Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.13, No.6, 2023

A Comparison of the Human Umbilical Cord's Histomorphometric and Histological Structure in Pregnant Diabetic and Non-Diabetic Women

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Abstract
This study aimed to explore the histomorphometrical and histopathological alterations of umbilical cord (UC) vessels caused by gestational diabetes mellitus (GDM) and pre-gestational diabetes mellitus (PGDM). A total of thirty UC samples were obtained from full term pregnant women without any complications. Ten out of thirty UCs were obtained from non-diabetic pregnant women (normal group), 10 from GDM and 10 from PGDM pregnant women. Segments from the placental attachment, center and fetal side of UCs were taken for each group. These segments were processed for paraffin blocks, sectioned, and stained with H&E, Masson trichrome (MT) and Periodic Acid Shift (PAS). The results of the histomorphometric study showed no significant differences in the UC mean weight among these three groups. In three different segments, GDM resulted in a significant decrease in artery and vein wall thickness compared to the control group. GDM and PGDM resulted in a non-significant difference in the diameter of artery and vein in fetal segment, the vein in placental segment, and artery in the central segment compared with normal. All the UCs in the three groups contained two arteries and one vein but only one cord recorded in the GDM group contained one artery and one vein. Histological study of diabetic UC segments showed extravasation of blood, artery discordance, degeneration of Warton's jelly (WJ) fibers with formation of honeycomb like empty spaces, formation of multiple spaces between smooth muscle cells of tunica media and detachment of the umbilical arteries from surrounding WJ. In both diabetic groups, there was a marked decrease in collagen fibers in tunica intima and media with their irregular arrangement in both arteries and vein especially in placental segment. The results also showed there was a rich carbohydrate content in the intima and media in all three groups. In conclusion, the current results proved that GDM and PGDM have an adverse effect on the structure of UC and its vessels.
Keywords: Umbilical cord; Diabetes mellitus; Histological study; Special stains; Blood vessels
DOI: 10.7176/JBAH/13-6-04
Publication date: April 30th 2023

1. Introduction

The umbilical cord (UC) is an important organ that runs from the fetal umbilicus to the fetal surface of the placenta and serves as a lifeline between the mother and the fetus. It works throughout pregnancy to secure the blood vessels that connect the baby and placenta, allowing for the flow of nutrients and gas (Salem *et al.*, 2019).

The umbilical cord has two umbilical arteries and one umbilical vein, which are suspended in an embryonic connective tissue known as Wharton's jelly (WJ). These blood vessels serve as conduits of communication between the placental and fetal circulatory systems. All these components are held together by a membrane known as the amnion (Lateef, 2013).

Diabetes mellitus (DM) is a well-known category of metabolic illnesses in which a person has excessive blood sugar levels, is caused by either insufficient insulin production by the pancreas or inefficient cell response to this insulin (Blanco *et al.*, 2011).

Pre-gestational diabetes mellitus (PGDM) is the term used for carbohydrate intolerance that existed before becoming pregnant, while gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of varied severity with onset or first detection during pregnancy (El Sawy *et al.*, 2018).

Gestational diabetes mellitus is a known risk factor for pregnancy complications such as macrosomia, shoulder dystocia, birth trauma, increased rate of cesarean delivery, and neonatal metabolic disorders. Obesity and impaired glucose tolerance are long-term health adverse effects in children born to mothers with GDM. Women who previously had GDM are more likely to develop diabetes later in life (Gagnon *et al.*, 2011; Tachibana *et al.*, 2013). Therefore, the present study was designed to evaluate the histomorphometrical and histopathological alteration of UC vessels caused by GDM and PGDM women in comparison with normal (healthy) pregnant women.

2. Materials and Methods

The present study was approved by the Medical Ethics Committee of the Duhok Directorate of General Health-Directorate of Planning –Scientific Research Division, Kurdistan Region-Iraq, with reference number 08032023-

2-7. After patient consent, the UC samples were collected from the Zakho Maternal Hospital and Duhok Maternal Hospital. The experimental work for this study was carried out in the Laboratory of Zoology, Department of Biology, Faculty of Science, University of Zakho.

Thirty pregnant women between the ages of 17 and 35, with gestational ages ranging between the 36th and 38th weeks, took part in this study and were divided into three groups, as follows:

- Group (1): Normal group (10 pregnant women with normal blood glucose levels and without any complications).
- Group (2): Diabetic group (10 pregnant women with GDM).
- Group (3): Diabetic group (10 pregnant women with PGDM).

All cords of full term looked healthy neonates were taken. After the delivery 5 centimeters (cm) from the abdominal wall, it was cut using scissors. The part of the cord from the cut end to the placenta insertion was taken and weighted using an electrical balance. Then they were placed in plastic containers, after that, three segments (1 cm for each segment) were taken from fetal, central and placental side of each UC. That's 30 segments for each group and in total 90 segments were collected for this study. These segments were placed in plastic containers filled with 10% of neutral buffered formalin and kept at room temperature for further preparations.

Three different staining protocols, namely and hematoxylin & eosin (H&E), Masson Trichome (MT), and Periodic Acid Shift (PAS) stains were used in this study to demonstrate and highlight the structure of UC vessels in three different groups (normal, GDM and PGDM).

2.1 Exclusion criteria.

According to Salem *et al.*, (2019), the pregnancies that experienced any complications before or during pregnancy such as smoking, hypertension, corticosteroid therapy, pre-eclampsia and thyroid dysfunction were eliminated.

2.2 Morphometric analysis of UCs section

Five sections for each segment (three segments per a sample = 150 UC sections per each group) were selected for examination. In each segment the following parameters were measured using the Dino-Eye camera:

1. Artery and vein diameter.
2. Wall thickness of the artery and vein.
3. Thickness of artery tunica intima, tunica media and vein (whole thickness).

2.3 Histological and histopathological studies.

The sections of UCs were examined microscopically in all groups in order to find out the histopathological changes that occur due to the effect of DM compared to a normal histological feature in the control group. Photographs were taken using (Dino-Eye microscopic camera).

2.4 Statistical analysis

The collected data was analyzed on a computer using GraphPad Prism (version 8). To find out the difference between three groups, one-way ANOVA (Analysis of Variance) was used (Keselman and Rogan, 1977).

3. Results

3.1 The effect of GDM and PGD on umbilical cord weight (macroscopic examination).

Table 0-1), indicated that there was no significant difference and alteration ($P > 0.05$) in UC weight in GDM and PGDM groups than in the normal group, although there is a slight increase in weight in the diabetic groups but it did not reach statistical significance.

Table 0-1: Mean ± S.E for the effect of GDM and PGDM on the weight of umbilical cord.

Umbilical cord weight (gm) Mean ±SE			
Normal group	GDM group	PGDM group	Level of significance
29.11±2.65	33.88±3.4	32.7±1.19	N vs GDM ns N vs PGDM ns GDM vs PGDM ns

ns refers to no significant difference at ($P > 0.05$). (*) refers to significance difference at ($P \leq 0.05$). (**) refers to highly significance difference at ($P \leq 0.01$) according to the Tukey multiple comparison test.

3.2 Histomorphometric measurements.

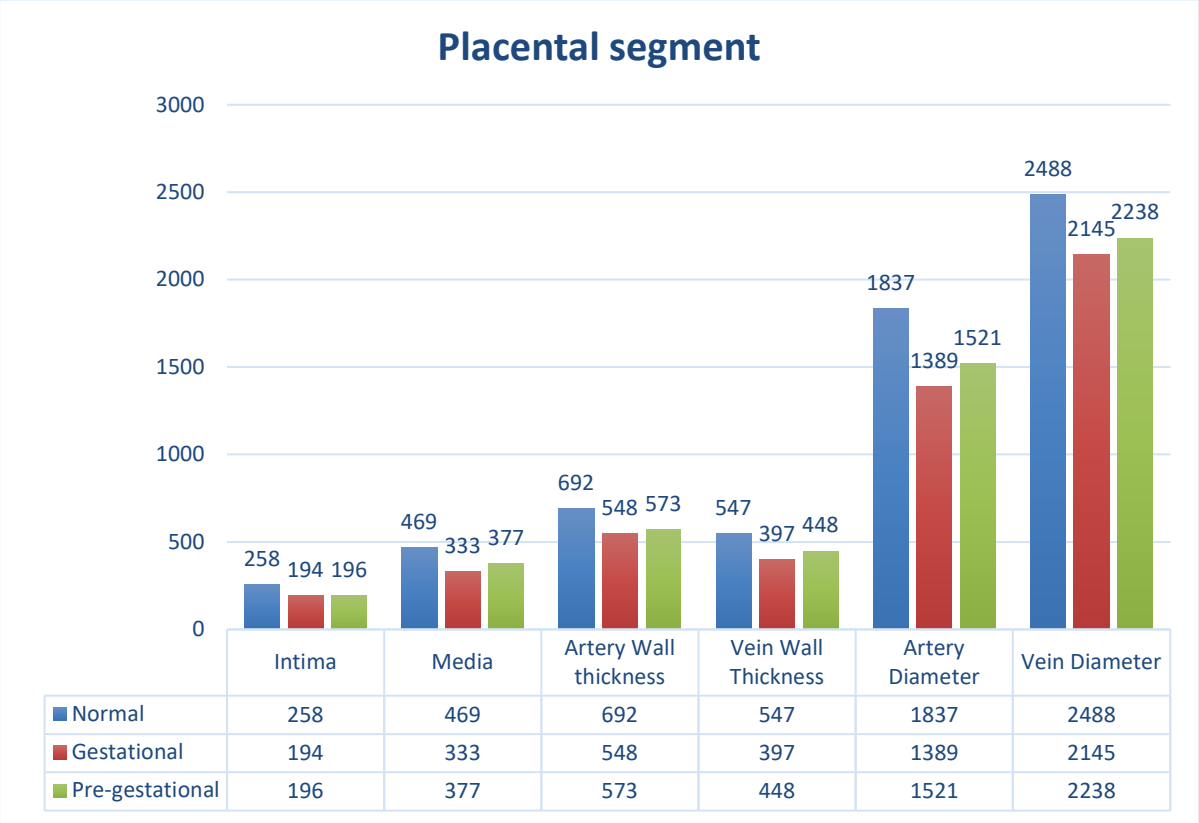
3.2.1 The effect of GDM and PGDM on the thicknesses of (tunica intima, media) and whole artery and diameter of the artery and vein in placental segment

As shown in diagram (1), the result of the placental segment indicated that GDM and PGDM caused a highly significant decrease ($P \leq 0.01$) in the thicknesses of the tunica intima, while these groups caused a significant decrease ($P \leq 0.05$) and a highly significant decrease ($P \leq 0.01$) respectively, in the thickness of the tunica media

compared to the normal group. But no significant difference was recorded ($P > 0.05$) in this thickness when comparing the GDM to the PGDM group.

In comparison with the control group, this diagram also indicated that GDM and PGDM caused a highly significant decrease ($P \leq 0.01$) and a significant decrease ($P \leq 0.05$) in the thickness of the (artery and vein) walls and artery diameter, respectively.

Regarding the vein diameter, both diabetic groups showed a non-significant difference ($P > 0.05$) compared to the normal group. No significant difference was found between the effects of the GDM and PGDM groups on the thicknesses of the (intima, media and whole artery and the diameter of the artery and vein.



	Intima	Media	Artery Wall thickness	Vein Wall Thickness	Artery Diameter	Vein Diameter
Normal	258	469	692	547	1837	2488
Gestational	194	333	548	397	1389	2145
Pre-gestational	196	377	573	448	1521	2238

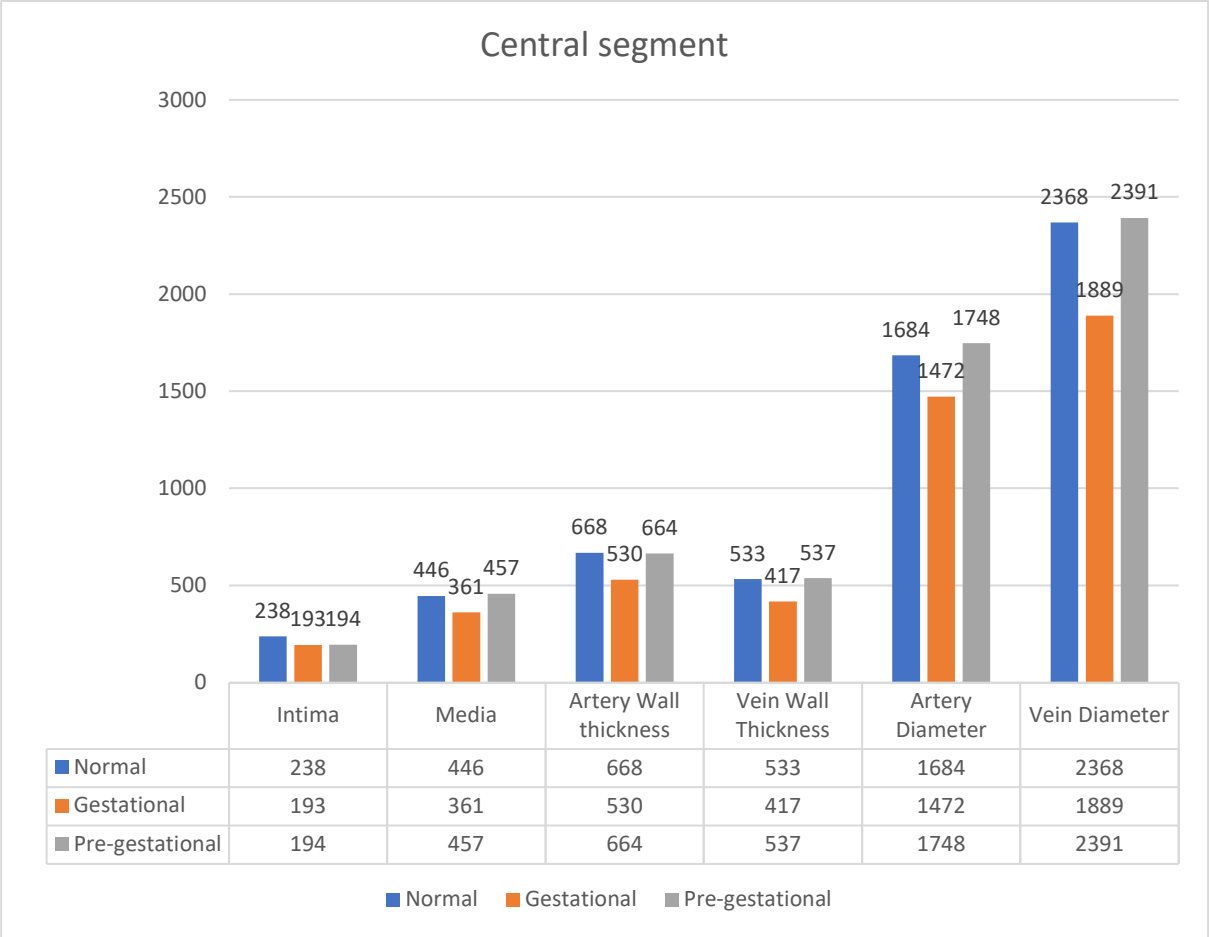
Diagram (1): Thickness and diameter of the artery and vein in the placental segment in the normal, GDM and PGDM groups.

3.2.2 The effect of GDM and PGDM on the thicknesses of (tunica intima, media) and whole artery and diameter of the artery and vein in central segment.

The result of the central segment (Diagram 2) revealed that both GDM and PGDM cause a statistically significant decrease ($P \leq 0.05$) in the tunica intima thickness compared to the normal group. But there was no significant difference ($P > 0.05$) documented between diabetic groups. The results also showed a highly significant decrease ($P \leq 0.01$) in the thickness of tunica media in the GDM group compared to other groups. In contrast, media thickness showed no significant difference ($P>0.05$) comparing the PGDM to the normal group.

Gestational diabetes mellitus caused a highly significant decrease ($P \leq 0.01$) and a significant decrease ($P \leq 0.05$) in the thickness of the artery and vein walls, respectively, in comparison with the normal group. The results also documented a highly significant decrease ($P \leq 0.01$) in the artery wall thickness and a significant decrease ($P \leq 0.05$) in the vein wall thickness in the GDM group compared with the PGDM group. But there was no significant difference ($P > 0.05$) recorded between the normal and PGDM groups in the wall thickness of the artery and vein.

As indicated in diagram (2), the UC artery in the central segment showed a non-significant difference ($P > 0.05$) in its diameter in both the GDM and PGDM in comparison to the normal group. Meanwhile, GDM caused a significant decrease ($P \leq 0.05$) in this diameter when compared with PGDM groups. The GDM group highlighted a significant decrease ($P \leq 0.05$) in the vein diameter compared to other groups. But PGDM showed no significant difference ($P > 0.05$) if compared with the normal group.



	Intima	Media	Artery Wall thickness	Vein Wall Thickness	Artery Diameter	Vein Diameter
Normal	238	446	668	533	1684	2368
Gestational	193	361	530	417	1472	1889
Pre-gestational	194	457	664	537	1748	2391

Diagram (2): Thickness and diameter of the artery and vein in the central segment in the normal, GDM and PGDM groups.

3.2.3 The effect of GDM and PGDM on the thicknesses of (tunica intima, media) and whole artery and diameter of the artery and vein in fetal segment.

A highly significant decrease ($P \leq 0.01$) was recorded in the thickness of the tunica intima in the GDM group compared to the normal group. On the other side, PGDM presented no significant difference ($P > 0.05$) in the intima layer thickness compared to both the GDM and normal groups.

The present study shows that a significant decrease ($P \leq 0.05$) in the thickness of tunica media was observed when both the normal and PGDM groups were compared to the GDM group. In contrast, PGDM highlights no significant difference ($P > 0.05$) in the media thickness in comparison to the normal group.

The effects of GDM and PGDM showed no significant difference ($P > 0.05$) in their effects on the diameter of arteries and veins and vein wall thickness compared to the normal group. While a highly significant difference ($P \leq 0.01$) was found in the fetal segment in the artery wall thickness if the GDM group was compared to other groups (Diagram 3).

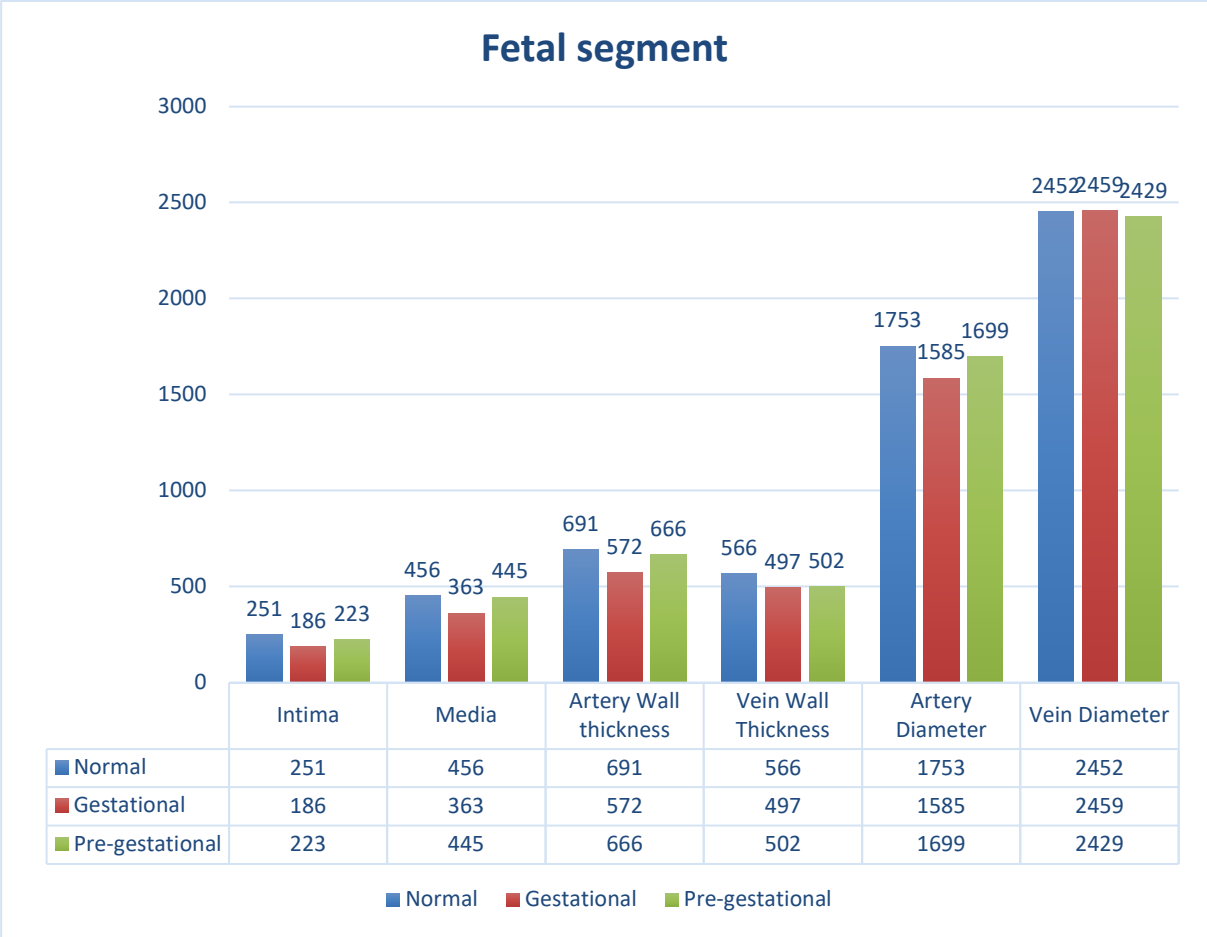


Diagram (3): Thickness and diameter of the artery and vein in the fetal segment in the normal, GDM and PGDM groups.

3.3 Histological and histopathological study of the effect of GDM and PGDM on the umbilical cord vessels.
Control (non- diabetic) Groups

The microscopic examination of the normal UC placental (Fig.1), central (Fig.2) and fetal (Fig.3) segments revealed the normal histological structure of the UC, which contains two arteries and one vein in addition to the allantoic channel, all of them were embedded in mucoid (embryonic) connective tissue (WJ). The UC artery was characterized by a constricted irregular branches lumen (star shaped lumen), and thicker tunica media than tunica intima. While the UC vein was characterized by a wider lumen and thinner wall, consisted of thin intima and media and was surrounded by a layer of the WJ. The WJ consists of fibroblast cells and is enclosed by the amniotic membrane, which formed of single layer of cuboidal epithelium.

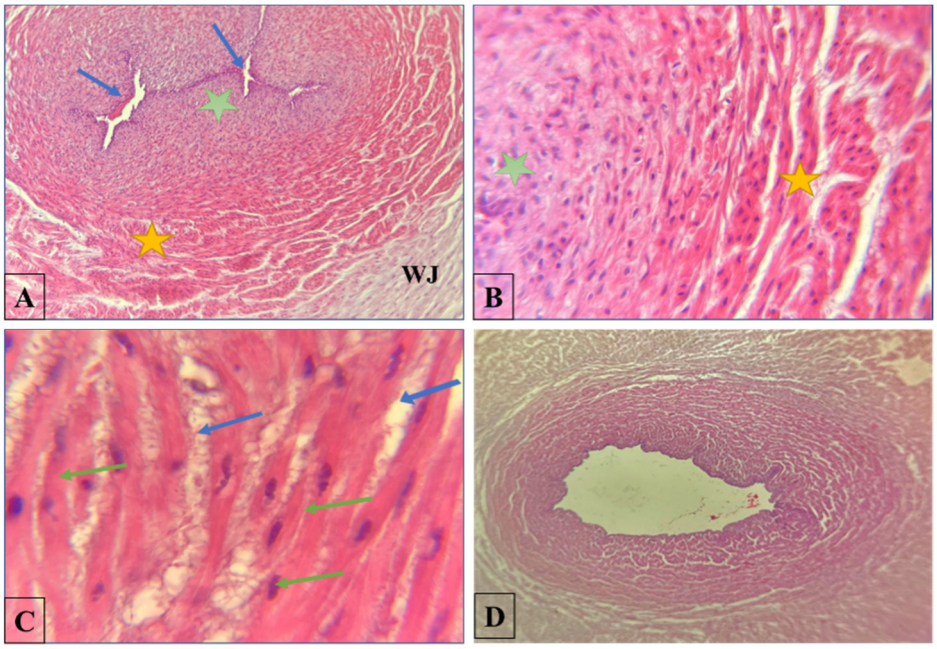


Figure (1): Transverse section of normal UC in placental segment showing: (A): Umbilical artery with narrow lumen This lumen was constricted with a typical irregular branched shape (blue arrows), thick intima (green star) and thick media (yellow star) with no adventitia, surrounded by WJ. (B) (high magnification of part of A) indicate the intima-medial junction of a normal artery. No elastin is present. Intimal muscle cells to the left (green star) are cut in transverse section and show fewer myofibrils than in the media (to the right, yellow star) where muscle cell layers are arranged in a circular wave. (C): Muscular layer illustrates mature myocytes (green arrow) with extracellular matrix (blue arrows). (D): Vein which showed normal structure with thinner wall and wider lumen than in arteries H&E. (A & D: 40x; B: 100x; C: 400x).

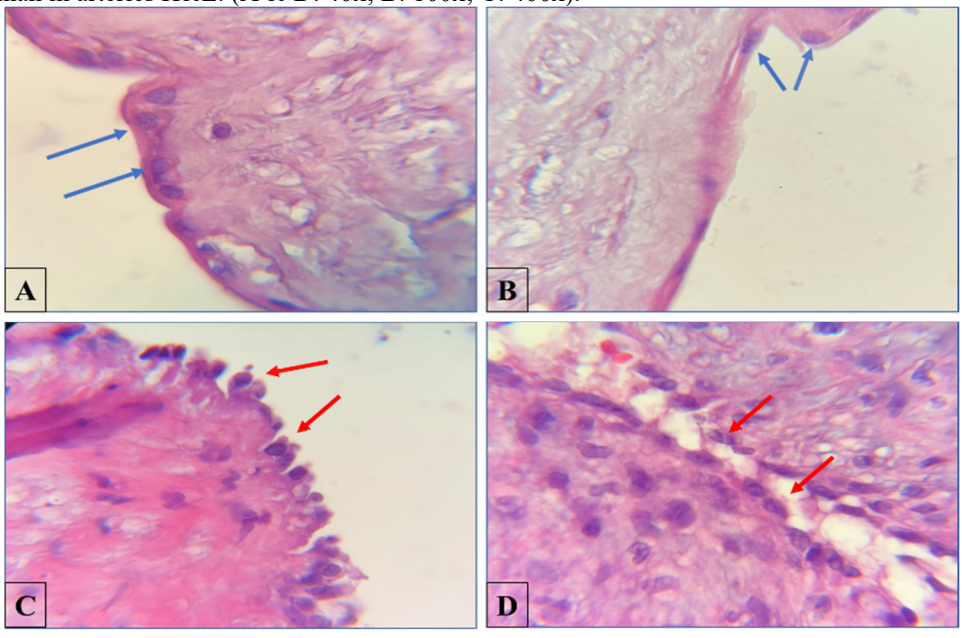


Figure (2): Transverse section of normal UC in central segment showing. (A): Amniotic membrane covering cord formed of cuboidal to squamous epithelium (blue arrows). (C): Inner layer of Vein composed of cuboidal to columnar endothelial cell (red arrows). (D): Inner layer of Artery composed of squamous endothelium (red arrows). (H&E; A, B, C and D 400x).

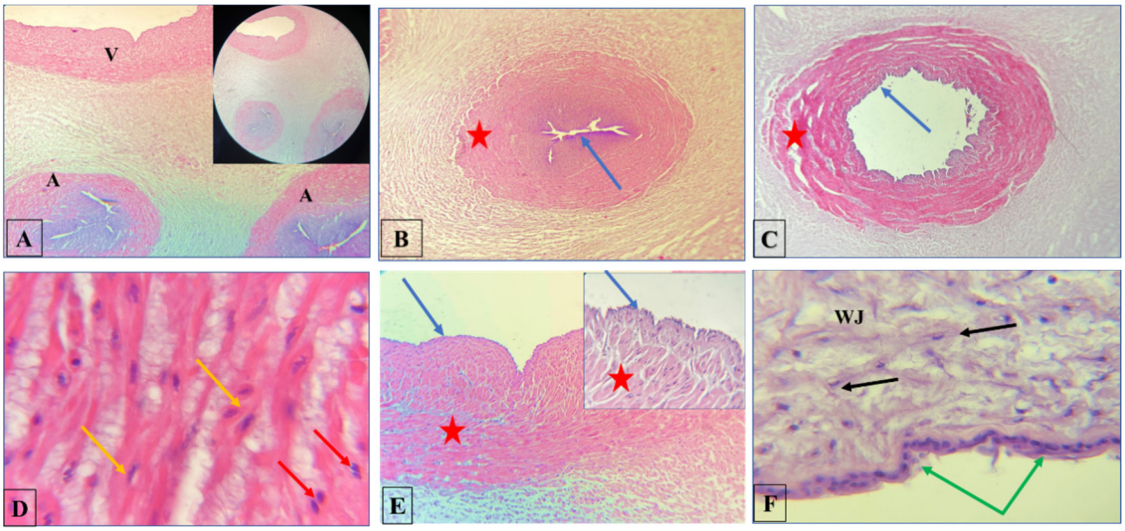


Figure (3): Transverse section of the normal UC in fetal segment showing, (A): Normal architecture of UC contains two arteries (A) and one vein (V). (B): Artery, having constricted irregular branched lumen and thicker tunica media than tunica intima. (C): Vein with thinner wall and wider lumen compared to the arteries. (D): Section at tunica media of artery showing mature smooth cell (yellow arrows) and dividing smooth muscle cell (red arrow) normal. (E): Section at endothelium layer and underneath muscle layer (inner longitudinal and outer circular muscle). (F): Normal structure of WJ, contains fibroblast cells (black arrows) and enclosed by amniotic membrane (green arrows). Red star: Tunica media, and blue arrow: Tunica intima. (H&E) (A, B, & C: 40x; E: 100x; D&F:400x).

Gestational diabetic mellitus (GDM)

As it is shown in figures (4;5(A,B,C&D) & 6 (A,B,C&D)) of three segments, GDM had an impact on the UC histological structure and caused extensive hemorrhage of the artery and surrounding area in placental and fetal segments, which was associated with an increase in the thickness of the arterial wall, in addition to the detachment of the artery from the WJ in the central segment. Huge dilation of the vein with enlargement of its lumen and thinning of its wall was recorded. Collagen fiber degeneration in vessel walls was also observed in the GDM group. Similar pathology was also documented in PGDM group as shown in Fig.5(E&F) and Fig.6(E,F,G&H) in addition, the erosion of the arterial endothelium and separation of smooth muscle were also documented in the PGDM group.

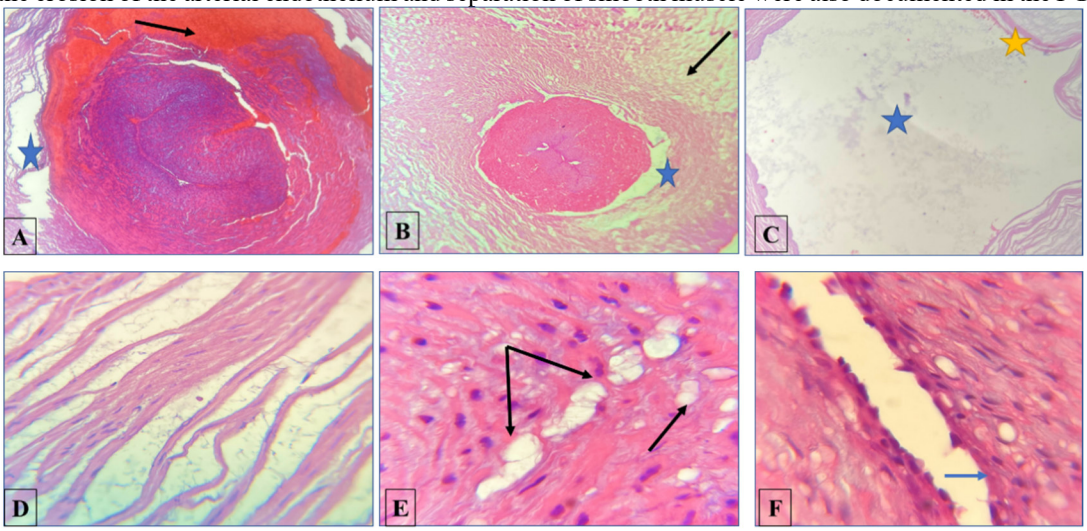


Figure (4): Transverse section of the GDM UC of the placental segment showing: (A): Extensive hemorrhage of WJ (black arrows). (B): reveals artery detachment from Wharton's jelly (blue star). (C): Highlighting hugely dilated vein with enlargement of its lumen (blue star) and thinning of its wall (yellow star). (D): Wide separation, thinning and destruction of smooth muscle in tunica media of the vein wall. (E): Spaces in media and intimal layers of arterial wall (black arrows). (F): Area of erosion of endothelial cell in some area of the arterial lining layer (blue arrows). (H&E) (A &D:100x; B&C 40x; E&F:400x).

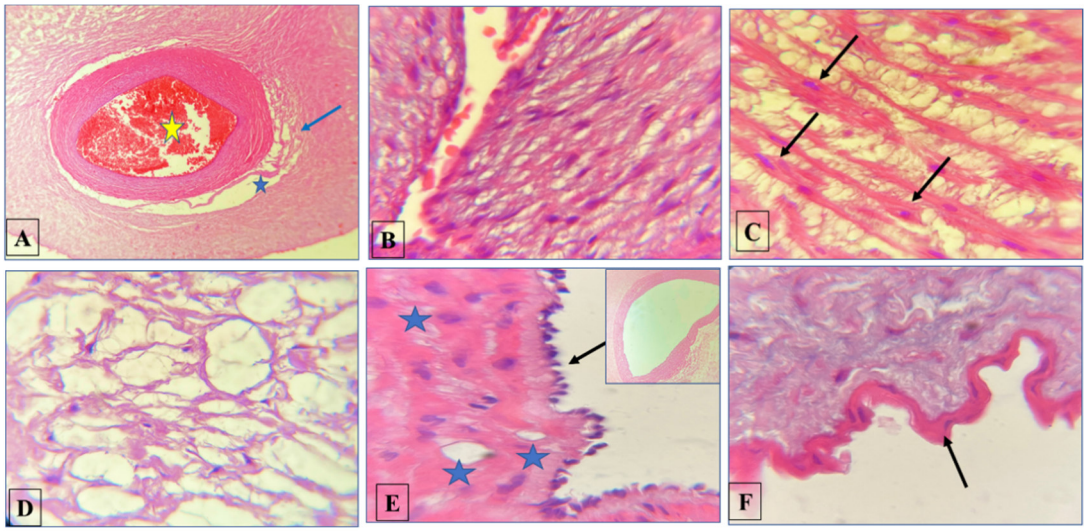


Figure (5): Transverse section of the central segment showing: (A,B,C&D: GDM): (A):Venous thrombosis (yellow star). (B): Spaces in the arterial intimal layer indicated degeneration of extracellular matrix. Notes: RBC infiltrate in its lumen. (C): The media layer showed widely spaces between mature smooth muscle cells (black arrow). (D): WJ, indicated honey combs formation. Figures (E&F: PGDM): (F): Vein with internal elastic lamina: the lining vein intima looked thin; the endothelium appeared tall with prominent nuclei (black arrow). The smooth muscle of tunica media was arranged in circumferential branched laminae which consist of 2 to 3 with scare myofibrils and separated by blebs (blue star). (F): Increasing in the thicknesses of amniotic membrane (black arrow). (H&E) (A:40x; B, C, D; E & F 400x).

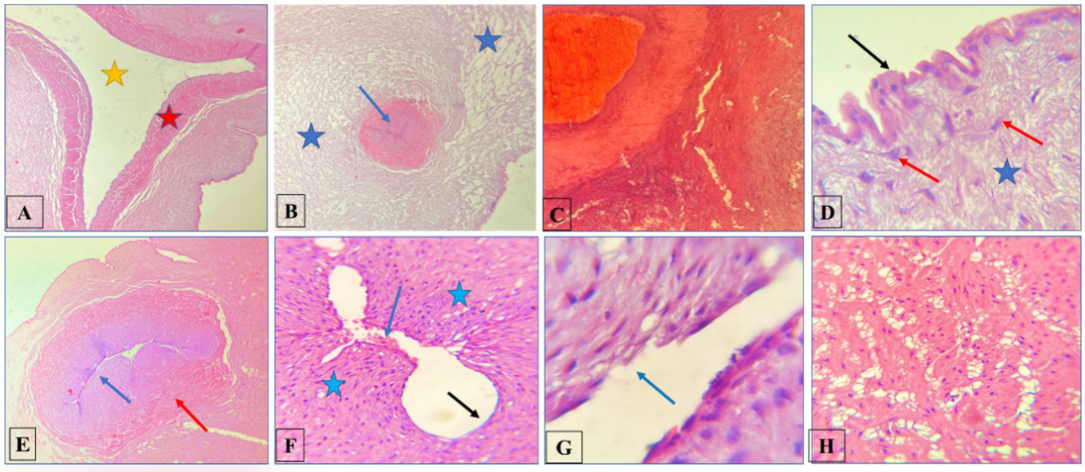


Figure (6): Transverse section of UC in fetal segment showing: (A,B,C&D: GDM). (A): Highlighting hugely dilated vein with enlargement of its lumen (yellow star) and thinning of its wall (red star). (B): The artery lumen appeared more constricted associated with obvious decreasing in its diameter and wall thickening (blue arrow) compared with vein. Blue star indicates the honeycomb formation in WJ. (C): Sever hemorrhage was seen in UC artery and WJ. (D): Thick amniotic membrane (black arrow), WJ (blue star), Fibroblast cells (red arrows). Figures (E,F,G&H:PGDM), (E): Thickening of intima layer of the arterial wall. (F): in the artery, the tunica intima is comprised of endothelial cells (simple squamous epithelium) (black arrow) and small endothelial spaces (blue star). Note: Presence of cells derbies and RBCs in the lumen (blue arrow). (G): Erosion of endothelium of intima of artery (blue arrow). Multiple spaces located between muscle fibers in the vein wall. (H&E) (A&F:100x; B,C&E: 40x; D,G&H: 400x).

Special Stain

Masson Trichrome stain

The results of the Masson trichrome stain revealed that in groups of GDM and PGDM, there was marked decrease in collagen fibers in both tunica intima and media layers with irregular arrangement in both arteries and vein especially in placental segment (Fig.7).

Periodic acid shift (PAS)

The UC segments in the GDM group stained with PAS stain showed a marked increase in PAS reaction on both the artery and vein walls. While in the PGDM group, the vessels walls showed a moderate to marked increase in PAS reaction when compared to the normal group (Fig. 8).

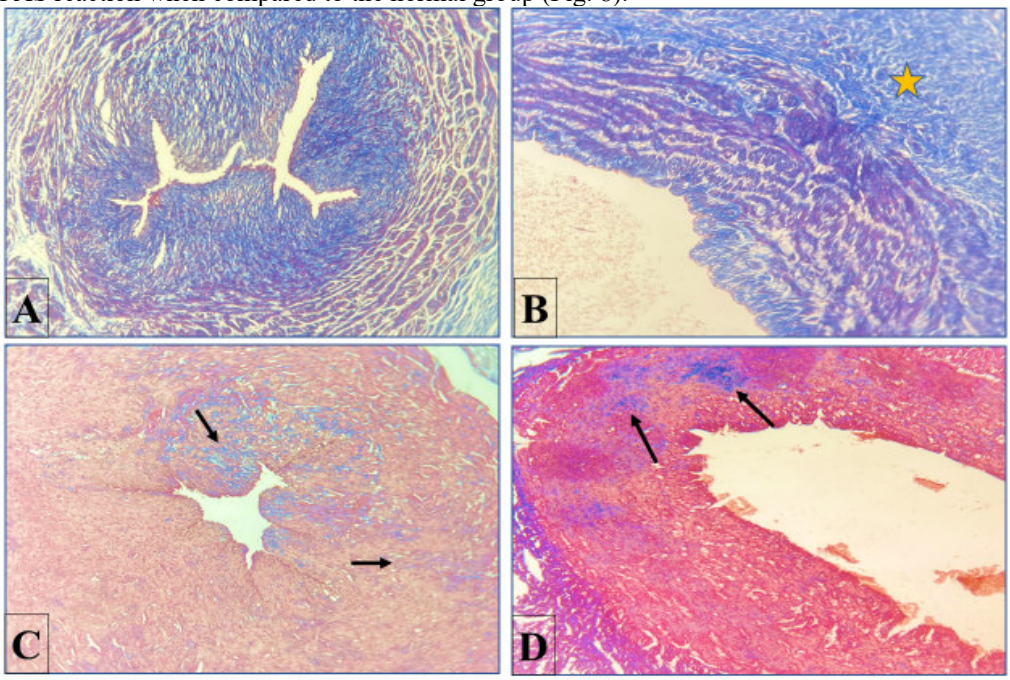


Figure (7): Transverse section of the UC stained with Masson trichome stain. (A) Placental segment in normal group showing normal distribution of collagen fibers (stained blue) in the arterial wall. (B) Fetal segment in normal group showing regular distribution of collagen fibers in vein wall, note more collagen fiber in WJ (yellow star). (C) Placental segment in the GDM group showing a marked decrease with irregular distribution of collagen fibers in the arterial wall (black arrows). (D) Fetal segment of the PGDM group showing a marked decrease with thick irregular distribution of collagen fibers in the tunica media between muscle cells of the vein wall (black arrows). (Masson Trichome Stain; A, B, C and D 100x,).

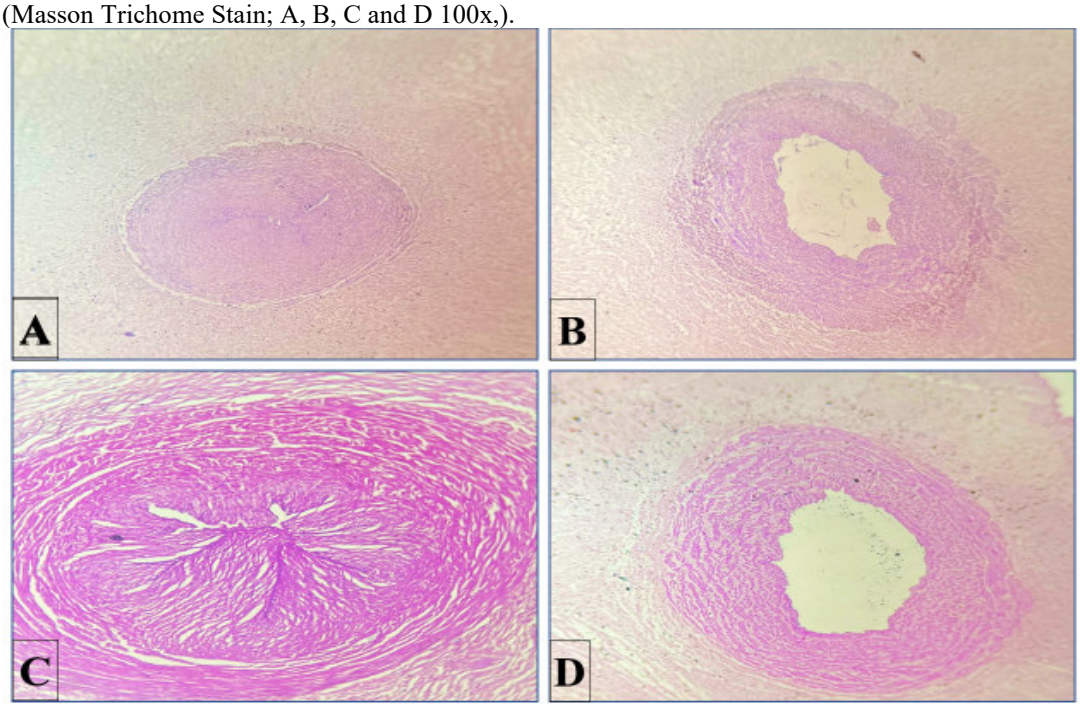


Figure (8): Transverse section of the UC stain with PAS stain. (A and B) Central segment of normal group showing mild PAS reaction of the artery and vein walls (A: artery), (B: vein). (C) Central segment of GDM group showing a marked increase in PAS reaction of the arterial wall. (D) Placental segment of the PGDM group showing a moderate increase in PAS reaction in the vein wall. (PAS; A, B and D: 40x, C 100x)

4. Discussion

The present study was aimed to find out the microscopic and macroscopic impact of PGDM and GDM on the UC in terms of histopathology and histological measurement, which may lead to the possibility of complications with adverse effects on both the placenta and fetus.

The macroscopic study showed that, there was no significant difference in the mean weight of UC comparing diabetic groups to the normal group. This result is in disagreement with the study of Aboud *et al.,* (2018) who stated that, UC weight in PGDM was significantly higher compared to GDM and the normal group. They demonstrated that this increase was brought on by the pathophysiology of weight gain, which indicated an increase in both the contents and density of WJ, but this feature was not recorded in the present study.

The present results showed that, there was a significant decrease in arterial wall thickness when comparing GDM to the normal group in three different segments. This reduction was due to a decrease in the thickness of both layers, the tunica intima and media. A similar reduction in this thickness was also observed in the vein wall of the GDM group compared to the normal group in the placental and central segments. While in the fetal segment, there was a decrease in this thickness, but it had not reached significant levels. These results are in agreement with Aboud *et al.,* (2018) they found that, thinness of the muscular layer of umbilical arteries with their largely dilated lumen was common in the fetal segment of the GDM group. A study carried out by Cetin *et al.,* (2002) stated that, the narrowing of conjunctive tissue that separates muscular layer could be an explanation of why muscle layers in vessels tend to be decreased in thickness. In another study carried out by Lateef in (2015), indicated that GDM is related to pathological alteration in tunica intima and tunica media, including sloughing of the endothelial layer, splitting of the internal elastic lamina and reduction in the quantity of smooth muscle fibers and fold. This indicated that the umbilical blood vessels of diabetic pregnancies had a tendency to increase luminal diameter and decrease their thickness. But the present results come into conflict with the results of Sarikabadayi *et al.,* (2012) and Salem *et al.,* (2019) , they clarified that, in the diabetic group, there was an increase in the thickness of both layers, the tunica media and the tunica intima, when compared to the normal group.

In the fetal and central segments of PGDM group, there was a slight decrease in arterial and vein wall thickness but it was not statistically significant compared with the normal group. These results are in agreement with the results of Alam *et al.*, (2015) they demonstrated that there were no significant differences in the wall thicknesses of UC blood vessels between the PGDM group and the normal group.

Results of the statistical analysis indicated that, there was no significance difference in the diameter of the artery and vein in the fetal segment in comparison of GDM and PGDM to normal group. Similarly, the vein in the placental segment and artery in the central segment also presented no significance differences in their diameter when normal group compared to the GDM and PGDM groups. This finding is in disagreement with the results of Salem *et al.*, (2019) they reported that the veins in the diabetic group showed an increase in diameter compared to the normal group, and their results indicated that there was an increase in the diameter of the vein lumen dilatation and a decrease in its wall thickness if compared to the control group.

In term of histopathological effect of GDM and PGDM on the UC, the extravasation of blood in the fetal segment and central segment within the tunica media and WJ in the GDM group was observed, as a consequence of the degenerative effect of DM. Similar findings were observed by Chakraborty and Banu, (2013) they observed extravasation of blood within the WJ as a result of rupture and severe erosion of vascular endothelium. These results were also in harmony with works of Blanco *et al.*, (2011), and Tahaoglu *et al.*, (2015). Another important pathological condition observed in present study was the presence of multiple spaces between smooth muscle cells of the tunica media in the placental segment, which was more common in GDM than PGDM, this outcome is in line with Aboud *et al.*, (2018) and Salem *et al.* (2019) they reported, wide separation of SMC in the media by an extracellular matrix composed of an irregular arrangement of collagen and elastic fibers.

The results of the Masson trichome stain revealed that in groups of GDM and PGDM, there was a marked decrease in collagen fibers in both the tunica intima and media layers, with irregular arrangement in both the arteries and veins, especially in the placental segment. These results were also recorded by Salem *et al.* (2019), who stated that "there were disruptions and a decreased amount of collagen fibers, with disorientation of their normal arrangement in the intima and in-between smooth muscle cells of the media of umbilical blood vessels in diabetic samples". Similarly, Chakraborty and Banu, (2013) and Koskinen, (2014), were also clarified that, collagen fibers were degenerated and its distribution patterns disturbed in GDM, and maternal diabetes causes the collagen fibers to rupture and cause edema in human UCs.

Periodic Acid Schiff (PAS) stain was another special stain that had been used in this study. The current study presented positive reactions for PAS stain in the intima and media of UC vessels in all three groups, which indicated rich carbohydrate content. In a comparison of the diabetic groups (PGDM and GDM) to the normal group, there was a moderate to marked increase in PAS positive reactions in the intima and media of vein and arteries. These results were in agreement with the work of Sexton *et al.*, (1996), who showed that endothelial cells of UC vessels maintain extra glycogen throughout gestation. Glycogen fills the region around the nucleus in both the endothelial.

5. Conclusions

In conclusion the current results proved that GDM and PGDM have an adverse effect on the structure of UC and its vessels cells and SMCs.

Acknowledgement: The authors are thankful for the Department of Biology, Faculty of Science, University of Zakho for providing some of the research facilities.
Conflict of Interest: The authors declare no conflict of interest.
Funding statement: This researched received no external funding.

6. References

Aboud, F. *et al.* (2018) 'Histopathological changes of umbilical cord blood vessels in diabetic pregnancies', *Tripolitan Medical Journal*, 7(2), pp. 1–6.

Alam, M. R. *et al.* (2015) 'Gross and Histomorphologic Study of the Umbilical Cord in Pre-gestational Diabetes Mellitus and Gestational Diabetes Mellitus', *Bangladesh Journal of Anatomy*, 12(1), pp. 25–29. doi: 10.3329/bja.v12i1.22615.

Blanco, M. V. *et al.* (2011) 'Histopathology and histomorphometry of umbilical cord blood vessels. Findings in normal and high risk pregnancies', *Artery Research*, 5(2), pp. 50–57.

Cetin, A., Kükner, A. and Öztürk, F. (2002) 'Ultrastructure of human umbilical vessels in pre-eclampsia', *The Journal of Maternal-Fetal & Neonatal Medicine*, 12(3), pp. 178–184.

Chakraborty, S. K. and Banu, L. A. (2013) 'Microscopic impacts of gestational diabetes mellitus on the umbilical cord.', *Mymensingh Medical Journal: MMJ*, 22(4), pp. 755–760.

Gagnon, A. J. *et al.* (2011) 'International migration and gestational diabetes mellitus: a systematic review of the literature and meta-analysis', *Paediatric and perinatal epidemiology*, 25(6), pp. 575–592.

Keselman, H. J. and Rogan, J. C. (1977) 'The Tukey multiple comparison test: 1953–1976.', *Psychological Bulletin*, 84(5), p. 1050.

Koskinen, A. (2014) 'The effects of maternal hyperglycemia on human umbilical vascular gene expression and neonatal rat lung development'.

Lateef, R. H. (2013) 'Histological study of umbilical cord at different stages of gestation', *Egypt J Exp Biol (Zoo)*, 9(1), pp. 75–78.

Lateef, R. H. (2015) 'Adverse effects of gestational diabetes mellitus (GDM) on measurements of the umbilical cord and its vessels', *Pakistan Journal of Biological Sciences*, 18(7), pp. 346–351. doi: 10.3923/pjbs.2015.346.351.

Salem, M. A. E. R. *et al.* (2019) 'Histological study of human umbilical cord in diabetic pregnant females', *Egyptian Journal of Histology*, 42(4), pp. 874–887. doi: 10.21608/EJH.2019.7717.1076.

Sarikabadayi, Y. U. *et al.* (2012) 'Umbilical artery intima-media and wall thickness in infants of diabetic mothers', *Neonatology*, 102(2), pp. 157–162. doi: 10.1159/000339278.

El Sawy, N. A. *et al.* (2018) 'Histomorphological study of placenta in gestational diabetes mellitus', *Int. J. Morphol*, 36(2), pp. 687–692.

Sexton, A. J. *et al.* (1996) 'A study of the ultrastructure of developing human umbilical vessels.', *Journal of anatomy*, 188(Pt 1), p. 75.

Tachibana, M. *et al.* (2013) 'Human embryonic stem cells derived by somatic cell nuclear transfer', *Cell*, 153(6), pp. 1228–1238. doi: 10.1016/j.cell.2013.05.006.

Tahaoglu, A. E. *et al.* (2015) 'Expression of PECAM-1 and E-Cadherin in the Umblical Cords of Gestational Diabetic Mothers', *Int. j. morphol*, 33(4), pp. 1277–1281.