ORIGINAL ARTICLE

Histomorphological Analysis of Placental & Umbilical Cord Blood Vessels in Gestational Diabetes Mellitus

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Abstract

Background: Gestational Diabetes Mellitus (GDM) is a primary health issue in our society. Diabetes during pregnancy presents various challenges that can impact the growth and development of the fetus, relying on the healthy functioning of the placenta and umbilical cord.

Objective: In this study, an evaluation of histomorphological changes in umbilical cord was done in gestational diabetes mellitus.

Methods: This comparative cross-sectional study comprised 58 subjects, having two groups: Group A consisted of 29 healthy subjects, and Group B of 29 gestational diabetes mellitus patients. The study duration was from July 2020 to July 2021. It was conducted by keeping the declaration of Helsinki as an ethical guide for research involving human subjects at Lahore General Hospital, Lahore, and the Experimental Research Laboratory of Postgraduate Medical Institute (PGMI) Lahore. History and examination were done regarding gestational diabetes mellitus. Umbilical cords were collected at the time of delivery and washed with normal saline. A gross examination was done, and samples were preserved for histomorphology.

Results: The diameter of the umbilical cords was significantly increased in Group B (case) as compared to Group A (control). There was no significant difference in the number of false knots in the two groups. Histologically, there was endothelial disruption of the umbilical artery and congestion of the umbilical vein in Group B, but no such increase in Group A.

Conclusion: This study showed that there were significant histomorphological changes in GDM umbilical cords.

Keywords: *Gestational diabetes mellitus, umbilical cord, umbilical artery, umbilical vein, Wharton's jelly, false knots, histomorphology, comparative study, Lahore.*

INTRODUCTION

The umbilical cord is a vital link between a mother and her developing fetus [1]. It spans from the fetal umbilicus to the fetal surface of the placenta and facilitates the transportation of nutrients, respiratory gases, and metabolites. The proper psychomotor development of the fetus relies on the mobility that the umbilical cord provides. To ensure proper fetal development and survival, the umbilical vessels and the entire cord must have correct morphology. The umbilical vessels are surrounded by Wharton's jelly, which comprises connective tissue with a large amount of extracellular matrix [2]. This jelly gives the umbilical cord its strength, and elasticity, and protects its vessels from compression. Throughout pregnancy, the umbilical cord safeguards the vessels that transfer nutrients and gases from the placenta to the fetus [3]. It is surrounded by the amnion and contains a single umbilical vein and two umbilical arteries, all held in place by Wharton's jelly. The diameter and length of the cord are typically 1 to 2 cm and approximately 55 cm, respectively [4].

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Diabetes Mellitus can be categorized as either pre-gestational or overt diabetes, which includes type 1 or type 2 DM, that occurs before the pregnancy begins [5]. The second type is gestational diabetes (GDM), which is characterized by any degree of glucose intolerance that occurs with the conception of pregnancy. GDM can be diagnosed during any time of pregnancy if specific criteria are met, which include fasting plasma glucose of 5.1-6.9 m mol/l (92-125 mg/dl), one-hour plasma glucose of >10.0 m mol/l (180 mg/dl), and two-hour plasma glucose of 8.5-11.0 m mol/l (153-199 mg/dl) followed by a 75 g oral glucose load. The increase in GDM cases among women of reproductive age can be attributed to various factors such as sedentary lifestyles, and childhood and adolescent obesity [6]. Diabetes affects at least 2% to 5% of all pregnancies. Pregnancy is considered a very sensitive state in terms of glucose metabolism, as the hormones secreted by the placenta, such as estrogen, progesterone, cortisol, human chorionic somatotropin, human placental lactogen, and prolactin, play a vital role in it [7]. Human placental lactogen, for instance, increases insulin resistance and acts as an insulin antagonist. During pregnancy, blood sugar levels are usually stable as women balance peripheral insulin resistance by enhancing insulin secretion *via* beta cells of the pancreas [8]. However, some pregnant women may fail to increase insulin secretion, leading to GDM [9]. GDM is associated with both early and late complications in both the mother and the fetus. Early complications include macrosomia, hypoglycemia, neonatal jaundice, preeclampsia, preterm labor, and cesarean section [10], while late complications include obesity, glucose intolerance, and adolescent diabetes [11, 12].

Gestational Diabetes Mellitus (GDM) can cause various umbilical cord malformations that include an increase in diameter, false knots, disruption of umbilical artery endothelium, and umbilical vein dilatation. These pathologic deformities in the umbilical cords of GDM mothers are significant risk factors leading to fetal anoxia during pregnancy. Unrestricted diabetes can disturb umbilical cord function, leading to growth and developmental disturbances, macrosomia, congenital abnormalities, and intrauterine growth restriction [13]. GDM affects the vascular formation of the placenta and umbilical cord, depending on the type of diabetes, whether pre-gestational (T1D and T2D) or gestational [14]. While overt diabetes may impact the entire placental and fetal development, GDM hyperglycemia appears clinically in the second trimester of pregnancy [15]. Thus, GDM may cause complications in later-stage umbilical cord events like angiogenesis and microvascular remodeling, but not early-stage events such as vasculogenesis [16]. Since the umbilical cord acts as a communication pathway between fetal vasculature and uterine musculature, any event that affects the uteroplacental vascular unit, such as GDM, may impact umbilical cord morphology and fetal wellness. Despite multiple available treatment options, GDM still poses a threat to normal fetoplacental development and umbilical cord structure [17].

SUBJECTS AND METHODS

The Advanced Studies and Research Board of the University of Health Sciences, Lahore, and the Ethical Committee of PGMI, Lahore, approved this cross-sectional comparative study. It was conducted under their guidelines, keeping the declaration of Helsinki as an ethical guide for research involving human subjects at Lahore General Hospital, Lahore, and the Experimental Research Laboratory of Postgraduate Medical Institute (PGMI) Lahore. The study was conducted between July 2020 to July 2021. Using the WHO calculator a sample of 38 patients per group (58 per group) was obtained using the power of study equal to 80% and 95% confidence level. The anticipated mean diameter of the umbilical cord in GDM cases as 1.303 with a standard deviation of 0.1884 and the Anticipated mean diameter of umbilical cord levels in healthy subjects as 1.163 with a standard deviation of 0.1885 [18].

Pregnant females were diagnosed with Gestational Diabetes Mellitus (GDM) at a gestational age of 24-28 weeks and followed until delivery and 36 healthy pregnant females were enrolled in this study. Informed consent was taken from each patient. The patients were stratified into two groups according to inclusion criteria; in Group A, Umbilical cords of healthy pregnant females were enrolled, while in Group B, Umbilical cords of self-reported Gestational Diabetes Mellitus patients were enrolled. Umbilical cords of patients with Any infection like respiratory or urinary tract infection *etc*, previous history of GDM, Known case of diabetes mellitus, and patients with any Family history of diabetes mellitus were excluded. Non-probability consecutive sampling technique was used.

58 umbilical cords were collected for this cross-sectional comparative study, including 29 from pregnant females diagnosed with Gestational Diabetes Mellitus (GDM) at a gestational age of 24-28 weeks and followed until delivery and 29 from healthy pregnant females. Both groups were selected based on their age, parity, family history of GDM, weight, height, and clinical examination. Non-probability purposive sampling technique was used to collect the data.

The umbilical cords were obtained from the labor room or operation theatre of the Gynecology and Obstetrics department at Lahore General Hospital, Lahore. The cords were collected from 2cm away from the umbilical stump, washed with normal saline, and examined for gross parameters such as diameter and false knots. The cords were labeled, stored in plastic jars with 10% neutral buffered formalin, and kept at room temperature for 48-72 hours. Later, they were dehydrated by gradually replacing alcohol concentration and then cleaned with xylene before being embedded in paraffin blocks.

Data was analyzed through SPSS version 25. Shapiro Wilk test was used for normality assessment. Qualitative variables, *i.e.* endothelial disruption of the umbilical artery and congestion of the umbilical vein, were presented in frequency and percentage. Fisher's exact test was used to compare the categorical variable of cases and controls. Mean ± standard deviation was given for quantitative variables, *i.e.* the diameter of the umbilical cord and the number of false knots. Independent sample t-test was used to determine the mean difference of quantitative variables between cases and controls. P-value ≤ 0.05 was considered as significant.

RESULTS

The study enrolled 29 patients in Group A and 29 patients in Group B. The socio-demographic characteristics of the two study groups, as presented in Table **1**, indicate that the mean age of participants in Group A (healthy subjects) was 29.08 ± 6.27 years, while in Group B (Gestational Diabetes Mellitus patients), it was 30.43 ± 5.66 years. The difference in age between the two groups was not statistically significant (p = 0.330). However, a significant difference was observed in the Body Mass Index (BMI), where Group B had a higher mean BMI (23.83 ± 2.94 kg/m²) compared to Group A (21.64 ± 2.19 kg/m²), with a p-value of less than 0.05.

Table 1: Socio-demographic features among two groups.

Characteristics	Group A Mean (SD)	Group B Mean (SD)	p-value
Age (years)	29.08(6.27)	30.43(5.66)	0.330
BMI (kg/m^2)	21.64(2.19)	23.83(2.94)	<.001*
Gravida/parity or abortion	2.00(1.04)	2.04(1.00)	0.899

Table 2: Comparison of other parameters between two groups.

Parameters	Group A	Group B	p-value
Diameter umbilical cord cm, (mean±SD)	20.1 ± 1.9	25.7 ± 1.6	< 0.001*
False knots in Umbilical cord, n(%)			
Present	2(6.9)	5(17.2)	0.227
Absent	27(93.1)	24(82.8)	
Endothelial Disruption of Umbilical Artery, n(%)			
Present	1(3.4)	27(93.1)	< 0.001*
Absent	28(96.6)	2(6.9)	
Congestion of Umbilical Vein, n(%)			
Present	2(6.9)	27(93.1)	< 0.001*
Absent	27(93.1)	2(6.9)	

*Significant at p<0.05

Gravida/parity or abortion history was similar between the two groups, with no significant difference (p = 0.899).

Table **2** presents the comparison of other key parameters between the two groups. The diameter of the umbilical cord was significantly larger in Group B (25.7 ± 1.6 cm) compared to Group A (20.1 ± 1.9 cm), with a p-value of less than 0.001. Although the presence of false knots in the umbilical cord was more common in Group B

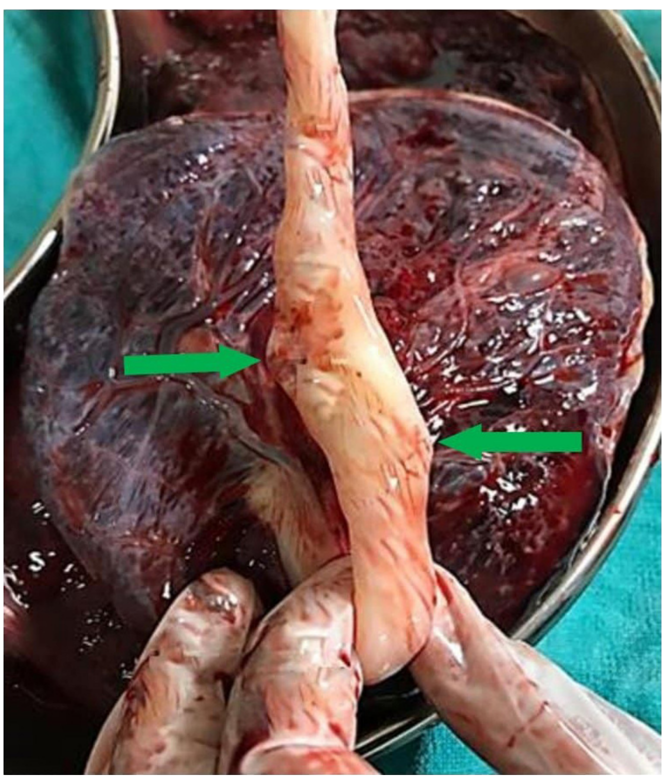

Fig. (1): Photograph showing false knots (green arrow) in the umbilical cord of a GDM placenta.

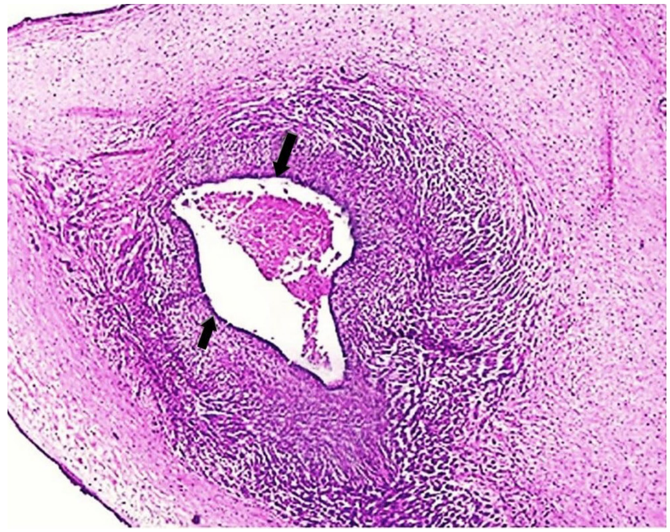

Fig. (2): Photomicrograph showing intact endothelium of the umbilical artery in Group A (H&E stain, 40x magnification).

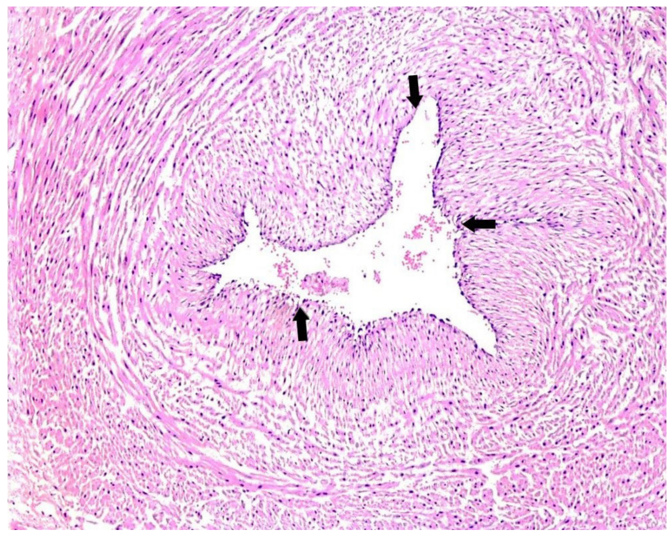

Fig. (3): Photomicrograph showing endothelial disruption in an umbilical artery in Group B (black arrows) (H&E stain, 40x magnification).

Fig. (4): Photomicrograph showing no umbilical vein congestion in Group A (H&E stain, 40x magnification).

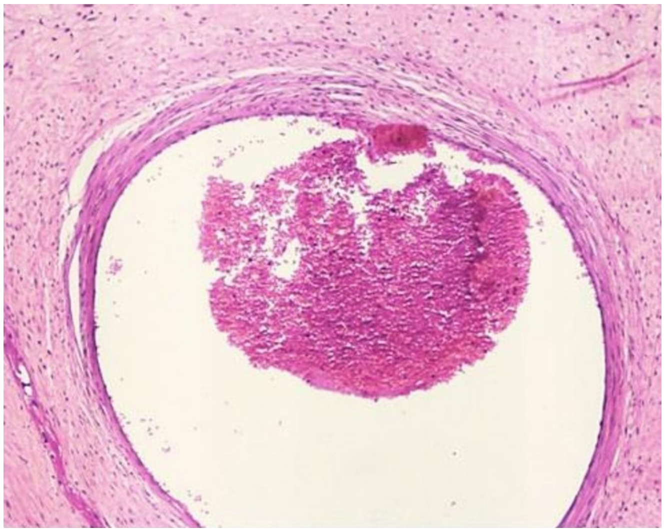

Fig. (5): Photomicrograph showing umbilical vein congestion in Group B (H&E stain,40x magnification).

(13.2%) (**Fig. 1**) compared to Group A (5.3%) (**Fig. 2**), this difference was not statistically significant (p = 0.430).

Histological analysis revealed significant differences in the vascular structure of the umbilical cord between the two groups. Endothelial disruption of the umbilical artery was present in 93.1% of the cases in Group B (**Fig. 3**), compared to only 3.4% in Group A in which the endothelium was intact mostly (**Fig. 2**), with a highly significant p-value of less than 0.001. Similarly, congestion of the umbilical vein was observed in 93.1% of Group B, while only 6.9% of Group A showed this feature, also with a p-value of less than 0.05 (**Figs. 4** and **5**).

DISCUSSION

The study found that BMI was significant statistically with a p-value of 0.002* [19], while parity and age were not significant. The gross appearance and histological features of the umbilical cords were also significantly different in GDM cases compared to normal subjects. The umbilical cords of GDM cases showed an increased diameter and endothelial disruption of the umbilical artery, as well as congestion in the umbilical vein. False knots were only observed in a small number of GDM cases and were not significant.

The umbilical cord plays an essential role in fetal survival, and this study compares various morphological parameters of the umbilical cord in GDM and normal pregnancies, including diameter and false knots of umbilical cords, and aspects of umbilical vessels necessary for fetal well-being such as the endothelial integrity of the umbilical artery and umbilical vein congestion.

The mean diameter of umbilical cords in GDM cases majorly increased than in normal subjects, which was consistent with previous study by Ennazhiyil *et al.* (2019). The possible mechanism for this was an increase in the substance of Wharton's jelly and lumen of umbilical vessels [20]. False knots were not significant in GDM cases [17], and endothelial disruption of the umbilical artery was significant, likely due to inflammation from hyperglycemic stress.

Other studies have also shown similar findings of endothelial damage in umbilical arteries in GDM cases, which can lead to increased vessel permeability and adverse pregnancy outcomes.

The present study found significant endothelial disruption of the umbilical artery, which is in accordance with the previous studies. Salem *et al.* (2019) reported point destruction of the endothelial lining in the umbilical arteries of GDM cases, which is believed to be due to inflammation resulting from hyperglycemic stress. Di Fulvio *et al.* (2014) also observed pro-atherogenic changes in the endothelial cells exposed to increased blood glucose in GDM [15]. Additionally, Alam *et al.* (2014) reported that disruption and erosion of the endothelial lining in the umbilical arteries lead to increased vessel permeability in diabetes [18]. Seval *et al.* (2019) also found increased endothelial damage in the umbilical artery when studying morphological changes in the umbilical cord in patients with GDM, preeclampsia, and HELLP syndrome [9].

LIMITATION OF STUDY

Further investigation is needed to determine the effects of achieving optimal glycemic control on both maternal and fetal outcomes. However, due to resource limitations, conducting a study with a larger sample size was not financially feasible. Additionally, exploring the genetic factors that contribute to feto-maternal complications in GDM, including poor nutrition, sedentary lifestyle, pollution, and stress, may provide additional insights.

CONCLUSION

The study has facilitated the establishment of a connection between GDM and umbilical cord histomorphometry. It is widely recognized that diabetes has detrimental effects on pregnancy outcomes; therefore, routine antenatal checkups, monitoring, and maintaining optimal glycemic control throughout pregnancy are crucial for the well-being of both mother and baby.

ETHICS APPROVAL

The study was approved by the Institutional Review Board of Lahore General Hospital and it conformed to the provisions of the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Informed consent was taken from each patient.

AVAILABILITY OF DATA

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Declared none.

AUTHORS' CONTRIBUTION

AHD: Study conceptualization and critical review of the initial draft; SN: Designing of the study and critical review of the initial draft; NI, SFD: Designing of the study and critical review of the initial draft; SS: Result analysis and investigation, manuscript drafting; AI: Manuscript critical review and revision of the initial draft. All authors read and approved the final manuscript.

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