Study of the ultrastructure of the placenta in gestational Diabetes mellitus

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ABSTRACT

The placenta is the organ situated between the mother and fetus. It plays critical roles during pregnancy and is essential for fetal growth and development. The placental functions are determined by the ultrastructure of the placental barrier which is an exchange surface area between the fetus and mother. Gestational diabetes mellitus (GDM) comprises unfit conditions for embryonic and feto-placental development and may result in placental abnormalities. This study was made to detect the ultrastructural changes of the placenta in women with gestational diabetes. The placentas of 10 control and 10 women with GDM were studied. Whole thickness placental samples were taken and prepared for light and transmission electron microscopy study. Light microscopic study of the control placenta showed numerous densely packed microvilli, thin walled blood vessels and narrow intervillous spaces. The placentas of GDM showed reduced number of microvilli, wide intervillous spaces, thick walled vessels, edematous spaces and areas of fibrosis and perivillous fibrinoid degeneration. Electron microscopic study of the placentas of control women showed terminal villi with a thick layer of syncytiotrophoblast (Sy) with a lot of cylindrical microvilli and a thin layer of cytotrophoblast (Cy). There were some endoplasmic reticulum cisternae beside few mitochondria. The underlying villus core was harboring fetal capillaries lined with flat endothelial cells and thin basement membrane. In GDM placenta, there was hypertrophy of Cy with atrophy of the Sy with multiple vacuoles and areas for glycogen storage. The microvilli were scarce and the villous core showed congested capillaries, edematous spaces, glycogen storage areas and fibrosis. All the changes in placentas of GDM are suggested to be attributed to the associated hypoxia and oxidative stress due to the associated decreased uteroplacental flow that was aggravated by the thick placental barrier.

Key Words: Gestational diabetes mellitus; Fetal; Syncytiotrophoblast; Cytotrophoblast; Placenta; Microvilli

INTRODUCTION

The placenta is the organ situated between the mother and fetus. It is essential for fetal growth and development (1). Placenta performs essential functions during pregnancy, including the exchange of nutrients, water, respiratory gases, and waste products together with the synthesis of various hormones which regulate the transport of maternal nutrients to the fetus and help maternal adaptation to different pregnancy stages. These functions are governed by the ultrastructure of the placental exchange barrier (2,3).

The fetal and maternal blood is separated by the placental barrier that allows the exchange between the fetus and mother with no mixing between both bloods. With the progress of pregnancy, it decreases in thickness to compensate for the increased fetal demands. The placental barrier is composed of different structures including a maternal-facing layer of syncytiotrophoblast (Sy) with multiple microvilli, a thin layer of cytotrophoblast (Cy) which gets thinner as pregnancy proceeds but persists until term, the endothelial lining and underlying basal membrane (BM) of the fetal capillaries, and the connective tissue of the villous core between them (3-5). A proper coordination of trophoblast proliferation, differentiation and invasion is required for placental development (5,6).

The physiological changes that occur during pregnancy necessitate proper nutrient supply to ensure placental and fetal development. During gestation, the placenta continuously undergoes changes in weight, shape and structure to make sure that the supply of nutrients is appropriate for fetal survival. Some forms of intrauterine fetal growth abnormalities have been attributed to abnormalities of placental blood flow and transfer of nutrients from the mother to the fetus (1).

Gestational diabetes mellitus (GDM) is defined as the glucose intolerance with onset or first recognition during pregnancy. It is associated with morbidity in both offspring and mother. Gestational diabetes mellitus (GDM) comprises unfit conditions for embryonic and feto-placental development and may result in placental abnormalities. This inconsistency was partly attributed to superadded preeclampsia and other associated complications that may change the metabolic environment and partly to the fact that the category of GDM women is not always homogeneous (1).

Despite the availability of treatment, GDM provides unfit conditions for feto-placental development and placental structure. However, reports on the placenta of GDM are usually controversial regarding the pathological features. Some researchers found no unusual features while others claimed that there were pathological changes. This inconsistency was partly attributed to the superadded preeclampsia and other associated complications that may change the metabolic environment and partly to the fact that the category of GDM women is not always homogeneous (1).

Moreover, few studies have investigated the ultrastructural changes in human term placentas (2). Thus, this study was made to detect the ultrastructural changes of the placenta in women with GDM.

MATERIALS AND METHODS

The placentas of 20 women; 10 control and 10 with GDM, were studied with a free consent approved by the committee of ethics, Faculty of Medicine, Alexandria University. Women with a history of pregestational diabetes or hypertension or other chronic diseases were excluded. The age ranged from 28 to 30 years for the control and 27 to 31 years for the GDM. Fasting blood glucose level was performed after an overnight fast of at least 10 hours at 24-28 weeks of gestation. The diagnosis of GDM was made based on the American Diabetes Association (2011) (7). The control women were within the accepted normal ranges of blood glucose level from 90 to 115 mg/dL.
throughout gestation while the GDM women initially showed levels ranging from 120 to 160 mg/dL. GDM women were asked to control diet beside insulin therapy in order that their fasting blood glucose levels were kept in the satisfying range from 100 to 150 mg/dL until delivery. Two GDM women had higher fasting blood glucose ranging from 140 to 165 mg/dL because of their poor diet control and interrupted therapy. The maternal weight at gestation ranged from 62 to 65.5 Kg for the control and from 65 to 68.5 Kg for GDM. All GDM women were delivered by cesarean section and the gestational weeks at delivery ranged from 36 to 38 weeks for the diabetics. 6 control women were delivered vaginally while 4 were delivered by cesarean section at a gestational age ranging from 36 to 39 weeks. All deliveries were done in Gynecology and Obstetrics department, Faculty of Medicine, Alexandria University. The fetal birth weight ranged from 2.80 to 3.15 Kg in the control group while in the GDM group, it ranged from 3.210 to 3.40 Kg. The embryonic membranes and the umbilical cords were trimmed from the placentas, In the control group, the placenta weight ranged from 450 to 480 gm while in the diabetic group, it ranged from 470 to 500 gm. Placental samples including the whole thickness of the placenta were taken from the center of the placenta beside the site of attachment of the umbilical cord. Paraffin-embedded blocks from both formaldehyde-fixed tissues were prepared and sections (thickness 0.5μm) were cut from each block and stained with hematoxylin and eosin (HE) to be examined by light microscopy (Optica-B-150). Other blocks were prepared for electron microscopy study. They were fixed in the fixative (3% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/l cacodylate buffer (pH 7.3) for 24 h at 4°C. After fixation in 1.0% OsO₄ in 0.1 mol/l cacodylate buffer (pH 7.3) for 2 h at room temperature, the tissue specimens were subjected to dehydration in graded ethanol. After immersion in propylene oxide (three times for 10 min each), the samples were immersed overnight in a mixture (1:1) of propylene oxide and Epon-812 resin to be finally embedded in Epon-812 resin (Spa, USA). Semithin sections (0.5 μm thick) were cut using ultramicrotome (Leica Ultracut; Leica, Berlin, Germany) that were picked on copper grids and counter stained with 2% uranyl acetate and lead citrate (12). The specimens were examined by transmission electron microscopy (JEOL- JEM- 100CX-JAPAN) and analyzed in the electron microscope unit, Faculty of Science, Alexandria University.

Statistical analysis: The results were analyzed statistically using SPSS software version 17 as mean± standard deviation and the 2 groups are compared using Paired-Samples T test and the level of significance was accepted as p<0.05.

RESULTS

1. Parameters of the studied cases: The gestational age was increased in the control than in GDM women with no significant difference. On the other hand, the neonatal weight was significantly increased in GDM than in control women. Regarding the maternal age, GDM women were older than the control with no significant difference. Comparing the maternal weight, it was significantly increased in GDM than in control women. Finally, the placental weight was significantly increased in GDM than in control women (Table 1).

2. Light microscopic study: Light microscopic study of the control placenta showed a large number of densely packed microvilli. The intervillous spaces were narrow and explicit and filled with blood cells. The microvilli showed syncytial knots at their poles. The villous core shows thin walled blood vessels (BV) containing blood cells. A H&E X200 B H&E X400 (Figures 5 and 7) The BM was too thin to be resolved. The capillaries were externally limited by concentric arrays of reticular fibers (Figures 5 and 7).

In the GDM placenta, there was hypertrophy of Cy with atrophy of the Sy (Figure 8). The cytoplasm of both types of trophoblast was occupied by a lot of membrane-bounded irregular vesicular structures that contained a low electron-dense material (Figures 8-10). Areas rich in glycogen granules and few mitochondria appeared in the Cy cells (Figure 9). In the Sy, there were inclusion bodies inside the nucleus that assumed a concentric lamellar appearance with a halo around (Figure 10). The microvilli on the surface were scarce and some had terminal club ends (Figure 10). The subtrophoblastic BM was thick and separated from the fetal capillary by a subtrophoblastic space (Figure 9). The fetal capillaries underneath the trophoblast were congested with much dark and few pale RBCs (Figure 8).

The endothelial cells lining the capillaries showed aggregates of glycogen granules near the nucleus together with the presence of dilated cisternae of endoplasmic reticulum (Figure 11). There were edematous clear spaces and well defined storage areas in the matrix of the villous core (Figures 12 and 13). These storage areas were well-circumscribed by coarse matrix fibers and enclosed scattered dense short fibrillar and granular content on a homogenous background of moderate electron density (Figure 13).

There was fibrosis inside a chorionic villous core where fibrinolasts and collagenous bundles were exceptionally prevalent (Figure 14). There were stromal macrophages with their lysosomes containing a material whose density was comparable to that of hemoglobin of the extra-vasated fetal RBCs (Figure 12).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>GDM</th>
<th>t-test</th>
<th>P value</th>
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<tr>
<td>Gestational age</td>
<td>36.90 ± 0.91</td>
<td>36.40 ± 0.60</td>
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</tr>
<tr>
<td>Maternal weight</td>
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<td>65.64 ± 0.53</td>
<td>5.807</td>
<td>0.001*</td>
</tr>
<tr>
<td>Maternal age</td>
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<td>29.20 ± 1.36</td>
<td>0.941</td>
<td>0.358</td>
</tr>
<tr>
<td>Placental weight</td>
<td>460.25 ± 8.96</td>
<td>485.25 ± 11.47</td>
<td>6.216</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Figure 11A,B): A photomicrograph of the control placenta showing numerous microvilli (V) with narrow intervillous spaces (red star) filled with blood cells. The microvilli show syncytial knots (S) at their poles. The villous core shows thin walled blood vessels (BV) containing blood cells. A H&E X200 B H&E X400
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Study of the placenta of the control group showed that the placental terminal villi were formed of a thick layer of Sy with multiple nuclei and a thin layer of Cy with a thin BM. The terminal villi bear regular and cylindrical microvilli. The trophoblast showed some rER and mitochondria. The villous core contained capillaries lined with flat endothelial cells with a thin BM. Same results were obtained by Meng et al. (3) for the structure of the trophoblastic covering and the core of the placental villi.

Placental growth might be considered a reflection of the coordination of trophoblast proliferation and differentiation. Moreover, the degree of trophoblastic maturation and microvillous density are determined by thickness of the trophoblastic layers that in turn affect the fetal-maternal exchange (3).

DISCUSSION

The placenta is a complex organ that has vital roles during fetal growth. Because of its unique position, the placenta is exposed to changes in both maternal and fetal environments. Diabetes mellitus affects both maternal and fetal environments with multiple effects on different body organs including the placenta that shows alterations from non-diabetic placenta (10).
In the control group, a thin layer of Cy is shown due to proper syncytial fusion. Sy cannot grow by itself but syncytial growth and surface expansion throughout pregnancy together with endometrial invasion depend on continuous proliferation and differentiation of the cells of Cy and finally fusing with Sy, a process known as syncytial fusion which is also important for the syncytial hormonal function (6). Thin Cy and hence thin placental barrier increases the trans-placental flow, thus ensuring adequate maternal nutrient supply to the fetus (3,6). Moreover, thin Cy cells ensure proper invasion to the endometrium and its spiral arteries as the Cy at the tips of the villi grows out to penetrate into the decidua basilata (6). Placental invasion and establishment of enough maternal blood supply represent the cornerstone in placental development while their dysregulation is associated with pregnancy troubles (3,5).

The placental barrier represents the site of interface between the maternal and fetal circulations. In the control group, it was formed of Sy, a thin layer of Cy and a thin layer of vascular endothelial cells with a thin BM together with a villous core made up of stroma. Gude et al. (13) and Meng et al. (3) mentioned that this structure is suitable to ensure proper oxygenation and nutrition of the embryo which are essential for a successful pregnancy (3,13).

Regarding the rER, it is the intracellular organelle where synthesis of proteins occurs prior to its eventual extrusion into the extracellular matrix to share in the production of placental hormones (6). Moreover, Mitochondria; by oxidative phosphorylation; adopt a lot of essential functions for placental survival and energy metabolism necessary for this active organ. By oxidative phosphorylation, the cells use enzymes to oxidize nutrients releasing energy. Under normal conditions, increased activity and oxidative phosphorylation of placental mitochondria throughout gestation results in overproduction of reactive oxygen species (ROS) as natural byproducts. Physiological ROS...
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levels play a crucial role in placental development through cellular signaling but increased ROS can inactivate certain enzymes thus exposing the placenta to oxidative stress even in normal pregnancies. This is dealt with by the placental antioxidant defense capacities which induce conversion of ROS to water and molecular oxygen (14,15).

The current study demonstrated certain structural changes in that GDM placentas. The trophoblast had thin Sy, thick Cy, scarce microvilli with thick trophoblastic BM and evident vacuoles. Moreover, The villous core showed obvious edematous spaces, fibroblasts and fibrin deposition and dilatation of endoplasmic reticulum together with stromal macrophages outside the villous capillaries. There were glycogen storage areas both inside the trophoblast and in the villous core. Also, there were evident areas of perivillous fibrinoid degeneration.

Regarding the thin Sy, thick Cy and scarce microvilli. Similar observations were found by Gul et al. (1), Meng et al. (3), Gheorman et al. (11) and Slukvin et al. (16) who found reduced number of or even absent placental microvilli with immature villous Cy with a dense material comparable to that of hemoglobin of the extra-vasated fetal RBCs (R). Magnification x 1500, bar – 500nm

Figure 10) An electron micrograph of Sy of GDM placenta. It demonstrates the appearance of an inclusion body (I) inside the nucleus (N); it assumes a concentric lamellar appearance with a halo around (crossed arrows). Microvilli (Mv) on the surface are scarce and some have terminal club ends (black arrows). There are cytoplasmic vesicular structures (forked arrows). Magnification x 4000, bar – 500nm

Figure 11) An electron micrograph with very high power magnification of a fetal capillary in GDM placenta. Aggregates of glycogen granules (arrows) are stored particularly near the nucleus (N) of an endothelial cell. Also, dilated cisternae of endoplasmic reticulum (forked arrows) are shown where a homogeneous material of moderate electron density is stored. Notice blebblings of capillary endothelial cytoplasm into the capillary lumen (crossed arrows). Magnification x 4000, bar – 500nm

Figure 12) An electron micrograph of the cone of a terminal chorionic villus of a GDM placenta. Notice, edematous clear spaces (forked arrows) and well-defined storage areas (*) inside the villous core matrix. A stromal macrophage (Ma) is seen that contains lysosomes (white arrows) with a dense material comparable to that of hemoglobin of the extra-vasated fetal RBCs (R). Magnification x 1500, bar – 500nm

Figure 13) An electron micrograph of the cone of terminal chorionic villus of a GDM placenta showing the detailed structure of the storage areas (*). These areas are well-circumscribed by coarse matrix fibers (white arrows) and enclose scattered dense short fibrillar and granular content on a homogenous background of moderate electron density. Notice, edematous clear spaces (forked arrows). Magnification x 1500, bar – 500nm

It was estimated that the maternal diabetic environment induces abnormal uncontrolled cell proliferation in the placenta with increased proliferative activity in the Cy layer with an influence on trophoblastic invasion (6,17). The cells of the thick Cy at the tips of the villi do not invade properly the endometrial vessels adding to the uteroplacental ischemia and hypoxia. Invasion is a complex process involving a range of invasion inhibiting and invasion promoting factors. The diabetic environment with the accompanying oxidative stress appear to shift the balance towards invasion inhibition (1,18). This improper invasion reduces the uteroplacental blood flow resulting in
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hypoxia and increased areas of placental infarction with degeneration of Sy layer leading to shedding and reduction of the number of the villi (3,16).

Also, villous Cy proliferation without syncytial fusion, as observed in severe hypoxia, might be accompanied by syncytial degeneration (5).

Oxidative stress and mitochondrial dysfunction are now emerging as key players in diabetic pathogenesis. It was reported that there is twofold increases in trophoblastic mitochondrial ROS production under hyperglycemic conditions compared to normoglycemic states together with the reduction of the placental antioxidant capacity. ROS with the resulting oxidative stress have a deleterious effect on all biological molecular structures leading to inactivation of specific enzymatic reactions together with loss of function and cell death. Thus, the trophoblast is affected by the hyperglycemic environment with reduction of its proliferation (19-21).

Regarding the thickening of the trophoblastic BM seen in GDM placentas. Similar results were obtained by Gheorman et al. (11), Mage et al. (22), Anshad et al. (23) and Meng et al. (3) in placentas of GDM women. The same was found by Gul et al. (1) in the placenta of diabetic rats. BM thickening might be attributed to mucopolysaccharide storage and it could be due to impaired villous trophoblastic activity such as increased production or decreased turnover of BM molecules, especially that the constituents of the BM are mostly produced by the trophoblast (11). BM thickening was also said to be due to fat droplet accumulation owing to the effect of diabetes on fatty acid oxidation (1,16). These changes may impair uteroplacental transfer further aggravating the hypoxic state (1).

Moreover, the vacuoles in both trophoblastic layers of GDM placentas detected in the current study were found by Meng et al. (3). It was hypothesized that slight vacuolation might be present in the normal human placenta as a natural and physiological form of cell degradation which promotes survival (24,25). On the other hand, GDM with its hyperglycemia and hypoxia may enhance the lysosome/vacuole functions of the trophoblast resulting in a widespread cytoplasmic vacuolation and altered transplacental exchange that may promote cell death (3,25,26).

Regarding the villous core, the present study showed obvious edematous spaces in GDM placentas. Similarly, Gauster et al. (27) and slukvin et al. (28) found edematous spaces in placental villi of GDM women. The same was mentioned by Sulaiman et al. (1) on diabetic rat placentas. Gheorman et al. (11) attributed the villous edema to the mucopolysaccharide deposits with diabetes that consist mainly of hyaluronic acid molecules that can retain water. This edema fluid is interposed as a barrier to the feto-maternal transport of oxygen and nutrients to the fetus (18,32).

Finally, this study showed that hyperglycemia and hypoxia are two key factors in the pathophysiological process of GDM complications and it is hyperglycemia that induces hypoxia and oxidative stress in the placenta. Also, the thickened placental barrier, edematous spaces, fibrin deposition and trophoblastic vacuoles, all of which potentially reduce transplacental transport and exchange with aggravation of ischemia and hypoxia.

CONCLUSION

REFERENCES


Figure 14) An electron micrograph revealing fibrosis inside a chorionic villus core of a GDM placenta; fibroblasts (F) and collagenous bundles (double arrows) are exceptionally prevalent. Magnification x 2500, bar – 500nm.


