasculature appeared mostly as longitudinal

vascular growth without remodeling, as had been recorded by some previous studies [48,61].

The increased villi of the diabetic placenta were covered by attenuated and thinned syncytial layer wherever there were underneath capillaries while senescent and/or necrosed cells were pushed apart in the form of syncytial knots. This actually what could happen normally in placental villi at the 3rd trimester and full-term [62] but seemed to be more adaptive in response to diabetes by minimizing the transport distance between fetal blood in the villous capillaries and the maternal blood at IVS.

Diabetic placentas showed greater number of young villi with increased choriogenesis and ramification of intermediate and terminal villi. Consequently, this could explain why the pregnant diabetic mother might have more detached syncytial sprouts which work together with polycythemia (erythrocytosis) and increased platelets aggregation making her more susceptible to lung embolization and deep venous thrombo-embolic events or even infarctions, as claimed before by different authors [11,40,63].

The increased chorionic villi vasculature by angiogenesis among diabetic placentas represented an adaptive response in a trial to increase the placental respiratory area through increased villous capillary number, surface area, diameter and length but without remodeling. This was also stated by some previous studies [20,48,64,65].

The diabetic placentas showed marked vascular congestion (plethora) and dilation among the main feeding fetal blood vessels (central, basal and subcapsular areas) as well as villous capillaries especially at basal and subcapsular areas. Parallel, the Intervillous Spaces (IVS) and sinuses were increased in volume and congested with maternal blood, which could be due to maternal secondary absolute polycythemia, uteroplacental endarteritis and the adjuvant relatively reduced uteroplacental blood drainage. These findings were in harmony with some previous studies [9,66-68].

The congested vessels and IVS with polycythemic fetal or maternal blood respectively, showed high percentage of mature RBCs which were best stained by Mallory's stain and Masson's Trichrome.

Congestion or plethora of fetoplacental blood vessels with could be attributed to exacerbation of secondary polycythemia that happen normally at a physiological level, but IDM might get increased red cell breakdown as a result of chronic intrauterine hypoxia, oxidative and nitrate stresses [69,70]. Diabetes might cause exacerbated hypoxia, oxidative stress, nitrate stress and impaired transport of glucose, amino acids and oxygen as well as secondary absolute polycythemia, congestion or plethora, hyperglycemic edema and hypo-calcemia, which all shared in insulting vascular smooth muscle leading to dilatation of fetal-placental vessels. This was in agreement with Metzger et al. [1] who stated that diabetes is a state of chronic oxidative stress, and the responses of the fetal-placental vasculature of diabetic placentas to vasoconstrictor and vasodilator agents are significantly attenuated when compared to those in normal control placentas.

Villous capillary vaso-dilation being increased at basal (subchorial) regions whereas villous capillaries number was increased at peripheral regions might be an adaptive response to the lower oxygen tension existed at these two regions as stated by some previous study [48].

The endothelial cells of the villous capillaries appeared flattened with normal-thickness intact basement membrane among the control group, as had been found by other authors [42,62,71]. The diabetic placentas' villous capillaries showed even more flattened endothelial

cells because of vasodilation and congestion (plethora), but their intact basement membrane appeared thickened, which had been attributed pathologically as diabetic microangiopathy (chorioangiogenesis or chorioangiopathy). These findings were in agreement with many previous diabetic research studies [14,19,33,34,60,72] who recorded endothelial basement membrane thickening, impaired capillary permeability and endothelial dysfunction as a result of all kinds of diabetes mellitus.

The present study manifested that the basement membrane was best explored for high power light microscopy by Masson's trichrome, Mallory stain, Van Gieson's stain and to a lesser extent by H&E stain. All stained the reticular lamina of the basement membrane with its content of collagen type I and its interacting type V but the polysaccharide moiety was faint. The histochemical PAS reaction was the best to explore the rest of basement membrane content as reticular fibers (collagen type III) which contain 6-12% hexoses but not collagen type I which contains only 1% hexoses.

PAS reaction also explored the polysaccharide moiety at the connective tissue ground matrix and the basement membranes as GAGs (Heparin SO₄, Dermatan SO₄, Hyaluronic acid and Chondroitin 6-SO₄) especially Heparan SO₄ which binds protein to form proteoglycan Perlecan in the basal lamina and other proteoglycans as decorin and biglycan. Also, PAS reaction explored the polysaccharide moiety of glycoproteins Laminin, Entactin and Fibronectin present at the basal laminae and connective tissue matrices. Periodic acid-Schiff (PAS) reaction to explore polysaccharides was based on the transformation of 1,2-glycol groups (present in the sugar molecules) into aldehydes which then revealed by Schiff's reagent producing purple or magenta red color. The ubiquitous free polysaccharide Glycogen was also best explored by PAS reaction in this study. These findings were in harmony with many other authors [25,41,73-77].

Diabetic thickening of the basement membrane of vascular endothelium could be explained as a consequence of metabolic derangements particularly hyperglycemia via three metabolic pathways. The first pathogenic pathway was the early non-enzymatic glycosylation (the chemical attachment of glucose to free amino groups of proteins without the aid of enzymes) of collagen and other long-lived proteins at the vascular wall leading to chemical rearrangements and forming irreversible Advanced Glycosylation End Products (AGEs). AGEs being formed on collagen lead to cross-links between polypeptides which might trap non-glycosylated plasma and interstitial proteins which in turn trap Low-Density Lipoproteins (LDL) and enhance cholesterol deposition in vascular intima ending by atherogenesis of arteries and arterioles. Whereas in capillaries, plasma proteins especially albumin bind to the glycated basement membrane leading to its thickening seen in diabetic chorioangiopathy (chorioangiogenesis).

The second pathogenic pathway was that diabetic hyperglycemia can stimulate de novo synthesis of Diacylglycerol (DAG) - from glycolytic intermediates - and hence worked as a second messenger after Ca ions (first messenger) in activation of protein kinase C (PKC). PKC activation lead to production of pro-angiogenic molecules as Vascular Endothelial Growth Factor (VEGF) implicated in diabetic chorioangiogenesis, and pro-fibrogenic molecules like transforming growth factor β (TGF β) implicated in increased deposition of extracellular matrix and basement membrane thickening. The third and least pathogenic pathway was intracellular disturbance of polyol (as sorbitol) pathway due to hyperglycemia where glucose being metabolized by aldose reductase to sorbitol then fructose which were both implicated to cause endothelial injury via increased intracellular

osmolarity and water influx. The latter pathogenic pathway together with the diminished antioxidant reserves (oxidative stress) occurred in the course of sorbitol metabolism would end by endothelial dysfunction and thickening of the basement membrane. These claims were similarly discussed by some previous authors [78-82].

Although there were thin-walled villous capillaries and the syncytial knots were pushed apart to minimize the syncytiovascular membrane distance, the trophoblast and hence the materno-fetal diffusion membrane appeared thickened among the diabetic group when compared with the control group. This could be attributed to the relatively increased villous core stroma, edema, and thickened basement membrane of the capillary endothelium as well as increased amount of syncytial knots which occupied a fair space of the villous surface area. These findings were sound with some previous studies [34,68].

Collectively, the increased amount of young, unspecialized and immature villi with poor syncytiovascular membrane might lead to impaired function and relative reduction in the respiratory and metabolic areas of the diabetic placenta which could enhance fetal hypoxia leading to increased fetal and neonatal morbidity and mortality. Also, some previous studies on diabetic placentas recorded impaired amino acids transporters (i.e. system A = a sodium-dependent transporter) and impaired oxygen conductance [22,82]. These findings put diabetes in the moderate to high risk factors of Vascular Placental Pathology (VPP) and the consequent obstetrical pathologies linked to placental ischemia [1,11,63]. This was also proved by the findings of some previous studies who recorded almost optimal growth and maturation of the chorionic villi with well-functioning syncytiovascular membrane among placentas of well controlled diabetic mothers [67,68].

The diabetic placentas showed a marked increase in villous stroma (non-parenchymal tissue) particularly at central areas with increased fibrous content of collagen and reticular fibers and stromal edema. These findings were in harmony with some previous researches [19,20,33,68] who recorded also the occurrence of fibrotic villi and fetal-placental sclerosis. Stromal edema could be attributed to the increased leak of plasma proteins out of the villous capillaries due to diabetic microangiopathy despite thickening of vascular basement membrane [10,11,20,33,79].

Although, Teasdale [20,33] had found diabetic villous immaturity with significantly increased non-parenchymal tissue (stroma) and only moderate increase of parenchymal tissue (syncytiovascular), he/she suggested that the placental function was not adversely affected in class A or B gestational diabetes and the perinatal morbidity associated with these conditions was probably the result of the existed materno-fetal metabolic abnormalities. As regard perinatal morbidity, the latter statement is in harmony to the claims of some other previous studies [30,82-85] who assumed that the placenta has an important role in fetal programming that could markedly affect the evolution of adult-life morbidity but does not markedly affect the perinatal morbidity despite the good degree of placental potentiality to adapt with diabetic derangements during pregnancy. Samuel et al. [60] suggested that adult type 2 diabetic vasculopathy has developmental origins in utero, as they observed pericyte detachment and endothelial cell irregularity in 76% gestational diabetes mellitus foetal blood vessels as compared with 10.4% non-gestational diabetes mellitus placental vasculature. On the other side, Saha et al. [23] concluded that placentae of women with GDM showed several changes that may be associated with impaired functioning, leading to bad perinatal outcome.

The last – but not least – assumed mechanism could be the retarded

and impeded normal physiological reduction of placental stroma in the third trimester approaching full-term [62], due to hyperglycemia metabolic derangements and the resultant oxidative and nitrate stresses. Eventually, this might lead to decrease in disposal of old and senescent fibers while new fibers formation was enhanced via the 1st and 2nd aforementioned assumed mechanisms. The latter assumed mechanism was rational with the expected decreased activity of Hofbauer phagocytes approaching full-term which could be more decreased in diabetic placentas by exacerbated hypoxia, oxidative and nitrate stresses [30,86,87]. This was clear in some previous studies who reported a significant decrease in acid phosphatase enzyme near term as the amount of hormonal by-products being already decreased particularly with the reduction in progesterone level near term to release myometrium from its inhibitory effect and prepare uterus for forcible contractions of childbirth [88].

The stroma of the control non-diabetic placentas showed the presence of a fair amount of fibrinoid material at the deep capsular areas, external layers of the trabeculae that hold the main feeding fetal vessels and around the big anchoring chorionic villi which were mainly present at central regions. The fibrinoid material had been best explored for light microscopy by Masson's trichrome and Mallory stain especially the fibrinoid material at the capsule, trabeculae and central big anchoring villi. Whereas Van Gieson's stain and PAS reaction were better to explore the fibrinoid material around intermediate and terminal villi even it was little. Hematoxylin and Eosin stain was not suitable to explore the fibrinoid material. These findings were in harmony with many other authors [41,42,74-77].

The fibrinoid material would be constituted from semi fluid - jelly like - hyaline mucous tissue with much content of hyaluronic acid matrix, fibrin and remnants of degenerating and liquefied cells. It may also represent Rohr's stria of fibrinoid which is an inconstant deposition of fibrin at the bottom of the intervillous space, deep to the capsule and surrounding the trabeculae and fastening villi. It may also represent Nitabuch's stria of fibrinoid degeneration (necrosis) occurring normally in the 1st and may be the 2nd trimester by invasion of the decidua basalis by the growing trophoblast, where the fibrinoid material would accumulate at the deep capsular areas and around basal trabeculae mainly at the central region of the growing placenta. This claim was in harmony with the claims of some previous authors [51,62,78,89,90].

The diabetic placentas showed reduction of the fibrinoid material at the central capsule, central basal trabeculae and around the big central anchoring villi while the peripheral region showed slightly thickened capsule with external deposition of fibrinoid material (fibrinoid necrosis) to cover the peripheral capsule. The intermediate and terminal villi showed much reduction of the surrounding fibrinoid material at central areas whereas the peripheral villi showed scarce amount of fibrinoid material around. These findings were mostly matching with some previous researches [10,11,19,78].

Despite exacerbated diabetic hypoxia, oxidative and nitrate stresses, the subsequent enhanced senescence of syncytial cells and increased syncytial clumps, the rate of degenerative fibrinoid deposition was lower than normal, because most of the villi were young (although functionally unspecialized) and hence more potent to resist degeneration than older villi.

The present study showed the peripheral region of the capsule of the diabetic placenta slightly thickened due to external deposition of fibrinoid material (fibrinoid necrosis) while the central area was saved. This could be attributed to diabetic exacerbation of hypoxia that already existed more at the peripheral placental regions in addition

to the degenerating decidua parietalis that merge with the peripheral capsular area but did not extend to the level of the central capsular area. Also, the peripheral region of the placenta might be exposed to some degree of pressure atrophy by the compressive forces of the macrosomic fetus (LGA) and its adjuvant polyhydramniotic hydrostatic pressure against the uterine wall. Whereas the central region of placenta was thick enough – and even more thickened in diabetes – to work as hydraulic cushion absorbing these experienced compressions to save the placental vasculature (villous, intervillous and subcapsular) from being occluded.

The present study observed the control (non-diabetic placentas) with mild-moderate PAS reaction among the villous core stroma and the syncytial cells, while lipid droplets (in the form of vacuoles) appeared abundant among the villous stroma. This mild-moderate PAS reaction might be attributed to the mild-moderate amount of glycogen inclusions and polysaccharides in the syncytial cells and the matrix of villous stroma as reported by some previous authors [71,78]. They recorded few glycogen inclusions at the syncytial cells while lipid droplets were abundant which were also seen in the core of the villous stroma extracellularly.

The diabetic placentas showed moderately high PAS reaction among villous core stroma due to increased glycogen and polysaccharides content while lipid droplets were decreased and seen as unstained vacuoles in the villous core stroma. The increased villous glycogen and polysaccharides content was also observed by some previous study [91] and could be attributed to fetal hyperglycemia due to maternal hyperglycemia which would produce fetal hyperinsulinemia with inability of the fetus to fully down-regulate insulin receptors. This condition would proceed to elevate insulin action with kept high affinity of insulin receptors yielding hyper-insulinism. This in turn might produce exaggerated insulin anabolic action resulting in increased glycogenesis and visceromegaly (organomegaly) especially in the liver, heart, muscles, viscera and placenta, but not the brain (which lacks glycogenesis).

Furthermore, the diabetic exacerbation of hypoxia and oxidative stress would reduce the activity of respiratory electron transport chain and Krebs' citric acid cycle enzymes particularly succinic dehydrogenase, which would lead to more accumulation of glycogen. These explanations were in harmony with some previous studies [88,92,93].

The lipid content in the villous core stroma of diabetic placentas was moderately decreased centrally and highly reduced peripherally when compared to the abundant lipid content among the normal control placentas. The decrease of lipid content in diabetic villi could be attributed to hyperinsulinism (antagonizes glucocorticoids) with impaired lipogenesis particularly phospholipids leading to fetal complications as low brain weight and deficient pulmonary surfactant with Respiratory Distress Syndrome (RDS). These findings were similarly recorded by some previous researches [3,94]. Furthermore, the diabetic exacerbation of hypoxia and oxidative stress might lead to enhanced reduction in syncytial cells secretion of 11HSD2 (11 hydroxysteroid dehydrogenase 2) more than its regular and normal reducing rate which normally inactivates cortisol to inactive cortisone to protect the fetus from high maternal cortisol which resulted in IUGR and smaller placenta. These finding were matching with some previous studies [24,30,95] and could also explain the more reduction of lipid content at the peripheral villi than that of the central villi.

In the present study, it was necessary to examine several preparations, each one stained by a different method to obtain a clear

and detailed idea of the whole composition and totality of placental structure.

By histochemical and histopathological bases and histophysiological explanations, despite these anatomical and histological changes, the placental functions were not severely adverted due to gestational diabetes as the compensatory adaptive responses had worked well. So, the elevated rates of perinatal morbidity (or even later adult life morbidity) and mortality were not primarily due to placental affection, but rather mainly due to metabolic abnormalities and derangements occurred in mother and fetus resulted of whatever kind of diabetes mellitus. These findings support earlier study [67] which indicated that essentially normal microscopical morphology is preserved in placentas from diabetic subjects with good glycemic control. Therefore, it is likely that fetal hypoxia associated with maternal diabetes mellitus is due to metabolic disturbances rather than abnormalities in the quantities or arrangements of maternal vascular spaces.

Moreover, further research is needed to investigate the impact of pathophysiology, glycemic control and clinical factors, such as infant sex, weight and race, on placental structure and function [96,97]. Furthermore and according to some different studies [98,99] epigenetics and epigenomics could present some explanations to materno-fetal insult due to gestational diabetes by studying a number of vascular risk factors, such as nutrition, smoking, pollution, stress, and the circadian rhythm, being associated with modification of epigenetic marks, which could determine whether the placenta - as a vascular organ - is a guardian or guilty. As stated by Gabbay-Benziv et al. [99-101], understanding placental changes and how they affect outcome is necessary in order to develop effective screening, prevention, and management approaches.

Conclusively, as the complications of gestational diabetes are manageable and preventable by advanced exogenous measures, the placenta itself is almost perfect, innocent and helpful in managing and preventing these complications through its optimal endogenous measures.

Lastly, the premium key in diabetic care is to apply the knowledgeable exogenous measures in harmony and according to the status of early diagnosed and strictly managed endogenous placental measures.

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