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 hyoxia 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...
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 gravidia, 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 ≤ 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 34.58 g.

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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
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 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
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
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 (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.
and enzyme activity [24]. The placenta of the over-nourished adult ewe - a model of IUGR - showed less proliferation of fetal trophoderm 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
In the present study, placental weight was insignificantly increased with IDM + LGA while the more affected (moderate or severe diabetes) IDM + AGA showed significant placental weight increase. 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 keep 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 nitrative 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 syncytiotrophoblast and become confluent together as a syncytial layer. This confluence or incorporation in one homogenous layer without basement membranes will potentiate 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 syncytiotrophoblast cells [43]. Their findings were not contradictory to our claim of confluence of both layers together, as the syncytiotrophoblast 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 syncytiotrophoblast 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 syncytiotrophoblast layers.

The syncytiotrophoblast 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 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].

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 syncytiotrophoblast 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 nitrative 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 chorangiosis 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-1α 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 choric 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, urotelial endotharteritis and the adjuvant relatively reduced urotelial placental 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 which happen normally at a physiological level, but IDM might get increased red cell breakdown as a result of chronic intrauterine hypoxia, oxidative and nitrative stresses [69,70]. Diabetes might cause exacerbated hypoxia, oxidative stress, nitrative stress and impaired transport of glucose, amino acids and oxygen as well as secondary absolute polycythemia, urotelial endotharteritis and the adjuvant relatively reduced urotelial placental blood drainage. These findings were in harmony with some previous studies [48,62,71].

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 vasodilatation 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 endotheal 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 Giesen'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 moieties 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 moieties 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 Perlecain in the basal lamina and other proteoglycans as decorin and biglycan. Also, PAS reaction explored the polysaccharide moieties 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 cellular 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 glyced 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 Dicacylglycerol (DAG) - from glycolytic intermediates and hence worked as a secondary 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 (TGFβ) implicated in increased deposition of extracellular matrix and basement membrane thickening. The third least pathogenic pathway was intracellular disturbance of polyl (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 finding 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 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 observed maternal-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 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 nitrative 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 trabeucle 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, trabeucle 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 trabeucle 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 trabeucle 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 trabeucle 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 nitrative stresses, the subsequent enhanced senescence of syncytiotrophoblasts 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 adjacent 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 a 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 hyper-insulinemia 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 corticone 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 matero-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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References
finding in placenta of insulin-dependent diabetic patients treated with continuous subcutaneous insulin infusion (CSI), Placenta 8: 153-165.


