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Gross and microscopic developmental study of the local rabbit's spinal cord

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Abstract

The aim of the study was to investigate developmental changes in the spinal cord in rabbit after birth till adulthood period. To conduct that, six local rabbits aged 2 weeks, 2 months and adults were selected.

Gross findings showed that the spinal cord was constructed from 37 segments which were compressed dorso-ventrally in all studied ages. The organ showed two distinct enlargements (cervical and lumbar). Out of all segments, the lumbar segments were the longest in all studied ages, whereas, the shortest were the sacral segments in the rabbits aged 2 weeks and the cervical segment in 2 months and adults.

Microscopically, each spinal cord segment revealed two main elements that were grey matter and white matter. The grey matter looks like a butter-fly in shape which has two thin dorsal horns and two other thick ventral horns. Three types of neurons were detected in the grey matter according to their sizes in addition to the presence of two types of glial cells.

In conclusions, highest changes in the spinal cord segments were clearly observed in the period extended between 2 weeks and 2 months of age.

Keywords: Spinal cord, Development, Neurons, Rabbit

1. Introduction

The spinal cord is the caudal part of the central nervous system, which extends through the vertebral canal. It is protected by the connective tissue membranes called meninges that are dura mater, arachnoid and the innermost pia mater. In most of the adult mammals, the spinal cord occupies only the upper two-thirds of the vertebral canal as the growth of the bones create the vertebral column which is proportionally growing more faster than that of the spinal cord. According to its rostrocaudal location, the spinal cord can be divided into four segments that are cervical, thoracic, lumbar and sacral. Two of these segments are marked by an upper (cervical) and lower (lumbar) enlargements ^[1].

Similarly to other organs, the spinal cord is macroscopically or microscopically affected by the processes of aging and the diseases. It was found that evaluation of the morphological changes in this organ by conducting quantitative data appeared more effective than using qualitative evaluation ^[2]. Limited information on the segmental morphometrical observations were obtained by previous techniques provided by researches performed on the spinal cord of some species such as rats ^[3], mice ^[4] and chickens ^[5]. In the late decades of the last century, sparse anatomical studies on the spinal cord were conducted. In this respect, ^[6-8] recorded valuable information on the anatomy of the spinal cord in donkey, buffalo and goat, respectively. Previous developmental study provided on the spinal cord of the rabbit however, this investigation focused only on the morphometrical aspect of the spinal cord via the quantitative measurements ^[9].

In the veterinary field, the diseases of spinal cord were of great interest. In the horse, diseases of the cervical spinal cord have a special importance among the central nervous system because of their high prevalence, clinical signs and often poor prognosis ^[10]. In fact, morphometrical evaluations of the spinal cord and its surrounding tissue are often used in medical imaging of different histopathologic studies ^[11].

The objectives of the current study were to determine macroscopically and microscopically the morphometrical features of the spinal cord of the local rabbit at different postnatal ages using gross morphometrical and histological approaches. The data obtained can be used to investigate possible relationships among the compartments of the spinal cord. However, the present study aimed to extend the knowledge of the morphometrical records of the spinal cord of the rabbit by means of the quantitative measurements.

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2. Materials and methods

2.1. Rabbit's collection

The study was performed on the spinal cord of eighteen local breed clinically health rabbits of different postnatal ages (2 weeks, 2 months and adults) and grouped as six animals for each age at the College of Veterinary Medicine / Baghdad University which was approved by the ethic committee for animal use and care. Animals were purchased from local animal's market of Baghdad city.

2.2. Sampling & morphometrical approach

The animals were anaesthetized by chloroform inhalation and killed under general anesthesia [12], afterwards 10% neutral buffered formalin solution was perfuse via the common carotid artery. The cadavers were then preserved in 10% formalin solution for 10 days before they manually dissected. Macroscopic measurements were achieved by the aid of a magnifying lens and Venire caliper. The gross morphometrical measurements that were carried out on the spinal cord includes measuring the entire length of the spinal cord, length of each segment, the dorsoventral and transverse diameters of each segment as well as the diameters of Intmuscentia cervicalis (cervical enlargement) and Intmuscentia lumbaris (lumbar enlargement).

2.3. Histological procedures

Representative specimens from each segment were taken for all ages and processed by routine histological methods to get histological sections of 6 µm thickness. Hematoxylin and

eosin stain was used to explore the general histological structure of spinal cord and to calculate the following parameters for each segment: area of white matter and grey matter, diameter of the whole spinal cord after staining, diameter of the central canal, and heights of Ependymal cells of the central canal, diameter and number of neurons and glial cells of dorsal, lateral and ventral horns of grey matter [13].

2.4. Statistical analysis

Computer package (Sigma plot V12.0 / SYSTAT software) was used to conduct the morphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed using one way analysis of variance (ANOVA) with significant level set on $P < 0.05$

3. Results and discussion

3.1. Gross and morphometrical findings

The spinal cord of the local rabbit of all studied ages was constructed from 37 segments that were divided according to the vertebral formula into eight cervical segments (C), twelve thoracic (T), seven lumbar (L), four sacral (S) and six caudal (CU) segments. Distinctly, in all studied ages, the spinal cord appeared compressed dorso-ventrally (Fig. 1) (Table 1). The present formula was in accordance with that stated recently by [14] in the spinal cord of Egyptian domestic rabbit. On the other hand [15] referred to presence of variable number of vertebrae in the New Zealand rabbit which were highly inbred for use in research and he stated only 43.8% of rabbits had the classic vertebral configurations.

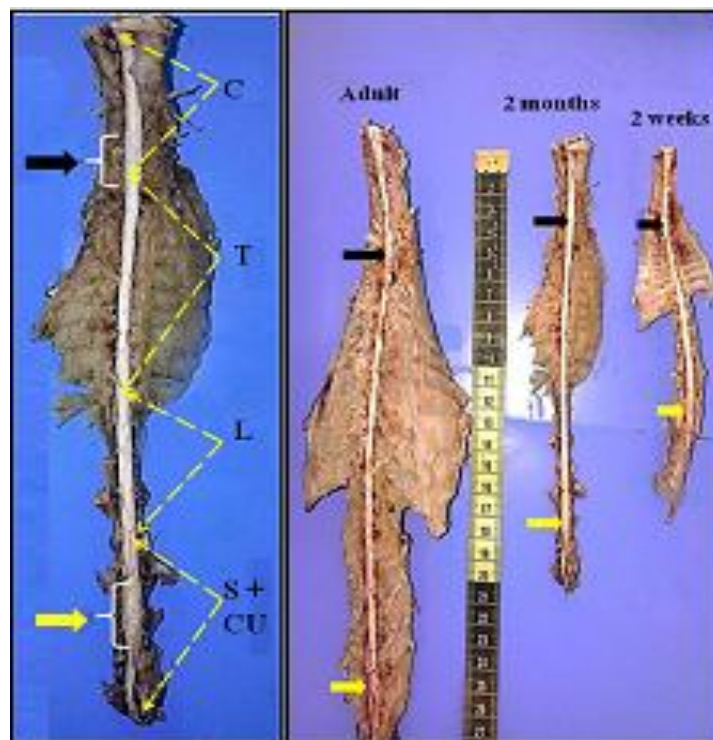


Fig 1: Spinal cords of adult, 2 months and 2 weeks old rabbits showed cervical (C), thoracic (T), lumbar (L) and sacro-caudal (S+CU) segments with the presence of cervical (black arrow) and lumbar (yellow arrow) enlargements

Table 1: It showed the formula of the spinal cord segments of the local rabbit

Formula of spinal cord segments				
C	T	L	S	CU
8	12	7	4	6

Note: C = cervical, T = thoracic, L = lumbar, S = sacral, CU = caudal

Grossly, two distinct enlargements were observed in the spinal cord of all studied rabbits, one in the cervical region and the other in the lumbar region (Fig. 1). In the rabbit of two weeks of age, the cervical enlargement extended from the beginning of the 7th cervical segments to the end of the 2nd thoracic segment. The lumbar enlargement was formed by the last two lumbar segments. In 2 months aged rabbits, the

cervical enlargement found extended from the middle of the 6th cervical segments to the middle of the 2nd thoracic segment and the lumbar enlargement was extending from the middle of 5th lumbar segments to the end of the 7th one. In rabbits of 6 months of age, the cervical enlargement occupied the last 3

cervical segments and extends to the 2nd thoracic segment. The lumbar enlargement was extended from the beginning of 5th lumbar segments to the end of the 1st sacral segment (Table 2).

Table 2: Regions of enlargements in the spinal cord of local rabbits

Locations of spinal cord enlargements			
Segments	2 weeks of age	2 months of age	adults
Cervical enlargement	Last 2 C + 1 st & 2 nd T	Last 3 C + 1 st and middle of 2 nd T	Last 3 C + 1 st , 2 nd T
Lumbar enlargement	6 th + 7 th L	Middle of 5 th + 6 th + 7 th L	5 th , 6 th , 7 th L + 1 st S

Note: C = cervical, T = thoracic, L = lumbar, S = sacral

In fact, [15] recorded in the New Zealand rabbit, L4, L5 and L6 to form the enlargement if the spinal cord possessed 12 thoracic and 7 lumbar segments but changed into L3, L4 and L5 in spinal cord having 13 thoracic and 6 lumbar. In other species such as donkey, such region was formed between second lumbar and first sacral [6], between six lumbar and first sacral in camel [16], between the last three lumbar and first two sacral in buffalo [7], between fourth lumbar and first sacral in sheep [17], between sixth lumbar and seventh lumbar in pig and fourth lumbar and first sacral in the dog [18].

Differently from present findings, [14] found in adult Egyptian rabbits that cervical enlargement occupied the vertebral canal from the caudal half of the fourth cervical vertebra to the first thoracic one whereas the lumbar enlargement extended between the caudal one - fourth of the fourth lumbar vertebrae and caudal three-fourths of the first sacral one. On the same region in pig's spinal cord it was formed between 7th cervical

and 8th cervical [18], in the dog's spinal cord between 6th cervical and first thoracic [19] in the buffalo between 6th cervical and 2nd thoracic [7], and between 5th cervical and 2nd thoracic in donkey [6], camel [16] and Indian sheep [17].

The data showed that cervical one was constructed from cervical and thoracic segments whereas; the lumbar was made from lumbar segments only. The cervical enlargements in all studied ages of rabbits were greater than those of the lumbar. The length of cervical enlargement was shorter than that of lumbar but in contrary the recorded diameter was greater to cervical than that of the lumbar enlargement (Tables 3 and 4). Similarly, [14] also stated that in adult rabbit the lumbar enlargement was longer and more voluminous than the cervical one; while [7] and [16] were observe in buffalo camel, respectively a controversial opinion as the cervical enlargement was longer than the lumbar one.

Table 3: Length and percentage of the length of each segment (cm) compared to the total length of the spinal cord in local rabbits

	2 weeks	2 months	adults
Cervical	2.86±0.011 (17.83%)	3.20±0.082 (13.72%)	4.80±0.047 (13.52%)
Thoracic	4.17±0.96 (25.99%)	6.81±0.96 (29.21%)	9.50±0.81 (26.76%)
Lumbar	5.81±0.091 (36.22%)	7.30±0.82 (31.31%)	12.20±0.86 (34.36%)
Sacro-caudal	2.70± 0.052 (16.83%)	4.50±0.086 19.30%)	6.00±0.75 (16.90%)
Total	16.04±0.94	23.31±0.99	35.50±1.07
Cervical enlargement	1.7±0.019	2.4±0.026	3.8±0.015
Lumbar enlargement	2.1±0.033	3.5±0.065	5.2±0.12

Table 4: Diameters of each segment of the spinal cord of different aged local rabbits

Gross transverse diameter of each segment in the spinal cord (mm)			
	2 weeks	2 months	adults
Cervical	4.49±0.033	4.58±0.088	5.25±0.045
Cervical enlargement	4.93±0.078	5.99±0.079	6.51±0.085
Thoracic	3.50±0.013	3.88±0.035	4.40±0.071
Lumbar	3.97±0.077	4.08±0.012	5.31±0.054
Lumbar enlargement	4.62±0.098	5.91±0.056	6.19±0.069
Sacro-caudal	2.40±0.014	2.86±0.022	3.75±0.013

Morphometrical measurements such as length and diameters of each segment of the spinal cord were well listed in Tables 3 and 4. The data of 2 weeks aged rabbits showed that the lumbar segment was the longest one (36.22% from total length of spinal cord) compared to the other segments of the spinal cord, whereas, the sacro-caudal segment was the shortest (16.83%). Thoracic segment was distinctly longer than cervical and sacro-caudal segments (25.99%) but still shorter compared to the lumbar one. Similarly, measurements in 2 months aged rabbits showed

that the lumbar segment remained the longest (31.31%) whereas; the cervical segment was the shortest in length (13.72%). The thoracic segment was lesser slightly compared to that of the lumbar segment (29.21%). Sacro-caudal segment was slightly higher than that of the cervical one (19.30%). In fact all segments were increased in length but it appeared poorly changed in cervical region, whereas, that of thoracic was well increased to be as that of the lumbar segment. In adults, the data revealed non distinct changes in both cervical (13.52%) and sacro-caudal (16.90%) segments.

In contrary, the lumbar and for lesser degree the thoracic were increased prominently (34.36%, 26.76%, respectively).

Characteristically, the data of table 3 explored elevated lengths of all segments of the spinal cord during the period extended between suckling (2 weeks of age) and post weaning (2 months of age) of these animals. In fact such period represents an important time in the life of the rabbit in which the animal feeding may include greenish and solid food. Such changes may satisfy and be parallel to the body requirements and organ development. This postulation was in agreement with previous records of [20] and [21]. The highest changes in spinal cord segments during this period were clearly observed due to the demand and development of the other body organs. Concerning the length of segments in different regions in the local rabbit, present findings agreed with those recorded by [14] in rabbit where they stated longest segment in the lumbar region. Differently, the longest segment was 3rd cervical in goat [22], buffalo [7] and donkey [23] or at 5th cervical in camel [16]. The present measurements reported that the average length of the spinal cord in adult local rabbits was about 35.5 cm. However, [9] and [14] recorded 39.5 cm and 34.7 cm, respectively in the same animal. [24] stated the total length of the spinal cord of Indian grey mongoose was 29.2 and 24.4 cm in males and females, respectively.

Measurements of transverse diameters were different in different segments of the spinal cord. Current records found that it has greater diameter in the cervical compared to those of other segments in all studied ages (4.49 ± 0.033 mm, 4.58 ± 0.088 mm and 5.25 ± 0.045 mm, in 2 weeks, 2 months and adults, respectively). In contrary it has lesser diameter in sacro-caudal segment which was 2.40 ± 0.014 mm, 2.86 ± 0.022 mm and 3.75 ± 0.013 mm in the same ages. The diameters of both thoracic (3.50 ± 0.013 mm, 3.88 ± 0.035 mm and 4.40 ± 0.071 mm) and lumbar (3.97 ± 0.077 mm, 4.08 ± 0.012 mm and 5.31 ± 0.054 mm) were nearly same but were lesser than those of the cervical segment.

3.2. Histological findings

Microscopic examination revealed that the spinal cord was bilateral symmetrical segmental structure. In general, its histological picture indicates the presence of two main elements that were grey matter and white matter. The grey matter which included bodies of different sized and types of neurons that were gathered in a butter-fly shaped structure of two dorsal horns and two other ventral horns in addition to the two intermediate lateral horns with a central canal located in the central point of this matter. The white matter forms the bulk part which surrounds the grey matter (Fig. 2). [25] mentioned that neurons tend to concentrate in these three main regions of grey matter mentioned above and current description of this matter on the spinal cord of local rabbit was similar to those described for other mammalian species such as rat [26-28], hamster [29], humans [30] and rabbit [31]. The butter-fly shape of grey matter and nerve cell existence and distribution in the local rabbit's spinal cord was in agreement with previous references [32].

In all studied ages, the sections of the spinal cord segments showed thinner dorsal horns compared to the ventral horns and they were extended up to reach the dorsal border of the spinal cord whereas the ventral horns were wider and shorter and they did not extend to reach the ventral border of the spinal cord. In cross sections, a dorsal sulcus and ventral

fissure were observed which were extended toward the central canal from the midline of the dorsal and ventral surfaces, respectively (Fig. 2). The relatively thicker and wider ventral horn than the dorsal one of the grey matter may be due to its coordination of motor neurons [33].

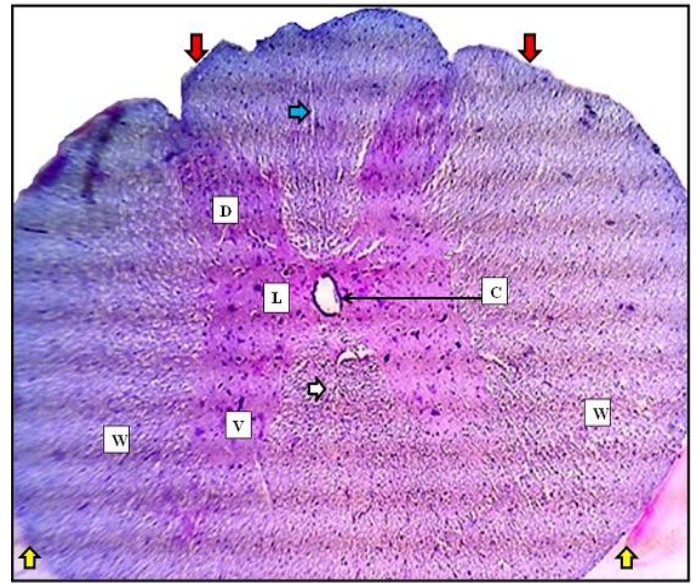


Fig 2: Cross section in the spinal cord at thoracic segment of 2 month aged rabbit showed dorsal (D), lateral (L) and ventral horns of the grey matter, central canal (C), white matter (W), dorsal surface (red arrows), ventral surface (yellow arrows), dorsal sulcus (blue arrow) and ventral fissure (white arrow). H&E stain, X40

Grey matter consists of different sized neurons, neuralgia and neuropils. The neurons were distributed all over the grey matter of both dorsal and ventral horns but with different number and sizes. Large neurons were the come type present in the ventral horns (for most segments) were a large and multipolar type with a diameter ranging between 25 to 29 μ m. However, with exception noticed in that of sacral segment where the small neurons were dominant in the ventral horns. These large neurons possessed pale central nuclei with prominent darkly stained nucleoli and their cytoplasm was fully filled with darkly stained blue granules (Nissl's granules). Other fusiform middle sized and small unipolar neurons were found in the central part of the ventral horns (17-24 μ m and 12-16 μ m, respectively) in contrast to the large neurons which located mainly at the periphery of ventral horns. Large neurons gave rise long processes or nerve fibers which extend within the white matter to form what is called the tracts (Table 5) (Fig. 3).

The current findings revealed the presence of two populations of neuralgia that were distributed all over the ventral horns which were the dark and light glial cells. The dark one was about 5 μ m in diameter located mainly near or around the neurons, while the light glial cells were observed among the clusters of neurons with mean diameter of 7 μ m (Fig. 3).

Neuropil, which was a framework area of unmyelinated nerve fibers of neurons in addition to the processes of glial cells located in the grey matter and appeared devoid of cells (Fig. 3). In fact previously was well known that the neuropil is more reactive in the dorsal horn of the cervical and lumbar enlargements, but not the thoracic segment [34].

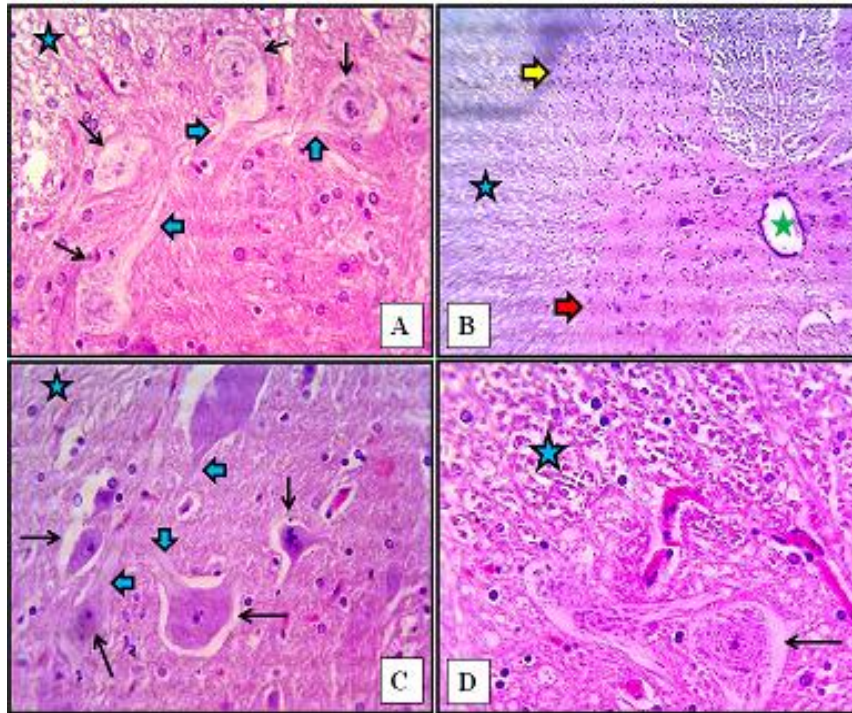


Fig 3: Ventral horns of the spinal cords of local rabbit. A: (2 weeks, cervical), B: (2 months, thoracic), C: (2 months, 1st enlargement and D: (adult, 2nd enlargement). It showed neurons (black arrows), tracts (blue arrows), central canal (green star), ventral horn (red arrow), dorsal horn (yellow arrow) and neuropil (blue stars). H&E stain. A, C, D: X400, B: X100

Table 5: Number of cells present in the grey matter of each segment of the spinal cord in adult local rabbits

Segment	parameters	Large sized neurons 25-29µm	Middle sized neurons 17-24 µm	Small sized neurons 12-16 µm	Total neurons	Glial cells
C	Dorsal horn	0	75	475	550	1200
	Ventral horn	175	150	100	425	1000
	Lateral horn	25	325	100	450	1125
Total					1425	3325
1 st	Dorsal horn	0	125	50	175	1075
	Ventral horn	150	100	100	350	875
	Lateral horn	25	100	100	225	1025
Total					750	2975
T	Dorsal horn	0	25	300	325	975
	Ventral horn	125	50	75	250	575
	Lateral horn	25	150	75	250	875
Total					825	2425
L	Dorsal horn	0	50	125	175	1125
	Ventral horn	175	25	50	250	725
	Lateral horn	25	25	50	100	575
Total					525	2425
2 nd	Dorsal horn	0	75	25	100	775
	Ventral horn	200	125	125	450	1000
	Lateral horn	50	75	125	250	1125
Total					800	2900
S	Dorsal horn	0	25	100	125	875
	Ventral horn	50	25	75	150	575
	Lateral horn	0	75	50	125	775
Total					400	2225

In contrast to the cells of ventral horns, most of neurons of dorsal horns were middle sized and small rounded neurons with mean the diameter of 17 to 24 µm and 12 to 16 µm, respectively. They have spherical light nuclei and prominent darkly stained nucleoli. Their cytoplasm contains a few number of Nissl's granules but some of the cells were devoid of them. The areas occupied by neuropils in the dorsal horns were more extensive than those in the ventral horns (Table 5)

(Fig 4 A-B).

Middle sized and small neurons in addition to fewer numbers of large types were detected in the lateral horns around the central canal supported with light and dark neuralgia. Interestingly in lateral horns, the blood vessels were relatively in large number represented by capillaries and small-sized arteries existed around the central canal (Fig 4 C-D).

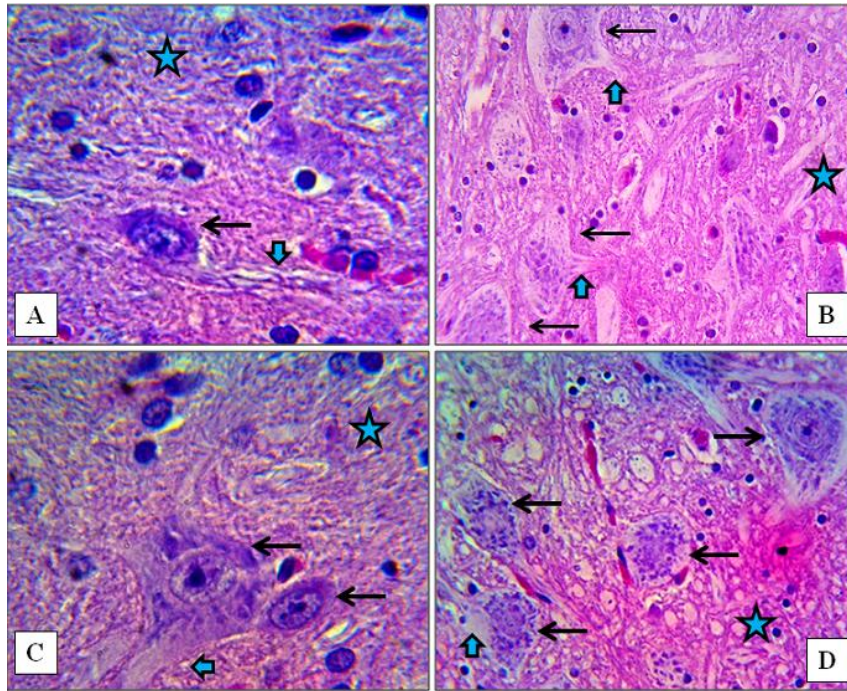


Fig 4: Dorsal horns (A & B) and lateral horns (C & D) of the spinal cords of local rabbit. A: (thoracic region, adult), B: (2nd enlargements, adult), C: (Lumbar, 2 months), D: (adult, 2nd enlargement). It showed neurons (black arrows), tracts (blue arrows) and neuropil (blue stars). H&E stain, A, C & D: X1000, B: X400

The canal was oval in shape in both cervical and thoracic segments but appeared rounded in lumbar and sacral segments. It was lined by high ciliated cuboidal ependyma cells (Fig 2, 3 B).

The data listed in table 5 which was highlighted on the total number of cells (neurons and neuralgia) distributed in the grey matter in different segments in the spinal cord during adulthood period. In fact current records detected no significant changes of these measurements compared to those of other ages (data not shown). Similar observation recorded by [35] whom found at birth, the distribution of motor neurons was similar to that seen in the adult rats. On the same time, in rat all types of cells were detectable in newborn rats, but there was significant intergroup variability in the intensity of their staining during early postnatal period [36]. These observations indicated absence of age effect on the number of neurons in the developing spinal cord in the rabbit postnatally. In general, the table revealed highest number of glial cells compared to the number of neurons in the grey matter of all studied segments of the spinal cord.

Concerning the neurons, it was found that their number in the cervical segment was approximately 2 folds higher than those counted in other segments, whereas, in contrary the number was so low in both lumbar and sacral regions. Current findings confirm those of [37] in the spinal cord of rabbit where the highest number of neurons was found in cervical region.

Analysis of neurons distribution revealed that their number in the dorsal horns (sensory nerve cells) of cervical and thoracic segments were higher than the case in the ventral horns, whereas, the case was in contrary lesser in those of 1st and 2nd enlargements as well as in lumbar and sacral segments. These findings expressed higher expression of sensory cells in the cervical and thoracic regions of the spinal cord and oppositely the motor cells were the higher in number in the other caudal segments. In fact, the rabbit uses its hind limbs for locomotion and more or less to jump and such complex motor acts like these require a greater number of neurons in the ventral horns of the caudal segments of the spinal cord and this postulation

was comparable to other rodents recently published [38]. Differently to the local rabbit, the ventral horn (motor neurons) of rat cervical segments showed an extensive area occupied mainly by large and medium sized motor neurons [39]. However, as in rabbit, the African great rat uses its tail to dig, defend itself and has been reported to stand on it the forelimbs shows high locomotor dexterity. This probably accounts for the ventral horns of lumbosacral regions being more developed than other segments [40].

4. Conclusions

In conclusions, highest changes in spinal cord segments were clearly observed in the period extended between 2 weeks and 2 months of age. The ventral horns of segments in all studied ages were included large-sized neurons whereas the dorsal horns were containing large number of small and middle-sized neurons.

5. Acknowledgment

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