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Histological and Morphological Study of the Placenta in Gestational: Pregestational Diabetic, and Normal Women's

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Abstract
This study was aimed at comparing the morphometric and histological features of the placenta in gestational diabetes mellitus (GDM), pre-gestational diabetes mellitus (PGDM), and non-diabetic normal pregnant women. Twenty-four placentas were obtained from non-diabetic pregnant women (control group), ten from women with GDM, and ten from PGDM women. Segments from the central part of the placenta were taken and processed for paraffin blocks, sectioned, and stained with H&E, Masson trichrome, and periodic acid-Schiff. The results of morphometric measurement revealed that the mean weight and diameter of GDM and PGDM placentae were significantly higher than in the control; placental thickness was a significant increase in PGDM; the number of cotyledons and fetal weights showed no significant difference in all these groups; the fetoplacental ratio was significantly higher in the PGDM; discoidal placenta shape, followed by irregular, oval, and kidney as the most common shapes recorded in these groups; the central umbilical cord insertion was most common in all three groups; followed by noncentral (eccentric) and marginal insertions. Diabetes caused several histopathological changes in the placenta, including an increase in the number of different sizes of villi with a decrease in the intervillous spaces, an increase in the: number of big syncytial knots, and fibrinoid necrosis; chronogenesis in the intervillous spaces and villous capillary congestion. Diabetic groups showed the presence of dense collagen fibers within the villous stroma (villous stromal fibrosis) and around fetal blood capillaries. GDM showed a mild thickening of the basement membrane (BM) of chorionic villi and a mild increase in the collagen in the villous stroma. While PGDM showed a moderate increase in the BM of syncytiotrophoblast and villous stroma and mild thickening of the BM of capillary blood vessels. In conclusion, both GDM and PGDM caused adverse effects on the histological and morphometrical features of the placenta.
Keywords: Placenta; Diabetes Mellitus; Morphometric analysis; Histopathology, Masson trichrome stain; Periodic acid shift reaction.
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1. Introduction

The placenta, a temporary organ of the fetus, is vital to the health of both the mother and the fetus (Cindrova-Davies & Sferruzzi-Perri, 2022), it is a membranous vascular organ that develops in female mammals and mediates materno-fetal exchange which supports intrauterine life (Saha *et al.,* 2014; Carrasco-Wong *et al.,* 2020). Anatomically, the human placenta is a discoid-shaped organ made up of both fetal and maternal components. The fetal components include the placental disc, the amniotic and chorionic membranes (often referred to as the fetal membranes), the chorionic villi, and the umbilical cord, while the maternal side is comprised of the decidua (Parolini *et al.,* 2008).

The placenta is an intrauterine fusion of both fetal and maternal tissues for the purpose of the physiological transfer of almost all nutrients and oxygen from mother to fetus the and transfer of waste products of metabolism from fetus to mother the for continuation of fetal life. Therefore, a fetus's intrauterine existence depends on this vital organ. In this case, the placenta has been considered a valuable indicator for fetal and maternal diseases and conditions. Many maternal diseases or disorders are associated with high perinatal morbidity and mortality and gross pathological changes in the placenta. An abnormal placenta adversely affects the fetal outcome (Tangirala and Kumari, 2015). Thus, the placenta as mentioned by Wang, (2017), achieves a fundamental role in sustaining adequate fetal growth, and it has been implicated in abnormal fetal growth such as it is well recognized as a problem in pregnancy complicated by type 1 diabetes.

Diabetes mellitus is a chronic, lifelong condition that affects on body's ability to use the energy found in food and it is a major complication of pregnancy regardless of whether it is pre-gestational (overt) or gestational in onset (Mondestin *et al.,* 2002; Lowe *et al.,* 2012).

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance of variable severity with onset or first recognition during pregnancy. GDM is important because more than half of women with GDM usually develop overt diabetes (Daskalakis *et al.*, 2008). Since the placenta is positioned between maternal and fetal circulation, it is exposed to the mother and fetus's diabetes-associated endocrine and metabolic derangements (Calderon *et al.,* 2007). GDM has been known to raise the risk for large gestational-age fetuses as the maternal glucose crosses the placenta and stimulates fetal insulin secretion, which acts as the growth factor (Benhalima *et*

al., 2015).

Several studies indicated different histopathological changes in the placenta of diabetic women. In 2008, Daskalakis and his colleague reported that histological abnormalities were observed more frequently in the diabetic placentas compared to the controls. These findings support the hypothesis that impaired placental function is one of the main reasons for the increased frequency of fetal complications in diabetic pregnancies. There was a tendency to increase in placental weight and weight of newborns in complicated pregnancies as compared to normal pregnancies (Tandon *et al.,* 2018). GDM caused significant morphological alterations in the placenta, which in turn may affect the fetus's development (EL Sawy *et al.,* 2018).

This study was designed to compare the morphometric parameters and histological features of the placenta in three groups of pregnancies: GDM, pregestational diabetic (PGD), and non-diabetic normal (healthy).

2. Material and methods

This study was approved by the Medical Ethics Committee of Duhok Directorate of General Health- Directorate of Planning –Scientific Research Division, Kurdistan Region-Iraq, with reference No 08032023-2-8

After patient consent, all placenta samples were taken. The placenta samples were collected from the Maternity Hospital in Zakho and from Duhok Obstruction and Gynecology Hospital. The experimental work for this study was carried out in the Laboratory of Zoology, Department of Biology, Faculty of Science, University of Zakho.

2.1 Placental samples collection

The present study was including 44 pregnant women with full-term (37-40 weeks) gestation periods. With ages ranging from (18-30) years old during the period from 4th October 2021 to 20 June 2022. These pregnant women were divided into three groups as follows:

Group (1): The control group includes (24) pregnant women with normal blood glucose levels

Group (2): GDM group, includes (10 pregnant women with GDM).

Group (3): PGDM Group, includes (10 pregnant women with pre-existing diabetes).

According to Tendon *et al.,* (2018), just after delivery all the placenta was collected and put in a clean tray. Then the placenta was gently expressed to remove its blood content, washed carefully under tap water, wiped with a dry cotton pad, and transferred to the lab in 10% formal saline-filled containers for further investigation.

2.2 Maternal and newborn information

From each maternal and newborn, medical records, demographic and clinical information was gathered. These include the following: Name, age, newborn weight, sex, newborn number, gestational age (which is determined by last normal menstrual period (LNMP) or ultrasonography, mode of delivery (normal vaginal delivery or cesarean section), drug use smoking, and alcohol use.

2.3 Exclusion criteria

Exclusion criteria include all abnormal conditions during pregnancy such as Rh-isoimmunization, cardiac disease, renal disorders, Rhesus incompatibility, smoking, corticosteroid therapy, pre-eclampsia, and pregnancy-induced hypertension.

2.4 Macroscopic examination

As mentioned above, soon after taking the placenta with its attached (fetal membrane and umbilical cord (UC)) to the lab, the membranes and UC were cut off from where it had been inserted then the following parameters were studied:

1- Diameter, shape, color, surface area, thickness of placentae, number of cotyledons, presence of infarction, calcification, and site of UC insertion, were recorded and photographed.

2- The placenta and infant were weighed using (Ohaus Balance) in order to estimate the placental ratio between infant birth weight and placental weight.

2.5 Histological studies

For histological studies, the placental tissue pieces were taken from the central region of each placenta (about 2cm thick), and fixed in 10% formal-saline for 3-4 days. Then these fixed pieces were routinely processed for histological studies. By using a rotary microtome, 5 µm thick sections were trimmed from the paraffin block and stained. Some of the prepared slides containing placental sections were subsequently stained with H&E and mounted in Canada balsam. These sections were examined microscopically to find out the histopathological changes in the placental tissues (Feldman &Wolfe, 2014). While other prepared slides were used for staining with special stains: Masson trichrome for detecting connective tissue, and Periodic acid -Shift reagent (PAS) to distinguish the basement membrane (Weli, 2021). Then photographed using a digital camera (Dino-Eye:

Microscopic Eye-Piece Camera).

2.6 Statistical analysis

The collected data was submitted to the SPSS program (SPSS,2019), in order to analyze it statistically. However, the means within ANOVA (both one two-way way) were separated using Duncan's multiple-range test (Duncan, 1955).

3. Results

Out of the total (44) placentas collected, 24 placentas were obtained from normoglycemic women (control group), 10 placentas from women with GDM, and 10 from women with PGDM. Neonatal gender distribution and maternal and morphometric parameters for pregnant women in the control, GDM, and PGDM groups were illustrated in Tables (1 and 2).

The genders of the neonates, as indicated in (Table 1), were as follows: in the control group, (13 males and 11 females); in the GDM group, (6 males and 4 females); and in the PGDM group, (5 males and 5 females). Table (2) showed maternal and neonatal demographic parameters for pregnant women in the control, GDM, and PGDM groups as follows:

The mean maternal age of the control, GDM, and PGDM groups were 26; 32; and 29 years, respectively. While the mean gestational period in all these groups ranged between (37- 40) weeks.

Regarding the type of delivery, in the control group, there were 20 normal vaginal deliveries (83%) and 4 cesareans (16%). While in GDM and PGDM, all cases are grouped as 100% cesarean deliveries. This table also showed the mean number of births in each group; these are in control (2), GDM (3), and PGDM (6).

According to the color of the placenta, the examination of the placenta in the control group revealed that it appeared in normal color, while in the GDM and PGDM groups, the macroscopic comparison of the placenta showed abnormal fibrinoid deposition. In the PGDM group, the maternal side appeared dark brown. It is worth noting that one case of placenta accreta was recorded in the GDM group.

Table (1): Neonatal gender distribution in pregnant women with GDM, PGDM, and non-diabetic pregnancies (control).

Groups	Male No. (%)	Female No. (%)	Total
Control	13 (54)	11 (45)	24
GDM	6 (60)	4 (40)	10
PGDM	5 (50)	5 (50)	10
Total	24	20	44

Table (2): Maternal and neonatal demographic parameters for pregnant women with GDM, PGDM, and non-diabetic pregnancies (control).

Demographic parameters	Control (mean)	GDM (mean)	PGDM (mean)
Mother's age (year)	26	32	29
Gestational period	37-40 weeks	37-40 weeks	37-40 weeks
Type of delivery	20 Vaginal delivery (83%) 4 Cesarean (16%)	Cesarean (100%)	Cesarean (100%)
Number of births	2 (mean)	3 (mean)	6 (mean)
Placental color	Normal	Abnormal Fibrinoid deposition and placenta accreta	Abnormal Fibrinoid deposition and dark brown maternal side
Infraction/ Calcification	None	None	None

3.1 Comparison of morphometric parameters of the placenta in the control, GDM, and PGDM groups

As indicated in Table (3) and Figure (1), the following morphometric parameters were determined after examining the placentas from the control, GDM, and PGDM groups. The mean weight and diameter of the placenta in groups of GDM and PGDM were significantly ($P<0.01$) higher compared to the control.

The statistical analysis showed there was no significant difference ($P>0.05$) in the placental thickness between the control and GDM. While PGDM caused a significant increase ($P<0.05$) in this thickness compared with other groups.

No significant difference ($P>0.05$) was recorded in the number of cotyledons and fetal weights in all these

groups. The fetoplacental ratio was significantly ($p<0.05$) higher in the PGDM group compared with the control. while a nonsignificant difference ($P>0.05$) was recorded between GDM and PGDM, and between GDM and the control group.

Table (3): Morphometric analysis of the placentas in the control, GDM, and PGDM groups. Mean± SE

Morphometric parameters	Groups	No.	Mean± SE	Sig. (p)
Diameter of placenta	**Control**	24	14.69 ±0.503 [b]	
	GDM	10	17.55 ±0.579[a]	**
	Pre- GDM	10	19.15 ±0.916[a]	(0.0001)
Weight of placenta	**Control**	24	430.96 ±16.267[b]	
	GDM	10	552.90 ±42.261[a]	**
	Pre- GDM	10	612.89 ±29.167[a]	(0.0001)
No of Cotyledons	**Control**	24	13.92 ±0.859[a]	
	GDM	10	14.00 ±0.856[a]	NS
	Pre- GDM	10	16.50 ±0.749[a]	(0.151)
Thickness of placenta	**Control**	24	2.03 ±0.107[b]	
	GDM	10	3.05 ±0.425[b]	*
	Pre- GDM	10	7.14 ±2.985[a]	(0.015)
Fetal weight	**Control**	24	3.64 ±0.100[a]	
	GDM	10	4.07 ±0.246[a]	NS
	Pre- GDM	10	3.95 ±0.205[a]	(0.119)
Feto_Placenta Ratio	**Control**	24	0.12 ±0.006[b]	*
	GDM	10	0.14 ±0.015[ab]	(0.020)
	Pre- GDM	10	0.16 ±0.0103[a]	

Means with different letters within each parameter differ significantly. (NS= non-Significant; *= Significant at level ($p<0.05$); **= Highly Significant at level ($p<0.01$). GDM=Gestational Diabetes Mellitus, PGDM=Pregestational Diabetes Mellitus

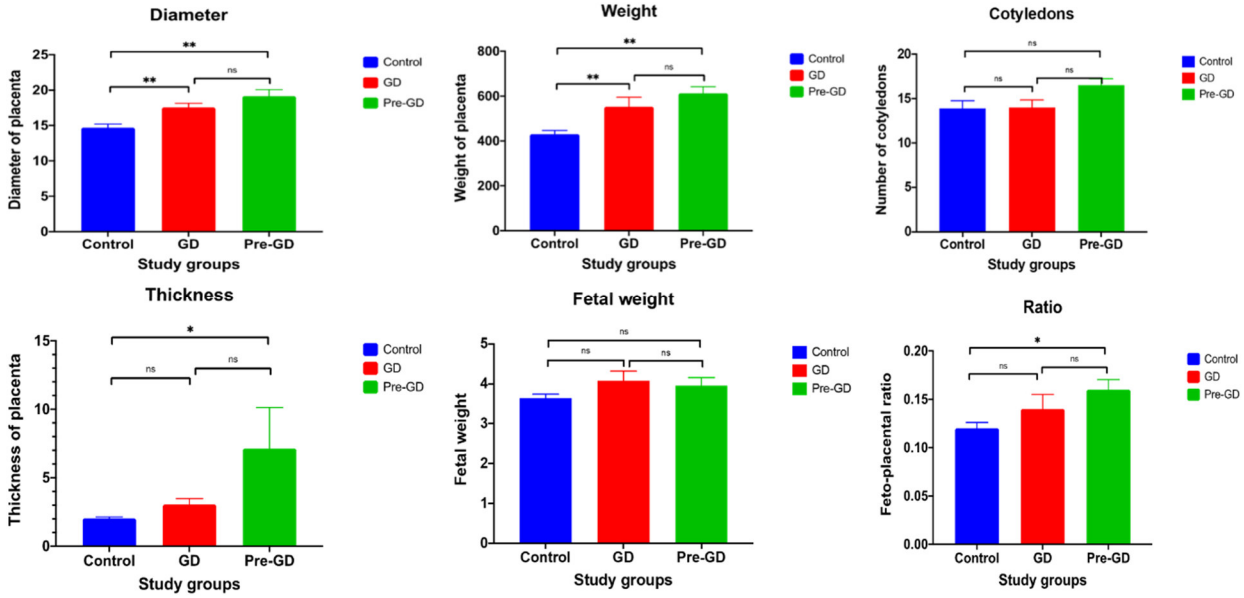

Figure (1): Morphometric parameter of the placentas in the control, GDM: Gestational Diabetes Mellitus, PGDM: Pregestational Diabetes Mellitus.

Table 4, indicated that the diameter of the placenta is correlated positively and significantly ($P<0.01$) with the weight of the placenta, No. of Cotyledons and Feto-Placenta Ratio, which means that any increase in the diameter of the placenta will be followed by the increase of the mentioned correlated parameters. The weight of the placenta also was positively and significantly ($P<0.05$) associated with No. of Cotyledons (0.368) and high significantly ($P<0.01$) with Feto-Placenta Ratio (0.829); and the final positive significant ($P<0.01$) correlation coefficient has been found between No. of Cotyledons and Feto_Placenta Ratio (0.393); while the unique negative significant correlation ($P<0.01$) is recorded between fetal weight and Feto-Placenta Ratio, which mean that any increase in the fetal weight will be followed by decreasing the Feto-Placenta Ratio.

Table 4. The correlation coefficients between the studied parameters for all studied groups (pooling).

		Diameter_placenta	Weight_placenta	No_Cotyledons	Thickness_placenta	Fetal_weight	Feto_PlacentaRatio
Diameter_placenta	Pearson Correlation	1	.689**	.404**	.080	.086	.553**
	Sig. (2-tailed)		.000	.007	.607	.577	.000
Weight_placenta	Pearson Correlation	.689**	1	.368*	.229	.078	.829**
	Sig. (2-tailed)	.000		.014	.135	.616	.000
No_Cotyledons	Pearson Correlation	.404**	.368*	1	.026	-.170	.393**
	Sig. (2-tailed)	.007	.014		.869	.270	.008
Thickness_placenta	Pearson Correlation	.080	.229	.026	1	.283	.025
	Sig. (2-tailed)	.607	.135	.869		.063	.873
Fetal_weight	Pearson Correlation	.086	.078	-.170	.283	1	-.481**
	Sig. (2-tailed)	.577	.616	.270	.063		.001
Feto_PlacentaRatio	Pearson Correlation	.553**	.829**	.393**	.025	-.481**	1
	Sig. (2-tailed)	.000	.000	.008	.873	.001	
**. Correlation is significant at the 0.01 level (2-tailed).							
*. Correlation is significant at the 0.05 level (2-tailed).							

The number and percentage of the type of placental shape and site of UC insertion were illustrated in Table (5). From this table, it appeared that the most common shape of the placenta in the three groups was discoidal, followed by irregular, oval, and kidney shapes. But the kidney and irregular shape placenta was not recorded in GDM and PGDM groups respectively.

The site of UC insertion among the normal, GDM, and PGDM placentas was mentioned in Table (6). This table showed that the central UC insertion was the most common in the control, GDM, and PGDM groups, followed by noncentral (eccentric or paracentral) and marginal insertions (Fig. 2&3).

Table (5): Percentage of human placenta shape in control (healthy), gestational and pregestational groups.

Groups	Discoid shape		Oval shape		Irregular shape		Kidney shape		Total
	No.	%	No	%	No.	%	No	%	No. of samples
Control	18	75	1	4	3	12.5	2	8	24
GDM	8	80	1	10	1	10	/	/	10
Pre-GDM	5	50	3	30	/	/	2	20	10

GDM=Gestational Diabetes Mellitus, PGDM=Pregestational Diabetes Mellitus

Table (6): Percentages of different types of umbilical cord insertion with the placenta.

Groups	Central		Non-central eccentric (or paracentral)		Marginal		Total (No. of samples)
	No.	%	No.	%	No.	%	
Control	12	50	8	33.4	4	16.6	24
GDM	5	50	3	30	2	20	10
Pre-GDM	4	40	3	30	3	30	10

GDM=Gestational Diabetes Mellitus, PGDM=Pregestational Diabetes Mellitus

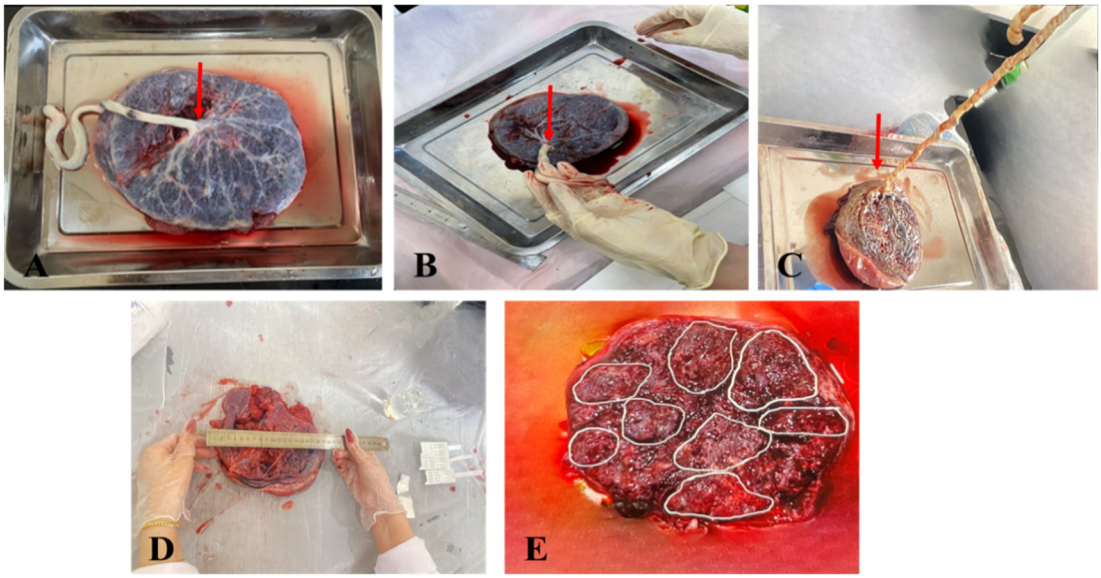

Figure (2): Photographed of human placenta shape and site of UC insertion with the placenta in the control (healthy) group. (A, B, C &D) The fetal surface of the placenta. (A): The oval shape of the placenta / Central insertion of UC. (B): Discoidal shape of placenta /Eccentric insertion of UC. (C): Discoidal shape of placenta / Marginal insertion of UC. (D): Irregular shape of the placenta. (E): The maternal surface of the placenta shows the chorionic cotyledons. The red arrow indicates the UC.

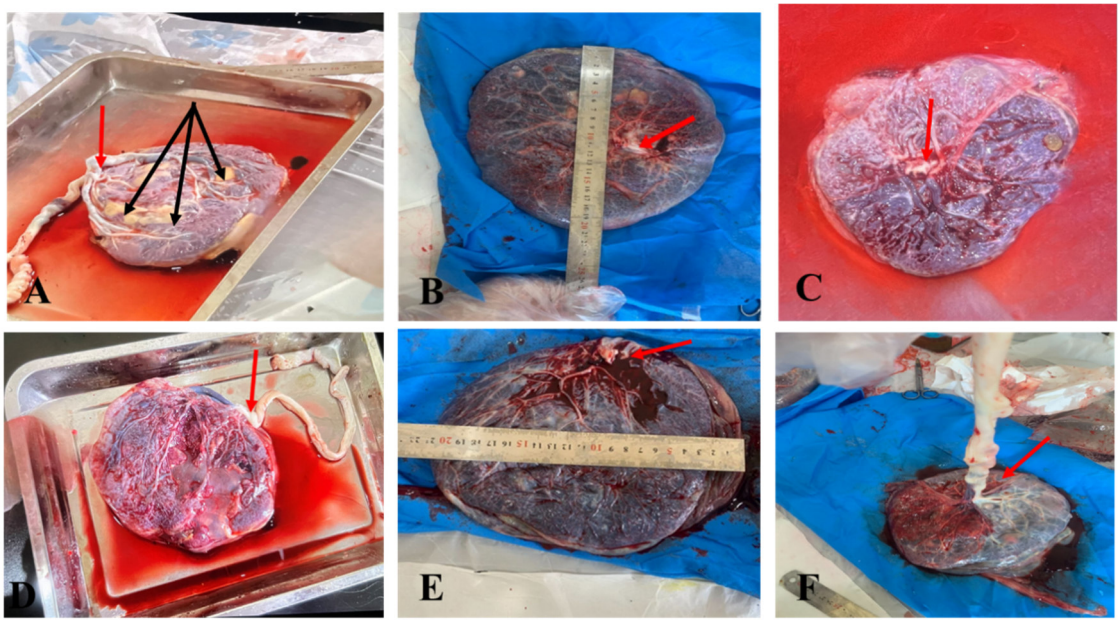

Figure (3): Photographed of human placenta shape and site of UC insertion with the placenta. (A, B &C): GDM group showing (A): Discoidal shape placenta/Marginal insertion of UC. Note: Fibrinoid deposition (black arrows). (B): Discoidal shape placenta/Eccentric insertion of UC. (C): Oval-shaped placenta/Central insertion of UC. (D, E &F) PGDM group showing (D&E): Kidney shape placenta/ Marginal insertion of UC. (F): Circular shape placenta / Central insertion of UC. The red arrow indicates the UC.

3.2 Histological and histopathological studies
3.2.1 Hematoxylin and Eosin stain results
3.2.1.1 Control group (normal placenta):

The light microscopic examination of full- termed placenta in the normal group (control), as indicated in (Fig. 4A, B & C), showed, a normal component of the placenta, in which the villi appeared in different sizes, and with varying in diameter from small to large. The large ones, the stem villous which is known as the fully mature villi, while the smaller ones, the terminal villi, were not fully mature and appeared oval or rounded in shape. Normally each of these villi was lined by trophoblasts and contain many blood vessels which peripherally placed in the villi. Some of these blood vessels were patent, while others contained fibrinoid thrombi. The trophoblast contained aggregates of nuclei, termed syncytial knots.

The terminal villi were consisting a core of connective tissues and are surrounded by a continuous syncytiotrophoblast layer. Each terminal villous was rich with fetal capillaries and separated from each other by different sizes of intervillous spaces filled with maternal blood. The results also showed the presence of small aggregates of fibrinoid material between villi.

The stem villous were distinguished from the terminal villous by their large size, the presence of one or more arteries and veins, or arterioles and venules, having distinctly discernible muscle walls, the fibrous stroma with fibroblasts and a significant amount of connective tissue stroma surrounding them, and also by decreasing the number of trophoblast cells on their periphery.

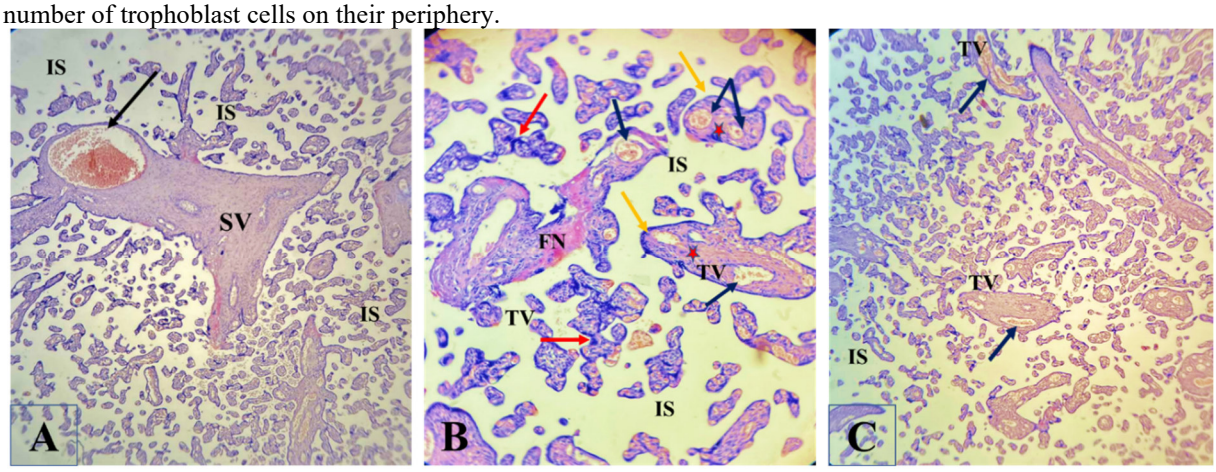

Figure (4): A photomicrograph of a central segment of the normal placenta generally shows the normal structure of villi which looked in different sizes separated by different sizes of intervillous spaces (IS). These villi contain trophoblast cells and peripheral thin-walled blood vessels. (A): showing large stem villous (SV), which contain blood vessels (black arrow). (B): showing terminal villous (TV) which is rich with fetal capillaries (black arrows) and separated from each other by different sizes of intervillous spaces (IS), also this figure indicated the syncytial knots (red arrow) and fibrinoid necrosis (FN), yellow arrow indicates syncytiotrophoblast and red star mesenchymal tissue. (C): Terminal villi (TV) rich with fetal capillaries. H&E stain. (A: 100x; B: 400x; C: 40x).

3.2.1.2 Gestational Diabetic Mellitus Group:

The histopathological findings of the sections from the placenta in the GDM group revealed many histological alterations, these include: crowding of villi (which appeared in different sizes) with decreased intervillous spaces (Fig. 5 A, B & C), but in other sections, some of these spaces appeared wider than in the normal placenta this occurred may be due to intervillous contain more maternal blood with increased of fibrinoid material (Fig.5 D, E&F). High number of big syncytial knots formation, and fibrinoid necrosis increased in the perivillous and intervillous also were recorded in Fig. (5 B, E&F). Villous capillaries were congested and dilated with fetal blood within terminal villi and stem villi.

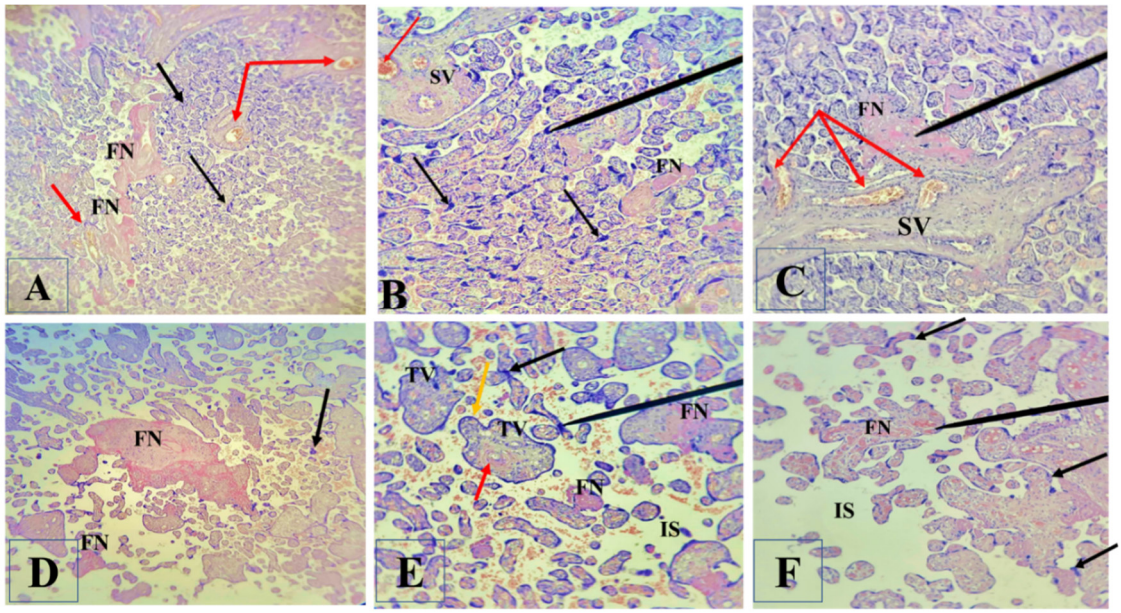

Figure (5): A photomicrograph of the central segment of the placenta in the GDM group all these figures showed: Crowded terminal villi (which appeared in different sizes) with fibrinoid necrosis (FN) and numerous big syncytial knots (Black arrow); Villous capillaries (red arrow) were congested and dilated with fetal blood within terminal villi (TV) and stem villi (SV). Notes: Figures (A, B & C), indicate the decrease of the intervillous spaces (IS). Other sections as shown in Figures (D, E & F) indicated mildly increased in (IS) which contains maternal blood and less crowded villi. (Yellow arrow) in Fig. (E), indicate the syncytiotrophoblast layer that surrounds villi, (H&E stain; A: 40x; B, C, D, E & F: 100x).

3.2.1.3 Pregestational Diabetic Mellites group:

The PGD groups also showed several histopathological alterations as indicated in (Fig.6) such as crowded villous capillaries, which were congested and dilated, in addition, to increase fibrinoid necrosis and chorangiosis. A high number of big syncytial knots and crowded of different sizes of villi also were recorded (Fig. 6A, B, C & D). Figures (6 E &F), showed fused large chorionic villi. In general, all these figures indicated the presence of numerous numbers of syncytial knots and crowded of different sizes of villi. villous capillaries and stem villi.

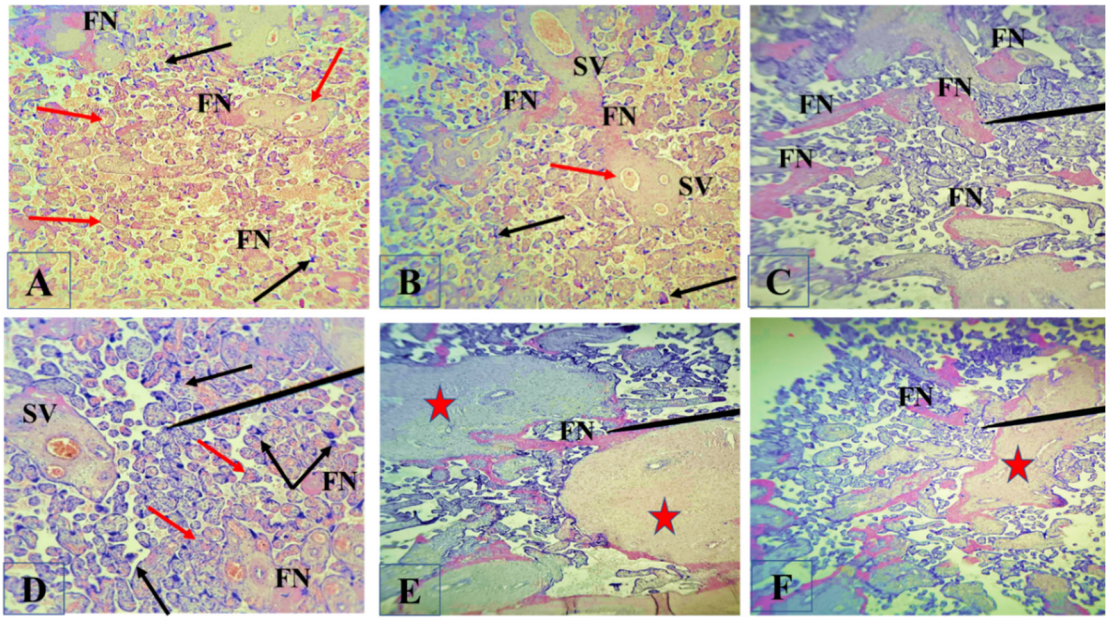

Figure (6): A photomicrograph of the central segment of the placenta in the PGDM group: (A&B): showing: crowded villous capillaries, which were congested and dilated with fetal blood, fibrinoid necrosis (FN), and chorangiosis. (C): demonstrates increased FN. (D): Congestion of villous capillaries and dilation with fetal blood, chorangiosis, and crowded villi and FN. (E &F): indicate fused large chorionic villi (red star). All these figures indicate the presence of numerous numbers of syncytial knots (black arrow) and crowded of different sizes of villi. Villous capillaries (red arrow); stem villi (SV). (H&E; A&B: 40x; C, D, E &F: 100x).

3.3 Special stain

3.3.1 Masson's trichrome (MT) stain

The result of MT stain in the control group showing normal distribution of delicate collagen fibers within the villous stroma and around fetal blood capillaries (Fig.7A). While in groups of GDM and PGDM, both of them showed dense collagen fibers within the villous stroma (villous stromal fibrosis) and around fetal blood capillaries (Fig. 7 B, C&D) GDM and (Fig. 7 E&F) PGDM.

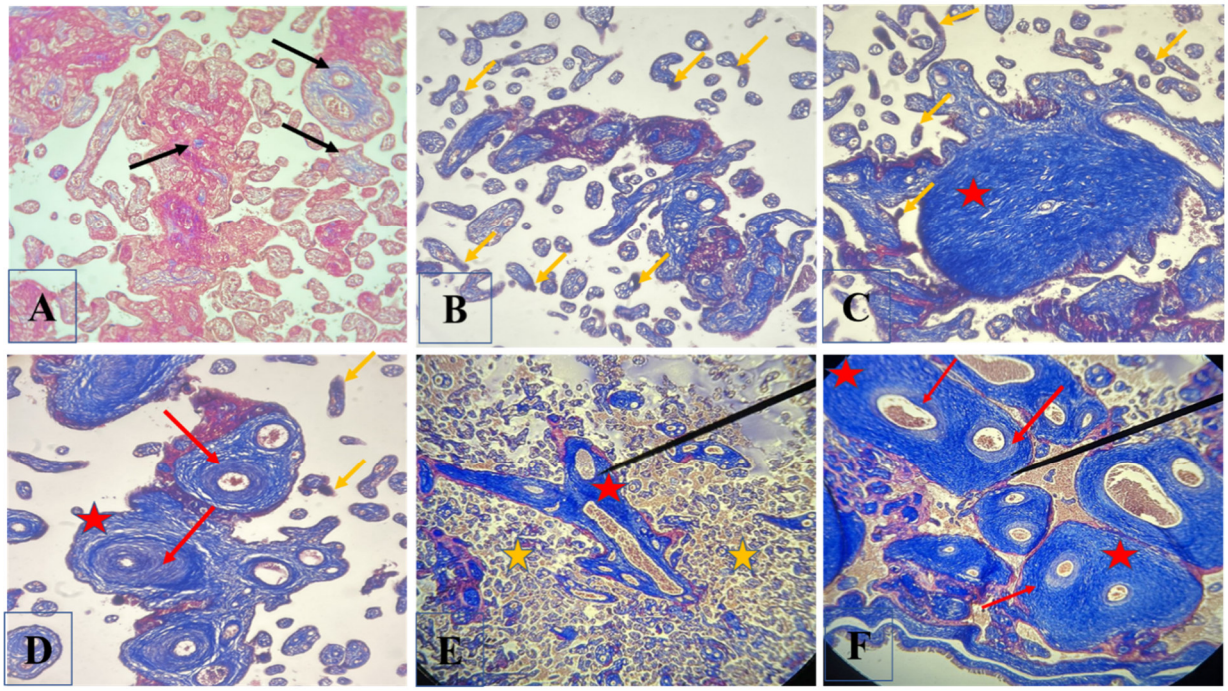

Figure (7): Photomicrograph of central sections of human placenta stained with Masson trichrome. (A): Control group, showing normal distribution of collagen fibers within the villous stroma (black arrow). (B, C&D) GDM and (E&F) PGDM: generally showing dense collagen fibers within the villous stroma (villous stromal fibrosis) (red star) and around fetal blood capillaries. Note: the increased thickness of the blood vessel wall (red arrow); the presence of a large number of syncytial knots (yellow arrow) and the crowded of different sizes of villi are sociated with the decrease of the intervillous spaces. (A, B&E: 40x; C,D&F: 100x).

3.3.2 Periodic Acid Shift stain (PAS)

The control group showed a faintly positive PAS stain reaction in the chorionic villi (Fig. 8 A&B). While the GDM group showed mild PAS reaction as indicated in the mild thickening of the BM of chorionic villi and a mild increase in the collagen in the villous stroma (Fig.8C &D). The results of PGDM showed a moderate increase in PAS in the BM of syncytiotrophoblast, mild thickening of BM of capillary blood vessels, and an increase in PAS moderately in the villous stroma (Fig.8 E&F).

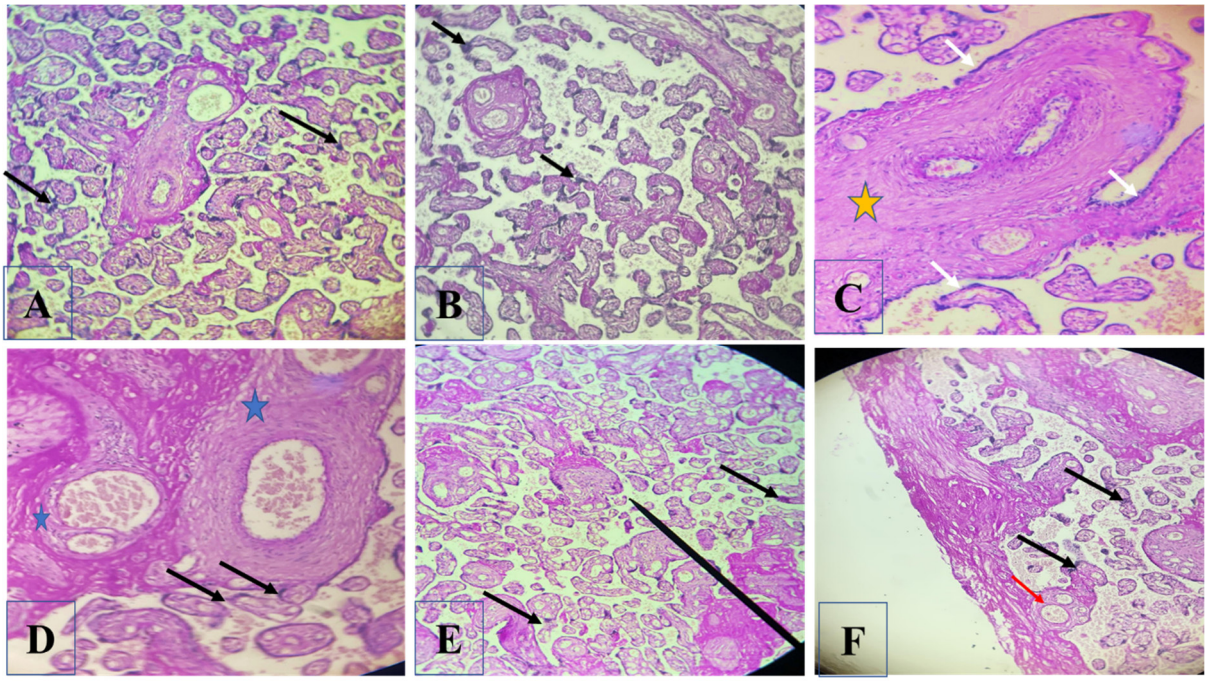

Figure (8): Photomicrograph of central sections of human placenta stained with Periodic acid shift reagent (PAS), showing: (A&B) control: a faintly positive PAS stain reaction in the chorionic villi. Note the presence of a large number of terminal villi close to each other, with their small diameters and thin walls. (C&D) GDM: showing mild PAS reaction, (C) mild thickening of the basement membrane (BM) of chorionic villi (white arrow) and mild increase collagen in the villous stroma (yellow star); (D) accumulation of collagen around the blood vessels (eosinophilic material) (blue star). (E&F) PGDM: moderate increase in PAS reaction in the BM of syncytiotrophoblast and mild thickening of BM of capillary blood vessels (Red arrow), and a moderate increase in collagen in the villous stroma. Black arrows indicate the syncytial knots. (A, B, E&F: 40x; D&C: 100x).

4. Discussion

Chronic hyperglycemia is a common metabolic condition known as diabetes. It is a major component in the morbidity and death produced by chronic diseases that affect essential organ systems, one of which is the placenta (Patric 1996). As a result, several alterations develop in the human placenta during DM-complicated pregnancies. These anomalies are closely related to intrauterine blood glucose levels, which disrupt maternal-placental blood circulation and maternal-fetal exchange (Salge *et al.,* 2012).

The present results of the timing and mode of delivery in three studied groups (control, GDM, and PGDM), showed that the mean gestational period in all these groups ranged between (37-40) weeks, this period comes beneficial to the fetus as mentioned by Gawlik *et al.,* (2015), waiting at least until 38 completed weeks' gestation enhances fetal outcome, particularly in GDM women. Additionally, the present results have shown an increase in the number of cesarean deliveries in diabetic groups (100%) in comparison to normal deliveries (16%). This finding was consistent with earlier research that found the cesarean section rate increased significantly among diabetic women, as Denguezli *et al.,* (2007) found that the cesarean rate in diabetic pregnant women (GDM, DM) is greater than in the control group. In national research, Hameed (2011) and Al-Maini *et al.* (2017) found that cesarean section was a prevalent method of delivery in Iraqi women with GDM. According to Aviram *et al.* (2016), women with GDM had a reduced rate of spontaneous delivery and a greater rate of cesarean delivery.

Furthermore, Boriboonhirunsarn and Waiyanikorn, (2016), discovered that the frequency of cesarean section rose considerably in GDM pregnant women when compared to typical pregnant women. In general, there are numerous variables factors that may influence the timing and method of birth in diabetic women, including biomedical, psychological, social, and environmental factors. Such a decision is best reached through the practice of active, informed debate with the patient and her family (Kalra *et al*., 2016).

The fetal weight was not statistically different in all three groups in the present research, which may suggest excellent antenatal care and optimum diabetes control. This finding was consistent with the findings of Salge *et al.,* (2012) and Alo Khudir, (2017), who found no substantial variations in neonatal weight between the DM and control groups. However, the current findings did not agree with the findings of Tandone *et al.,* (2018) and Weli, (2021), who discovered a clear significant increase in birth weight in the DM group compared to healthy women, owing to the effect of diabetes, which causes disturbances in the transport of nutrients across the placenta, particularly hyper transposition of glucose through the glucose family.

The current findings also revealed no significant rise in the number of cotyledons in the DM groups when compared to the control group. This finding contradicted the findings of Bhattacharjee *et al*. (2017), who discovered a substantial rise in the number of cotyledons in diabetic groups (GDM, mild hyperglycemic, and overt diabetics) when compared to the normoglycemic group. Weli, (2021), also revealed that the diabetic group had a significantly higher number of cotyledons than the control group.

According to the findings, the mean weight and diameter of the placenta in the GDM and PGDM groups were considerably greater than in the control group. While there was no notable variation in placental thickness between the control and GDM, PGDM produced a substantial increase in this thickness when compared to other groups. This finding contradicted the findings of Bhattacharjee *et al*. (2017) and Tondon *et al*. (2018), who discovered that the difference in placental (weight, girth, and thickness) between the normal and diabetic groups was not statistically significant. However, the current findings concurred with those of El Sawy *et al*., (2018), who found that the mean weight of the placenta, as well as its diameter and thickness, were considerably higher in the GDM group than in the normal placenta. Kurrey *et al*. published research (2017), that found a rise in the placental weight of PGDM women compared to normal and GDM women; this result is congruent with the present study.

Several studies have found various placental morphological anomalies in women with GDM, including a rise in placental weight and size, among other things. However, as Edu *et al*., (2016) and Ruud *et al*., (2017), indicate, the impact of metabolic and hormonal changes, particularly hyperinsulinemia, appears to be more important.

According to Huynh *et al*. (2015), the main reason for the increase in placental weight and growth is non-parenchymal substantial hyperplasia (decidual and chorionic plates, the connective tissue of villi, inter-cotyledonary septa, fetal vessels, fibrin deposits, and infarcts). As a consequence of the continuing hyperglycemia, the placenta develops macrosomia and substitutionary hyperplasia. As a result, the current study backed a national study that discovered diabetes groups' placentas had increased weight, central thickness, breadth, and the number of cotyledons (Martino *et al*., 2016; Castillo-Castrejon and Powell, 2017).

In terms of the embryonic placental ratio, the current study found that it was considerably higher in the PGDM group than in the control group, but there was no significant difference between the GDM and PGDM groups. This finding was consistent with the findings of Alo Khudir (2017), who found that the fetoplacental ratio was not substantially higher in the GDM and PGDM groups. However, the current finding did not agree with (Ganer *et al*., 2017), who stated that the neonatal/placental weight ratio was lower in the two diabetes groups, but the difference was not significant.

In terms of placental shape and UC insertion, the current study found that the most common shape of the placenta in the control group was discoidal, followed by irregular, oval, and kidney shapes. These shapes were also recorded in the GDM and PGDM groups, with the exception of the kidney and irregular shapes, which were not recorded in these groups. Other scholars, including Yampolsky *et al*., (2011), Kurrey *et al*., (2017), and Weli, (2021), had similar findings. Diabetes alters the growth of placentae at the beginning of gestation and has a long-term effect as diabetes buffers excess maternal glucose and increases vascular resistance (Kurrey *et al*., 2017), as well as the abnormal branching of the blood vessels that make up the vascular network in the placenta. Furthermore, a decline in the placenta's effectiveness in its functions indicates abnormal changes in its shape (Yampolsky *et al*., 2011).

The most frequent location of UC insertion in the three groups in the current research was central insertion, followed by noncentral (eccentric) and marginal insertions. Weli, (2021) also found this finding, while EL Sawy *et al*., (2018) found that the eccentric variety is the most prevalent, followed by the marginal and central sites of UC insertion in both normal and diabetic groups.

According to Chang and Aw, (2019), this variation in UC insertion with the placenta is due to the Trophotropism theory, which confirms that the placenta is formed in response to the location of the optimal vascular supply, which results in the movement of the placental disc, leaving the UC to emerge as a branch in chorionic membranes rather than penetration into the placenta.

The current study's findings revealed the typical component of the placenta, the villi, which emerged in various sizes and were separated from one another by intervillous spaces. These villi had few blood vessels that were mostly located on the periphery. These blood tubes had thin walls. The fetal blood is contained in the villous blood vessels, while the mother’s blood is contained in the intervillous areas. In this instance, the trophoblast layers and the thin wall of the blood vessels divide the fetal and maternal blood. According to Laurini *et al*., (1987), this is the placental barrier that separates the fetal and maternal blood and plays a critical role in the simple transmission of oxygen and nutrients between the fetal and maternal blood.

The present findings showed that diabetic groups' placentas had distinct histopathological changes. These include rises in the number of various villi sizes linked with either a decrease or an increase in the intervillous spaces. The quantity of big syncytial knots has increased. The villous trunk is surrounded by syncytiotrophoblastic cells that vary significantly in thickness, structure, and nuclei distribution (Blackburn, 2018). These localized clumping’s or aggregations of syncytial nuclei on the distal villous surface create a multinucleated projection from the villous surface. Within the terminal villous, these are tiny clusters of structureless, homogeneous, and

eosinophilic compounds. (Augustine *et al*., 2016). The presence of an increased number of syncytial knots, bridges, and seedlings is referred to as syncytial knotting or Tenny-Parker alterations (Benirschke *et al*., 2012). Other authors, including Mishra *et al*., (2017), and Weli, (2021), have also suggested the current finding.

The current study found increasing fibrinoid necrosis and chronogenesis in the intervillous spaces, as well as villous capillary congestion and other histopathological alterations. Fibrinoid necrosis is defined as a non-cellular homogeneous eosinophilic substance within the villi (Benirschke *et al*., 2012; Augustine *et al*., 2016). Hypoxia or relative stagnation caused by higher sugar levels may promote fibrinoid degeneration of villi (Tewari *et al.,* 2011). Because of the greater diffusion distance between the intervillous space and the fetal capillaries, fibrinoid necrosis may also impact oxygen exchange. (Daskalakis *et al*., 2008). In conclusion, both GDM and PGDM caused adverse effects on histological and morphometrical features of the placenta.

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