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HISTOPATHOLOGICAL CHANGES OF THE UMBILICAL CORD IN COMPLICATED PREGNANCIES

*Seval KAYA¹, Yusuf NERGİZ*², A.Kadir TURGUT³*

Orcid: 0000-0001-6251-6529; Orcid: 0000-0002-9988-9385; Orcid: 0000-0002-3156-2116

¹Dicle University Faculty of Medicine, Department of Histology & Embryology

²Dicle University Faculty of Medicine, Department of Histology & Embryology

³Dicle University Faculty of Medicine, Department of Obstetrics and Gynecology

Corresponding author : yusufnergiz21@gmail.com

Abstract: *Preeclampsia, HELLP syndrome and gestational diabetes are quite common complication in pregnancy. In this study, it is aimed to compare the thickness of basal lamina and vascular structure of umbilical cord. By comparing preeclampsia, HELLP syndrome, gestational diabetes and normal pregnant women. In the study, samples were taken from postpartum umbilical cords of normal pregnant women, preeclampsia, gestational diabetes and pregnant women diagnosed with HELLP syndrome. Umbilical cord samples taken during maternity, followed by routine paraffin through fixing 10% neutral formalin. In this performed as totally sixty facts through taking fifteen umbilical cords from each of four groups, Hematoxyline-eosin, Masson trichrom, periodic acid Schiff, and toluidine blue stain were applied on to the serial histological sections obtained from paraffin blocks. The obtained slides were evaluated with Zeiss Imager A 2 Microscope by taking micrographs. Morphometric measurements were statistically analyzed with SPSS software. In cases of gestational diabetes due to increased collagen production, thickening and excessive narrowing of the lumen and differentiation of Wharton jelly were observe in the umbilical artery. Differentiations are observed in the vacuolization of smooth muscle cells and muscle fibers that make up the media while camber and damage are observed in endothelial. The most significant structural changes of preeclamptic placentas are obtained as the thickening and the endothelial injury. In the umbilical cord artery medial layer, due to the growth of collagen fiber. In HELLP cases due to the increase in media diffuse edema between muscle fibers and in collagen, the thickening of umbilical artery wall and the narrowing of luminal are seen as extremely significant. Another striking symptom with HELLP cases is the observing of intramuscular hemorrhages in the umbilical artery wall as an unattained case in preeclampsia and gestational diabetes. In most of the cases, differentiation in Wharton jelly, metaplastic changes in the amniotic epithelium and thickening of the basal lamina are observed.*

Key words: *Umbilical cord, Preeclampsia, Gestational diabetes, HELLP syndrome*

1. Introduction

The umbilical cord plays an important role in interactions between mother and fetus during pregnancy. The folded umbilical cord that connects the fetus to the placenta holds the placenta in centric or eccentric positions, its length being approximately 55 cm in length.

The two umbilical arteries and a vein are located within the Wharton jelly [1].

Gestational diabetes, HELLP syndrome and preeclampsia are very common complications during pregnancy.

Gestational Diabetes (GD) is defined as the deterioration of glucose tolerance that occurs or is detected in pregnancy. It is mostly the most common metabolic disorder [2]

Preeclampsia, vasospasm and secondary organ perfusion decrease in pregnancy is a disease belonging to [3].

HELLP syndrome is generally considered a severe form of preeclampsia. HELLP syndrome is associated with high maternal and perinatal morbidity and mortality, characterized by hemolysis, elevated liver enzymes and decreased platelet count [4-5].

The aim of this study was to examine and compare the morphological changes in the umbilical cord, such as vascular structures and basal lamina thickness in patients with gestational diabetes, preeclampsia and syndrome.

2. Material and Methods

2.1. Tissue follow up for histopathological examination

The study was started after the approval of Dicle University Medical Faculty Non-interventional Clinical Research Ethics Committee. This study was performed on the umbilical cord obtained from 15 normotensive, 15 preeclampsia, 15 gestational diabetes (GD) and 15 HELLP syndrome cases. Tissue of the umbilical cord 1 cm in size were taken by the placenta. Tissue samples were fixed in 10% buffered neutral formalin. Routine histological tissue was followed by paraffin embedded.

5 micrometer thick sections were taken from each paraffin block with the help of the fully automatic rotary microtome (Leica RM 2265, Germany). Hematoxylin-Eosin (H&E), Masson trichrome and Periodic Acide Schiff (PAS) were applied to the paraffin sections. The preparations were then evaluated by Zeiss Imager A2 light microscope and photomicrographs were taken.

2.2. Morphometric Measurements

The cross-sectional area, umbilical artery lumen, vein lumen and tunica media thickness were measured by Zeiss axio imager 2 microscope using the Zen 2012 Sp2 (blue edition) program.

Furthermore, macroscopically, each sample was measured with umbilical cord total circumference length and total diameter planimeter.

In each section, the mean of the maximum and minimum diameter values for the measurement of the artery and vein lumen were taken into account. In the measurement of thickness of the tunica medial layer, 1 artery and 1 vein were selected separately for each sample. Five different regions were measured in the media layer and the mean values obtained were measured as the mean thickness of

tunica media layer. For all samples, total umbilical cord total circumference length, umbilical cord total diameter as well as both artery and vein tunica media layer thickness and lumen diameter averages were obtained.

2.3. Statistical Analysis

One-way analysis of variance (ANOVA) test was used to compare the averages of the umbilical cord total measurements, arterial and vein parameters, and in the case of statistical significance, multiple comparisons Dunnett and Tukey HSD multiple comparison tests were used. A 95% confidence interval was applied in all tests and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Umbilical cord histopathological results

3.1.1. Histopathological results of the control group umbilical cord

Normal histological structure was observed in the umbilical cord sections of the control group. The outermost single-row cubic amniotic epithelium and the Wharton jelly under it observed 2 arteries and 1 vein (Figure 1).

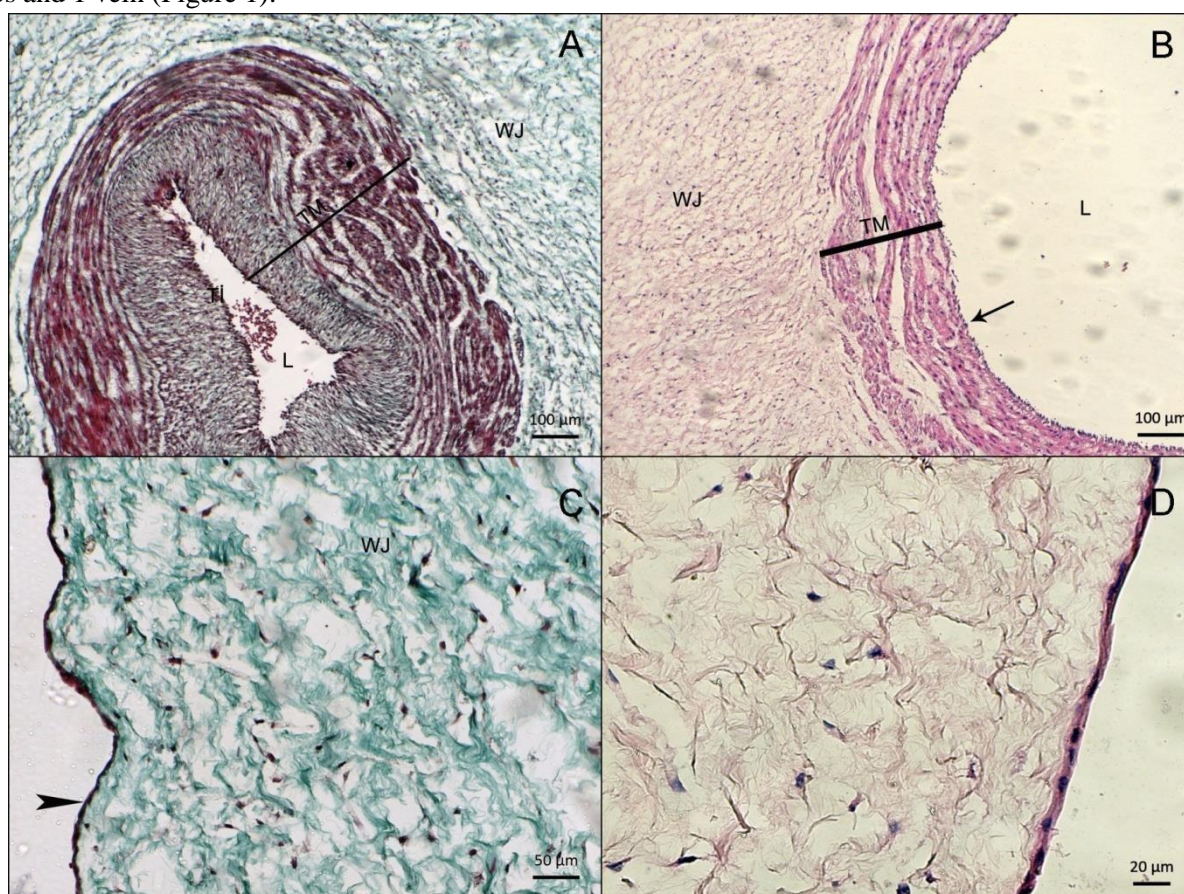


Figure 1. Normotensive section of the umbilical cord sections. a) View of the umbilical artery (Masson trichrome, Bar: 100 μ m). b) Umbilical vein appearance (H&E, Bar: 100 μ m). c) Amnion epithelium surrounding the umbilical cord and Wharton gelly. (Masson trichrome, Bar: 50 μ m). d) Single-row cubic amnion epithelium in the umbilical cord. (H&E, Bar: 20 μ m). L: Lumen, TIA: Tunica intima, TM: Tunica media, WJ: Wharton gelly, Arrow: Vascular endothelium, Arrow head: Amniotic epithelium

3.1.2 Histopathological results of the gestational diabetes group umbilical cord

Excessive thickening and collapse of the lumen were observed in the umbilical artery wall due to increased collagen (fig. 2a). At tunica media were observed vacuolization and disruption of muscle fibers, while the endothelium showed bulging into lumen and damage (figure-2b). Normally, the circumference of the umbilical vein was observed in the smooth muscle cell line (Figure-2c). Another structural change in the umbilical cord sections of the GD group was metaplasia in the amniotic epithelium (Figure 2d).

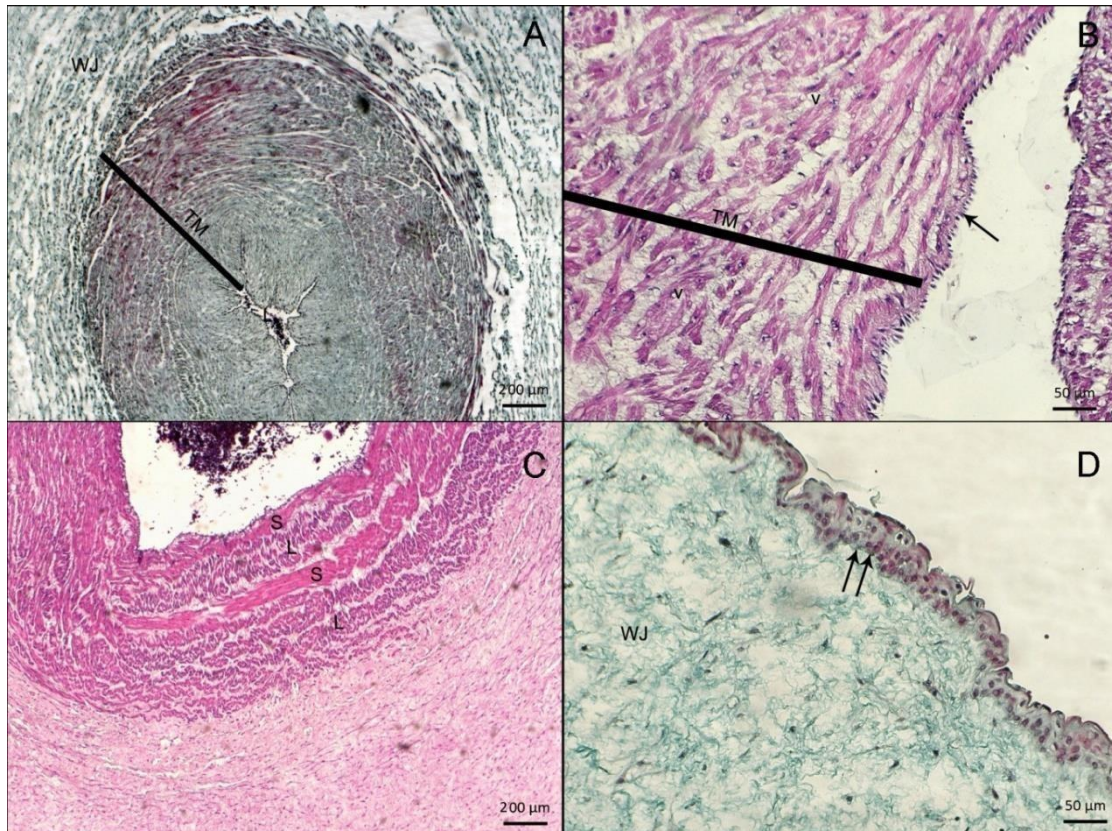


Figure 2. Umbilical cord sections of gestational diabetes group. a: Umbilical cord excessive thickening of the umbilical artery wall (Masson trichrome, Bar: 20 μ m). b: Columnar shape in endothelial cell nuclei of umbilical vein, vacuolization and dissociation in tunica medial muscle cells (H-E, Bar: 50 μ m). c: Disorganization status in umbilical vein muscle array. (H&E, Bar: 200 μ m). d: Metaplasia in the umbilical cord amnion epithelium (Masson trichrome, Bar: 50 μ m). L: Lumen, TM: Tunica media, WJ: Wharton's jelly, Arrow: Vascular endothelium, V: vacuolization, S: Circular muscle alignment, Double arrow: Metaplasia, WJ: Wharton's jelly.

3.1.3 Histopathological results of the umbilical cord in the preeclampsia group

The most significant changes in preeclamptic pregnancies, endothelial injury, and asymmetric thickening due to collagen fiber increase in the media layer of the umbilical artery (Figure 3A).

In some umbilical cord sections, umbilical artery media layer showed a snake-like appearance in smooth muscle cell nuclei due to edema, dissociation and contraction (Figure 3B). In the endothelial basal lamina, fragmentation, swelling and undulant laminar changes were observed in all

umbilical cord veins (Figure 3C). A significant thickening was observed on the amniotic basal lamina (Figure-3D).

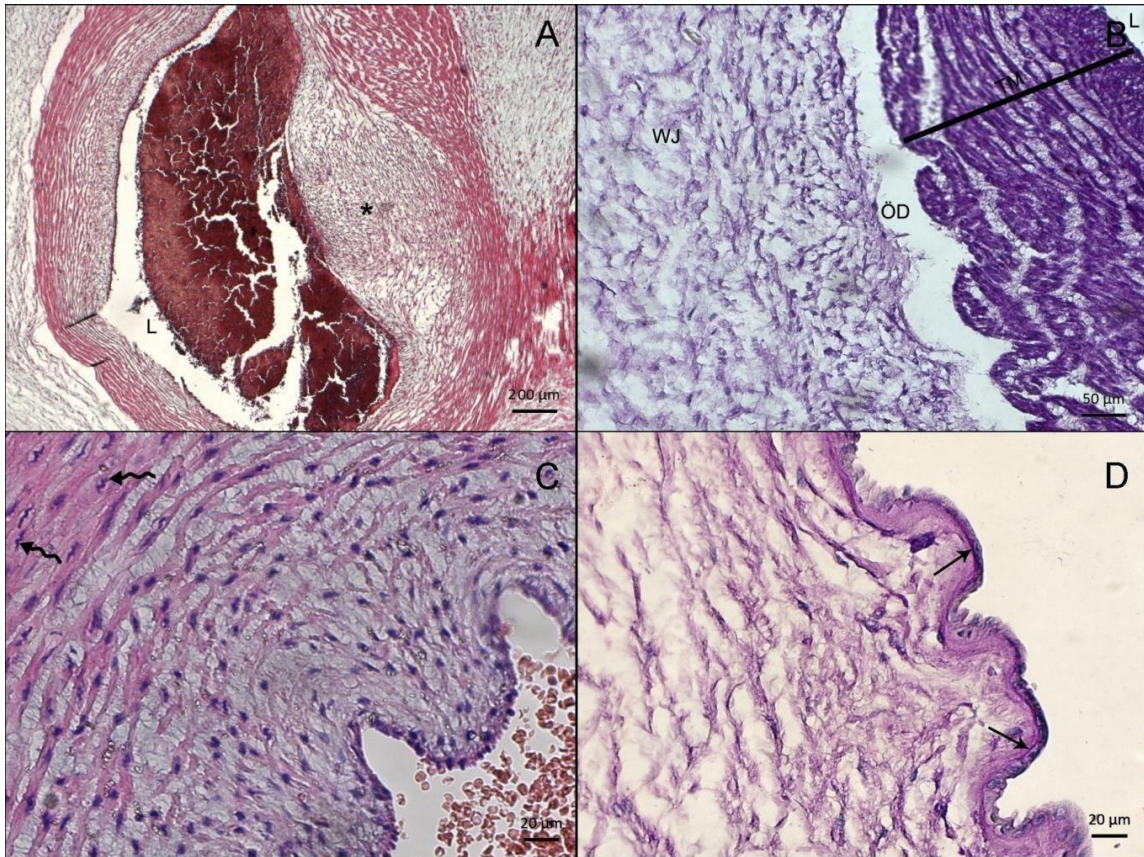


Figure 3. Preeclamptic umbilical cord sections. A: Asymmetric thickening of umbilical artery wall, (Masson trichrome, Bar: 200 μ m). B: Muscular disorganization and perivascular edema in the umbilical artery wall, (PAS, Bar: 50 μ m). C: Destruction of the umbilical artery endothelium and a snake-like in the muscle cell nuclei, (H&E, Bar: 20 μ m). D: Thickening of amniotic basal lamina, (PAS, Bar: 20 μ m). Asterix: Asymmetric thickening, TM: Tunica media, L: Lumen, ÖD: Perivascular edema, Curved arrow: Muscle cell nuclei, Thick arrow: Amniotic basal lamina.

3.1.4. Histopathological results of HELLP group umbilical cord.

Due to diffuse edema and collagen fiber increase in muscle fibers, excessive thickening and luminal narrowing in the umbilical artery wall was very prominent (Figure 4A). In most of the cases, Wharton gelly dehiscence, metaplastic changes in amniotic epithelium and obvious thickening of the basal lamina were detected (Figure 4B). Another striking finding was intramuscular and perivascular hemorrhage in the umbilical artery wall, which was not seen in preeclampsia and GD groups (Figure-4C). While endothelial nuclei of the umbilical vessels were prismatic and bulging towards the lumen, in all cases basal lamina was seen to fragment, swelling and ondulations (Figure 4D).

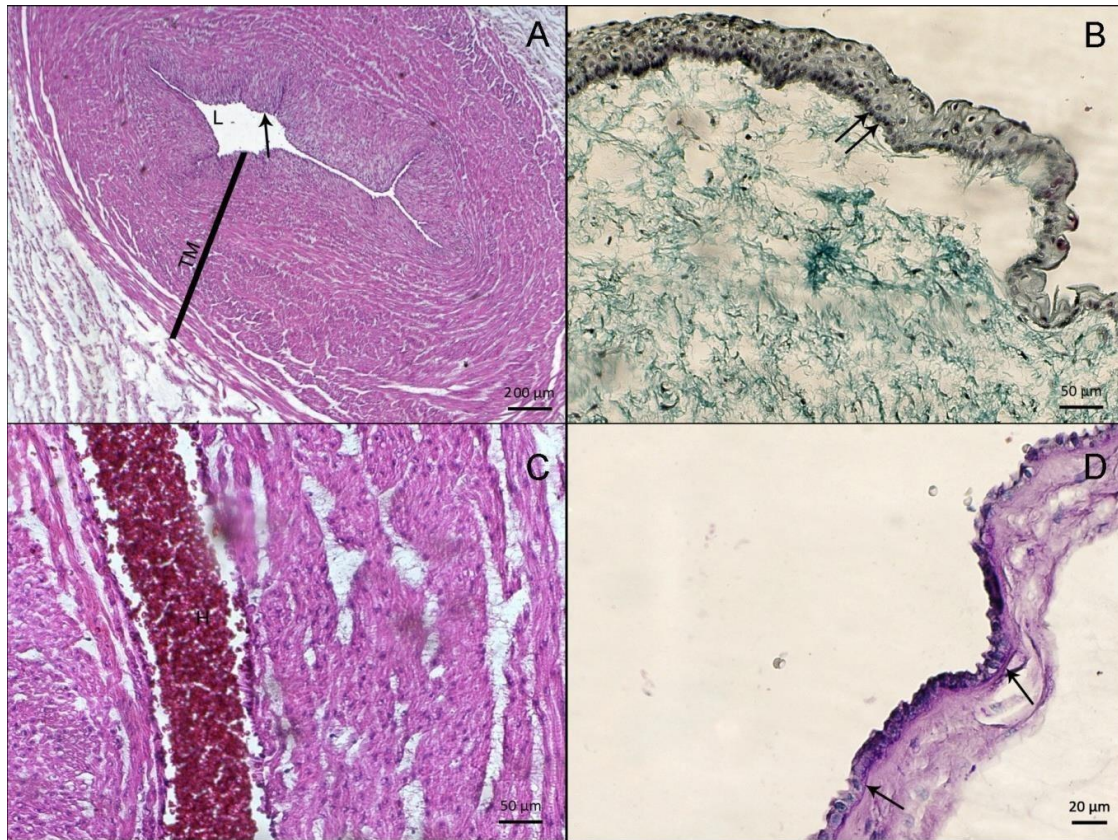


Figure 4. HELLP group umbilical cord sections. A: Advanced thickening of the umbilical artery wall (H&E, Bar: 200 μ m), B: metaplasia in amniotic epithelium (Masson trichrome, Bar: 50 μ m), C: Hemorrhage in the umbilical artery tunica media (H-E, Bar: 50 μ m), D: Thickening of amniotic epithelium basal lamina (PAS, Bar: 20 μ m). L: Lumen, Arrow: Endothelium, TM: Tunica media, H: Hemorrhage, Thick arrow: Amniotic basal lamina.

3.2. Umbilical cord statistical analysis

The measurement data of the umbilical vessels are shown in table-1 and the significance status is shown in Table 2. As a result of the measurements, it was observed that there was narrowing of umbilical artery and artery lumen diameter in all three groups according to the control group (Figure 1). A significant thickening of the HELLP, GD and preeclampsia groups was observed in the umbilical artery media (Figure 6).

In the evaluation of umbilical cord vein lumen diameter, it was concluded that only the GD group had dilatation and this measurement was significant (Figure-7). There was a significant thickening ($p=0.000$) in the GD group compared to the control group but the HELLP and preeclampsia groups were similar to the control group (Figure 8).

An increase in the perimeter length was found in the GD group compared to the control in the total circumference measurement of the umbilical cord (Figure 9).

There was a significant increase in the umbilical cord diameter in the GD group compared to the control group (Figure 10).

Table 1: Morphological measurement data of umbilical cord diameter, circumference and umbilical vein.

	Groups		Mean	Standard deviation	Standard error	Minimum	Maximum
Artery lumen diameter mean. (μm)	Control		668,5429	41,60216	15,72414	604,20	722,60
	Hellp		329,8857	64,54449	24,39552	243,20	418,60
	Preeclampsia		292,8286	71,41017	26,99051	197,80	380,90
	GD		325,1000	56,82693	21,47856	227,50	393,50
Artery media thickness mean. (μm)	Control		330,4571	35,28290	13,33568	277,90	374,80
	Hellp		476,4571	35,19990	13,30431	434,60	518,70
	Preeclampsia		472,8857	44,10493	16,67010	412,00	529,40
	GD		734,7286	72,15060	27,27036	627,30	853,50
Vein lumen diameter mean. (μm)	Control		1030,0429	118,76648	44,88951	884,00	1239,50
	Hellp		995,5714	282,84704	106,90613	630,20	1306,50
	Preeclampsia		814,4000	433,45071	163,82897	150,60	1583,00
	GD		1582,6571	247,92722	93,70768	1156,50	1882,00
Vein media thickness mean. (μm)	Control		363,5757	62,32031	23,55486	307,80	482,92
	Hellp		359,1114	113,46184	42,88455	208,54	479,70
	Preeclampsia		299,7800	76,61616	28,95819	210,12	412,16
	GD		610,8200	77,83889	29,42033	500,62	742,38
Total circumference length. (cm)	Control		3,0571	44293	16741	2,50	3,90
	Hellp		2,6571	,17182	,06494	2,40	2,90
	Preeclampsia		2,8000	,48305	,18257	2,30	3,80
	GD		4,0943	,12998	,04913	3,90	4,30
Total diameter length. (mm)	Control		73,0000	6,02771	2,27826	67,00	85,00
	Hellp		67,2857	9,01322	3,40668	57,00	82,00
	Preeclampsia		78,2857	11,49948	4,34640	62,00	95,00
	GD		110,2857	3,03942	1,14879	107,00	116,00

Table 2. Multicenter multiple comparison chart of umbilical cord diameter, circumference and umbilical vein.

	Intergroup pairwise comparisons		P
Artery Lumen F=61,713 p=0,000	Control	Hellp	0,000
	Control	Preeclampsia	0,000
	Control	GD	0,000
	Hellp	Preeclampsia	Ns
	Hellp	GD	Ns
	Preeclampsia	GD	Ns
Artery Media F=82,416 p=0,000	Control	Hellp	0,000
	Control	Preeclampsia	0,000

	Control	GD	0,000
	HELL	Preeclampsia	Ns
	HELL	GD	0,000
	Preeclampsia	GD	0,000
Vein Lumen F=8,973 p=0,000	Control	HELL	Ns
	Control	Preeclampsia	Ns
	Control	GD	0,005
	HELL	Preeclampsia	Ns
	HELL	GD	0,005
	Preeclampsia	GD	0,000
Vein Media F=18,614 p=0,000	Control	HELL	Ns
	Control	Preeclampsia	Ns
	Control	GDM	0,000
	HELL	Preeclampsia	Ns
	HELL	GD	0,000
	Preeclampsia	GD	0,000
Total circumference F=24,820 p=0,000	Control	HELL	Ns
	Control	Preeclampsia	Ns
	Control	GD	0,000
	HELL	Preeclampsia	Ns
	HELL	GD	0,000
	Preeclampsia	GD	0,000
Total diameter F=40,036 p=0,000	Control	HELL	Ns
	Control	Preeclampsia	Ns
	Control	GD	0,000
	HELL P	Preeclampsia	Ns
	HELL	GD	0,000
	Preeclampsia	GD	0,000

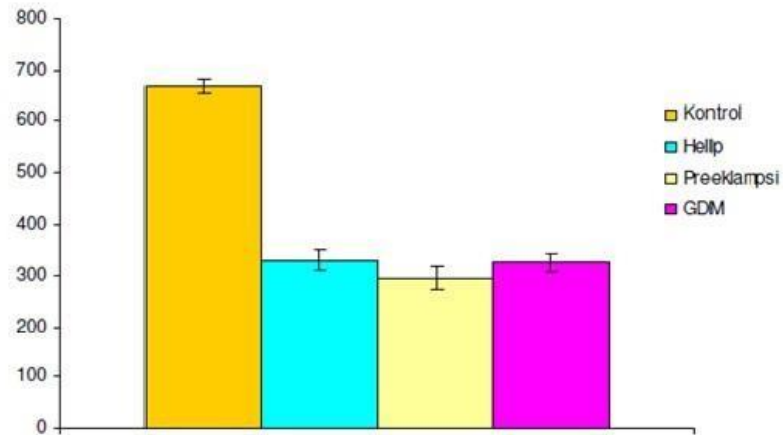


Figure 5: Mean diameter of umbilical artery lumen

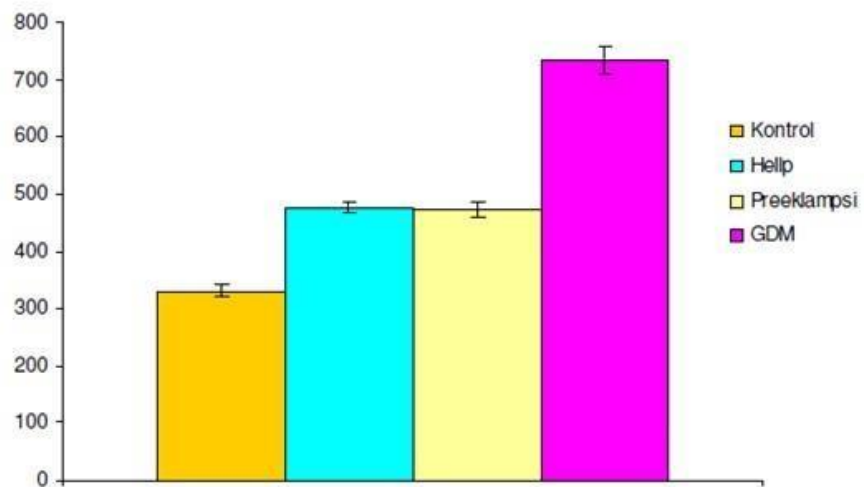


Figure 6. Mean thickness of the umbilical artery tunica media layer

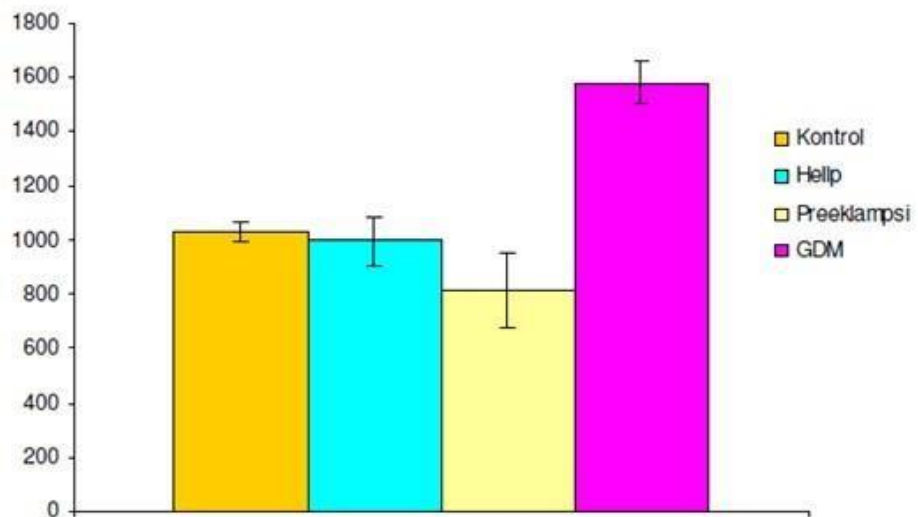


Figure 7. Mean diameter of umbilical vein lumen

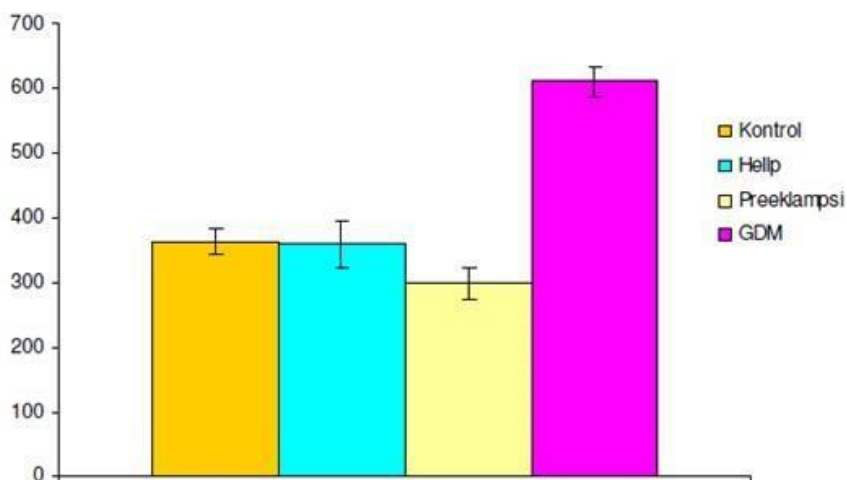


Figure 8. Mean thickness of umbilical vein tunica media layer

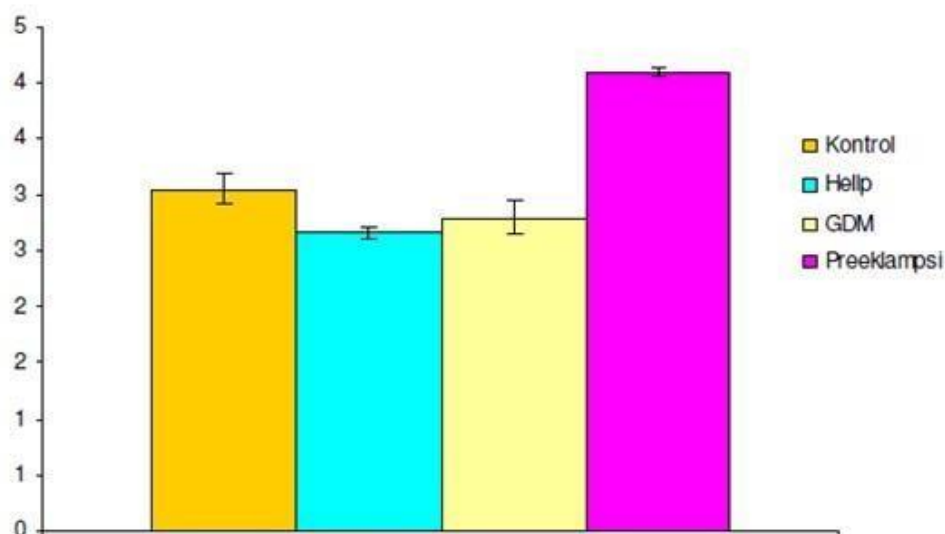


Figure 9. Total circumference of umbilical cord according to the groups

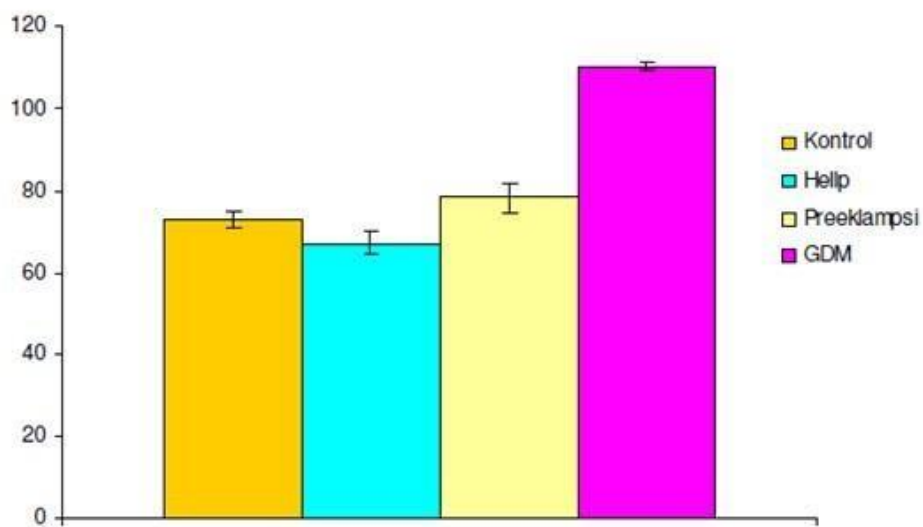


Figure 10. Total diameter of the umbilical cord according to the groups

4. Discussion

In this study, histopathological and morphometric changes in the umbilical cord of patients with gestational diabetes, preeclampsia and HELLP syndrome were examined.

In our study, when we compared the control group, we found that Wharton jelly was more in the GD group and it had many samples. This is in line with the findings of Weissman et al. [6]. Although 2 arteries were found in all the umbilical cord groups (% 100) and control group (% 94) in our study groups; 1 patient in the control group and 1 artery in 2 arteries (6%). Similar phenomena were recorded in Nadkarni as 4%. Excess umbilical vein is very rare [7-8].

Excess vein structure is generally reported as a vein in publications. Excess vein is the right vein structure that does not undergo atrophy. This structure is the remnant of vitelline veins when the excess vein is in the arterial structure [9-10]. In our case, more veins were more rarely seen in the arterial structure.

Stoker et al. [11] noted that preeclamptic arteries were 15% thicker than normal pregnancies, but Junek [12] reported this rate as 20%. Our study found this rate to be 50%. This thickness is the result of both the tunica intima and the expansion of the tunica media.

Weissman and Jakobi [6] emphasized that the umbilical cord was thicker in diameter due to the increase in Wharton jelly in GD cases in comparison with the umbilical cord in cases with gestational diabetes. In our study, we found similar results in both GD and HELLP cases

The thickening of the artery intima is due to the accumulation of smooth muscle cells, collagen increase and glycosaminoglycans in the vessel wall. These findings are correlated by the study of Bertrand [13].

It is known that the mother and placenta vascular system affect the intrauterine development of fetus. Preeclampsia is an important pathological syndrome related to pregnancy and affects both the fetus and the mother's vascular system [14].

In a pressure increase in the lumen of the umbilical arteries, the arterial wall is stretched, an increase in tactical structures, and consequently a little thickening in the artery wall compensates itself [14].

In our study, we found similar findings in umbilical arteries. On the other hand, the compensatory state of the umbilical vein is limited in the pressure increase, so that lumina are dilated and the wall was thinner [15].

In our study, although almost all sections of the umbilical cord obtained from cases with preeclampsia, GD and HELLP syndrome had increased endothelial damage and collagen fiber increase in umbilical artery media layer, intramuscular hemorrhage in the media layer of umbilical artery was considered as an interesting finding in patients with HELLP syndrome (Figure-4).

As known, amniotic epithelium surrounding the umbilical cord was simple cubic layer in normal pregnancies, while metaplasia and basal laminar changes (rupture, swelling, ondulation) were observed in GD and preeclamptic cases. However, we would like to emphasize the advanced metaplasia and basal lamina thickening in the amniotic epithelium of the umbilical cord of our cases with HELLP.

Inan et al. [16] emphasized the decrease in wall thickness in umbilical vessels in patients with pre-eclampsia with doppler. These morphological changes were not found to be due to irregularity of blood flow or decreased blood flow. In our study, the vascular wall thickness of the preeclampsia

umbilical cord was found to be considerably thicker than normal. These results are in contrast to the work of Inan and his friends.

In our study, arterial wall thickness was found more striking than normal in gestational diabetes and HELLP cases. We believe that intense collagen fiber increase observed in umbilical artery wall of our cases with gestational diabetes, preeclamptic and HELLP syndrome will decrease the umbilical blood flow by decreasing the lumina and will have a negative effect on the development of fetus.

Acknowledgment

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