

Histological in View on the conus medullaris and filum terminale in rabbits

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Abstract: The terminal end of the spinal cord and filum terminale had significant roles in spinal cord pathophysiology, therefore, the present study was designed to investigate the structural characteristics for that areas. Ten white adult male and female New Zealand rabbits were used in this experiment. Transcardial perfusion technique was made and the spinal cord terminal ends and fila terminale were collected and processed for histological examination by light and electron microscopes. The results revealed that the spinal cord transformed into conus medullaris at the end of the second sacral segment at which a characteristic lateral and ventral neuronal interdigitations between the white and gray matter was evident. The filum terminale internum had a different zonal organization to the white and gray matters. The filum terminale externum formed from fibrous stroma and characterized by an absence of neurons, glia and ependyma.

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Key words: spinal cord, conus medullaris, rabbits, electron microscopes.

1. Introduction

The spinal cord has an indispensable connection and undertaking information between the brain and the body and unaccompanied commands many reflexes (Hill et al., 2012 and Hall 2015). Animals' spinal cord histological studies had indicated by many kinds of literature (Abbadie et al., 1999, Yamamoto et al., 2001 and Uehara et al., 2012). Little interest had been paid for the transmission of spinal cord tissue into filum terminal and it's accompanied with cauda equina from the histological point of view (Fontes et al., 2006 Attia and Shehab 2010). Anatomically, preceding to the lumbar region the spinal cord became diminished in size (Wall et al., 1990) and its termination as conus medullaris continues to descend filum terminale and bundles of lumbar and sacral nerve roots. Conjointly this bundles called the cauda equine (Dhokia and Eames 2014). The filum terminal divided into two parts; filum terminale internum that represented the upper two-third of the filum and covered by dura and arachnoid and extradural attached filum terminale externum (also known as coccygeal ligament) that represented the lower third of the filum and fix to the dorsal coccyx (Standring 2015). The exact histological structure of the filum terminale still contradictory: some researcher finding main basic elements of the spinal cord tissue; ependyma, neurons and neuroglia (Réthelyi et al., 2004). Others records neurons in the dorsal horn some of them linked to the substantia gelatinosa and others substantia gelatinosa-independent (Réthelyi et al., 2008). Others recorded neurons distribution in small groups dorsal and bilateral to the central canal while glial were astrocyte

and oligodendrocytes besides axonal arborization were distributed at the whole grey matter but mainly at the lateral nuclei (Boros et al., 2008) in contrary some literatures mentioned filum terminal majority contents were found as thick longitudinal bundles of collagen incorporated elastic and elaunin fibers but the other contents and capillaries were secondary represented (Fontes et al., 2006). Other species like frogs filum terminal structure was recorded to be neuronal devoid and only contain neuroglia (Glusman et al., 1979).

2. Material and methods:**Animals**

In the present study, ten healthy adults New-Zealand male and female rabbits were used. The animals were obtained from laboratory animals' farm, faculty of agriculture, Zagazig University, Egypt. All animals were kept for two weeks under strict hygienic conditions and fed synthetic ration besides, fresh water ad-libitum before the beginning of the experiment.

Tissue preparation:

The animals were injected by heparin i/v in ear vein 2.0 ml/B.W then the animals were deep anaesthetized with sodium pentobarbital i/v 0.1 ml/B.W (from a concentrated solution of Sodium pentobarbital 65mg/ml). Transcardial perfusion was applied to all animals with solution of 1% Para formaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer according to the technique described by (Millonig, 1976 and Spencer et al., 2000). After perfusion, the spinal cord terminations and fila terminale was taken out and fixed in Karnovsky's

fixative (Bozzola and Russell, 1998). After complete fixation, the spinal cords terminations and fila terminale were removed and processed for paraffin and plastic sections for light and transmission electron microscopes and other samples were processed for scanning electron microscope (Bozzola and Russell, 1998 and Suvarna et al., 2013). For paraffin sections some samples were collected with its enclosed bony vertebrae and firstly decalcified in EDTA for two months then all samples were washed, dehydrate, cleared and prolonged impregnated in paraffin; Finally, the samples were embedded and sectioned into (5-7u) and serial sections were taken and stained with H&E for the boney enclosed tissues. Other samples were collected without its bony vertebrae and processed for paraffin sections then stained with Cresyl violet, Luxol fast blue Periodic Acid-Schiff Hematoxylin (P.A.S.HX), silver impregnation, methyl green Nile blue sulfate and Hematoxylin and Eosin (Suvarna et al., 2013). For plastic sections the segments were fixed in glutaraldehyde 4% and processed for resin preparation; spinal cord tissue samples washed in phosphate buffer, post-fixation in 2% osmium tetroxide, then washed again in the same buffer, dehydrated in ascending grades of alcohol followed by propylene oxide. Finally, infiltrated in a mixture of 50% propylene oxide and 50% resin then embedded in 100% resin. Semithin sections were obtained using Leica ultra-cut ultratome and stained with toluidine blue. Ultrathin sections were obtained from different areas after using semithin sections as a guide, then mounted and stained with uranyl acetate and lead citrate and studied at (Zeiss 10 CA) TEM electron microscope (Hayat, 1990 and Bozzola and Russell, 1998).

3. Results

A- Anatomical findings:-

Gross examination of the rabbit's spinal cords revealed that the spinal cords gradually decrease in diameters at the last lumbar vertebra and turned into cone-shaped structure, conus medullaris at cranial part of the sacrum while the slender filament; filum terminale appeared at the middle of the sacrum, which extended caudally to the base of the tail. The filum terminale was distinguished into two parts: bluish white intradural part, filum terminale internum, and thinner glistening white extradural part, filum terminale externum. The posterior spinal nerves (lumbers, sacrals and the coccygeal) were collected and encircle the terminale end of the spinal cord conus medullaris and the filum terminale in form of nerve bundles, quada equina which continued along the whole length of the filum terminale (Fig.1).

B- Histological findings:

1- Transformation of the spinal cord into conus medullaris:

Microscopically, the spinal cord transformed into cone-shaped structure, conus medullaris, at the end of the second sacral segment of the spinal cord. This conus medullaris continued till the end of the fourth sacral segment (Fig.2).

Transformation of the spinal cord into conus medullaris was distinguished microscopically by the discontinuity of the superficial dorsal horn but, the basic histological components of the spinal cord still represented. Caudally, the dorsal horn gradually disappeared. A very interesting histological structure gradually appeared in the conus medullaris in the form of interdigitation between the gray matter neuropil and white matter columns laterally and ventrally. The lateral interdigitation located at the gray matter intermediate zone between the dorsal and lateral horns and bilaterally to the central canal (Fig.3), while the ventral one located between the ventral horn gray matter and the ventral column ventromedial (Fig.4). The lateral interdigitation characterized by presence of some neuronal cells with extensive neuropil attachments invaded the lateral column (Fig.5) Some of these neurons appeared with synaptic connection to both grey matter and white matter cells (Fig.6) while other neurons invaded deeply inside the white column and revealed extensive synaptic connections in many forms; axo-axonic, axodendritic and dendrodendritic (Fig.7) The latter neurons had large rounded nuclei with prominent centrally located nucleoli and clear slightly basophilic karyoplasm and coarse basophilic Nissl's granules (Fig.8) these neurons have typical ultrastructure of the multipolar neuronal cells; central pale nuclei with prominent nucleoli and characteristic Nissl's granules in form of extensive rough endoplasmic reticulum and many ribosomes (Fig.9). Parallel to these changes the ventral horn motor nuclei gradually diminished caudally and discontinuously but, few large motor neurons were occasionally seen at some sections (Fig.10). With the exception of the above-mentioned regions, the rest of the white matter columns had only nerves fibers and glia cells (Fig.11) At the end of the conus medullaris, the lateral and ventral interdigitations reached their maximum, the dorsal horn became greatly diminished, the perfect shape of the H-gray matter disappeared and the white and gray matter organized into central and marginal zones. (Fig.12) The central canal was found along the whole length of conus medullaris running centrally but, its lumen gradually became more elongated till became slit like at filum terminale internum.

2- Transformation of the conus medullaris into filum terminale internum:

The apex of the conus medullaris transmitted caudally into a filamentous structure intradural filum

terminale internum. This distinguished microscopically by turning off the low ovoid-shaped conus medullaris cross-sectional areas into small rounded ones and presence of dorsal and ventral nerve fiber bundles that closely encircling the filum terminale internum with an appearance of bilateral fusiform ganglia. (Fig.13) the filum terminale internum had the same structural organization of the conus medullaris terminal part in the form of central and marginal zones. At the central zone, the neuronal elements greatly decreased and the majority of cells were neuroglia (fig.14) the neuronal elements were distributed in small groups dorsolateral to the central canal. Concerning the site of the previously described ventral horn in the spinal cord, a very few multipolar cells rarely were seen. Little neuropil was noticed in the central zone. The glial cells were distributed all over the central zone and sometimes periependymal glial cells clusters, mostly oligodendrocytes and astrocytes were noticed. At that level the central canal consisted of slit-like structure that still lined with simple layer of evenly arranged cuboidal to columnar cells with rounded to oval nuclei with uniformly distributed chromatin (fig.15) the cross section of the filum terminale internum at its first third revealed; the marginal zone area became almost equal to the area of the central zone, and the marginal zone was still kept glial cells and nerve fibers. Caudally the cross sections revealed the diminished area of the marginal zone and become smaller than the central one. More caudally the marginal zone became more and more rudimentary (Fig.16). After that the central zone gradually diminished and the central canal continued singly with very little surrounding indefinite tissue ruminants at the end of the filum terminale internum for very short distance that become dilated and split at one end

3- Transformation of the filum terminale internum into filum terminale externum:

The filum terminale internum terminated directly when it merged with the the cul-de-sac of the dura mater at the midline. The outer fibrous coat of the dural cul-de-sac appeared attached to the fibrous stroma of the filum terminale externum. The final terminal externum appeared as connective tissue stroma in form of fibrous bands, that invested with fibroblast cells and few peripherally nerve fibers that run in different directions (Fig.17) the final terminale externum end part was only fibrous stroma without any nerve fibers (Fig.18).ultrastructure's of this part revealed fibrous bands that run in longitudinal, oblique and cross directions with few wavy undistinctive fibroblasts (Fig.19) along the filum

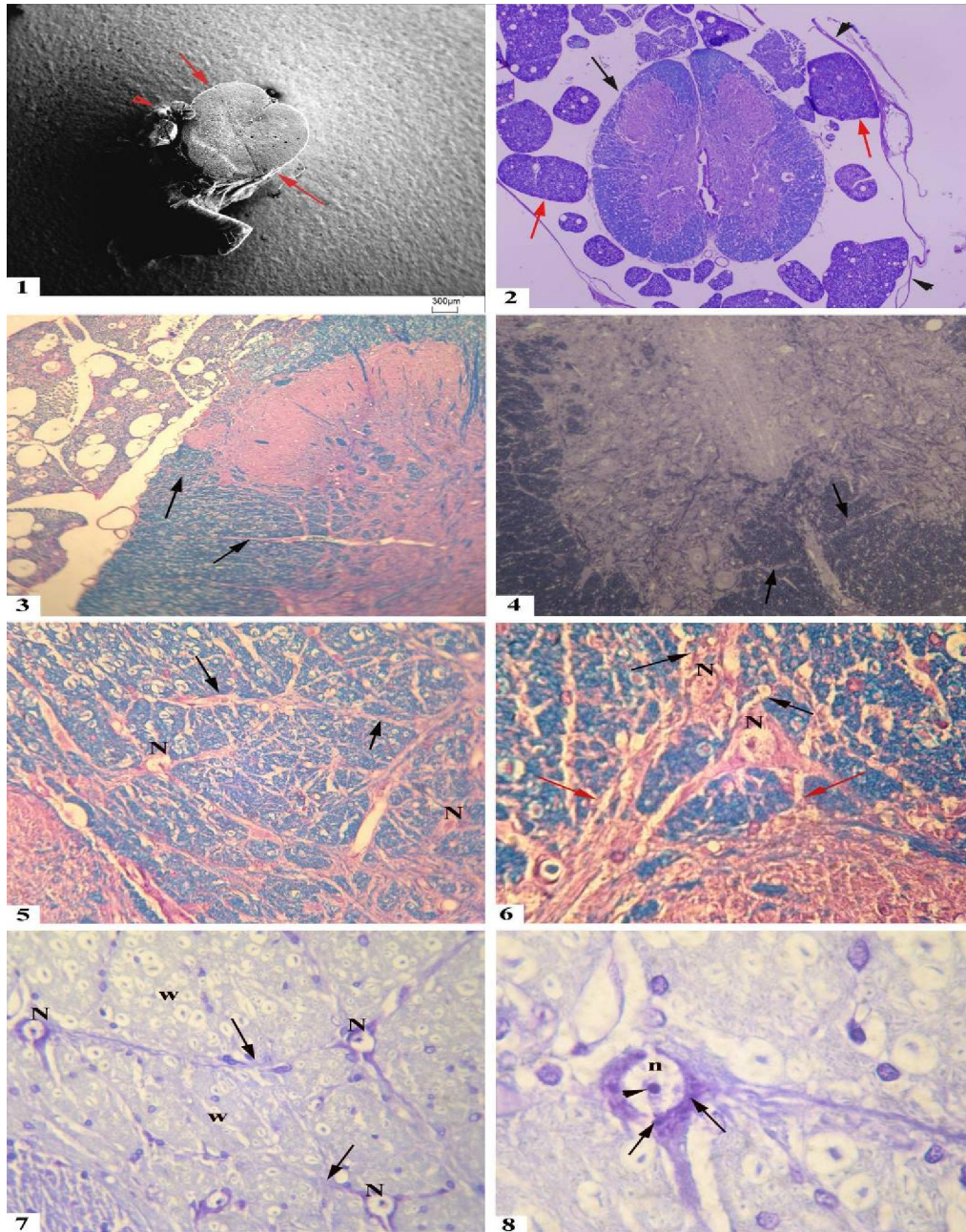
terminale externum; the filamentous structure of the filum terminale internum, glial cells and ependymal were not found.

4-The quada Equina:

The quada equina at the level of conus medullaris and filum terminale internum appeared as bundles of nerve fibers of variable sizes (Fig. 20) these nerve fibers appeared with well-developed myelin sheaths associated with their Schwann cells (fig.21). The axoplasm of these nerve fibers appeared argyrophilic by silver stains with well-developed blood supply (Fig.22) and all enclosed in a dural sheath. The quada equina continued along the filum terminale externum but, appeared surrounded with perineurium and endoneurium.

4. Discussion:

The terminal end of the spinal cord and filum terminale was generally overlooked during the routine anatomical or histological examinations and has received relatively little attention in histological literatures, therefore; authoritative information about the fine structural details of the conus medullaris and filum terminale particularly in laboratory animals is basically not available (Choi et al., 1992). This area had significant roles in spinal cord pathophysiology as it have numerous functional correlations including neurological and neuro-musculoskeletal features (Selçuki et al., 2003), and are involved in many pathological conditions including paralytic and both glial and ependymal neoplasms (Sonnehed et al., 1985, Specht et al., 1986 and Koeller et al., 2000 and Bulsara et al., 2001). For that, the present study was designed to investigate the structural characteristics for that area. Longtime ago, it was established that the terminal end of the spinal cord is a just fibrovascular attachment between the spinal cord and the coccygeal vertebrae (Tarlov 1938), but recently histological studies confirmed that the filum terminale had neurological elements (Choi et al., 1992 and Tubbs et al 2005). The main histo-anatomical observation was a transformation of the spinal cord into conus medullaris which continue from the end of the second sacral segment till the end of the fourth sacral segment of the spinal cord then it transformed into a tubular intradural filum terminale internum which continued distally into extradural filum terminale externum. The same anatomical finding was reported in cats by (Miller 1968). The central canals ended with the end of the filum terminale internum. This is the same in cats but there is no distal central canal in monkeys (Miller 1968).



Fig(1): SEM of rabbit conus medullaris at second sacral segment termination showing cone shaped cross section (arrows) and some bundles of quada equina (arrowheads) (X20).

Fig.(2): Rabbit conus medullaris at third sacral segment level showing, low ovoid cross- sectional area (black arrow) surrounded by quada equina (red arrows) and all surrounded by the dura mater (black arrowheads) (Luxol fast blue P.A.S Hx. X4).

Fig.(3): Rabbit conus medullaris at the third sacral segment level, showing lateral interdigitation from the gray matter into the white matter (arrows) (Luxol fast blue P.A.S Hx. X10).

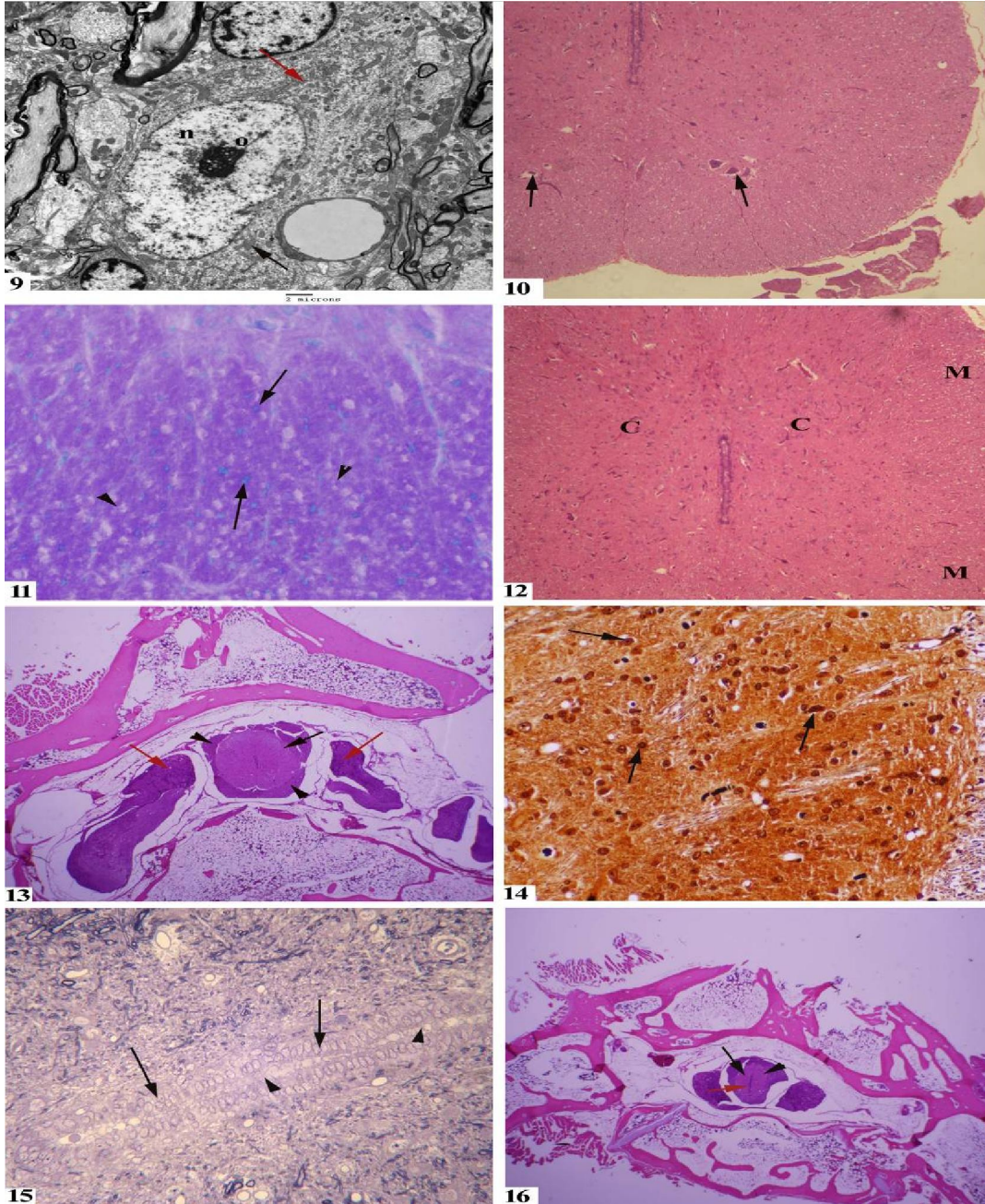
Fig.(4): Rabbit conus medullaris at the third sacral segment level showing ventromedial interdigitation from the gray matter into the white matter column (arrows) (Toluidine blue plastic section. X10).

Fig.(5): Rabbit conus medullaris at third sacral segment level revealed extensive lateral neuropil extension inside the lateral white column (arrows) with the presence of some neurons (N) (Luxol fast blue P.A.S Hx. X20).

Fig.(6): High magnification of the previous figure showed some neurons (N) that have a synaptic attachment to both white matter (black arrows) and gray matter (red arrows) (Luxol fast blue P.A.S Hx. X20).

Fig.(7): Rabbit conus medullaris at the beginning of fourth sacral segment level, had deeply extended neuronal cells(N) with prominent neuropil (Arrows) inside the lateral white column (W) (Cresyl violet stain X40).

Fig.(8): High magnification of the previous figure reflect the structural detail of that neurons; with central rounded nuclei (n), prominent centrally located nucleoli (arrowhead) and coarse basophilic Nissl's granules (arrows) (Cresyl violet stain X100).



Fig(9): TEM of lateral interdigitation neuronal cell showing central pale nucleus (n) with prominent nucleolus (o) and characteristic Nissl's granules (arrows) (X 5700).

Fig.(10): Rabbit conus medullaris at fourth sacral segment level revealing few large multipolar neurons in the ventral motor nuclei (arrows) (H&E X10).

Fig.(11): Rabbit conus medullaris at third sacral segment showing ventral white column as nerve fibers (arrow heads) and numerous glial cell nuclei (Arrowheads) (Methyl green blue sulfate X20).

Fig.(12): Rabbit conus medullaris at the end of the fourth sacral segment showing organization arrangement in the form of central zone (C) and marginal zone (M) (H&E. X10).

Fig.(13): Rabbit filum terminal internum showing rounded cross -sectional area (black arrow) encircled with nerve fibers bundles (arrowheads) and bilateral ganglia (red arrows) (H&E. X4).

Fig.(14): Rabbit filum terminal internum showing numerous neuroglial nuclei (arrows) as a majority of cell type in the central zone (Silver impregnation X20).

Fig.(15): Rabbit filum terminal internum showing slit-like central canal lumen (arrowheads) lined with simple layer of evenly arranged cuboidal to columnar cells with rounded to oval nuclei (arrows) (Toluidine blue plastic section X40)

Fig.(16): Rabbit filum terminal internum at its end showing reduced tissue cross section (black arrow)and rudimentary marginal zoon (arrowhead) with the central canal at the middle(red arrow) (H&E. X4).

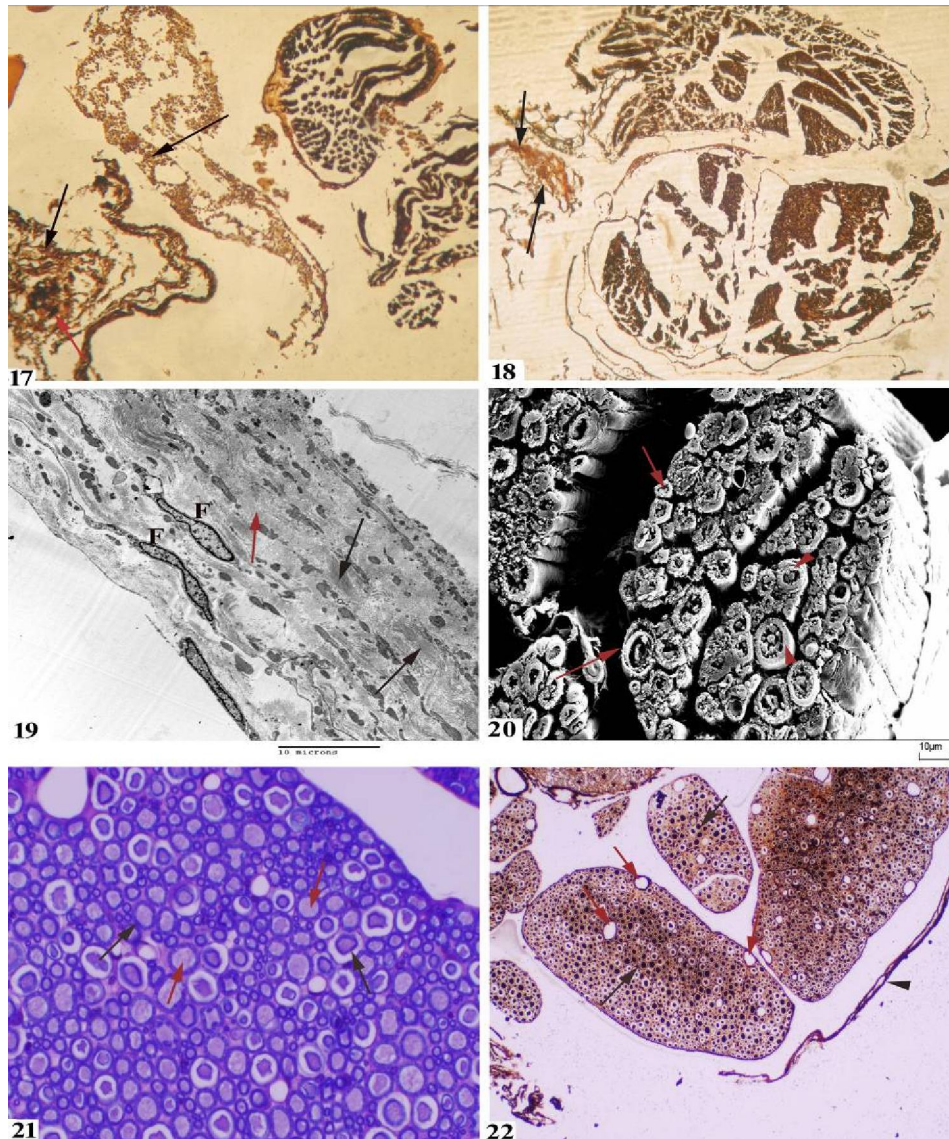


Fig.(17): Rabbit final terminal externum appeared as connective tissue stroma (black arrows) that invested some peripherally nerve fibers (red arrow)

Fig.(18): Rabbit final terminal externum end part was only fibrous stroma without any nerve fibers (arrow) (silver impregnation X4)

Fig.(19):TEM of rabbit filum terminale externum terminal part showing fibrous bands cross sections (red arrow), longitudinal sections (black arrows) and fibroblasts with elongated nuclei (F) (X 4520).

Fig.(20): SEM of The quada equina at the level of filum terminale internum appeared as bundles of nerve fibers of variable sizes (arrows) with prominent myelin sheaths (arrowheads) (X765)

Fig.(21): Rabbit quada equina at the level of conus medullaris nerve fibers appeared with light basophilic axoplasm (red arrows), well-developed deep basophilic myelin sheaths (black arrows) (Toluidine blue plastic section X100)

Fig.(22): Rabbit quada equina at the level of filum terminale internum showing The argyrophilic axoplasm nerve fibers by silver saint (black arrows) that surrounded with the dura mater (arrowhead) with well-developed blood supply (red arrows) (silver impregnation X 40).

The foremost histological findings in the present study was that neuronal, glial and ependymal elements are all present in conus medullaris and filum terminale internum while filum terminale externum is simply

connective tissue elements containing few nerve fibers at its cranial part and just a connective tissue elements devoid from any nerve fibers at its caudal end. The conus medullaris revealed a very characteristic

interdigitation between the gray matter and white matter that located at the transition zone and ventral horn. This interdigitation reported previously in the sacral region of the spinal cord at the transitional zone and the ventral horn cell borders in white leghorn chickens by (Yamamoto et al., 2001 and Uehara et al., 2012), and in the cervical and lumbar segments of the spinal cord of mouse, cat and rhesus monkeys and in cervical segment of the Zebra finch bird by (Abbadie et al., 1999). The functions of these cells are not yet declared but, they were positive for neuronal markers but not immunostained for glial fibrillary acidic protein (Abbadie et al., 1999). Electron microscopic examination of the filum terminale externum showed that it lacks the classic ligamentous structure as it consisted of irregular fibrous bands running in different directions plus the presence of few nerve fibers in its cranial part. The same finding was reported by (Tubbs et al., 2005). The absence of meningeal covering and neurons from filum terminale externum related to the embryological development (Kernohan 1925 and Bale 1984). The exit of some posterior spinal nerves came more posteriorly to their origin forming the quada equina that were continued along the whole length of the filum terminale (Craigie 1966 and Manning et al., 1994).

Conclusion:

- 1- The radical change in the classical histological view of the spinal cord was at the end of the second sacral segment, at which it transformed into conus medullaris.
- 2- The main difference between conus medullaris and filum terminale internum was the organization of the white and gray matters plus the great size reduction.
- 3- The main difference between filum terminale internum and filum terminale externum was the absence of neurons, glia and ependyma.
- 4- The neuronal interdigitations in the conus medullaris are a unique structure, histologically proven to be typical multipolar neurons with extensive neuropil.

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