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Original Article

Ultrastructure of human umbilical artery and vein

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Abstract

Aim: Our objective in this study was to investigate ultrastructure of endothelial and muscle cells of human umbilical vessels.
Material and Methods: Ten umbilical cords were used for this study. These cords represented periods of gestation from sixteen weeks to full-term. The specimens were fixed in 5 % buffered glutaraldehyde for four hours at 4oC. The sections were postfixed for two hours in a solution of 1% osmium tetroxide prepared with Millonig phosphate buffer. Sections were dehydrated in ethanol and propylene oxide and then embedded in araldite. They were stained with uranyl acetate, examined electron microscopically.
Results: Rod- shaped bodies were observed in the arterial endothelium in the early stage as described by Weibel-Palade. Large numbers of mitochondria and increased lipid and vacuoli structures and increased Weibel-Palade bodies were also noted in full-term. the muscle cells of the umbilical vein were characterized by prominent crystalized structures of mitochondria at sixteen weeks.These structures were degenerated at full-term, and myelin figures were observed.
Conclusion: The endothelial and muscle cells of the umbilical vessels from early stage to full-term showed morphological changes.

Keywords: Ultrastructure; Umbilical Artery; Vein.

INTRODUCTION

The umbilical cord contains two arteries and one vein (1,2). The lumen of umbilical vein is large and has an irregular shape. Regarding the umbilical artery wall, this is greater than the umbilical vein wall (3). It has been previously reported in an ultrastructural study that the nuclei usually contain well-developed nucleoli in the umbilical arteries endothelium at ten weeks of gestation. Mitochondria, Golgi apparatus, rough endoplasmic reticulum, and large glycogen accumulation were seen in the cytoplasm. The paucity of glycogen was noted in the venous endothelial cells rather than in the arteries. Lipid droplets were not encountered. It was further stated that mitochondria were numerous and wide spread and that rough endoplasmic reticulum was more numerous in veins than in arteries (4).

A decrease of glycogen in the arterial endothelium was noted at fifteenth week. The most prominent feature in the venous endothelium was a very well-developed endoplasmic reticulum. Most of the rough endoplasmic reticulum was in the form of widely dilated channels. Weibel-Palade bodies were far more numerous in vein than in the corresponding artery. Rough endoplasmic reticulum was seen to be well-developed in the arterial endothelium at twenty week of gestation as was Golgi apparatus (4). Rough endoplasmic reticulum, mitochondria and Golgi apparatus, found in the arterial endothelium were well-developed in full-term specimens (5,6,7). Pinocytotic vesicles were scattered within the cytoplasm. The glycogen granules formed were either gathered in clusters or were scattered among the organelles. Because of the lack of an internal elastic lamina, the intima-media boundary was vague in the umbilical arteries. Either scattered or well-defined clusters of glycogen were seen in the media layer (5,7). Golgi apparatus was well-developed and Weibel-Palade bodies were quite numerous in venous endothelium(4,8). The basal membrane of vein was better defined than that of the arteries (7,9). Generally, in the studies that have been done, the development of the endothelial cells of the umbilical vessels was observed, but the media layer was not considered. The purpose of this study was to investigate the umbilical vessels, both in intima and media layers during gestation.

MATERIAL and METHODS

In this study, ten human umbilical cords were used. These cords were from sixteen weeks to full-term gestation. The number of cords used were 1,2,2,5 for 16,23,25 week, and

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full-term, respectively. All of the full-term cords were from normal deliveries. The umbilical cords were collected from pregnant women immediately after vaginal delivery at the Department of Obstetrics and Gynaecology, Medical Faculty of Firat University. All patients were examined before delivery for cytomegalovirus (CMV) infection. Infected patients were excluded from the study. The specimens were fixed immediately after delivery in 5% buffered glutaraldehyde for four hour at 4 oC. The sections were postfixed for two hours in a solution of 1% osmium tetroxide prepared with Millonig phosphate buffer. They were washed twice with the Millonig phosphate buffer for ten minutes. Sections were dehydrated in ethanol and propylene oxide and then embedded in araldite. Sections from the araldite blocks were sliced at 500°A. They were stained with uranyl acetate and lead citrate in 70% methanol.

Thin slices were examined and photographed with a Zeiss E.M. 10 B electron microscope. The micrographs were blinded and reviewed by an observer.

RESULTS

At sixteen week

At the light microscopic level lumen of umbilical arteries have spherical and regular shape. The periphery of tunica media was surrounded by Wharton's jelly . The lumen of umbilical vein was narrow and has a cylindirical shape. It was surrounded by Wharton's jelly. Following electronmicroscopic examination umbilical vein;In the endothelial cells rough endoplasmic reticulum was quite well-developed and dilated. Intensely grouped endocytotic vesicles were present around the RER. Multivesicle bodies were seen in the cytoplasm. Myelin figures that we observed within the structures were considered to be mitochondria. Scattered Weibel-Palade bodies, glycogen and lipid were also observed. Accumulated fibrin was present in the subendothel (Figure 1). At this stage; mitochondria, and grouped glycogen were prominent around the nucleus and rough endoplasmic reticulum of the muscle cell of vein. Large amounts of collagen fibrils were present in the inter-mediary tissue. Accumulated fibrin was observed outside the muscle cell.

At twenty-three week

Artery; Large amounts of vacuolization were encountered in the arterial muscle cells and fibrin accumulation was observed between the cells.

Vein; Rough endoplasmic reticulum occured as narrow cisternae and a large amount of glycogen accumulation was indicated in the media layer of the vein around the nucleus of muscle cell. Vacuolization was seen similar to that found in the arterial muscle cells .

At twenty-five week

Artery; With the light microscopic examination tunica intima of the arteries contained squamous epithelium. Its tunica media was thicker compared to vein and contained fibres which were longitudinal in the innermost layer, elastical in the middle layer whereas circuler in the outermost layer.

Following electronmicroscopic examination;mitochondria and rough endoplasmic reticulum were strikingly prominent in the arterial endothelium. And also Weibel-Palade bodies were seen (Figure 2).

Collagen fibres were observed in tunica media. One notable feature in the media layer of the umbilical arteries was that an increase in content of RER was observed and also moderate glycogen was observed.

Vein; Following light microscopic examination its tunica media was thin and elastic fibres were not observed. Intense mitochondria and scattered rough endoplasmic reticulum were observed around the nucleus of the venous endothelial cells. An important feature at this stage; was the presence of lysosomes. Lipid was found in the venous endothelium but not in the arteries. An internal elastic lamina was prominent under the endothelium (Figure 3). The vacuolization, which had been observed in the media layer of the vein at this stage, was not present. Mitochondria and rough endoplasmic reticulum cisternae were present and glycogen was plentiful and scattered.

At full-term

Artery; Following light microscopic examination it was found that lumens of artery was narrow and usually has a star-like shape. Squamous epithelium was observed in thetunica intima.Tunica media was surrounded by Wharton's jelly. Mitochondria, which contained prominent cristae structures, rough endoplasmic reticulum and lipid were observed sections of the endothelial cell of the umbilical arteries in the cytoplasm. Vacuoli structures were present in the endothelium and the subendothelium. Degenerated areas and accumulated fibrin were encountered in the subendothelium (Figure 4). Enlarged and degenerated mitochondria, Golgi apparatus, vacuoli structures, rough endoplasmic reticulum, and dense glycogen were observed in the arterial media layer in the cytoplasm. Especially, dense and osmiophilic formed bodies were appearent around the nucleus. Mitochondria without crista were observed in tunica media. Large amount of glycogen was found.

Vein; Following light microscopic examination lumens of vein were larger and has a more regular shape compared to that of arteries. Its tunica media was thinner .Tunica media was surrounded by Wharton's jelly .

The cell nucleus of the venous endothelium was euchromatic. Mitochondria, which contained quite prominent cristae structures, were also observed. Rough endoplasmic reticulum was scattered in the cytoplasm and Weibel-Palade bodies were more numerous. Internal elastic lamina was visible as wavy structures in the subendothelium (Figure 5). Degenerated mitochondria were prominent around the nucleus in the media layer of the veins. Myelinated figures were observed within some mitochondria while rough endoplasmic reticulum was seen as usual. Glycogen granules were scattered in the cytoplasm and vesicles in the boundaries of the muscle cells.

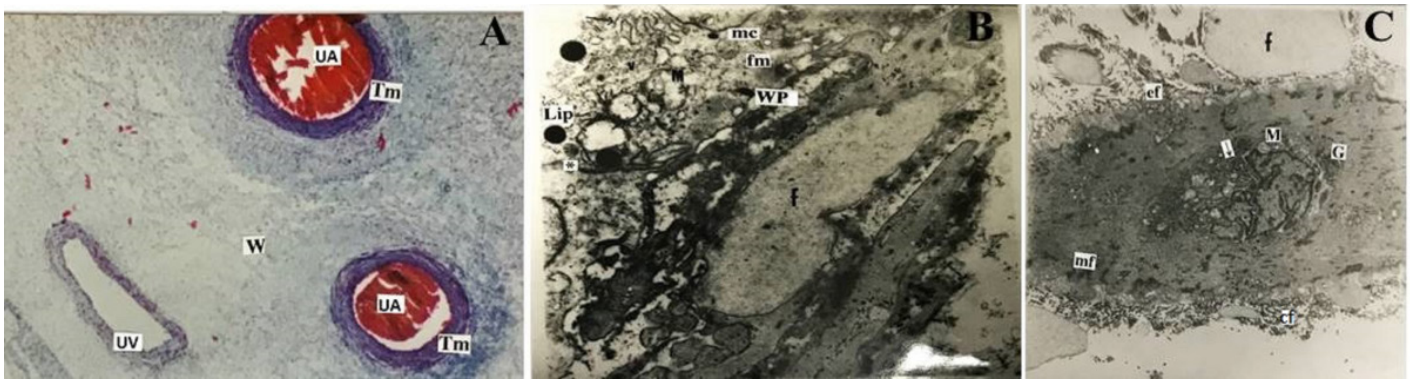

Figure 1. Vein (16 weeks).A: Light microscopic structure of the umbilical cord is seen. It has two Umbilical artery and one Umbilical vein surroundedby Wharton's jelly.UA: Umbilical artery, UV: Umbilical vein , Tm:tunica media, W: Wharton's jelly. Crossman trichrome staining. X4. B: Endothelial cells are seen. Mitochondria (M) are largened. Rough endoplasmic reticulum cisternae (arrow) are dilated. Weibel-Palade bodies (WP), vesicles(v), lipid(Lip), fibriler material (fm), multivesiculer body (mc), fibrinoid accumulation (f),intercellular junction (*). X 14000. C:Muscle cells of the Umbilical vein is seen. M: mitochondria, G: glycogen, ef:exocitotic figurs, f:fibrinoid accumulation, arrow: rough endoplasmic reticulum, mf: myofibril,cf:collagen fibres.X8800

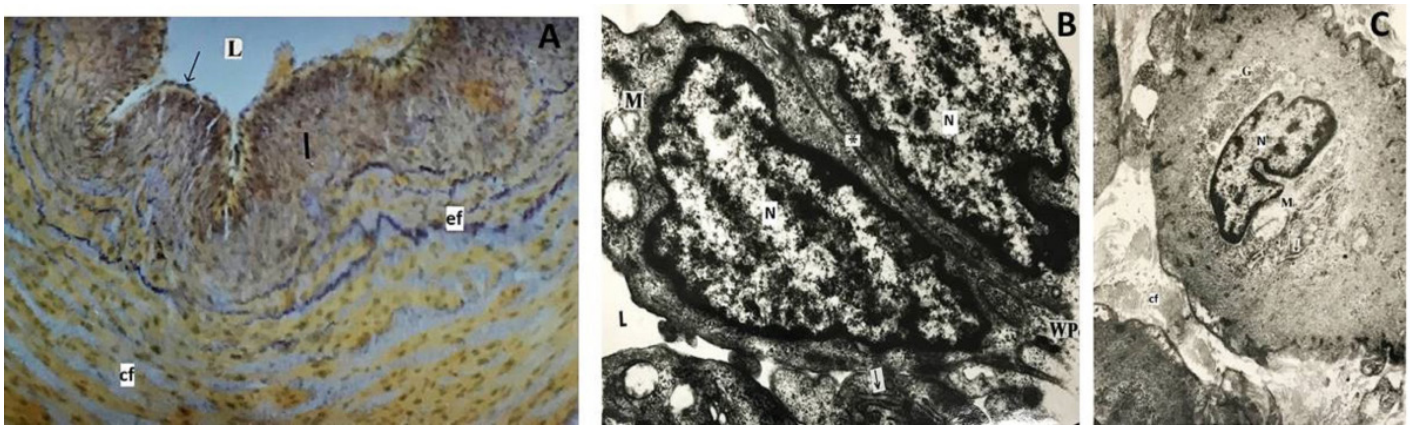

Figure 2. Artery (25 weeks). A: Light microscopic structure of the umbilical artery is seen. Arrow : endothelial cells of tunica intima, L:lumen, l:longitudinal fibres, ef:elastic fibres, cf:circular fibres of tunica media. Resorcin fuchsin staining. X20. B: Endothelial cells are seen. Mitochondria (M), rough endoplasmic reticulum cisternae (arrow), Weibel-Palade bodies (WP), nucleus (N), intercellular junction (*), lumen (L). X 24000. C: Muscle cells of the umbilical artery is seen. N:nucleus, M: mitochondria, G: glycogen, cf:collagen fibres, arrow: rough endoplasmic reticulum. X14000

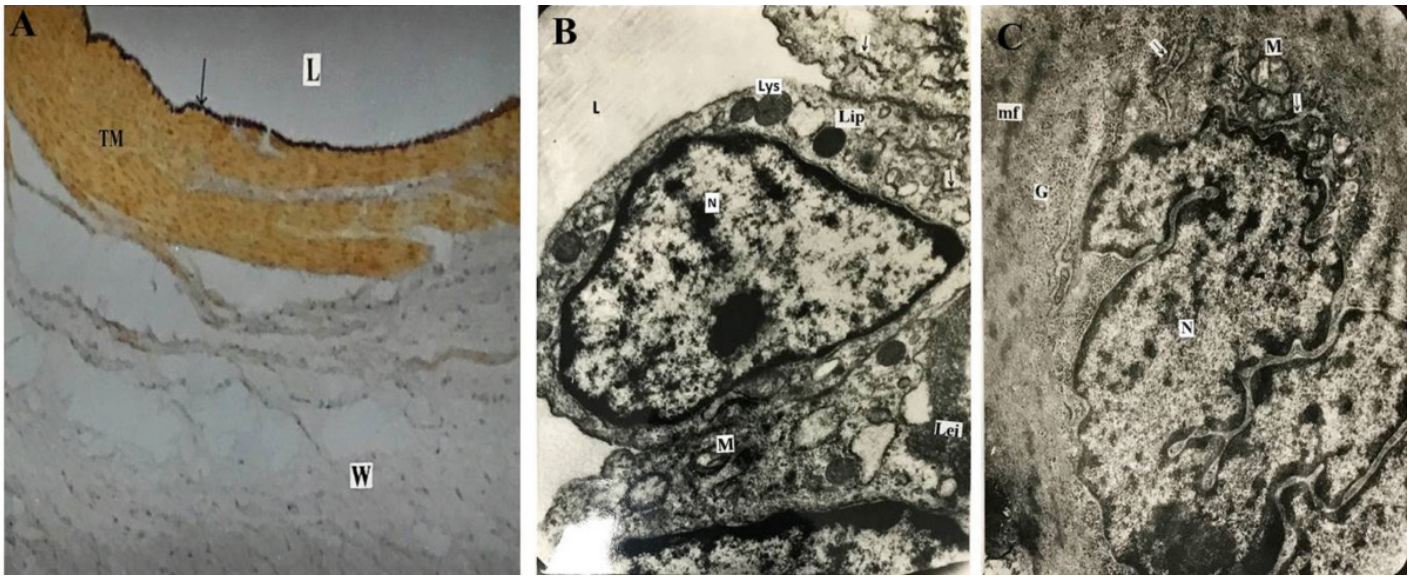

Figure 3. Vein (25 weeks). A: Light microscopic structure of the umbilical vein is seen.Elastic fibres of tunica media are not seen. Endothelial cells (arrow) of tunica intima, L: lumen, TM: tunica media, W: Wharton's jelly. Resorcin fuchsin staining. X10. B: Endothelial cells contain dilated rough endoplasmic reticulum. Lysosome was prominent. Internal elastic lamina (Lei), rough endoplasmic reticulum (arrow), mitochondrion (M), lysosome (Lys), lipid (Lip), lumen (L). X 24000. C: Muscle cells of the umbilical vein is seen. N:nucleus, M: mitochondria, G: glycogen, arrow: rough endoplasmic reticulum, mf: myofibril.X24000

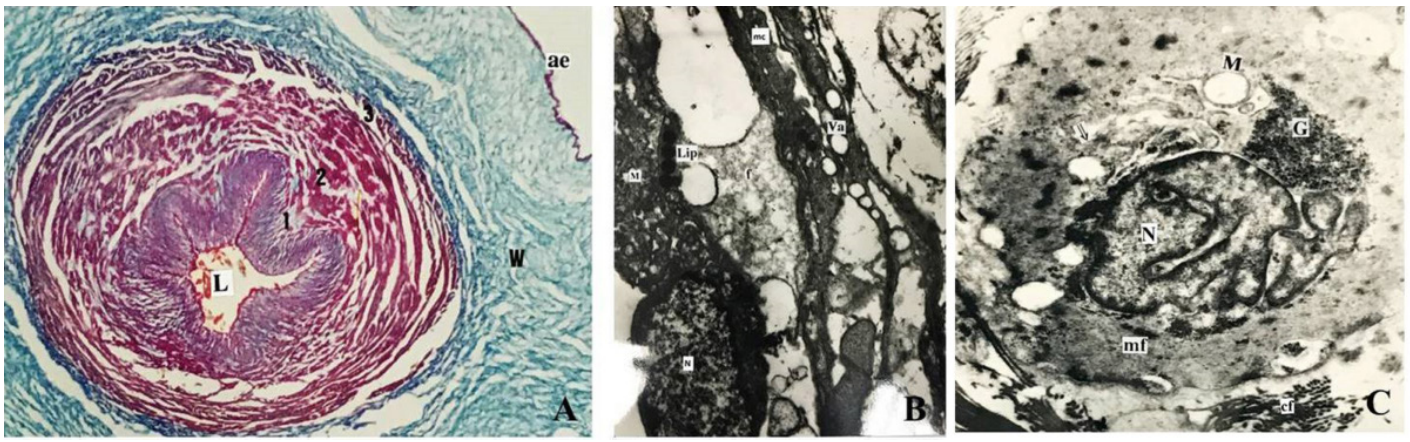

Figure 4. Artery (full-term). A: Light microscopic structure of the umbilical artery is seen. Its narrow and star-shaped lumen (L) is remarkable.Tunica media of its is quite thicker than the umbilical vein. Muscle fibers are longitudinaly inside (1), circuler fibres on the middle (2), longitudinaly fibres outside (3). W: Wharton's jelly, ae:amnion epithelium. Crossman trichrome staining. X4. B: Artery (full-term). Endothelium and subendothelium are seen. Degenerated area is observed in subendothelium. Nucleus (N), mitochondrion (M), fibrinoid accumulation (f), lipid (Lip), muscle cells (mc), vacuole (Va). X 8800. C: Muscle cells of the umbilical artery is seen. N:nucleus, M: mitochondria, G: glycogen, doublearrow: rough endoplasmic reticulum, mf: myofibril, cf:collagen fibres. X 24000

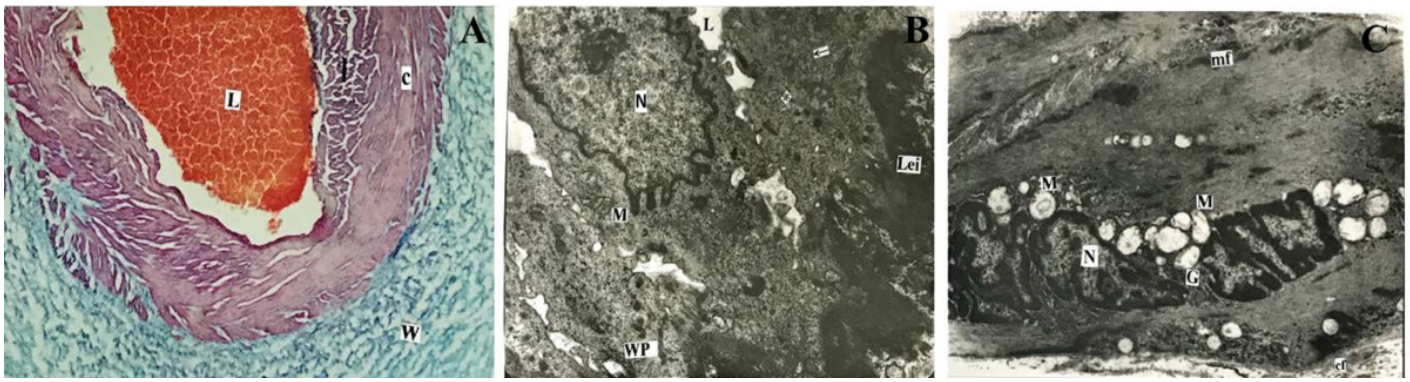

Figure 5. Vein (full-term). A: Light microscopic structure of the umbilical vein is seen. Muscle fibers are longitudinaly (l) inside, as circuler(c) outside. L:lumen, W: Wharton's jelly. Crossman trichrome staining. X4. B: Endothelium is seen. Nucleus (N), mitochondrion (M), lumen (L), Weibel-Palade bodies (WP), Internal elastic lamina (Lei), rough endoplasmic reticulum (arrow), intercelluler junction (*). X 14000. C: Muscle cells of the umbilical vein is seen. N:nucleus, M: degenerated mitochondria involving myelin figures, G: glycogen, mf: myofibril, cf:collagen fibres. X 14000

DISCUSSION

The rough endoplasmic reticulum, in the venous endothelium was enlarged at sixteen-week gestation. The mitochondria structures had become quite enlarged and Weibel-Palade bodies were observed. Takagi et al. (9) have shown that cells contained some bodies like lysosome, large amounts of rough endoplasmic reticulum and a few glycogen granules in the venous endothelium at the eighteen week stage. In this study, Weibel-Palade bodies were found in the periphery of cells. An increased number of rough endoplasmic reticulum and turn-shaped dilated channels were reported in the venous endothelium at the fifteen week stage (4). Same study reported far more numerous Weibel-Palade bodies in the veins than in the corresponding arteries. Our study confirmed the same results. We also found that rough endoplasmic reticulum was shaped as dilated channels. The striking property of this stage was the finding of mitochondria and rough endoplasmic reticulum in the media layer.

It was reported that sparse collagen fibers were found, but almost no elastic fibers in the media layer was observed (9). Our study also confirmed this. The most prominent characteristic of the media layer of the umbilical arteries was the much higher occurence of vacuolization at twenty-three week. A higher degree of vacuolization was also observed in the media layer of the vein. We were not able to locate any previous study that have determined the characteristics of media layer of the arteries and veins at this stage. Rough endoplasmic reticulum was enlarged and Weibel-Palade bodies were encountered in both arteries and veins.

It was reported that rough endoplasmic reticulum of the arterial endothelium was usually well-developed and Golgi apparatus was observed at twenty-three week. Also it was reported that sparse rough endoplasmic reticulum and numerous Weibel-Palade bodies were present in veins (4). An important feature of the umbilical arteries media layer at this time was the increase in rough endoplasmic reticulum content. However, in a previous study a decrease in the amount of rough endoplasmic

reticulum and glycogen was reported in the arterial media layer at twenty-five week (9). The important finding of our research was the increase in rough endoplasmic reticulum content and the amount of glycogen in the venous media layer at twenty-five week. We were not able to find any study which reported the characteristics of the media layer of umbilical vein at this stage.

In our study in term three layers were found in tunica media of the umbilical artery. These are longitudinal in the innermost layer, circuler in the middle layer , longitudinal in the outermost layer. Tunica media was observed to have large amount of collagen and elastic fibres. In the literature it was reported that tunica media of umbilical artery was circuler in innermost layer and longitudinal in outermost layer. In contrast to what was reported by Arpi, we found that tunica intima of the umbilical vein contained internal elastic lamina (3).

One of the most important features of the arterial endothelium at full-term was the increased lipid content and the presence of degenerated areas in the subendothelium. We observed cytoplasmic vacuoles in endotelial cells in line with that reported in the literature. We also found tight junctions between endothelial cells as other workers stated (10). The amount of rough endoplasmic reticulum in the arterial endothelium increased in mid-gestation and become much more prominent at full-term (4). In another study, it was reported that rough endoplasmic reticulum, mitochondria, plentiful Golgi apparatus and rare lysosomes were present in the cytoplasm of the arterial endothelium (7). The same research reported the Weibel-Palade bodies, clustered or scattered forms of glycogen granules among the organelles in all cells. It was observed that cells contained lipid in the distal portion of the intima of the umbilical arteries (9). However, they did not encounter these cells in the fetal period. It was thought that the lipid droplets might be the products of phagocytose. In our study, lipid droplets in both arterial and venous endothelial cells were observed from the early stage of gestation up to full-term. The prominent feature were the increase in the venous endothelium, Weibel-Palade bodies and internal elastic lamina. In another study, it was reported that Weibel-Palade bodies in the venous endothelium were more numerous than in the corresponding arteries (4). And also reported the improved development of more Golgi apparatus and of endothelial basal membrane in the veins than in the arteries (4,9). It was reported that rough endoplasmic reticulum reached the greatest amount in the venous endothelium at fifteenth week and became sparse by twenteeth week (4). In another study, it was observed that large numbers of vesicles, smooth and rough endoplasmic reticulum, Golgi apparatus, clusters of free ribosomes and irregular mitochondria were present in the umbilical venous endothelium (11). The researchers demonstrated that umbilical vein endothelium showed an endothelial cell with cytoplasmic protrusion extending into the vascular lumen and scarse rough endoplasmic reticulum (12). They also noted the presence of a regularly structured inner elastic lamina with characteristic wavy course. In our study, we observed the narrowing of the rough endoplasmic reticulum cisternae, regularly structured inner elastic lamina as described. But on the contrary this researchers (12) we found numerous rough endoplasmic reticulum and at no time ribosome loss was observed during gestation. One of the most distinctive features of the arterial media layer between early gestation and full-term was the presence of osmiophilic structures in the muscle cells. At this time, we are unable to comment on the origin of these structures or their functions. A previous study reported that the media layer of the umbilical artery was characterized by large amounts of rough endoplasmic reticulum and glycogen (9). A study also found scattered or clustered glycogen and numerous pinocytotic vesicles in the arterial media layer (7). In our study it was found thatumbilical vein and arteries were surrounded by mucous connective tissue called Wharton's jelly as mentioned in the literature (3). In our study, we especially observed clustered glycogen in the arterial media layer. The important characteristic of the venous media layer was the enlarged and degenerated mitochondria and observed of pinocytotic vesicles (7). Our study confirmed this result. A study reported that, the smooth muscle cells of the distal vein contained no lipid vacuoles at four days after birth, but by the sixth day, many lipid containing cells were transmurally present (9). Another study reported that the elongated smooth muscle cell of umbilical vein was surrounded by bundles of longitudinal and cross sections of collagen and was lying on a rather thin basement membrane. It was also demonstrated that the smooth muscle cell cytoplasmic membrane contained pinocytotic vesicles and caveola (12). In our study, we could not observed basement membrane in the muscle cell of the umbilical vein. But we confirm that observing pinocytotic vesicles and caveola.

CONCLUSION

The endothelial and muscle cells of the umbilical vessels from early stage to full-term showed morphological changes.

Competing interests: The authors declare that they have no competing interest.
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Ethical approval: The study was conducted in1995. At that time no approval from an ethical committe was required.

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