

Histological and histometrical measurement study on human newborn spinal cord.

*Dr. Sami Ibrahim Abdullah, Dr. Abdul-Jabbar J. Mahdi, Dr. Abdul-Kareem S. Mahood
University of Tikrit College of Medicine*

Summary

Cadavers were collected from the Forensic Medicine Unit of Kirkuk and Tikrit Teaching Hospital between October (2012) to December(2013). Twenty Iraqi male neonate cadavers with age ranging from 0-28 days were obtained to study the histological features of their spinal cord.

Laminectomy of the cadavers and dissecting the meninges were performed. The cords were immersed in 10% formalin for hardened and fixation. Histological study showed the cross section area of the neonate spinal cord was enveloped by thin layer of pia matter and separated from under lying white matter by sub pial space.

The white matter divided into three regions anterior, lateral and posterior funicului. White matter consisted of a large number of longitudinal nerve fibers, the diameter was range from(4-10 μ m), and blood capillary diameter (5-14 μ m) and dark glial cells(2-6 μ m), which produce a myelin sheath around the nerve fibers to give supporting and isolating the fibers from each other. The largest number of longitudinal nerve fibers present in the anterior funicului of white matter.

The gray matter shape was similar to the (H) letter, and consisted of anterior and posterior horns connected with transverse gray commissure and contained circular to oval-shaped central canal which was surrounded by a single row of 70-100 cells long ciliated columnar ependymal cells. The central canal length and width were 23.6 and 15.7 μ m respectively. The gray matter consisted of different sizes of neurons with diameter ranged from(7-22 μ m). The largest number of neurons were found in the cervical region, followed by the lumbar, thoracic and then sacral segment. A multipolar(pyramidal) neurons with round nucleus, the cytoplasm and dendrites containing small dark Nissl body, whereas the hillock and axon lack from Nissl substance, were also clearly seen in the section.

Introduction

The human spinal cord is a major part of the central nervous system (CNS) lodged in the foramen magnum at the base of the brain to a point in the lumbar or sacral vertebrae, depending on the species and considered to be one of the important organ in the our body(1). The spinal cord is the only part of the human central nervous system that has an external segmental organization; which

divided in to 31 segments. Each spinal segment contains a pair of nerve roots called the dorsal (sensory) and the ventral (motor) roots which travels through the intervertebral foramina of vertebral column.(2) The spinal cord (Medulla Spinalis) situated within the vertebral canal of the vertebral column it is surrounded by three meninges; the dura mater, the arachnoid mater and the pia matter. The spinal cord is divided

into internally gray matter(H shape) and peripherally white matter. The central canal filled with cerebral spinal fluid(CSF), as well as the subarachnoid space which provide further protection with meninges to the spinal cord.(3,4)

Materials and Methods

Neonates without any congenital cranio - vertebral catastrophes were selected for this study. Laminectomy was performed to open the vertebral canal from behind to approach the neonate spinal cord. The present study the involved the histology of neonate spinal cord based on post-mortem study of transverse section of each segment to obtain the normal and accurate measurements. This included length, transverse and sagittal section of each segment. The central canal of the four regions of neonate spinal cord cervical(C),thoracic(T),lumber(L) and sacral(S) were included in the measurements. Measurements were also included the shape and number of neurons, glial cells and nerve fibers, the shape of anterior and posterior gray horn in each segment. (5,6)

Laminectomy was performed by dissecting the back and the spinal cord of each neonate cadaver were rapidly removed then cleaned from the fat and meninges using scissors and forceps. Each specimen of the spinal cord was divided in to four parts cervical (C), thoracic (T) , lumber (L) and sacrum (L), and each part was sectioned with a razor blade into 5mm length of each piece . Each tissue fragment was the subjected to the following procedure : (7,8)

Fixation in 10% formalin , then processed for histological procedures and 5µm thick sections were obtained and stained, using three stains as follows:- Haematoxylin Eosin (routine stain), Bielschowskys - Silver PAS stain

and Toluidine blue stain (special stains).(9,10)

The slides were used for descriptive and histometrical study using light microscopic (Olympus and dissection light microscope), with 40x objective lens and oil immersion 100x , all that by using, micrometer type calibrated stage (Olympus) and calibrated ocular lens (Reichert) (7,9) was used for the measurements.

Results

I. Descriptive histology:

The microscopic investigations of transverse sections taken from neonate involved the morphometric and descriptive histology. The spinal cord was covered and closely adherent with very thin transparent membrane composed of loosely arranged connective tissue called pia mater. The thickness ranged from 2 – 5 µm , which sends a thick vertical or longitudinal prolongation fold to the bottom of the anterior median fissure . Also the pia mater separate from spinal cord and form subpial spaces which contained a bundle of fibers and longitudinal blood vessels which form the sub pial plexus blood vessels, and the diameter of anterior spinal artery ranged from 5 – 20 µm are seen in Figure (1).

The substance of white matter contains profuse longitudinal arrangement of myelinated and unmyelinated nerve fibers (ascending and descending tracts), with transverse diameter ranging (4 – 10 µm) under high magnification (100x) in the four segments (C, T, L and S) (Figure 2). Moreover, in the cross section of white matter the myelin sheath was completely surrounded the transverse and longitudinal nerve fibers and splits the nerve fibers from each other. Under high magnification the glial cell (oligodendrocytes cell)

produced large amounts of thin processes or fibers of myelin sheath around the nerve fibers (Figures 3). As well as, it shows a large number of different shape and size dark color of neuroglia cells ranging from (3- 8 μ m) present around and attached to the nerve fibers and around continuous blood capillary which lined with dark flattened elongated endothelial cells in wall of capillaries. The diameter of perforated blood vessels capillary ranged from (5 - 14 μ m) which traversed the subpial space to enter deeply inside the white matter then distributed in gray matter substance, the penetrated capillary surrounded by thin layer of pia matter as seen in (Figure 2).

In this study showed small branches of blood vessels from pial plexus, similar to branches from anterior spinal artery enter the white and gray matter of spinal cord and carry with them an adventitial connective-tissue sheath derived from the pia mater, this indicated that the pia mater can be seen inside the substance of the spinal cord with blood in (Figure 4).

The present study shows the gray matter is located centrally or deep to the white mater and forms an H-shaped or butterfly mass; the gray mater formed by two horns connected centrally by gray commissure with a mean vertical diameter thickness range of four segments were $31.7 \pm 6.25 \mu\text{m}$ (Figure5)

The gray matter substance was seen densely packed with neuron cell bodies and glial cell. The neurons showed several variability in shapes (i.e. fusiform, triangular and polygonal) and size (large, medium and small), with cell size ranging from (7-20 μ m). Generally the largest neurons found in the anterior group than the lateral group of ventral horns in cervical and lumbar regions than the thoracic and sacral segments. The

anterior horn nerve cells are generally well-differentiated (Figure 5), while the neurons in the intermediolateral regions are smaller, slender, and frequently bipolar(Figure 6).

Furthermore, externally the large and medium size neurons in the ventral horn of gray matter has apparent dendrites and axon. Whereas internally the neuron possesses round large nucleus, dark prominent nucleolus and its cytoplasm are extremely rich in basophilic Nissl substance which arranged around the nucleus and extend inside the dendrites while the hillock and axon are deficient a Nissl substance ,whereas the small dark supporting cells in gray and white mater are rich and was found in close contact with the cell body of neurons or with their processes, also interposed between each neuron and the blood capillaries and around the nerve fibers (axons) (Figures 6 and7).

Furthermore, the traversed centrally gray commissure was occupied by a small rounded or oval shape foramen called central and canal was opened in all spinal segments which include cervical (C), thoracic (T), lumbar (L) and sacral (S).The mean transverse diameter of central canal was ranged $23.63 \pm 2.85 \mu\text{m}$ in the four segments. The central canal was lined with continuous single layer of ciliated columnar epithelium of ependymal cells with different shapes and sizes and it's nuclei was elongated shape generally placed in a central position of ependymal cell and long axis of nucleus was vertical reach to the luminal surface of the cell. In addition, the central canal contains cerebrospinal fluid (CSF) as seen in (Figure 9). Also, under the high magnification of digital camera revealed a small, thin

and abundant processes microvillus projected from the apical surface of columnar epithelia toward the lumen of central canal (Figure 10).

II. Histometrical measurements :

1. Transverse and Sagittal diameter of neonate spinal cord:-

The statistical analysis of transverse and sagittal (vertical) diameter of these results revealed a non significant ($P>0.05$) variations found between segments (C and L), while, there was significant difference ($P<0.05$) was found between (T and S) segments, also a significant ($P<0.05$) variation found between (C) segment and the segments T and L of neonate spinal cord. Additionally, a significant ($P<0.05$) variation was found when the (C and L) segments was compared with the (T and S) segments (Table 1).

2. Transverse and vertical diameter of central canal :-

The transverse and vertical diameter of central canal revealed of observable differences correlated to segments. The mean values of transverse diameters of central canal in the four segments C, T, L and S were $24.48 \pm 1.55 \mu\text{m}$, $21.98 \pm 2.28 \mu\text{m}$, $27.28 \pm 1.79 \mu\text{m}$ and $20.84 \pm 1.05 \mu\text{m}$ respectively. While vertical diameter of the central canal in the above segments were $15.38 \pm 1.57 \mu\text{m}$, $16.40 \pm 0.95 \mu\text{m}$, $15.92 \pm 1.92 \mu\text{m}$ and $15.26 \pm 0.81 \mu\text{m}$ respectively (Table 2).

The statistical analysis of transverse diameter revealed of no significant ($P>0.05$) variations between T and S segment, while there was significant ($P<0.05$) variations between C and L segments. There was significant ($P<0.05$) variations when C and L segments compared with T and

S segments. In addition, statistical analysis of vertical diameter of central canal, showed no significant variations ($P>0.05$) among all segments (C, T, L and S) of neonate spinal cord.

3. Number of longitudinal nerve fiber in white matter of neonate spinal cord:

The statistical analysis of the total number of longitudinal nerve fibers in anterior, lateral and posterior funiculi of neonate spinal cord revealed of no significant variations ($P>0.05$) between anterior and lateral funiculi, while there was significant variations ($P<0.05$) between the anterior and posterior funiculi and between the lateral and posterior funiculi of neonate spinal cord (Table 3).

4. Number of Nerve cell in gray matter of neonate spinal cord :-

The statistical analysis correlation between the number of neurons in right and left gray horns of cross - section of the four regions of neonate spinal cord showed no significant variations ($P>0.05$) between (C and L) segment, while there was significant reduction ($P<0.05$) in number of neurons in (T and S) compared with (C and L) segments. There was highly significant difference ($P<0.01$) between segment (S and C) in both columns of right and left horns in gray matter of neonate spinal cord (Table 4).

Concerning, the total number of neurons in four segments (region) (C, T, L and S) of neonate spinal cord statistically showed there was no significant ($P>0.05$) variations (C and L) segments, while there was significant ($P<0.05$) difference among (T, L and S) segments, also there was highly significant difference ($P<0.01$)

between (S) and (C and L) in the same column of neonate spinal cord.

Discussion

Microscopical examination showed that the outer surface of spinal cord was covered by innermost layer of pia matter which was a very thin continues layer of fibrous tissue. Additionally, the subpial space situated between the pia matter and outer surface of spinal cord (white matter), which contains bundles of collagen fibers surround the spinal cord, and separates the pia mater from the neural tissues of the neonate spinal cord. The pia matter reflected and surrounded the arteries which traversed the subpial space and penetrate the spinal cord deeply, first and foremost supply of white matter then gray matter area, as well as the arterial branches from subpial plexus enter the white matter at right angle, also the pia matter produced longitudinal vertical median fold which contain a single branch of anterior spinal artery called central artery which descend inside the anterior median fissure then reaches to anterior white commissure, where it diverge to the left side and vertically to enter the gray matter of the spinal cord. This result agree with the Nicholas et al(11) and Weller, (12) who stated that the pia matter composed of connective tissue, which completely surround the spinal cord and subpial space and contains thick bundle of collagen fibers, blood vessels enclosed by layer of pia matter which form arterial pial plexus then branches of this plexus enter inside the white and gray matter substance of spinal cord.

The present results agreed with Lasjaunias et al(13) and Perese et al(14) who stated that, the gray matter vascularity distribution and capillary

anastomoses are greater than the white matter substance and the type of capillary was contentious type due to endothelial cells found in the wall of capillaries which distribute inside the white and gray matter in transverse section of spinal cord. The central arteries leave the anterior spinal artery singly, not in pairs, and, in the depths of the anterior median fissure, turn alternately to the right and to the left. Furthermore, in present findings, the diameter of anterior spinal artery was ranged from(5 - 20 μ m), while Perese et al(14) stated that the diameter in the adult was range from (2 -3 mm). This difference can be age related.

In present study under the dissecting microscope (5x) of the four (C,T, L and S) segments showed the largest value of the transverse and vertical (sagittal) diameters 2.64 ± 0.72 mm and 1.92 ± 0.71 mm respectively was established in the cervical segment and then become decreased in the thoracic segment, while in the lumber segment diameters turn into larger. The transverse and vertical diameter turn into decreased in the sacral region to 1.45 ± 0.78 mm and 1.16 ± 0.61 mm respectively in neonate spinal cord. This result is similar to Donaldson et al (15) and Barson et al (16) they confirmed the greatest transverse and vertical diameters was in the middle cervical and upper lumber segments, while the minimum dimension was in thoracic and sacral segments of spinal cord. This means that the transverse and vertical diameters shows significantly($P < 0.05$) the largest measurements in the lumber, followed by cervical, then thoracic and finally the sacral region of neonate spinal cord.

The present results revealed that the means and SD of the large number of neurons was in the cervical and lumber segments, compared to the thoracic and sacral segments which contained

fewer number of neurons. Statistically the number of neurons in the right and left ventral and slightly dorsal gray horns revealed that there was no significant variation ($P>0.05$) between the left and right horns of (C and L) regions, but significant reduction ($P<0.05$) in number of neurons in (T and S) which compared with (C and L) regions, where as highly significant difference ($P<0.01$) showed between (S and C) regions in the both columns of right and left horns of gray matter of neonate spinal cord. This findings was in agreement with the findings of Craw(17) and Pullen et al (18) where they mentioned the difference in the number of neuron was not significant ($P>0.05$) between the right and left side of cross - section of spinal cord. Furthermore, the total number of neurons in cervical (116.0 ± 8.77) and lumbar (114.80 ± 6.09) segments were highly significant difference ($P<0.01$) than the number of neurons in thoracic (96.80 ± 7.19) and sacral (80.40 ± 2.1) segments of neonate spinal cord (Table 4).

The statistical analysis of total number of nerve fibers values showed no significant variations ($P>0.05$) between anterior and lateral funicului, while there was significant variations ($P<0.05$) between the anterior (839 ± 7.69) and posterior (707.9 ± 7.34) funicului and between the lateral (799.8 ± 6.84) and posterior funicului as mentioned above. While Zahang et al (19) and Keen et al (20) mentioned that in adult spinal cord the number of longitudinal nerve fibers was greater in the posterior funiculus than the anteriolateral funicului of white matter.

The transverse diameter of central canal of four the segments (C, T, L and S) was found in the lumbar segment while the small diameter was in sacral segment of transverse section. Whereas the range of vertical

diameters of central canal of the four segments was ($15 - 16 \mu\text{m}$) and there was no significant variations ($P>0.05$) among the four segments of neonate spinal cord. While Nakayama et al (21) reported that the transverse and vertical diameter of central canal of adult spinal cord was ($72 \mu\text{m}$ and $80 \mu\text{m}$) respectively, while in present study the range of transverse and vertical diameter of central canal was ($20 - 27 \mu\text{m}$) and ($15 - 16 \mu\text{m}$) respectively.

References

1. Haneline M.T., Robert C., Morgan D. and Justin R. : Determining spinal level using the inferior angle of the scapula as a reference landmark: a retrospective analysis of 50 radio graphs. *J. Can Chiropr. Assoc.* 2008; 52(1): 24-29.
2. Dum RP. and Strick PL. : The spino thalamic system targets motor and sensory areas in the cerebral cortex at monkeys . *J Neurosci* . 2009; 45: 14223 - 14235.
3. Wolpaw J. R.: What can the spinal cord teach us about learning and memory? *Neuroscientist. J.* 2010; 16 (5):532-49.
4. Talal A., Mohammed H. and Ali A. : Deep Vein Thrombosis in Iraqi spinal cord injured patients. *Iraqi post. Med. J.* 2009; 1: 21.
5. Sadullah B., Durmus B., and Muhammet L. S. : The Segmental Morphometric Properties of the Horse Cervical Spinal Cord: A Study of Cadaver. *Scien. World J.* 2013; 73: 1-9.
6. Durmus B., Sadullah B. and Emrah S. : Selective Gray and White Matter Staining of the Horse Spinal Cord. *Kafkas Univ. Vet. Fak. Derg.* 2012; 18(2): 249 - 254.
7. Luna, G : Manual of histological staining institute of pathology USA (1968) .

8. Mowry R.W. and Manus J.F.: Staining methods (Histological). Hitt Intern 1998 ; 14; 47-50.
9. Johan DB. , Suvarna SK. and Christopher L. : Theory & practice of histological techniques.7th Edition. London, Elsevier Limited. 2013;70 - 130.
10. Suzanne L. and Zsuzsanna N.: New temperature modification makes the Bielschowsky silver stain reproducible. Acta. Neuropathol. 2001;101:17-21.
11. Nicholas, D.S. and Weller R.O.: The fine anatomy of the human spinal meninges. Journal of Neurosurgery. 1988. 69(2).276-282.
12. Weller R.O. : Microscopic morphology and histology of the human meninges. Morphologie,2005; 89; 22-34.
13. Lasjaunias P. and Berenstein A. : Surgical Neuroangiography. Functional vascular anatomy of brain, spinal cord, and spine. New York.Springer-Verlag,1990; 3:16-68.
14. Pereš D. M. and Fracasso J. E. : Anatomical considerations in surgery of the spinal cord. A study of vessels and measurements of the cord.1959; 314 - 325.
15. Donaldson H. H., and Davis D. J. : Description of charts showing areas of the cross-sections of the human spinal cord at the level of each nerve. J. Comp. Neur. 1903; 13: 19 - 40.
16. Barson A. J. and Sands J. : Regional and segmental characteristics of the human adult spinal cord. J. Anat. 1977;123: 3,797-803.
17. Craw C.H.: The distribution of the nerve cells in the ventral columns of the spinal cord. J. Comp. Neuro. 1945; 45; (1).283 - 299.
18. Pullen A.H., Tucker D. and Martin J.E. : Morphological and morphometric characterization of Onufin nucleus in the spinal cord in man. J. Anat.1997;191: 201 - 213.
19. Zahang C. and Goto N. : Morphometric analysis and aging process of nerve in human spinal posterior funiculus. Okajimas Folia Anat.Japn.1995;83:342-347.
20. Keen M. F. : Myelination in human central nervous system. Neuropathology. J. Anat. 1938; 3: 1-18.
21. Nakayama Y. and Kohnonumber K. : Number and polarity the ependymal cilia in the central canal of some vertebrates. J. of Neur.1974; 3: 449-458.

Table (1): Means \pm SD the transverse and vertical diameter in transverse section the four segments of neonate spinal cord.(The area was calculated per microscopic field 5 x).

Segments	Transverse diameter of spinal cord(mm)	sagittal diameter of spinal cord(mm)
Cervical (C)	2.64 \pm 0.72*	1.92 \pm 0.71 "
Thoracic (T)	1.89 \pm 0.68"	1.32 \pm 0.85 "
Lumber (L)	2.25 \pm 0.88 "	1.77 \pm 0.63 *
Sacral (S)	1.45 \pm 0.78 "	1.16 \pm 0.61 *

Table (2): Means \pm SD of the transverse and vertical diameter of central canal in four segments of neonate spinal cord.(The number was calculated per microscopic field 40X) :-

Segments	Transverse diameter of central canal(μ m)	vertical diameter of central canal(μ m)
Cervical (C)	24.48 \pm 1.55*	15.38 \pm 1.57 "
Thoracic (T)	21.98 \pm 2.28"	16.40 \pm 0.95 "
Lumber (L)	27.28 \pm 1.79*	15.92 \pm 1.92 "
Sacral (S)	20.84 \pm 2.1* "	15.26 \pm 0.81 "

Table (3): Means \pm SD of the number of longitudinal nerve fibers in anterior, lateral and posterior funiculus in transverse cross-section of the four segments in white matter of neonate spinal cord and shows the correlation between the segments (The number was calculated per microscopic field, oil immersion 100x) :-

Segments	No. of nerve fibers in anterior funiculus of white matter	No. of nerve fibers in lateral funiculus of white matter	No. of nerve fibers in posterior funiculus of white matter
Cervical (C)	$380.4 \pm 6.44^{**}$	$408.7 \pm 7.15^{**}$	$402.1 \pm 7.30^{**}$
Thoracic (T)	$85.8 \pm 4.78^*$	$121.3 \pm 4.29^{n*}$	$105.6 \pm 6.53^{n*}$
Lumber (L)	$202.4 \pm 5.57^{n*}$	$110.2 \pm 5.63^{n*}$	$98.8 \pm 5.86^{n*}$
Sacral (S)	$170.4 \pm 4.09^{n*}$	$159.6 \pm 4.91^*$	101.4 ± 5.13^n
Total	$839 \pm 7.69^*$	799.8 ± 6.84^n	$707.9 \pm 7.34^*$

Table (4): Means \pm SD of the number of neurons in right and left horns of gray matter in four segments of neonate spinal cord and shows also correlation between the segments (The number was calculated per microscopic field X40) :

Segments	No. of neurons in right horn of gray matter	No. of neurons in left horn of gray matter	Total No. of neurons in gray matter
Cervical (C)	$61.20 \pm 4.1^{n*}$	$54.80 \pm 3.3^{n*}$	$116.0 \pm 8.77^{n*}$
Thoracic (T)	$51.20 \pm 3.1^*$	$45.60 \pm 4.3^*$	$96.80 \pm 7.19^*$
Lumber (L)	$59.80 \pm 2.6^{n*}$	$55.00 \pm 4.4^{n*}$	$114.80 \pm 6.09^{n*}$
Sacral (S)	$42.40 \pm 2.1^*$	$38.00 \pm 1.6^*$	$80.40 \pm 2.1^{**}$
Total	214.6 ± 4.71	193.4 ± 4.18	408 ± 1.67

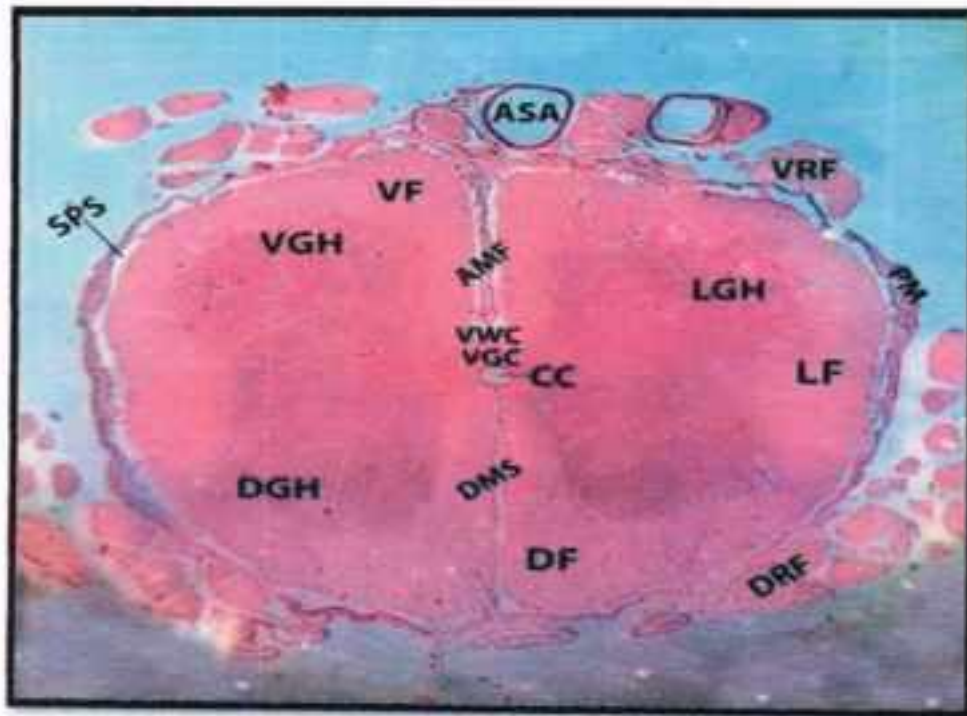


Fig.(1). Transverse section of neonate spinal cord shows the white and gray matter, the ventral and dorsal gray horns (VGH) (DGH) and small lateral gray horn (LGH) with the ventral gray (VGC) and white commissure (VWC) adjacent to the central canal (CC) and the ventral, lateral and dorsal funiculi (VF), (LF) and (DF) respectively. The dorsal root fibers (DRF) and ventral root fibers (VRF). The anterior median fissure (AMF), dorsal median sulcus (DMS) and anterior spinal artery (ASA) also, subpial space (SPS) can also be seen (H&E, 5x).

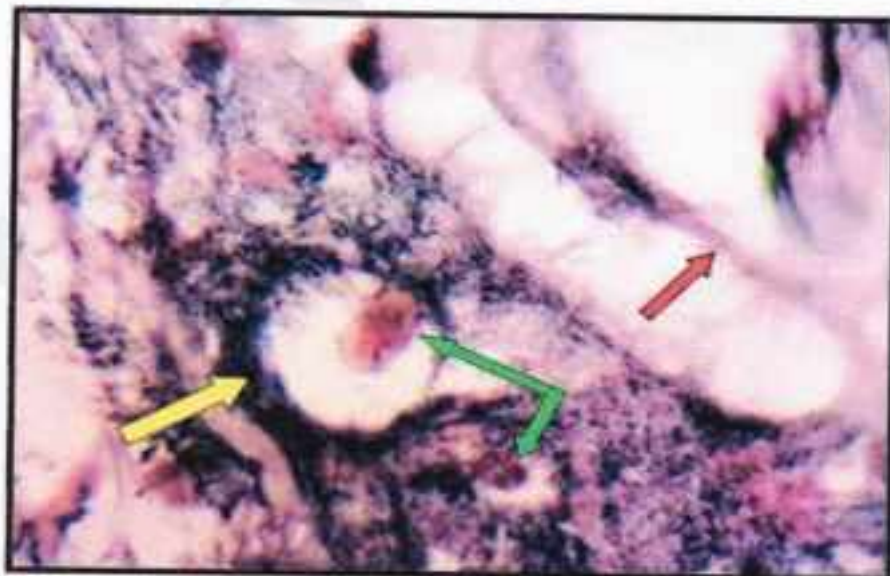


Fig.(2). Single longitudinal nerve fiber encircled by dark myelin sheath (yellow arrow) and small red color glial cells with its centrally red nucleus (green arrow) in white matter of cervical segment of neonate spinal cord. (Bielschowsky's Silver PAS 100X).

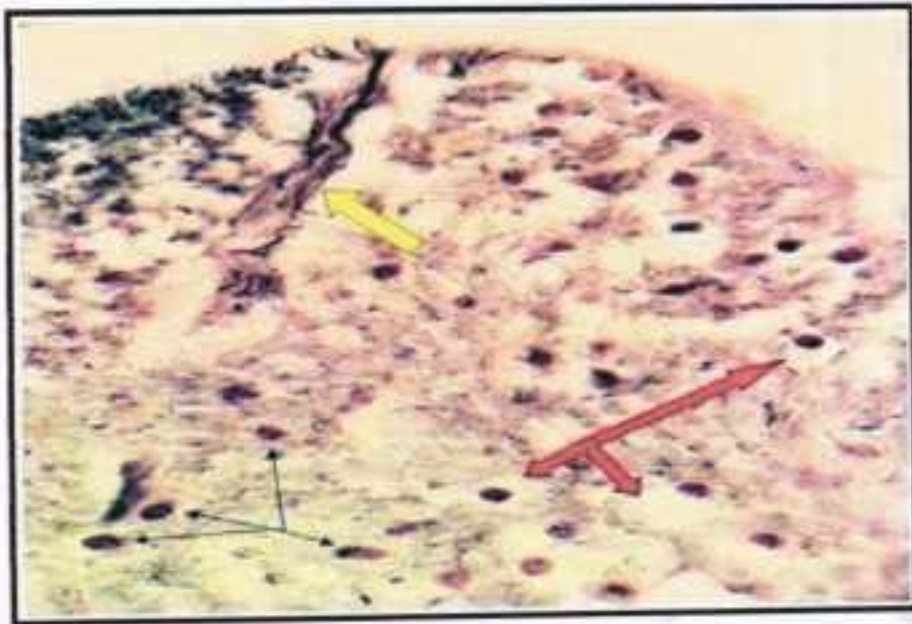


Fig.(3). White matter of cervical segment show the anterior funiculus, a large number of longitudinal myelinated nerve fibers (red arrows) and plentiful of small dark brown glial cell (black arrows) and blood vessel (yellow arrow) which perforate the white matter of neonate spinal cord.(Bielschowskys Silver PAS 40X).

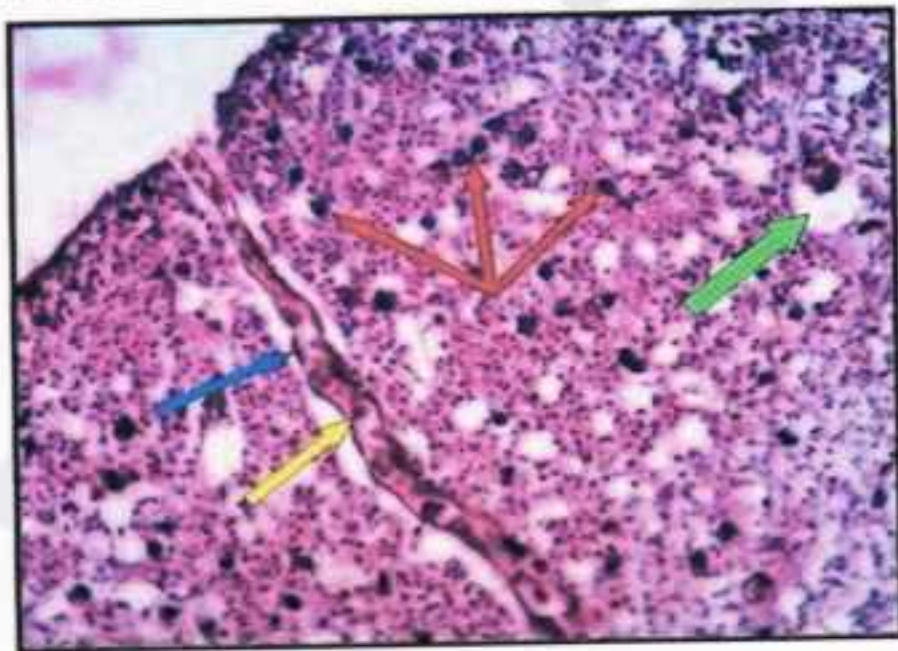


Fig.(4). Contentious blood capillary type lined with endothelial cells(blue arrow) enter inside the white matter substance, RBCs (yellow arrow) numerous small dark support cells(red arrows) with myelinated nerve fibers (green arrow) (H&E, 40X).



Fig.(5). The gray commissure (yellow arrow) centrally connected the two horns of gray matter which traversed by central canal (blue arrow) and posterior funiculus (green arrow) and anterior white commissar (black arrow) connected the two anterior funiculi of neonate spinal cord.(H& E 25 X).

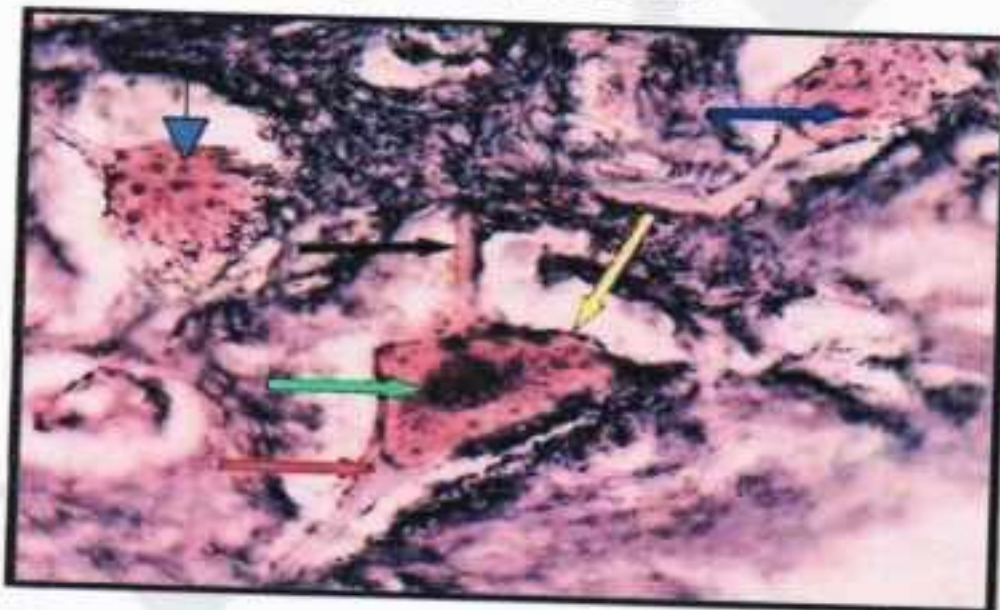


Fig.(6). Ventral gray horn of lumbar segment shows many well differentiate neurons, polygonal, pink in color(yellow arrow), hillock and axon are lacks of Nissl bodies(red arrow),dendrite (black arrow), round dark nucleus (green arrow) and abundant Nissl bodies within the cytoplasm of the nerve cell body (blue arrow) (Bielschowskys Silver PAS 100X).



Fig.(7). Ventral horn of lumbar segment shows the bipolar lateral group neurons, slender shape, dark red color neurons (black arrow), axon with out Nissl substance (yellow arrow), elongated nucleus with central round dark nucleolus (green arrow) and a mass of dark color nissl bodies in the cytoplasm of neuron (red arrow) (Bielschowskys Silver PAS 100x).

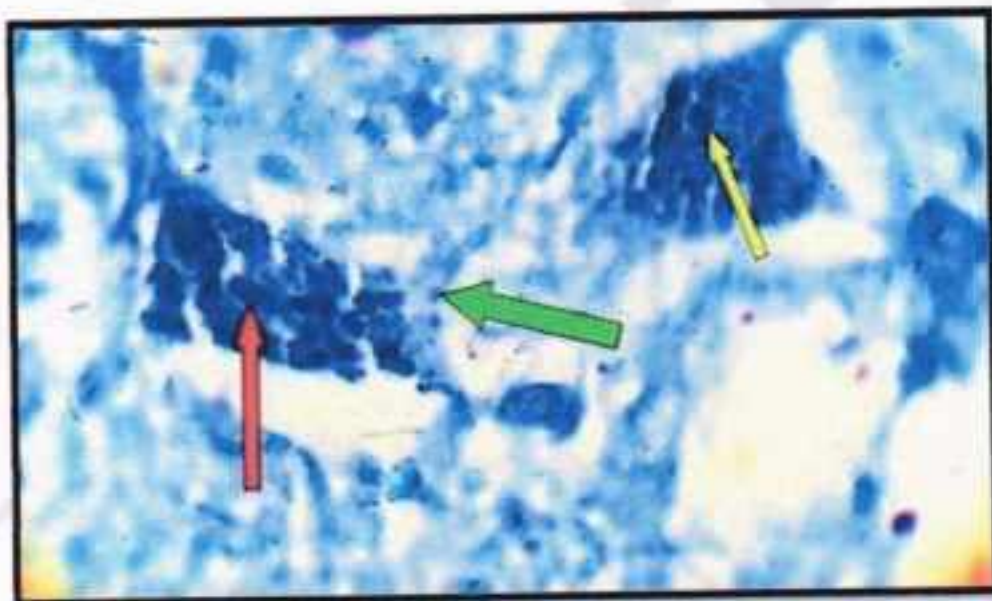


Fig.(8). The cytoplasm of a multipolar neuron contains centrally round nucleus (red arrow) and small dark Nissl bodies (yellow arrow), hillock and axon (green arrow) (Toluidine blue 100X).



Fig.(9). Gray commissure of cervical segment shows the central canal lined with ependymal cell (black arrow) , the thickness of apical surface (yellow arrow) and filled with CSF (pink in color) (H&E,100X)

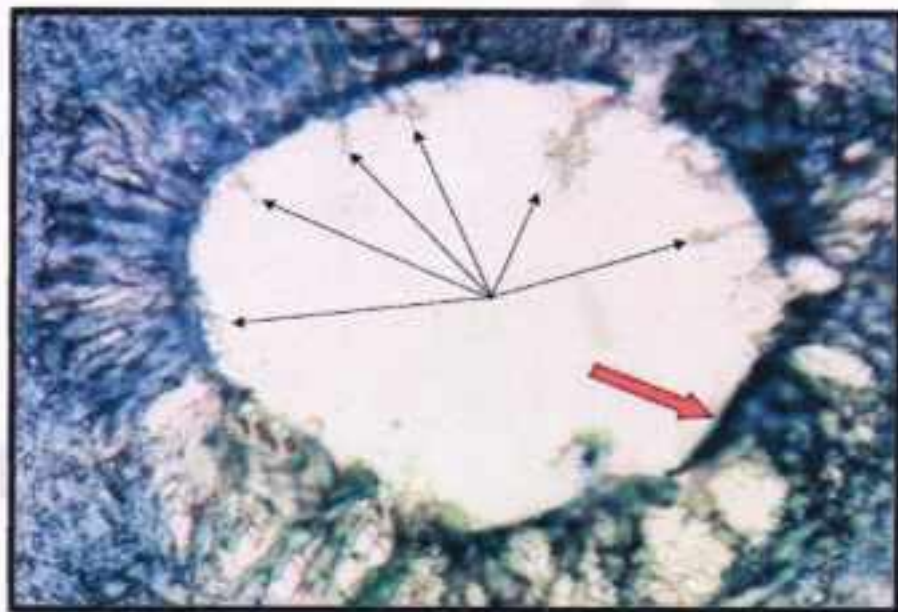


Fig.(10). Central canal of thoracic segment show the elongated cilia and microvillus(black arrows) processes originated from the apical surface(red arrow) of ependymal cells towered the lumen of central canal of neonate spinal cord (Bielschowskys Silver PAS, 100X).