Histological changes in the motoneurons induced at L6 & L7 segments of spinal cord in different age group in rabbits

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Abstract:
The aim of the present study was to observe the tissue changes that occur in motoneurons in the L6 and L7 segments of the ventral horn of the spinal cord in different age groups in rabbits. Fifteen Rabbits were divided into three groups of equal number according to their age (weaning, maturation, Adult). L6 and L7 were removed (after sacrifice the rabbits) from the spinal cord and slice histologically to be examined under light microscope. Comparison was made in diameters of neurons and their numbers in different age groups and the results showed a significant decrease in the number of motor neurons and a significant increase in diameters with advancing age.

Introduction:
During the past years, an increasing amount of interest has been focused on the effects of age on the locomotor system, the mechanisms underlying this so-called "aging atrophy," [1]. Evidence now exists that one of the major factors leading to the structural and functional changes in human muscle with aging is the progressive degeneration of the nervous system. Alterations similar to those observed in humans also take place in the motor unit of other mammals[2]. Verdu et al[3] were clarified that aging deeply influences several morphological and functional feature of the peripheral nervous system. Morphologic studies have reported a loss of myelinated and unmyelinated nerve fibers in elderly subjects, and several abnormalities involving myelinated fibers, such as demyelination , remyelination and myelin balloon figures. Aging also affected functional and electrophysiologic properties of the peripheral nervous system including a decline in nerve conduction velocity, muscle strength, sensory discrimination, autonomic responses. The present study was designated to evaluate the changes occur in the momtoneurons of spinal cord at L6 & L7 levels in different age groups in Rabbits.

Meterials & Methods:
Total number of rabbits used in this study was fifteen. The rabbits divided into three groups [five in each] according to their ages:

1- Weaning group [W]: Their ages range between 1 -2.5 months[4].

2- Maturation group [M]: Their ages range between 5-8 months.[5].

3- Adult group [A]: Their ages range between 12-18 months. [6].

All animals were sacrificed after using overdose inhalation of chloroform inside plastic jar. Each animal was laid on its dorsal surface and dissected by making a longitudinal incision in the midline of its ventral surface. All the abdominal and pelvic viscera were removed. The Psoas major muscles were removed to expose underlying part of vertebral column The pedicles of the lower 3 lumbar vertebrae and upper 2 sacral were dissected. The bodies of vertebral column were removed to expose the underlying spinal cord [7] . Removal of lumbar segment of spinal cord from its place in the vertebral canal was manipulated by separating it from the remains part of spinal cord.

Preparation of tissues for light microscopy: The lumbar segment was removed as one part and immersed in 10% formalin for 24hs, then dehydrated in graded alcohols [30%, 50%, 70%, 90% and 100%] 1/2h for each, cleared in xylene, and finally embedded in paraffin wax. After removing the tissue from paraffin path and before blocking, separation of L6 segment from L7 segment of spinal cord was made.

The procedure was made under vision of surgical loupe. The posterior limit of each segment was detected by emergence of posterior fibers of both ventral and dorsal roots of spinal nerve corresponding to the segment. The anterior limit was detected by the emergence of the posterior fibers of both ventral and dorsal roots of the spinal nerve of the segment infront [8]. After blocking, sections were made using rotary microtome. Dewax the sections in xylene. Rehydrated the sections through graded alcohol [100%, 90%, 50%, 30%] and then in distal water 5min for each, staining with H&E, dehydration, cleared in xylene, and mounting by Canada balsam.

The number of motoneurons in the spinal cord were counted in 10 microscopical field at objective lens [40X] in both right and left sides. The diameter of 20 motoneurons were measured at objective lens [40X] multiplied by factor[2.5], in both right and left sides. Morphometric data were taken only from those neuronal somas that showed a delineated shape and a distinguishable nucleus [9]. Comparison was made among the three age groups and evaluated statistically.

The statistical analysis of the experiment results were performed using the general linear model within a ready statistical program (SAS) and Duncan test was performed to determine significant differences between averages at a probability level $p < 0.05$ [10].

Results:
In the weaning groups, most motoneurons aggregated in groups and few scattered throughout the ventral horn of spinal cord [fig 1]. most motoneurons intensely stained by H&E and it was difficult to

recognize Nissl granules in the cytoplasm , while in the maturation and adult groups the staining of neurons were normal and Nissl granules appeared clearly [fig 2].

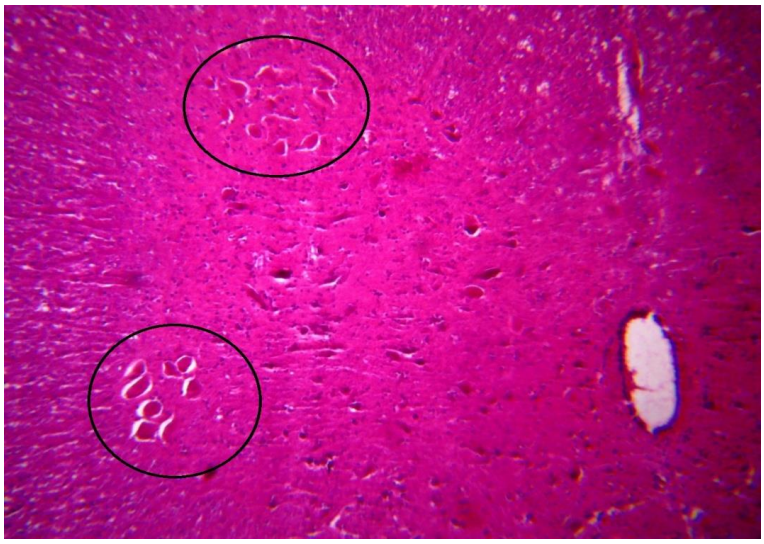

Fig [1]: Weaning group. Motoneurons aggregated in groups [circle] and darkly stained with H&E. 100X.

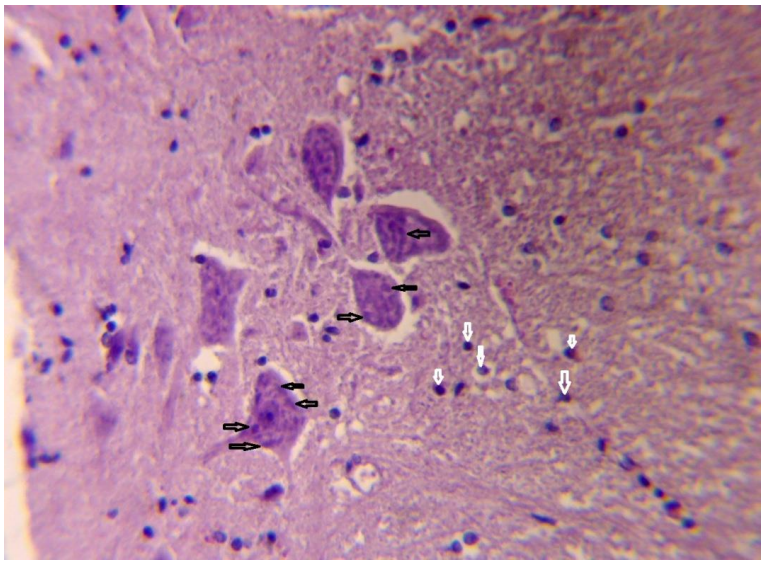

Fig [2]: Maturation group. Motoneurons at L6 segment of spinal cord, Nissl granules [black arrows]and nucleus of glial cells [white arrows]. [H&E] 400X.

The number of motoneurons in the ventral horn at L6 segment was 16.65 and 16.00 in the right and left sides respectively in the weaning group and 12.81 in the right and 13.76 in the left side in the maturation group and reduced to 10.99 and 10.95 in the right side and left side respectively in the adult group. Table [1] fig [3]

Table [2] revealed the number of motoneurons in the ventral horn at L7 segment which was 15.74 and 15.70 in the right and left sides respectively in the weaning group and was 12.04 and 12.18 respectively in maturation group and 11.40 in the right side and 11.13 in the left side in adult group. Fig [4]

The results clarified that the number of motoneurons was significantly decreased with advancing age in both L6 and L7 segments of spinal cord.

Concerning the diameter of motoneurons, table [3] showing and comparing the mean values of the three groups at L6 segment, which was 22.69µm in the right side and 22.35 µm in the left side in case of weaning group and 23.29 µm and 23.18 respectively in the maturation group. In the adult group the values were 25.32 and 25.54 µm respectively. Fig [5]

At the level of L7 segment of spinal cord, the diameter of motoneurons in the right and left sides were [24.06 and 23.85 µm] in the weaning group, [25.62 and 24.96 µm] in the maturation group and [28.56 and 27.85 µm] in the adult group respectively. Table [4], fig [6].

The diameter of motoneurons was significantly increased with advancing age in both L6 and L7 segments of spinal cord.

Table [1] Mean and ±SE of motoneurons number in L6 segment of spinal cord at weaning, maturation and adult ages

Parts	Cells Number (Mean±SE)									P<0.05
	W			M			A			
Right	16.65	±	0.75	12.81	±	0.24	10.99	±	0.11	*
Left	16.00	±	0.62	13.76	±	0.21	10.95	±	0.13	*

Table [2] Mean and ±SE of motoneurons number in L7 segment of spinal cord at weaning, maturation and adult ages

Parts	Cells Number (Mean±SE)									P<0.05
	W			M			A			
Right	15.74	±	0.65	12.04	±	0.42	11.40	±	0.08	*
Left	15.70	±	0.70	12.18	±	0.33	11.13	±	0.10	*

Table [3] Mean and ±SE of motoneurons diameter in L6 segment of spinal cord at weaning, maturation and adult ages

Parts	Cell Diameter [µm] (Mean±SE)									P<0.05
	W			M			A			
Right	22.69	±	1.42	23.29	±	1.22	25.32	±	1.08	*
Left	22.35	±	1.40	23.18	±	1.19	25.54	±	1.11	*

Table [4] Mean and ±SE of motoneurons diameter in L7 segment of spinal cord at weaning, maturation and adult ages

Parts	Cell Diameter [µm] (Mean±SE)									P<0.05
	W			M			A			
Right	24.06	±	1.60	25.62	±	1.42	28.56	±	1.25	*
Left	23.85	±	1.12	24.96	±	1.41	27.85	±	1.83	*

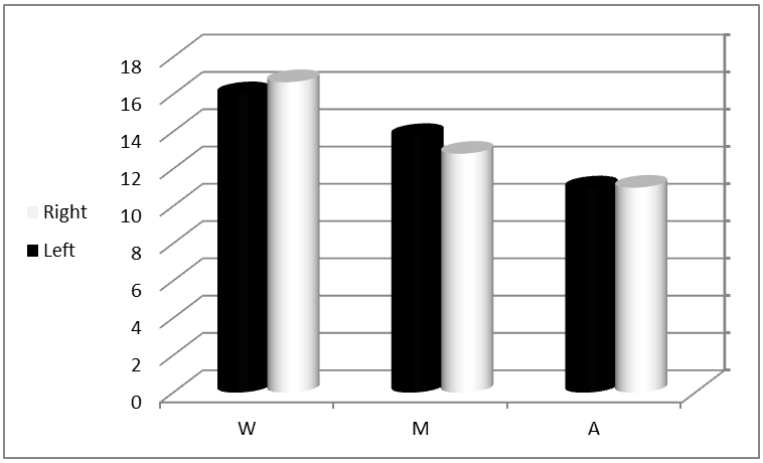

Fig [3]: motoneurons number in L6 segment of spinal cord at weaning, maturation and adult ages

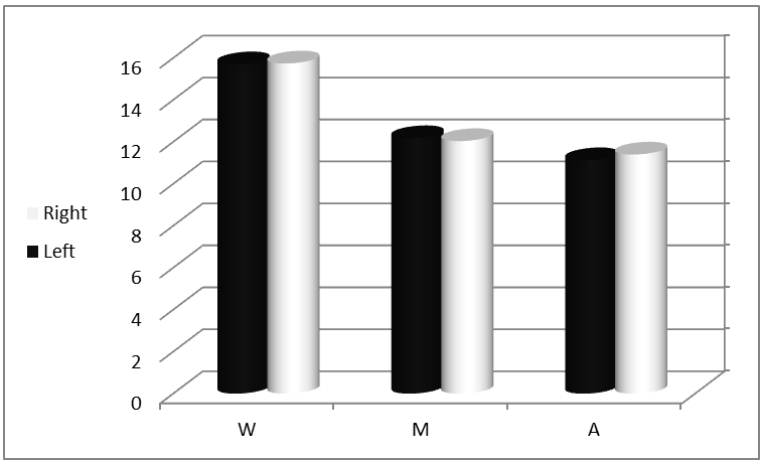

Fig [4]: motoneurons number in L7 segment of spinal cord at weaning, maturation and adult ages

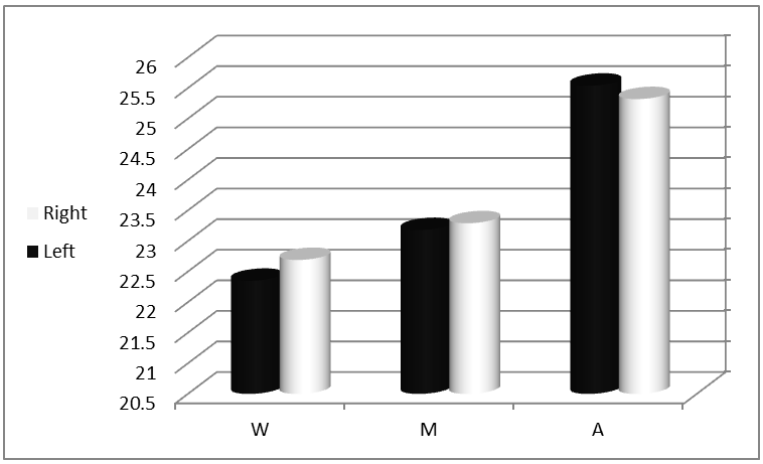

Fig [5]: motoneurons diameter in L6 segment of spinal cord at weaning, maturation and adult ages

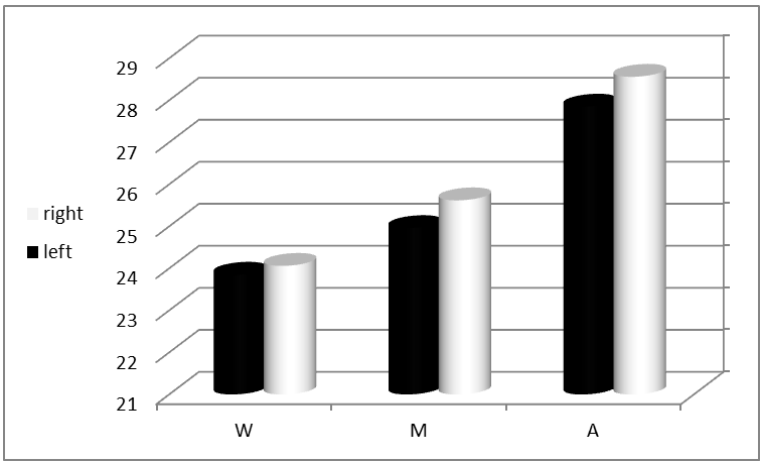

Fig [6]: motoneurons diameter in L7 segment of spinal cord at weaning, maturation and adult ages

Discussion:

Ageing (British English) or aging (American English) is the accumulation of changes over time [11]. Skeletal muscle undergoes major structural and functional changes with advancing age. A progressive degeneration of the nervous system is now considered a major factor underlying these alterations [12]. In the present study the number and diameter of motoneurons in the ventral horn of spinal cord at L6 and L7 were compared among different age groups. The results clarified a significant decrease in number of motoneurons in both L6 and L7 segments of spinal cord with advancing age. While the diameter of motoneurons were increased significantly with advancing age.

Motor neuron death occurs naturally during early embryonic development, apparently as a result of failure in a competition between motor neurons for a limited supply of putative trophic factors derived from muscle[13] [Neurotrophins belong to a family of

growth and trophic factors that function during development to promote axonal growth and neuronal survival][14]. In rats and mice, although this naturally occurring death is largely complete at birth, the survival of motor neurons continues to be dependent on support from the muscle for some time.[13] So, Motor neurons require neurotrophic factor(s) for their survival during development and for maintenance of function in adulthood. In vivo studies have shown that motor neurons respond to a variety of molecules, including ciliary neurotrophic factor, members of the neurotrophin family, and the insulin growth factor IGF-1] [15]

Ptacek & Dubin [16] were observed postnatal changes in neuron cell body diameter and neuron density in newborn, two-week old, and eight-week old golden hamsters. Quantitative data indicated that in both the visual cortex and superior colliculus, neuron cell body diameter increased nearly twofold during the first two-week period following birth and reached maximum size by approximately day 14. Neuronal density was highest in the newborn and decreased with age in both visual centers. The decreased neuronal density and, thus, increased neuropil area may reflect a greater number of synaptic connections mediating visual function.

Another study was made by Baulac & Meininger[17] on the motoneurons of sciatic nerve in the lumbar segment of spinal cord of mouse at various postnatal stages, 1-3 days, 6-7 days, three weeks, and 2-3 months. Horseradish peroxidase (HRP) was applied at the cut end of the distal sciatic nerve, and, after retrograde transport, transversal and longitudinal histological sections of the cord were made in order to compare various features of the motor pool such as cell number and cell size. Cells volume increase and a 31% decrease in the number of labeled cells was observed between birth and 2-3 months. The rate of cell death was not constant, since 17% disappeared during the first week and 14% thereafter. The 31% decrease was lower than that observed in previous studies in the rat, 50-80%. The difference in species may explain this discrepancy. The previous studies were in agreement with the present study.

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التغيرات النسيجية في العصبونات الحركية حفزت في القطع ل6 و ل7 من الحبل الشوكي في فئات عمرية في الارانب

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الملخص

الهدف من هذه الدراسة الحالية هو ملاحظة التغيرات النسيجية التي تحدث في العصبونات الحركية الموجودة في القطع ل6 ول7 في القرن البطني للحبل الشوكي في فئات عمرية مختلفة في الارانب. قُسم 15 ارنب الى ثلاث مجاميع متساوية بالعدد حسب العمر(فطام, نضوج, بلوغ). رُفِعت القطع ل6 و ل7 (بعد التضحية بالأرانب) من الحبل الشوكي وقُطِعت نسيجيا لغرض فحصها بالمجهر الضوئي. قورنت اقطار العصبونات واعدادها في الفئات العمرية المختلفة وأظهرت النتائج نقصان معنوي في عدد العصبونات الحركية وزيادة معنوية في اقطارها مع التقدم بالعمر.