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Morphological and ultrastructural changes in the placenta of the diabetic pregnant Egyptian women

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease in which the body fails to produce enough insulin or increased tissue resistance to insulin. The diabetes may have profound effects on placental development and function. This study was designed to detect the placental changes in pregnancy associated with DM comparing these changes with normal placenta. The study was carried out on sixty full-term placentae; divided into three equal groups; control group (group I): placentae of normal pregnancy, uncontrolled diabetes (group II): placentae from pregnant women whose blood glucose is poorly controlled during pregnancy. Controlled diabetes (group III): includes placentae from diabetic women whose blood glucose is controlled during pregnancy. The placentae from group II tend to be heavier and exhibited immaturity of villi, villous edema, fibrosis, excessive syncytial knots formation and infarctions. In addition to, fibrinoid necrosis, increased thickness of vasculosyncytial membrane, syncytial basement membrane, microvillous abnormalities and vascular endothelial changes were demonstrated. The syncytial multivesicular knots were present in placentae of group II. The nuclei within these syncytial knots display condensed chromatin, either dispersed throughout the nucleus or in the form of dense peripheral clumps with and numerous cytoplasmic vacuoles. The syncytial basement membrane showed focal areas of increase in its thickness and irregularity. Villous cytotrophoblasts showed increased number and activity in the form of numerous secretory granules, abundant dilated RER, larger distorted mitochondria. Villous vessels showed various degrees of abnormalities in the form of endothelial cell enlargement, folding, thickening and protrusion of their luminal surfaces into vascular lumen making it narrower in caliber. In placentae of group III, most of these abnormalities decreased. In most of placentae of group III, the VSM appeared nearly normal in thickness and showed nearly normal composition of one layer of syncytiotrophoblastic cells, one layer of smooth, regular capillary endothelium and the space between them. Mild microvillous abnormalities were noted in few placentae as they appeared short and blunted with mild decrease in their number per micron. The electron picture of syncytial knots appeared nearly normal containing aggregations of small, condensed hyperchromatic nuclei, minimal vacuoles could be seen in the cytoplasm of syncytial knots. Syncytial basement membrane appeared regular and nearly normal in its thickness and composition coming in direct contact with fetal blood capillaries but mild abnormalities were noted in the basement membrane in few placentae as increased its thickness and deposition of fibers or fibrinoid. Regarding cytotrophoblasts in the terminal villi of placentae with controlled diabetes, these cells appeared nearly normal. They were scattered beneath the syncytium and were active containing mitochondria, rough endoplasmic reticulum, free ribosomes and a large nucleus with fine dispersed chromatin. The vascular ultrastructural pattern in terminal villi of placentae of this group showed no significant abnormalities and was normally distributed in the villous tree. The luminal surface of the vascular endothelium appeared regular smooth in the majority of placentae of this group. The endothelial cells appeared connected to each other with tight junctions. It could be concluded that whether if long-term diabetes is controlled or not, placentae of diabetic mother showed a variety of significant histological structural changes seen more frequently than in the placentae of pregnant women without diabetes.

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1. Introduction

The placenta acts as natural barrier between the maternal and fetal blood circulations and fulfills a wide range of endocrine and transport functions. This location between the two blood streams makes the placenta not only a crucial regulator of fetal nutrition, gas exchange and maternal immune tolerance, but it becomes also a target for maternal and/or fetal metabolic alterations associated with pregnancy pathologies (Gauster et al., 2012).

Diabetes mellitus (DM) is a chronic metabolic disease in which the body fails to produce enough insulin or increased tissue resistance to insulin. The diabetes may have profound effects on placental development and function. The diabetic pregnancy is characterized by numerous disturbances in pregnant mother herself as well as in the fetus and in the placenta. The fetal growth and development show various abnormalities as fetal macrosomia, congenital malformations, intrauterine growth retardation, spontaneous abortions, hypoxia and polycythemia with neonatal jaundice which are commonly seen in poorly- controlled diabetes (Hiilesmaa et al., 2000). These abnormalities are due to either direct effect of diabetic environment on the fetus or on the placenta as disturbance in trophoblast invasion and dysfunction of glucose, lipid and amino acid transport as well as oxidative stress and inflammation associated with diabetic environment (Staff et al., 2000; Gauster et al., 2012).

Some authors believe that, whether if long-term diabetes is controlled or not, placenta of diabetic mother presents a variety of significant histological structural changes seen more frequently than in the placenta of pregnant women without diabetes (Evers et al., 2003; Abeer et al., 2015).

It has been postulated that the diabetic environment may have profound effects on placental development and function. However, the morphological and histopathological changes in diabetes mellitus placenta are inconsistent and even somewhat controversial.

2. Methods

Sixty placentae were collected immediately after delivery from the Department of Obstetrics and Gynecology, Cairo University Hospital (Kasr Al-Ainy), Egypt and were examined at the Departments of Anatomy and Histology, Egypt. Informed consent for research use of blood samples and placental tissues from all women was obtained prior to the time of labor. The present study was approved by the institutional ethical committee at Kasr Al-Ainy Faculty of Medicine. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki http://www.wma.net/en/30publications/10policies/b3/index.

2.1. Inclusion criteria

- Women during childbearing period (20–38years old)
- Pregnancies reaching full term (which is defined by completed 37 weeks gestation)
- Diabetic patients (controlled and uncontrolled diabetes).
- Singleton pregnancies (pregnancy with single fetus)
- Delivered vaginally or by cesarean section.

2.2. Exclusion criteria

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  - ○ Pregnancies not reaching full term.
  - ○ Twins or multifetal order pregnancies.
  - ○ Associated medical disorders other than diabetes mellitus.

2.3. Experimental design

The placentae were divided into three equal groups; 20 placentae for each group:

Group I (control group): including placentae of normal pregnancy.

Group II (controlled diabetes): this group of placentae was collected from diabetic women whose blood glucose is controlled during the pregnancy. Hemoglobin A1C is less than 7% (Roszyk et al., 2007).

Group III (uncontrolled diabetes): this group of placentae was collected from pregnant women whose blood glucose is poorly controlled during the pregnancy. Hemoglobin A1C is more than 7% (Roszyk et al., 2007).

The level of HbA1C in each trimester was less than 7 in controlled DM but it was 7 or more in uncontrolled DM (Roszyk et al., 2007).

2.4. Gross morphological assessment

Gross observation of placentae and following parameters were examined: weight of placenta, diameter of placenta, thickness of placenta, shape of placenta, the site of attachment of umbilical cord, any associated anomalies in placenta as: placental hematomas and calcifications and neonatal birth weight.

2.5. Histological study

Placental tissue samples were immediately collected after labor from the central part of placenta. Specimens $1cm^3$ in size was processed for light microscopic examination. Subsequently, 5 μm thick sections were stained with: (Bancroft and Gamble, 2008).

- Hematoxylin–eosin stain as usual classical method.
- Periodic Acid Schiff (PAS) techniques for highlighting basal membranes and extracellular matrix. components of mucopolysaccharides and placental villous stroma.
- Masson's trichrome stain for highlighting connective tissue and collagen fibers.

Histological appearance of the villi of placenta was examined and the following parameters were assessed by light microscope: villous edema (fluid- filled spaces within villi), villous immaturity, Syncytial knot formation, Abnormal vascularization, Glycogen deposits, Villous fibrinoid necrosis, Thickness of vasculosyncytial membrane and Villous fibrosis.

2.6. Histomorphometric study

Morphometric measurements of the digitalized images were carried out using the Image Analysis Computer System in Faculty of Dentistry, Cairo University and the following parameters were assessed: Percentage area (%) of fibrosis was measured in Masson's Trichrome-stained sections. In addition, fibrinoid and glycogen in PAS sections were also measured, in 5 different microscopic non overlapping fields (x400) from 10 placentae in each group.

2.7. Transmission Electron Microscopic (TEM) examination

Other placental sections from each group were prepared and exposed to Transmission Electron Microscopic (TEM) examination for ultrastructural changes in TEM laboratory in Cairo University Research Park (CURP), Faculty of Agriculture, Cairo, Egypt.

Tissue samples were sliced into ~1 mm slices. Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 500–1000 μm thickness with a Leica Ultracut (UCT) ultramicrotome. Thin sections were stained with Toluidine blue (1X) then sections were examined by camera Lica ICC50 HD. Ultrathin sections prepared at approximately 75–90 μm thickness and were stained with uranyl acetate and lead citrate, then examined by transmission electron microscope JEOL (JEM-1400 TEM) at a suitable