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Histopathological Changes of Umbilical Cord Blood Vessels in Diabetic Pregnancies

Fouzi Aboud[@1], Fairouz Torjman[2], Mohammed Sultan[3] and Ahmeda Benjama[4]

[1]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli- Libya.
[2]Department of Pathology, Tripoli Medical Center, Faculty of Medicine, University of Tripoli - Libya.
[3]Department of Obstetrics and Gynecology, Aljala Maternity Hospital, Tripoli - Libya.
[4]Department of Histology and Genetics, Faculty of Medicine, University of Tripoli, Tripoli - Libya.

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ABSTRACT

Histopatological changes of umbilical cord (UC) functions due to diabetes mellitus (DM) are resulting in fetal hyperinsulinemia, which in turn stimulates hematopoiesis and fetal erythema. This may increase metabolic rate and oxygen requirements in the response of several factors such as hyperglycemia, ketoacidosis and vascular diseases. The study aimed to evaluate the histopatological changes of UC blood vessels in diabetic pregnant women.
A cross- sectional, analytical study was designed. A total of 75 UC samples were collected from Aljala Maternity Hospital, Tripoli-Libya, from March to December 2017. Out of them 25 were from non - complicated pregnancies, 25 were pre-gestational diabetes mellitus (PGDM group) and 25 were gestational diabetes mellitus (GDM group). Segments of UC were taken at 5 cm from fetal, central and placental attachments for each group. All tissue segments were stained by special stains and examined under light microscope.
The mean weight of UC was larger in PGDM than GDM and control groups respectively. The tissue segments of PGDM in comparison to GDM showed widely edematous spaced smooth muscle cells, more increased amount of collagen and elastic fibers, glycogen, proteoglycans (PGs) and glycosaminoglycan (GAGs) molecules, mostly in central segments. Hugely dilated and discordant umbilical arteries were observed in fetal segments of GDM. Histopatological changes revealed that diabetic pregnancy had a higher effects on PGDM than GDM.

Keywords- *Histopatological; Umbilical Cord; Blood Vessels; Diabetic Pregnancies.*

INTRODUCTION

Perinatal morbidity and mortality remains a serious problem threat to fetuses' life of diabetic pregnancies. Despite the enhancements made in diagnosis and management of DM, diabetic pregnancies are still exposed to spontaneous abortion and stillbirth, with an increased risk of congenital malformations.[1] DM in pregnancy has shown an increasing rates of disease and its effects such as preeclampsia, primary cesarean delivery, macrosomia, birth injury and clinical hypoglycemia.[2] Recent studies showed that PGDM pregnancies are associated with higher morbidty and mortality rates than GDM.[3] The UC is a cylinder vascular system, plays a vital role in continuation of pregnancy and any disruption of its functions is considered a prime source of damage to normal growing of fetus[4], where it provides an interrupted blood flow from the placenta to the fetus during its development.[5] The cells within the UC possess plasticity and ability for differentiation, they may be use to assess the biological responses that are associated with diabetic pregnancy.[6]

The Royal College of Pathologists has reported that any sample of diagnostic value removed from the human body should be histologically examined.[7] After childbirth, the UC alongside placenta is disposable as medical waste, which may additionally for the relative lack of knowledge and interest inside UC that may improve pregnancy outcome.[8]

Histopathological changes of UC functions due to DM are resulting in fetal hyperinsulinemia, which in turn stimulates hematopoiesis and fetal erythema. This may increase metabolic rate and oxygen requirements in response of several factors such as hyperglycemia, ketoacidosis and vascular diseases.[9] Histological examination of UC is considered an essential component for evidence of cord occlusion and hypoxia; which resulted in postnatal morbidity and mortality.[10] Therefore, this study was designed to evaluate histopathological changes of UC blood vessels in PGDM and GDM.

@ Author to whom correspondence should be addressed. Dr. Fouzi Aboud; Tel.+21891 5981973; E-mail: Fouzi.aboud.1987@gmail.com

MATERIALS AND METHODS

Study design, setting and duration:

A cross-sectional, analytical study, was done in the Department of Obstetrics and Gynecology at Algalaa Maternity Hospital, Department of Histology and Genetics, Faculty of Medicine, University of Tripoli; Tripoli-Libya, in the period from March to December 2017.

Study population:

880 pregnancies, aged between 17 and 45 years of gestational age ranged within 36th and 41th weeks, participated in this study; 75 pregnancies were selected according to strict criteria; 25 of non- complicated pregnancies, 25 were PGDM and 25 were GDM. The pregnancies that experienced any complications before or during pregnancy such as hypertension and thyroid dysfunctions were excluded.

Study samples:

A total of 75 segments of 5 cm were taken from fetal, central and placental attachments of UC for each group. Then the specimens placed in plastic containers filled with buffered formalin 10% and kept at room temperature for further preparations and examinations.

Study tools:

The UC weight (UCW) was measured in grams by directly placing the cord on digital scale. Two centimeters of tissue segments were dehydrated in ascending levels of alcohol and xylene, followed by embedding in soft paraffin in oven at 60ºC overnight, followed by embedding in hard paraffin then 4 µm serial sections were cut by rotator microtome. The tissue sections were stained by special histological and histochemical stains by Bancroft and Gamble, (2008).[11]

Data management and analysis:

The UCW was entered and analyzed in a computer using Statistical Package for Social Science (SPSS) (Version 16.0). Analysis Of Variance (ANOVA). One Way test was used to compare the variable means. *P*- value ≤ 0.05 was considered as the level of significance.

Ethics consideration

All the participants were fully informed and explained about the nature of study and written consents were taken.

RESULTS

Macroscopic examination:

The UCW showed highly significant difference between study groups ($P < 0.001$). The mean weight of the UC was 53.52 ± 17.17g, 50.24 ± 20.56g and 34.96 ± 11.20g in PGDM, GDM and control respectively (Table 1).

Table 1: Results of macroscopic examination of the UC.

Range	(22 – 58)	(23 – 104)	(27 – 106)	
Mean ± SD	34.96 ±11.20	53.52 ± 17.17	50.24 ± 20.56	<0.001

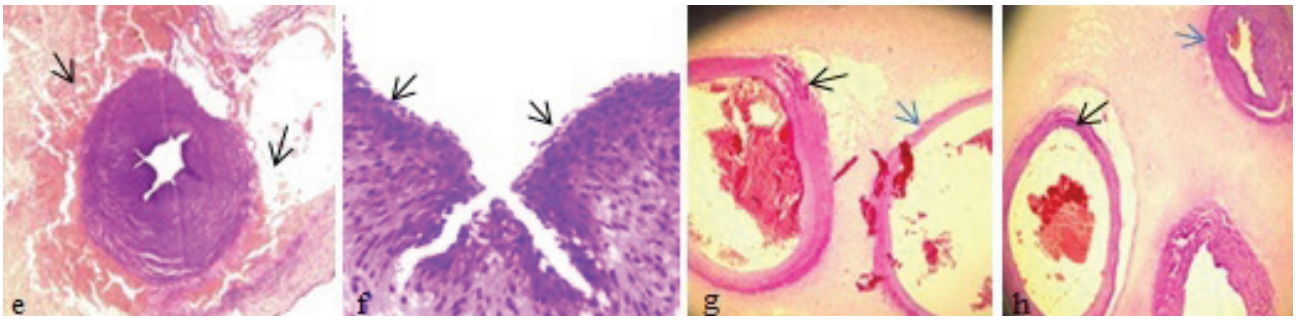

Figure 1A: Photomicrographs of control group showing

The general architecture of the UC consisting of two arteries (As) and one vein (V) (H&E, 25×). (b) A higher magnification of umbilical artery showing the luminal endothelium appears with flattened pale stained nuclei (arrows), (H&E, 400×). (c) A umbilical vein consisting of an inner longitudinal (IL) and outer circular (OC) smooth muscle layers (H&E, 100×). (d) The general architecture of umbilical WJ covered by amniotic membrane (arrows (H&E, 400×).

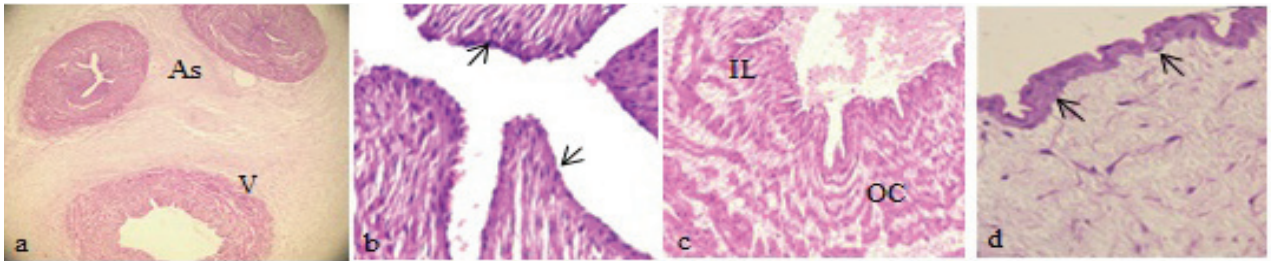

Figure 1B: Photomicrographs of PGDM group showing.

(e) Central segment of umbilical artery showing; an extensive hemorrhage of WJ (arrows) (H&E, 40×). (f) A higher magnification of previous micrograph showing endothelium of the artery with flattened dark stained nuclei and acidophilic cytoplasm (arrows) (H&E, 400×). Photomicrographs of GDM group showing: (g) Fetal segment showing hugely dilated umbilical arteries (arrows) (H&E, 25×). (h) Fetal segment showing discordant umbilical arteries (arrows) (H&E, 25×).

Microscopic examination:

Haematoxylin and eosin (H&E):

***Control group*:**

The histological findings of normal UC samples showed, two arteries and one vein embedding in the Wharton›s Jelly (WJ) (Figure 1A-a). They lined by endothelial cells with pale stained nuclei resting on subendothelial connective tissue (Figure 1A-b). They also showed, a double muscle layer consisting of inner thin longitudinal smooth muscle cells and outer thick circular spiraled muscle cells (Figure 1A-c). The WJ covered by a single layer of cubical epithelium (Figure 1A-d).

***PGDM group*:**

Central segment of umbilical artery showed**,** an extensive hemorrhage of degenerative WJ (Figure 1B-e), appeared with dark pyknotic stained nuclei and highly acidophilic cytoplasm (Figure 1B-f).

***GDM group*:**

Fetal segments of umbilical arteries showed, hugely dilated thinness muscular layers of umbilical arteries (Figure1B-g), luminal discordant with normal structure of the umbilical vein (Figure1B- h).

Mallory's trichrome:

***Control group*:** Placental segments compared to central and fetal segments showed, a higher amount of collagen fibers in subendothelial layer and between smooth muscle cells of the umbilical blood vessels.

***PGDM group*:**

Central segment of umbilical artery showed, an increased amount of collagen fibers with an extensive hemorrhage of WJ (Figure 2A-a). Central segment showed an extensive hemorrhage of WJ adjacent to the umbilical vein with diminution its lumen and degeneration changes of external layers (Figure 2A-b).

***GDM group*:**

Central segment of umbilical artery showed, increased amount of collagen fibers in subendothelial layer and around the artery with sever extensive hemorrhage of the WJ (Figure 2A-c).

Fetal segment of umbilical vein showed, an increased amount of collagen fibers in subintimal layer (Figure 2A-d).

Van Gieson stain:

***Control group*:** The umbilical blood vessels showed, a few collagen fibers in Subendothelial layer, between smooth muscles and around the blood vessels.

***PGDM and GDM groups*:**

Staining the sections of umbilical blood vessels from diabetic groups by Van Gieson stain confirms the presence of fibrin and fibrosis that obtained by Mallory's Trichrome (Figure 2B-e,f,g,h).

Orcein stain:

***Control group*:** Elastic fibers appeared as dark brown and thin wavy lines within the media of the umbilical artery and subintimal layer of umbilical vein.

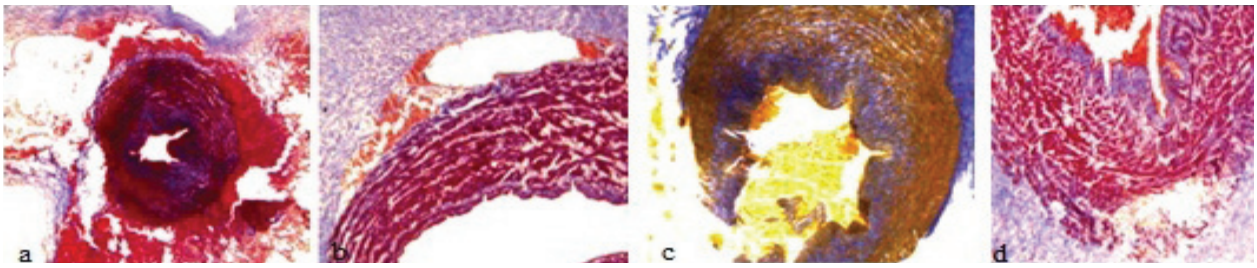

Figure 2A: Photomicrographs of PGDM group showing.

(a) Central segment of umbilical artery showing; an increase of collagen fibers (stained blue) (Mallory's trichrome, 40×). (b) Central segment of umbilical vein showing; an increase of collagen fibers between smooth muscle cells (stained blue) (Mallory's trichrome, 100×). Photomicrographs of GDM group showing: (c) Central segment of umbilical artery showing; an increase of collagen fibers in subitimal layer and around artery (stained blue) (Mallory's trichrome, 100×). (d) Fetal segment of umbilical vein showing; an increase of collagen fibers in subitimal layer (stained blue) (Mallory's trichrome, 40×).

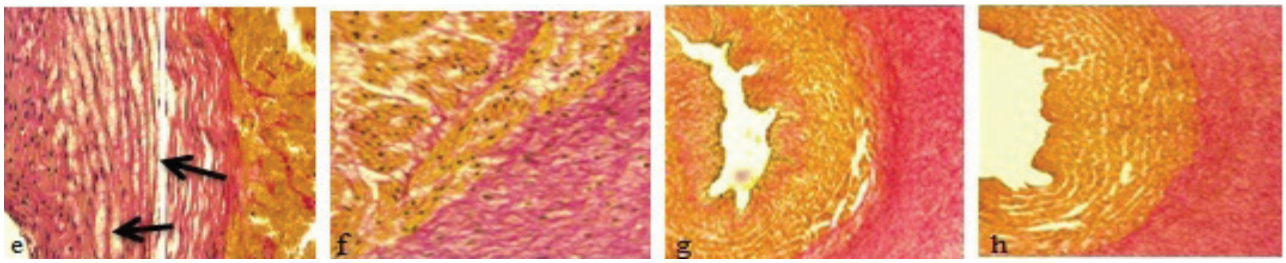

Figure 2B: Photomicrographs of PGDM group showing.

(e) Central segment of umbilical artery showing; multiple spaces separating muscle layers (arrows) (Van Gieson, 200×). (f) Central segment of umbilical vein showing; an increase of collagen fibers in between smooth muscle and around vein (red stained) (Van Gieson, 200×). Photomicrographs of GDM group showing (g) Central segment of umbilical artery showing; an increase of collagen fibers in subendothelial layer and around the artery (red stained) (Van Gieson, 100×). (h) Placental segment showing; an increase of collagen fibers around the vein (red stained) (Van Gieson, 100×).

***PGDM group*:** More pronounced of the elastic fibers were observed within the media and subintimal layers of umbilical artery and vein (Figure 3a and 3b).

***GDM group*:** Much more pronounced of the elastic fibers were observed within the media of umbilical artery whereas less pronounced observed in subintimal layer of umbilical vein (Figure 3c and 3d).

Periodic-acid shiff stain (PAS):

***Control group*:**

Significant deposition of glycogen granules was observed more in subendothelial layer and cytoplasm of smooth muscle cells of placental compared to central and fetal segments respectively.

***PGDM group*:**

Significant deposition of glycogen granules with strong PAS reactivity was observed more in central segments (Figure 4a). Marked thickening of the basement membrane of amniotic epithelium with strong PAS reactivity was seen in the tissue sections (Figure 4b).

***GDM group*:**

Significant deposition of glycogen granules varied between moderate and strong PAS reactivity was observed more in central segments (Figure 4c). Normal thickening of the basement membrane of amniotic epithelium was seen in the tissue sections (Figure 4d).

Methyl green pyronin reaction:

***Control group*:**

Methyl green pyronin reaction showed, variations in deposition of PGs molecules varied between weak and moderate in the extracellular matrix (ECM) of the tissue sections.

***PGDM group*:**

Methyl green pyronin reaction showed, strong deposition of PGs molecules in the ECM of the tissue sections (Figure 5a).

***GDM group*:**

Methyl green pyronin reaction showed, moderate to strong deposition of PGs molecules in the ECM of the tissue sections (Figure 5c).

Toluidine blue reaction:

***Control group*:** Toluidine blue reaction showed, weak to moderate deposition of GAGs molecules in the ECM of the tissue sections.

***PGDM group*:**

Toluidine blue reaction showed, strong deposition of GAGs molecules in the ECM of the tissue sections (Figure 5b).

***GDM group*:**

Toludine blue reaction showed, moderate to strong deposition of GAGs molecules in the ECM of the tissue sections (Figure 5d).

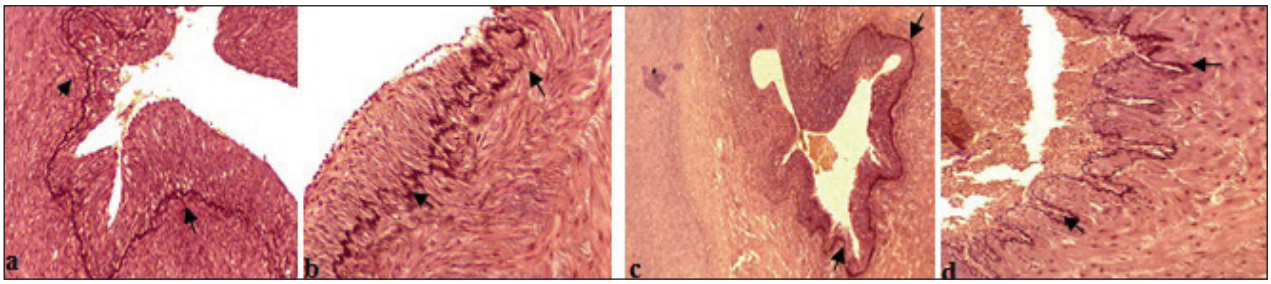

Figure 3: Photomicrographs of PGDM group showing.

(a) Central segment of umbilical artery showing; more pronounced of thick elastic fibers within the media layer (arrows) (Orcein, 40×). (b) Fetal segment of umbilical vein showing; more pronounced of thick elastic fibers in subintimal layer (arrows) (Orcein, 40×). Photomicrographs of GDM group showing : (c) Central segment of umbilical artery showing; much more pronounced of thick dense elastic fibers within the media layer (arrows) (Orcein, 40×). (d) Placental segment of umbilical vein showing; less pronounced of the elastic fibers within the media layer (arrows) (Orcein, 40×).

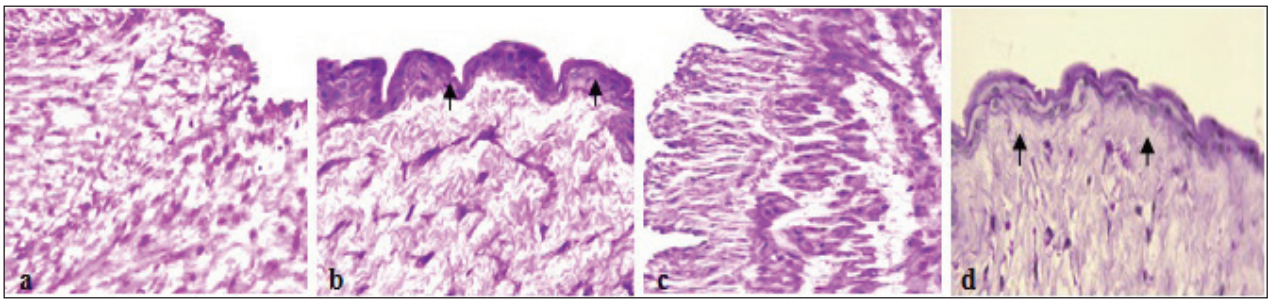

Figure 4: Photomicrographs of PGDM group showing.

Central segment of umbilical artery denoting; stronger periodic acid-schiff (PAS) reaction (200×). (b) Marked thickened basement membrane of amniotic epithelium (arrows) (PAS, 200×). Photomicrographs of GDM group showing: (c) Central segment of umbilical vein denoting; moderate periodic acid-schiff (PAS) (200×). (d) Normal thickened basement membrane of amniotic epithelium (arrows) (PAS, 200×).

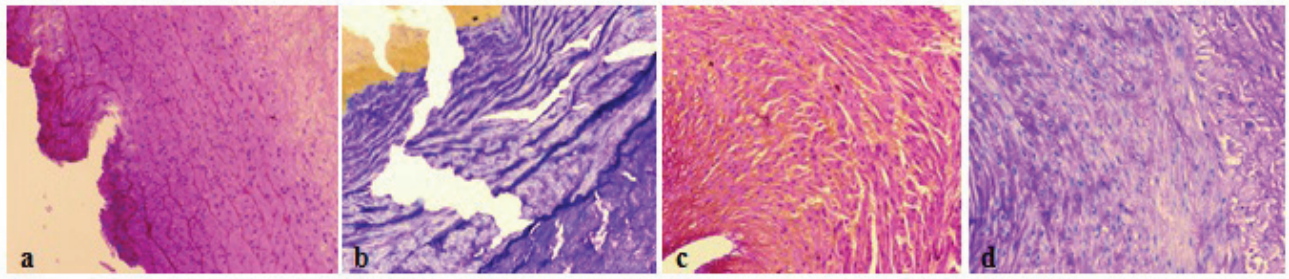

Figure 5: Photomicrographs of PGDM group showing.

(a) Central segment of umbilical vein showing; strong Methyl Green Pyronin reaction (red color) (200×). (b) Fetal segment of umbilical artery showing; strong Toluidine Blue reaction (red-purple color) (200×). Photomicrographs of GDM group showing: (c) Central segment of umbilical artery showing; moderate Methyl Green Pyronin reaction (red color) (200×). (d) Central segment of umbilical vein showing; moderate Toluidine Blue reaction (red-purple color) (200×).

DISCUSSION

Diabetic pregnancy has shown that changes of UC are still occurring even in an intensive recent management of DM. In order to explain that, it should be understood the histopathological changes of UC quantitatively in diabetic pregnancies have not been mentioned in details in previous studies. The macroscopic examination revealed that mean weight of UC from PGDM was significantly larger than GDM and control groups. The pathophysiology behind weight gain indicated the increase of both contents and density of WJ. Accordingly, this may affect the fetal growth as result of prolonged effects of DM. In this study, abnormal accumulation of blood within the WJ was observed in central segments of PGDM and GDM groups as result of degenerative effects of DM. In a Bangladeshi study, by Chakraborty and Banu (2013)[12], they found severe erosion and rupture of vascular endothelium resulting in extravasation of blood within the WJ. These findings were consistent with the study of Tahaoglu *et al*. (2015)[13], in Turkey. An Italian study, by Di Fulvio *et al*. (2014)[14], stated that degeneration of vascular endothelium in diabetic pregnancies could have originated from oxidative stress and over-expression of inflammatory cytokines such as nitric oxide synthase and nitrotyrosine. In GDM group, hugely dilated lumen with thinness muscular layers of umbilical arteries were observed in samples taken from fetal segments. A Turkish study, by Cetin *et al*. (2002)[15], found that thinness of muscular layer may be attributed to narrowing of conjunctive tissue separating muscular layers. Similar findings were observed by Alam *et al*. (2014)[16], in Bangladesh. On the other hand, an Iraqi study by Lateef (2015)[17], she found that splitting of internal elastic lamina in tunica intimal and degeneration of endothelial layer reducing the number of smooth muscle fibers and affect the total thickness of umbilical blood vessels. This indicated that umbilical blood vessels of diabetic pregnancies had a tendency for increase luminal diameter and decrease its thickness. Another important histological change observed in GDM group, was luminal discordant of umbilical arteries in sections taken from fetal segments. Predanic and Perni (2006)[18], stated that luminal discordant of umbilical arteries has direct influence on fetal blood flow. In New York, they found that larger umbilical artery produced lower resistance to blood flow, whereas smaller artery could not equally produce higher resistance to blood flow because of presences of Hyrtl's anastomosis. An increased amount of fibrinoid deposition identified between smooth muscle cells and around the umbilical blood Vessels. In PGDM and GDM groups, collagen fibers deposition found more associated with rupture muscle layers, mostly in central and fetal segments. There are many explanations for the deposition of fibrinoid in the UC. The most acceptable one explained by Rampersad and Nelson (2007)[19], in Washington; which may attributes to mechanical defect in blood flow to UC as consequence of maternal blood stasis. The elastic fibers were found more pronounced in PGDM group. On the other hand, in GDM group, highly dense and thick elastic fibers were found much more pronounced in the umbilical arteries compared to umbilical veins. Increased of collagen and elastic fibers, particularly in umbilical arteries caused decreasing in the elasticity and made the umbilical blood vessels more stiff which may have effects on luminal dilation and constriction mechanism of blood vessels during blood flow between placenta and fetus. Another histological change recognized in this study, multiple spaces were separating smooth muscle layers in the central segments of PGDM and some of GDM. These findings were inconsistent with Jain *et al*. (2014)[20], in India, where they found many empty spaces within the WJ which indicates degeneration process of DM. Thickening basement membrane of UC amniotic epithelium was observed in diabetic groups, particularly in PGDM. In an American study, by Brace and Wolf (1989)[21], a rapid bidirectional exchange diffusion was observed between fetus and an amniotic fluid across the UC. The main reason behind multiple spaces, may be due to increase of fluid between muscle cells as result of thickening basement membrane and might explain associated edema. An important histological change identified in this study was deposition of glycogen, PGs and GAGs molecules. In PGDM group, an accumulation of large amount of glycogen was observed in the cells of intima and media layers of the blood vessels. On the other hand, GDM group, showed significant variations of glycogen deposition varied between small and large amounts. Similar results were observed by Asmussen, in (1980)[22], in Denmark, where he found a great amount of glycogen granules distributed in cytoplasm of smooth muscle cells of intima layer. In PGDM group, significant strong reaction of PGs and GAGs was observed

in the tissue sections. Whereas in GDM group, significant variations of PGs and GAGs reaction varied between moderate and strong among tissue segments. A study achieved by Galewska *et al*. (2008)[23], in Poland, they found that an accumulation of PGs and GAGs molecules may be attributed to increase biosynthesis of those molecules which affects the biological process of UC and the solubility of collagen fibers. It suggested that an accumulation of such molecules may be enhanced by several growth factors mainly insulin-like growth factor.

CONCLUSION

Histopatological changes revealed that diabetic pregnancy had a higher significant effects on PGDM than GDM, particularly in the segments taken from central attachments, this may be attributed to UC hypoxia due to excessive UC coiling at central segment as result of increased contents and density of the WJ.

RECOMMENDATIONS

Evaluate the effects of various pharmacologic agents of DM on the maternal and UC to identify which drug can maintain normal histological structure of UC and improve fetal blood flow.

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