INTRACELLULAR INCLUSIONS
IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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We examined two families which developed amyotrophic lateral sclerosis in two generations. In one of the families, the mother and two daughters suffered, while in the other family, the father and daughter did (for greater details, please be referred to Korsakov Neuropathology & Psychiatry Magazine, 1960, issue 9). Later, we were able to conduct a morphological examination of the central nervous system of the deceased members of the families (patient P from the first family and patient M from the second family). In addition to the damage typical of amyotrophic lateral sclerosis, intracellular inclusions were discovered which were of interest.

The morphological changes were identical in both cases except for a slightly more acute process in one of them. For that reason, below are given the results of microscopic investigation of the spinal cord and the cerebrum of the case P only.

Patient P, 60 years old, was observed by the Neurology Institute from March 10th to October 16th, 1955. She became sick in July 1954 when she suffered fever heat, catarrhal signs, puffiness in right facial side, neck and shoulder for several days. Shortly after, she developed speech and deglutition disorders, then also numbness and inertia of the right hand. Speech worsened quickly and 5 months later, the patient stopped speaking, could hardly swallow, had excessive salination and inertia in left hand and legs. Legs and hand palsy worsened rapidly, and a year later, the patient became physically immobile.

By then, the patient was emaciated, while the overall physical deterioration was followed by muscle atrophy in the neck, thoracic girdle, hands, chest, and back, while tonus in leg muscles was high, and amyotrophia was relatively mild. Aphonia and anarthria were observed. Mouth half-open. Soft palate hung down, tongue was akinetic and had atrophy and fibrillation, the patient could swallow only water, suffocating. Bulbar disorders intensified and breathing malfunctioned in the subsequent months. The patient died on October 16th, 1955.

Spinal cord. The dura mater thickened with some areas goffered or defibered and rarely hyalinized. Proliferation of cellular elements closer to the subdural space. Dilated veins often with expressed perivascular infiltrates.

The pia mater is thickened and sclerosed, represented by gelatinous fibers with some fibers glued together; having unstructured appearance in some areas; abundant with cells forming focal polymorphic conglomerates where lymphocytes, histiocytes, and single segmental leukocytes were found. The pia mater is especially thickened around the roots where it was hyalinized and tightly integrated with the roots which made them appear heavily covered up. Mostly larger infiltrates are found here. The pia mater veins are varicose, sclerosed and with perivascular infiltrates.

Ventral roots are expressly deformed and have cellular structure due to numerous cavities; most of them are sharply sclerosed and with small foci of demyelinated nerve fibers. Some roots have substantial focal conglomerates of lymphoid elements. Veins are varicose, sclerosed, often with expressed perivascular infiltrates.

Rear roots are much less damaged but also have demyelination and sclerosis foci found.

Anterior horns are atrophied along the entire spinal cord. Very distinct dropouts of larger motor cells. In the cervical, their quantity is very insignificant, while utterly devastated in the thoracic segment and relatively many
cells in dorsolumbar segments. Nearly all surviving motor cells suffered major changes and hardly any retained a normal appearance. Most cells are small in size with thin elongated bodies and sulcated, sometimes fragmented process. Some cells appear somewhat puffed and orbited, while their nuclei are often deformed, and relocated towards outside or even protruded outside the cell; nucleoli are pyknotic, often with 1-3 vacuoles. Tigroid mass is degenerate in many cells, and whenever tigroid mass is preserved, it is located peripherally in rough clods; protoplasm has much lipofuscin, sometimes larger vacuoles. It is observed that the deformed cells are surrounded with expressed glial reaction with astrocyte nodules in places of dead cells.

Pericellular spaces are sharply widened.

The quantity of cells in the lateral horns and Clarke’s columns slightly decreased, but many of them are deformed – without processes, withered, having tigrolysis and ectopia of nucleus, while protoplasm is overloaded with lipofuscin.

Along the entire spinal cord, there is a rough demyelination in the anterior and especially the lateral columns. Sudan staining revealed that myelin sheaths in the lateral columns are filled with chromophanes. Similar though less expressed changes are also observed in the anterior columns, while no fat was found in the posterior columns. At demyelination areas with greater rarefaction, individual granular balls, astrocytes in large quantities (including gemistocytes), and frequent glial nodules are found.

Veins are distended, sanguine and sclerosed both in white and grey matter of the spinal cord. In addition to the sclerotic changes in veins, walls are swollen too. Homogenous substance fills the lumen in some of the smaller veins (hyaline thrombi). Expressed perivascular reactions in the form of lymphoid conglomerates were noted, occasionally forming relatively large infiltrations. Some veins have traces of vasculitis, such as decondensation of walls and significant proliferation of wall elements, which makes it impossible to identify wall structure and vein lumen. These changes are found more frequently in the anterior and lateral columns of the spinal cord and are more expressed in the cervical and the thoracic part.

Small hemorrhages in white and grey matter.

Cerebrum. The cerebrum substance is hydropic with sharply widened perivascular and pericellular spaces and agglutinated capillaries. At the molecular layer surface, the cortex has rarified cerebrum substance and fibrous astrocytes in large quantities, which proportionally increase against rarefaction level.

The pia mater is unevenly sclerosed, while some areas reveal substantial proliferation of cellular elements. The pia mater veins are varicose and sclerosed, while some were wrapped in lymphoid elements; changes in vascular walls are frequent, which may be considered as vasculitis. Veins are sanguineous. Subarachnoidal hemorrhages were observed in limited numbers.

Similar changes are found in veins of white and gray matter, and those are more expressed in the pons Varolii and the rachidian bulb.

Changes are diffuse in cerebral cortex. Those manifest themselves in dropout of ganglion cells, their abnormal location and shape, smaller size of the cells, tigrolysis, excessive lipofuscin deposits with ectopia and darker cellular color. Cells in the acute swelling stage are found too. The motor unit was especially damaged, mostly layers III and V, where numerous desolate fields were found. Only individual Betz cells survived in irregular polygon shape and rarely in circular shape; some cells are withered while others are acutely swollen. Shadow cells are frequently observed. Sharply expressed satellitosis and neuronophagy; glial elements in places of dead cells.

Caudate and lenticular nuclei are marked by dropout of larger cells, while remaining cells are vacuolated and swollen, having ectopia of nucleus and pyknosis of nucleolus; and shadow cells are not uncommon. Sharply expressed glial reaction, and frequent true neuronophagia. Glial nodules are seen in places of dead cells. Most smaller cells are pyknotic. More severe changes are observed in caudate nuclei.

No significant changes in nuclei of optic thalamus. Still, it needs to be mentioned that cells are overloaded with lipofuscin, shadow cells are found, and moderate glial reactions are observed with formation of nodules.

Cerebellum. Uneven distribution of Purkinje cells. Some areas have but individual cells; those are in the swelling stage, withered, sometimes enlarged, pale, and void of nuclei. Macroglia proliferation in dropout spots. Same changes are found in the cells of the dentate nucleus, though expressed milder.

Scapus. Significant changes in the nerve cell of the red nucleus; withering, sharp swelling, tigrolysis, ectopia of nucleus and pyknosis of nucleolus. Satellitosis.

Lesser cell dropouts are found in the substantia nigra; the quantity of pigment is decreased. Many atrophied cells.
Image 1. Oxyphyle Inclusions in Motor Cell Protoplasm

\(\alpha\) – Cells of the anterior horn of the spinal cord (L\(_2\)), hematoxylin-eosin staining, immersion.
\(\beta\) – Cells of the sublingual nerve, Mann staining, immersion.
\(\gamma\) – Motor cell of the anterior horn of the spinal cord (L\(_1\)), Unna staining, immersion.
\(\delta\) – Motor cell of the anterior horn of the spinal cord (L\(_1\)), Romanovsky-Gimza staining, immersion.
Image 1. X-ray of skull of the patient F.
Calcification Area
Spinal bulb and pons Varolii are examined not entirely (parts of the material are taken for virologic analysis).

Among nuclei of the cranial nerves, the nuclei of the sublingual facial nerve and the trigeminal nerve nucleus were damaged the most. Barely anything left of those nuclei but individual cells where changes occurred in different times. Remains of some cells are represented merely by shapeless protoplasm blobs. Several darker cells are found with agglutinated nuclei – the nuclear membrane is dark blue, while the nuclei are approximately three times larger and surrounded by glossy pale rims. There are cells with expressed tigrolysis, overloaded with lipofuscin, having ectopia of nucleus and pyknosis of nucleolus. Glial multiplication and neuronophagia in places of dead cells.

The sensitive nucleus of the trigeminal and the dorsal nucleus of vagus nerve were less damaged. Insignificant cell dropouts though their dystrophic changes are distinct (withering, swelling, tigrolysis), with expressed glial reaction and neuronophagia founds.

Smaller changes in the cells of the nucleus of the oculomotor nerve – much lipofuscin; withered and swollen cells are rarely found.

Nuclei of the trochlear and abducens nerves are relatively undamaged.

Decreased quantity of cells in olivas, those are smaller in size and overloaded with lipofuscin. Many areas are void of cells and have significant glial multiplications.

In white cerebral matter, pyramidal fibers are found to be severely damaged. The anterior limb of the internal capsule has no significant changes (smaller demyelination foci). Significant changes are observed in the posterior limb, where the internal capsule resembles a grid plate with cavities of irregular shapes. At the same time, in some areas, the demyelination process is at the initial stage – as individual fibers are undistinguishable and integrated into a shapeless mass of granular homogenous appearance. The destruction process of the myelin fibers is followed by expressed proliferation of oligodendria and astrocyte cells.
Especially rough changes are found in pyramidal fascicles of white matter in the pons Varolii, with an enormous number of cavities and entire fields of dead fibers. Similar changes are noticed in the middle and superior cerebellar peduncles as well as in the area of the fasciculus longitudinalis dorsalis. Sharply expressed demyelination in the roots of the facial and trifacial nerves, which are appearing as glomerular mass with few surviving fibers. Such fibers are roughly deformed (uneven width, numerous varicose swellings, deformation). Sclerosis in places of dead myelin fibers. Diffuse glial reactions are observed along the veins and massive polymorphic infiltrates are seen. Own fibers of the pons endured.

It was already mentioned that, in addition to the described damage to the central nervous system, there were peculiar intracellular changes discovered – oxyphyle inclusions found in the motor cells of the anterior horns of the spinal cord and the nuclei of cranial nerves (pair VII and XII). Those were revealed by the methods of Mann, Unna, Romanovsky and Gimza as well as by hematoxylin-eosin staining. The said inclusions are always round and different in size (from 5µ to 20µ). They are stained red by the Unna and Romanovsky-Gimza methods, dark red by the Mann method, and bright pink by hematoxylin-eosin staining (image 1). Usually, they are located on the lighter background, within a sort of halo of rarified cytoplasm. Relatively large individual inclusions (image 1, a) are often found in the cells that retained nuclei and relatively well preserved; groups of 2-3 cells are found sometimes (image 1, b & c; image 2, a-b-c), and groups of 4 cells are found more rarely – in the latter event, those retain same reciprocal positions (image 2, c). Inclusions appear differently in nerve cells that suffered substantial changes, both with and without ectopia of nucleus, loaded with lipofuscin – such inclusions are relatively small (image 1, e; image 2, e).

Image 3. Death of motor cells in the anterior horns of the spinal cord and the nuclei of cranial nerves.

a – Cervical of the spinal cord, anterior horn, death of the motor cells. b – Nucleus of the nerve pair VII. c – Nucleus of the nerve pair XII, thionin staining, 108x zoom
grouping up to 6-8 in number, mostly forming chains with 4-6 links, having about same size (8µ - 10µ). We never found inclusions in withered cells.

What is the nature of these inclusions? Could they be cell organelles? The latter circumstance is not likely as the inclusions had been found by special methods designed to identify inclusions, while their shape, size and reciprocal position are always constant; they have a distinct silhouette, and usually surrounded by light-colored halo, thus being sharply separated from protoplasm.

Intracellular inclusions are described for viral diseases of the central nervous system. Some researchers consider them to be true viruses or microcolonies of viral particles, while others refer to them as to manifestations of pathologic response on the part of cells against viral intrusion.

The presence of inclusions in the cases of our research is of a special interest since those are familial cases with “inherited” amyotrophic lateral sclerosis.

The patient P (from the first family) fell sick when her body temperature grew to 39°C within several days, followed by disorders of speech and swallowing. The sickness progressed quickly. The patient died 1 years and 2 months later.

The patient M (from the second family) fell sick with similar disorders of speech, which progressed quickly in subsequent 6 months. Then swallowing became difficult, weakness and muscle atrophy occurred in hands, walking faculty disrupted. Mobility decreased gradually which lead to utter immobility. Her physical condition steadily worsened, speech disorders developed into anarthria, the patient could not swallow and was connected to nutritional

Image 4. Degeneration of Pyramidal Tracts

a – Cervical of the spinal cord, degeneration of anterior and lateral columns, myelin staining (Spielmeyer's method).  

b – Cervical of the spinal cord, degeneration of anterior and lateral columns, fat staining.

c – Demyelination focus in rear thigh of the internal capsule, myelin staining (Spielmeyer's method).
support. During her last months, breathing disorders became sharp, which resulted in death 1½ years after the illness began.

Thus, both cases were characterized by an acute onset and rapid progress of the sickness. The process steadily spread from a limited area (spinal bulb) into the entire central nervous system with dominant damage to the motor neurons, while genetic degenerative diseases are known to be characterized by slow, so called positive [доброкачественный] course of progress.

Pathomorphological examination of the spinal cord and the cerebrum revealed the typical picture of this disease where the anterior horns are severely damaged (image 3, а) at all levels of the spinal cord, accompanied by devolution of the pyramidal tracts (image 4, а-б-в) and by death of nuclei of the motor cerebral nerves (image 3, б-в).

In addition to the damage of the motor neurons, there was major diffuse damage of the central nervous system observed as well as expressed changes of veins in the form of vasculitides (image 5, а) and perivascular infiltrates (image 5, б). Vascular-infiltrative reactions were observed both in dura as well as cerebral gray and white matter, while those reactions were dominant in parts with most expressed pathological process.

The indicated histological characteristics of the damage to the central nervous system in the given cases and also clinical data conform to the popular statements of several authors [1-5] on infectious origins of the amyotrophic lateral sclerosis.

Based on the above, we assume that the inclusions, discovered in the motor cells of the anterior horns of the spinal cord and the nuclei of cranial nerves, may be due to the presence of a neurotropic virus. In order to make any resolute judgments on the nature of such inclusions, further research is required, using histological method (Brache and Feulgen reactions) and experiments.

Image 5. Vascular reaction in the white matter of the spinal cord and scapus.

а – Cervical of the spinal cord, white matter of the lateral column, vasculitides, thionin staining, 80x zoom.
б – Tectum of the pons Varolii, perivascular infiltrate, hematoxylin-eosin staining, 260x zoom.
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