

Stereological and Histological Assessment of the Umbilical Cord in New-Born Rat

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Abstract

Background: Umbilical cord plays a crucial role in the continuation of pregnancy by transferring nutrition and oxygen across the placenta to the fetus. We aimed to investigate the morphometrical and histological features of the umbilical cords in new-born rats. **Materials and Methods:** The adult male and female rats were chosen for matting purpose in the present study. Briefly, ten adult *Wistar albino* rats (female, $n = 5$; male, $n = 5$) were randomly assigned into five groups of two animals (female, $n = 1$; male, $n = 1$). Immediately after parturition, two umbilical cords of new-born rats (0-day-old) from each group were randomly selected. Finally, ten umbilical cord samples were examined using the histological and stereological methods in the light and electron microscopes. **Results:** The total numbers of Hofbauer cells and mesenchymal stromal cells was estimated statistically. We also calculated the mean volume of umbilical cords, arteries and veins, as well as arterial and venous lumens. Our histological findings also exhibited the histological features of Hofbauer cells, mesenchymal stromal cell cells, and blood vessels. **Conclusion:** Our findings showed more detailed information about umbilical cord tissues and their components, and that may contribute to the diagnose of umbilical cord complications in the developing fetus.

Keywords: Hofbauer cell, mesenchymal stromal cell, new-born rat, stereology, umbilical cord

INTRODUCTION

The umbilical cord, the fetal supply line, is a conduit between the placenta and embryo. This birth cord also contains two prominent arteries and a vein buried into Wharton's jelly (embryonic connective tissue). Wharton's jelly consists of rich collagen-containing ground substance, as well as mesenchymal stromal cell, fibroblast, and macrophage.^[1] Furthermore, Wharton's jelly that substitutes for the adventitia of UC vessels can prevent the natal preincidence of mortality through supportive function.^[2] In the absence or deficiency of Wharton's jelly, umbilical cord tends to compression, leading to fetal complications.^[3-6] Weissman and Drugan^[7] reported that the umbilical cord abnormalities caused the chromosomal aberration. The importance of umbilical cord study may also derived from the presence of potential multipotent stem cells in this vital organ.^[8-10] Mesenchymal stromal cells can be used for therapeutic purposes, which can potentially differentiate into any

type of cells.^[11,12] For example, mature types of neurons and glial cells can be differentiated from umbilical cord mesenchymal stromal cell.^[13] In the central nervous system, cord blood stem cells can be used in the treatment of the brain injury, stroke, and Cochlear damage.^[14-16] Modified fibroblasts that synthesize collagen fibers contributes to the elasticity of Wharton's jelly, as well as contraction of the vessels.^[17] Hence, understanding the architecture features of the umbilical cord helps us to identify its development process and associated abnormalities.

The placenta connects the developing fetus to the uterine wall and provides a natural defense against internal infection through Hofbauer cells. Furthermore, Hofbauer cell, as a placental macrophage, has substantial function in placental pathophysiology.^[18]

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Despite the importance of the umbilical cord, there are few studies regarding morphometrical assessment of its main structures. We therefore decided to investigate the morphometrical and histological features of the umbilical cords and its main components such as arteries and vein, as well as their lumens. Moreover, fine structures of blood vessels and Hofbauer cells, as well as mesenchymal stromal cells were examined using the electron microscope and light microscope.

MATERIALS AND METHODS

Experimental procedure and animal care

Ethical approval of the present study was granted by the Experimental Animal Research and Application Centre of Ataturk University, Erzurum, Turkey (No. 1). Ten adult *Wistar albino* rats (five females and five males), weighing 200–250 g and 12-week-old, were purchased from the Experimental Animal Research and Application Centre of Medicine Faculty of Ataturk University. In each plastic cages, two rats (one female and one male) were housed for 2 days. After the visualization of vaginal plug on the 3rd day, female rats diagnosed as pregnant were removed, then placed individually in 5 cages during the 21-day gestation period. After the delivery, the umbilical cords of two pups from each mother were randomly selected and immediately dissected. Finally, a total of ten umbilical cords were obtained for the stereological and morphometrical analysis. During the experiment period, all rats were maintained under 12:12 h night/day cycle at 50% ± 5% humidity and 22°C ± 2°C. Animals had *ad libitum* access to food and water.

Histological study

Dissected umbilical cord samples were fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer, followed by the postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer. These samples were dehydrated through graded acetone series, then washed in propylene oxide. Subsequently, samples were embedded in Araldite CY 212.^[19,20] Semi-thin (1 µm thick) and ultra-thin sections (80 nm thick) were cut using a Leica RM2125RT microtome (Gaintenbain Comp., Ankara, Turkey) and ultramicrotome (Nova LKB, Bromma, Sweden), respectively. Semi-thin sections were stained with toluidine blue for the stereological and histological examination in the light microscope.^[21,22] Furthermore, ultra-thin sections were stained with uranyl acetate and lead citrate, then analyzed using a Jeol 100 SX electron microscope (Jeol; Tokyo, Japan).^[19]

Stereological study

The point-counting grid and Cavalieri methods were used to estimate the mean volume of the regions of interest [Figure 1a and b].^[23-25] For this purpose, a pilot study was designed to survey whether the point density of a grid was valid. The coefficient of variation and coefficient of error were estimated according to the formulas as described by Kurtoglu *et al.*^[26] Briefly, all sections were photographed, then transferred to the private computer. The calibrated grid was randomly superimposed on the photographs, and sum of

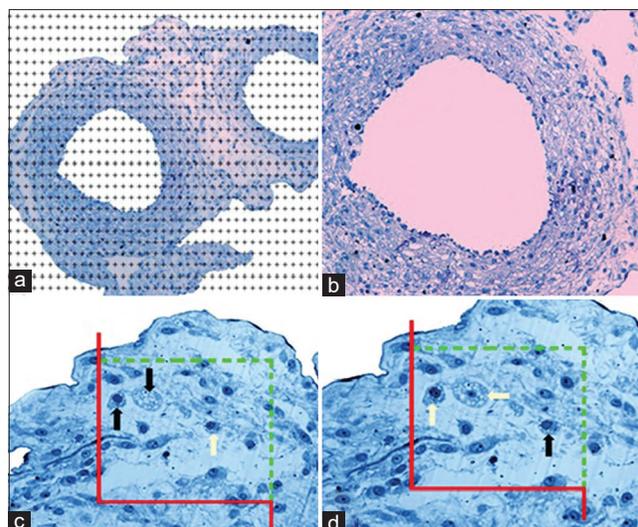


Figure 1: Representative micrographs showing application of the Cavalieri principle (a and b) and physical dissector (c and d). (a and b), point-counting grids for applying the Cavalieri principle; (c and d), consecutive pair sections for applying the physical dissector; c, reference section; d, look-up sections; White arrows, countable Hofbauer cell profiles as dissector particle; Black arrow, uncountable profiles according to the rule of unbiased counting frame

the points hitting the sections were counted. Finally, the mean volume of the region of interest was calculated as:

$$V(\text{total}) = t \times \Sigma A$$

Where, “t” is the total thickness of all sections plus intervals, and “ΣA” is the total area of interest region in all sections.

$$\Sigma A = a(p) \times \Sigma P$$

Where, “a(p)” is the area of point interval, and “ΣP” is the number of points hitting the region of interest.

The total number of Hofbauer and mesenchymal stromal cells was calculated by means of the physical dissector method [Figure 1c and d].^[25] Consecutive pair sections were chosen using the systematic random sampling technique, which the first section of pair was reference and the other was look up.^[27] After photographing the dissector pairs, an unbiased counting frame was placed on micrographs. Then, the profiles of the cells were counted according to the physical dissector rules using ImageJ soft program.^[23] The numerical density of Hofbauer and ME cells was calculated as:^[28]

$$N_{(v)} = \frac{\Sigma Q}{\Sigma V_{Dissector}}$$

Where, “ΣQ” is the Hofbauer and ME cell number and “ΣV” is the total volume of the dissector frames in reference sections.

Finally, the total number of Hofbauer and ME cells were calculated using the following formula:

$$TN_{(Total)} = N_V \times V_{Ref}$$

Where, “ N_V ” is numerical density of Hofbauer and ME cells and “ V_{Ref} ” is the mean volume of umbilical cord.

Statistical analysis

Statistical analysis was performed using the IBM version 25.0 SPSS software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were applied for obtaining the mean and standard deviation (SD) values. The results were expressed as mean \pm SD.

RESULTS

Stereological results

Our stereological results are given in Tables 1 and 2. The physical dissector and Cavalieri methods were used for estimating the total number of Hofbauer and mesenchymal stromal cells, as well as the mean volume of umbilical cords.

We found that the mean volume of the umbilical cords was 0.58 cm³. Moreover, the mean volumes of arteries and veins were 0.1 cm³ and 0.06 cm³, respectively. Furthermore, the mean lumen volume of arteries and veins was 0.04 cm³ and 0.03 cm³, respectively. We found that the mesenchymal tissue volume was 0.36 cm³ [Table 1].

The total number of Hofbauer and mesenchymal stromal cells were 6327 and 29678, respectively [Table 2].

Histological results

Ultra-structures of blood vessels, Hofbauer, and mesenchymal stromal cells were examined using the electron microscope. We detected the spindle-shaped endothelial cells of blood vessels with prominent ovoid nuclei. The cells observed in tunica media also consisted of a nucleus and distinctive nucleolus, as well as abundant cytoplasm [Figure 2]. Mesenchymal stromal cells (Fibroblast-like cells) of mesenchymal tissue exhibited abundant cytoplasm and

euchromatic nucleus [Figure 3]. In Hofbauer cells, the marginalized nucleus and cytoplasmic vacuoles with central location were evident [Figure 4].

DISCUSSION

Umbilical cord contributes to establish the bidirectional bloodstream between the fetus and mother during pregnancy. Numerous studies have been conducted on the characteristics of the umbilical cord, but comprehensive information regarding the histological structure of umbilical cords and its cells is very limited.^[29-32]

Wharton’s jelly that originates from extraembryonic mesoblast is composed of large amounts of extracellular matrix and low number of cells.^[1,33] Stromal cells of Wharton’s jelly

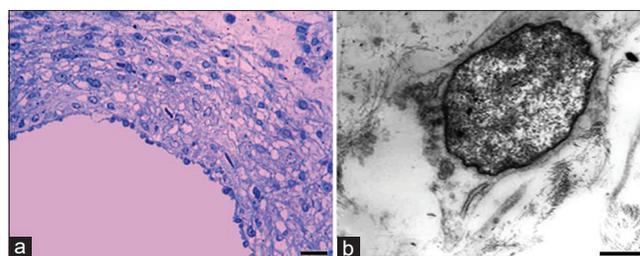


Figure 2: Representative micrographs of semi-thin section (a; $\times 40$; bar, 12.5 μm) and ultra-thin sections (b, $\times 6000$; bar, 1 μm) obtained from blood vessel wall of umbilical cord. a, wall of the umbilical artery; b, mesenchymal stromal cells

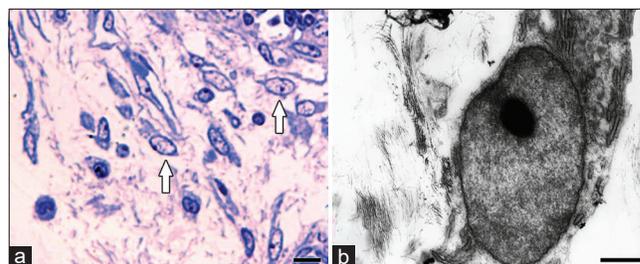


Figure 3: Representative micrographs of semi-thin (a, $\times 40$; bar, 12.5 μm) and ultra-thin (b, $\times 6000$; bar, 1 μm) sections obtained from the umbilical cord. Arrow, mesenchymal stromal cells; a and b, mesenchymal stromal cells at the light and electron microscopic levels, respectively

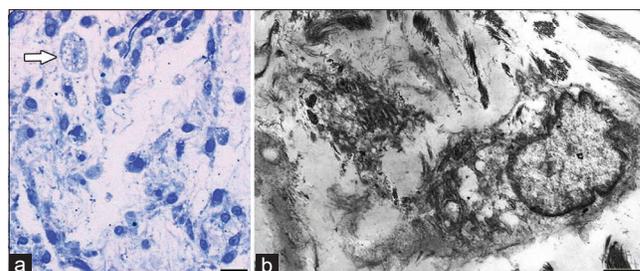


Figure 4: Representative micrographs of semi-thin (a, $\times 40$; bar, 12.5 μm) and ultra-thin (b, $\times 6000$; bar, 1 μm) sections obtained from the umbilical cord. Arrow, Hofbauer cells; a and b, Hofbauer cells at the light and electron microscopic levels, respectively

Table 1: The mean volume of umbilical cords, arteries, and veins as well as arterial and vein lumens

Estimation	Value
Mean volume of mesenchymal tissue (cm ³)	0.36 \pm 0.07
Mean volume of the umbilical cord (cm ³)	0.58 \pm 0.10
Mean volume of the artery (cm ³)	0.10 \pm 0.02
Mean volume of the vein (cm ³)	0.06 \pm 0.01
Mean volume of arterial lumen (cm ³)	0.04 \pm 0.01
Mean volume of the venous lumen (cm ³)	0.03 \pm 0.01

Data: Means \pm SD. SD: Standard deviation

Table 2: The total number of Hofbauer and mesenchymal stromal cells

Estimation	Value
Total number of Hofbauer cells	6327 \pm 849
Total number of mesenchymal stromal cells	29,678 \pm 1749

Data: Means \pm SD. SD: Standard deviation

regulate the umbilical cord blood flow and possess multipotent properties than other stem cells.^[34] Lack of Wharton's jelly, which plays a substantial role in fetal growth, can cause fetal death.^[13,35,36] Hew and Keller^[37] suggested that morphological alterations of small laboratory animals occurred faster than human.

Earlier studies that are performed on umbilical cord structures have been based on more clinical approaches such as ultrasonography.^[38-40] Moreover, less quantitative investigations have been carried out on the umbilical cords by means of the unbiased stereological method. Hence, in this study, stereological investigation and histological examination was conducted on the umbilical cord tissues to obtain the novel findings.

To the best of our knowledge, this is the first stereological study that estimates the number of Hofbauer cells and mesenchymal stromal cells, as well as the mean volume of umbilical cords in the rat. Furthermore, there are a few studies concerning histological assessment on the umbilical cord tissues at the electron microscopic and light microscopic level. Our morphometric results provided important information about the volume ratios of all parameters to umbilical cord. This ratio was approximately 62% for mesenchymal tissue, 0.17% arteries, 10% vein, 0.7% arterial lumen and 0.4% vein lumen. We also calculated the ratio of Hofbauer to mesenchymal stromal cells, which was approximately 21%.

Evaluation of the histological architecture of umbilical cord is very important due to the vital role in the fetal development. The previous studies showed that the average diameter and circumference of the umbilical cord after birth were 1.5 cm and 3.6 cm, respectively.^[41,42] It has been reported that there are a relationship between umbilical cord diameter and change in placental features. Proctor *et al.*^[43] reported that thin walled umbilical cord caused some complications such as fetal distress and low placental weight, as well as low infant birth weight. Togni *et al.*^[44] documented that any alteration in the mucous connective tissue of umbilical cord resulted in fetal disorders. Besides, architectural change in the umbilical cord may cause fetal intrauterine growth retardation, leading to undesirable pregnancy outcomes.^[45] Accordingly, umbilical cord as a crucial organ acts as a life-saving structure for embryo during pregnancy.

Our histological findings exhibited normal appearance of structures in the umbilical cord tissues. We observed the healthy ultrastructure of blood vessels and mesenchymal stromal cells, as well as Hofbauer cells. The spindle-shaped endothelial cells with ovoid nucleus were detected in blood vessels. The cells in the tunica media possessed the prominent nucleus, nucleolus, and a large amount of cytoplasm. We also found mesenchymal stromal cells (Fibroblast-like cells) with the euchromatic nucleus, a distinct nucleolus and abundant cytoplasm. Ceylan *et al.*^[46] reported the high transcriptional activity of mesenchymal stromal cells due to their spheroid polygonal morphology. These cells exhibited

the large number of free ribosome, Golgi complexes, mitochondria, and polestomes. They also detected the enlarged granular endoplasmic reticulum around the nucleus. Qiao *et al.*^[47] documented two different ultrastructural features of mesenchymal stromal cells in human umbilical cords. The one contained a large and oval or round nucleus with one prominent nucleolus, as well as organelle-poor cytoplasm. The other possessed one or two nuclei, organelle-rich cytoplasm, and expansion of mitochondria. Leeson and Leeson^[48] investigated the ultrastructure of rat umbilical cords mesenchymal stromal cell at different days gestation. At 17 days gestation, ribosomes attached to well-developed endoplasmic reticulum were observed as polysomal aggregates. At 21 days gestation, they also found less prominent ribosomes than the earlier stage, and these were in the form of certain bundles.

In Hofbauer cells, we found the cytoplasmic vacuoles, as well as marginalized nucleus with central location. The earlier studies have reported important information about the architectural features of Hofbauer cells. These large cells possess both smooth and rough endoplasmic reticulum, ribosomes, numerous rod-shaped mitochondria, and a poorly developed Golgi complexes.^[49] Some properties of Hofbauer cells are similar to macrophages in some futures, such as abundant micro- and macro-pinocytotic vesicles, cytoplasmic processes, larger vacuoles (phagosome), and very dense granules.^[49,50] Cytoplasmic granules are thought to be lysosomes due to the activity of phosphatase acid.^[51] This system of phagosomes and pinocytotic vesicles plays an important role in pinocytosis and phagocytosis.^[49]

We believed that increasing our knowledge of the umbilical cord structures had an important role in the prevention of fetal malformation and abnormality due to histological disorder in the umbilical cord; in this regard, further examinations should therefore be carried out.

CONCLUSION

The present study showed more detailed information regarding the morphometrical and histological features of umbilical cord than earlier reports. We found the quantitative data about the total number of Hofbauer and mesenchymal stromal cells in the healthy animal group. Moreover, the mean volumes of the umbilical cords, mesenchymal tissue, arteries, and veins were estimated.

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Conflicts of interest

There are no conflicts of interest.

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