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Case Report

Amyotrophic lateral sclerosis with appearance of many skein-like inclusions in anterior horn cells

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Abstract: We report an autopsy case of amyotrophic lateral sclerosis (ALS), in which an abnormally large number of skein-like inclusions (SLIs) was found in anterior horn cells. The patient was a 73-year-old man, who presented with dysarthria. His motor neuron symptoms were predominantly of the upper-neuron type, and cognitive impairment was also noted. He died of septic shock 13 months after onset of the first neurological symptoms. Autopsy revealed marked loss of upper motor neurons, severe degeneration of the pyramidal tract, mild to moderate loss of anterior horn cells, and the appearance of many SLIs, which were immunoreactive for both pTDP-43 (phosphorylated transactivation responsive DNA-binding protein of 43 kDa) and ubiquitin, in anterior horn cells. Intra-axonal pTDP-43-positive granules arranged in a bead-like fashion were also found. The appearance of pTDP-43-positive intracytoplasmic inclusions in the brain was mostly restricted to the motor cortex. An Alzheimer type tau-pathology was found mainly in the hippocampus (Braak stage III), and many argyrophilic grains were distributed in the limbic area. Atypical ALS showing a rapid clinical course associated with cognitive impairment and predominant involvement of the upper motor neurons has recently been reported. The present case shares some clinical and pathologic findings with this type of atypical ALS. The appearance of a large number of SLIs is an unusual finding. Although its pathologic significance remains unknown, it cannot simply be ascribed to the relative preservation of anterior horn cells.

Keywords: Amyotrophic lateral sclerosis, upper motor neuron degeneration, anterior horn cell, skein-like inclusion, pTDP-43

Introduction

Sporadic amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder whose pathogenesis is closely related to the abnormal intracytoplasmic aggregation of pTDP-43 (phosphorylated transactivation responsive DNA-binding protein of 43 kDa) [1-4]. It forms one end of the wide spectrum of "TDP-43 proteinopathy", which ranges from sporadic ALS to fronto-temporal lobar degeneration (FTLD) at the other extreme [1-4].

In ALS, the disease process affects both upper and lower motor neurons, but the degrees of pathologic involvement of both neurons vary among cases. "Primary lateral sclerosis (PLS)", a motor neuron disease in which upper neurons are almost solely affected, was demonstrated to have abnormal aggregation of pTDP-43 in some patients, suggesting that at least a part of PLS is a variant of ALS [5, 6]. Most patients with PLS show a slowly progressive, relatively indolent clinical course [5, 6]. However, the presence of rare cases of the upper-neuron-predominant type of ALS has recently been recognized, in which patients pursue a rapidly progressive course and show cognitive impairment [4, 7]. Their neuropathologic findings differ from those of typical PLS, and this group of patients may represent a distinct variant of ALS [4, 7].

We report an autopsy case of sporadic ALS, in which the clinical course was short and the cerebral motor cortex and pyramidal tract were markedly degenerated. Anterior horn cells of the spinal cord were relatively well preserved, and an abnormally large number of remaining neurons had pTDP-43-positive, intracytoplasmic skein-like inclusions (SLIs).

Clinical history

The patient was a 73-year-old man with a history of diabetes mellitus and hypertension but no relevant family history of neurologic diseases. He consulted our hospital 10 months before death, complaining of a gradual progression of speech difficulty for 3 months. Neurological examination demonstrated dysarthria and left hemiparesis, and a neuroradiological study revealed an old cerebral infarction scar in the right occipital lobe. Babinski reflex was positive on the left side. Weakness of upper and lower extremities and swallowing difficulty appeared 5 months later, and these symptoms and dysarthria rapidly progressed. Impairment of cognitive function appeared, and the consciousness level also lowered to that of somnolence. Accurate evaluation of the cognitive impairment became difficult due to consciousness disturbance and decreased spontaneous speech. He repeatedly suffered from aspiration pneumonia from 7 months before death, and tracheostomy was performed. The mechanical support of ventilation and gastric tube feeding were introduced, but he died of septic shock 13 months after onset of the first neurologic symptoms. Throughout the clinical course, upper motor neuron signs predominated. Neuroradiologic examination demonstrated progressive atrophy of the hippocampus, and a high intensity of the pyramidal tract at the level of the pontine base on MRI-FLAIR images gradually became prominent.

Neuropathologic findings

General autopsy revealed acute bronchopneumonia, intrahepatic cholangitis, and multiple liver abscesses. Skeletal muscles including the diaphragm and tongue showed neurogenic atrophy with adipocytic infiltration. The brain weighed 1,310 grams and showed mild cortical atrophy of the frontal and temporal lobes. Localized atrophy of the motor cortex was not apparent. The coronal sections disclosed moderate atrophy of the hippocampus with dilatation of the lateral ventricles. Small, old infarction scars were present in the right frontal and occipital lobes. The spinal cord was slightly attenuated, but otherwise appeared normal.

Immunohistochemical study was performed employing antibodies against pTDP-43 (pS-409/410, clone 11-9, Cosmo Bio, Tokyo, Japan, 1:3,000), ubiquitin (clone 1B3, MBL, Nagoya, Japan, 1:200), phosphorylated tau protein (clone AT8, Innogenetics, Ghent, Belgium, 1:200), β-amyloid protein (clone 6F/3D, Dako, Glostrup, Denmark, 1:50), phosphorylated neurofilament (clone SMI31, BioLegend, San Diego, CA, USA, 1:500), and CD68 (clone KP-1, Ventana Medical System Inc., Tucson, AZ, USA, prediluted). For antigen retrieval, CC1 solution (proprietary buffers containing EDTA, Ventana Medical System Inc.) or formic acid was applied prior to immunostaining.

The spinal cord showed marked loss of axons and myelin sheaths accompanied by an infiltration of foamy macrophages in the lateral and anterior corticospinal tracts (**Figure 1A**). The anterior horn showed slight atrophy and a decrease of number of large motor neurons, which was accompanied by rarefaction of the neuropil, activation of microglia, and reactive astrocytosis. Whereas neuronal loss was moderate in the upper cervical cord, most of the anterior horn cells were preserved in the thoracic and lumbo-sacral cord (**Figure 1B**). Neuronophagia of anterior horn cells was rarely observed in the lumbar cord, and a small number of Bunina bodies were detected. Anterior horn cells showing chromatolytic changes were not observed, and axonal spheroids were few. Areas other than the anterior horn and pyramidal tracts retained their normal appearances.

Immunostaining of the spinal cord demonstrated an abnormally large number of SLIs, which were immunoreactive for both pTDP-43 and ubiquitin, in anterior horn cells (**Figure 1C** and **1D**). The number of SLIs was counted on the pTDP-43-immunostained sections taken from each segmental level. In total, 243 anterior horn cells with SLIs were identified in 26 examined sections from C2 to S2 (**Table 1**). At the lower lumbar cord, where the largest number of SLIs was found, 26 among 64 large anterior horn cells (40.6%) and 40 among 147 large anterior horn cells (27.2%) at the L4 and L5 levels, respectively, contained SLIs. SLIs frequently extended into the dendrites (**Figure 1Dc**). In addition, pTDP-43-positive fine granules were dispersed throughout the perikarya in some neurons (**Figure 1Dd**). No pTDP-43-positive round inclusions were observed. pTDP-43-positive coarse granules were occasionally aligned in a beads-like fashion in axons of anterior horn cells up to the nerve roots (**Figure 1E**). pTDP-

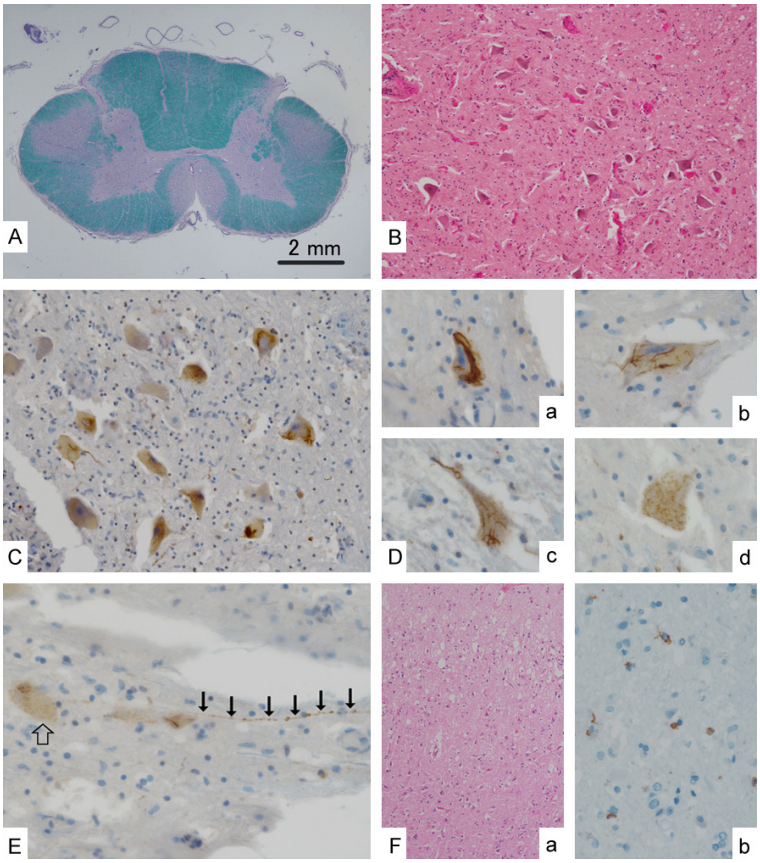

Figure 1. (A) The spinal cord showed marked degeneration of the anterior and lateral corticospinal tracts. (C8, Luxol fast blue-periodic acid-Schiff stain). (B) Anterior horn cells were relatively well preserved, especially in the lumbo-sacral segments. (L2, H&E stain). (C) Many anterior horn cells contained pTDP-43-immunoreactive skein-like inclusions (SLIs) in the cytoplasm. (S1, Immunostain for pTDP-43). (D) SLIs took various shapes, ranging from relatively thick, stout fibrils (a) to intricately entangled, thin filaments (b). Some SLIs were found within the primary dendrites (c). pTDP-43-immunoreactive fine granules were noted in some anterior horn cells (d). (a-d: Lumbar cord, Immunostain for pTDP-43). (E) Many pTDP-43-immunoreactive coarse granules (small arrows) were arranged in a bead-like fashion within the axon of an anterior horn cell (large arrow). (L2, Immunostain for pTDP-43). (F) (a) The motor cortex showed marked loss of neurons and rarefaction with microvacuolation of the neuropil. (b) Several pTDP-43-immunoreactive neuronal cytoplasmic inclusions were seen. (a: H&E stain, b: Immunostain for pTDP-43).

43-positive dystrophic neurites and glial inclusions were also frequently observed, but no intranuclear inclusions were noted.

In the brain, the motor cortex showed marked neuronal loss accompanied by rarefaction of the neuropil and astrocytosis (**Figure 1Fa**), and Betz cells had almost completely disappeared. A few remaining pyramidal neurons contained SLIs. Degeneration of the underlying white matter with the infiltration of foamy macrophages was evident. The distribution of pTDP-43-positive neuronal cytoplasmic inclusions or dystrophic neurites was mostly restricted to the motor cortex (**Figure 1Fb**). In the cerebral neocortex, many senile plaques (Thal's phase 2), tau-immunoreactive neuropil threads, and scattered neurofibrillary tangles (NFTs) were observed. The hippocampus was atrophied, and many NFTs and numerous neuropil threads were distributed from the CA1 sector to subiculum, corresponding to Braak stage III, but prominent neuronal loss restricted to the transitional zone from the CA1 to subiculum was not noted. No pTDP-43-positive inclusions were observed in dentate granule cells or pyramidal cells of the hippocampus (Nishihira's type 1) [8].

Many tau-immunoreactive, argyrophilic grains were distributed in the hippocampus, entorhinal cortex, ambient gyrus, and amygdala. The amygdala showed moderate neuronal loss and astrocytosis. The striatum, globus pallidus, and thalamus showed no remarkable changes. The substantia nigra exhibited mild neuronal loss but no pTDP-43-positive inclusions. The medulla oblongata showed marked degeneration of the pyramids. The hypoglossal nucleus exhibited moderate neuronal loss, and a few remaining neurons contained SLIs or Bunina bodies. The inferior olivary nucleus did not contain pTDP-43-positive inclusions. The cerebellum was normal, and no anoxic changes were found.

Discussion

In most cases of ALS, degeneration of the pyramidal tract is more pronounced at the lower level and considered an axonopathy that slowly progresses upwards from the lower levels [6]. In the present patient, whereas anterior horn cells were relatively well preserved, especially

Table 1. Number of skein-containing anterior horn cells

C2	0	T1	6	L1	12
C3	0	T2	6	L2	10
C4	4	T3	7	L3	15
C5	1	T4	2	L4	26
C6	27	T5	2	L5	40
C7	14	T6	4		
C8	10	T7	9		
		T8	2	S1	18
		T9	3	S2	17
		T10	7		
		T11	5		
		T12	6		

Total 243/26 sections (levels).

in the lumbo-sacral region, the pyramidal tract had markedly degenerated up to the level of the subcortical white matter despite the short clinical course. This finding suggests that upper motor neurons rapidly degenerated in the early phase of disease progression, while the spread of pTDP-43 pathology still remained in stage 1, according to the staging proposed by Brettschneider et al. [9]. On the other hand, the degeneration of lower motor neurons is considered to have progressed much slower than that of the upper neurons, or it began later than upper neuron degeneration. In this context, it is noteworthy that the findings regarded as early lesions in ALS, that is, chromatolytic changes of anterior horn cells and axonal spheroids [10], were almost absent in the present patient.

Nishihira et al. classified sporadic ALS into types 1 and 2 on the basis of the clinico-pathologic features and the distribution pattern of pTDP-43-immunoreactive inclusions [8]. Takeuchi et al. subclassified type 2 ALS into 2a and 2b, based on the pTDP-43-pathology of the cerebral cortex and basal ganglia [7]. The subtype 2b is characterized by poor prognosis despite less severe loss of lower motor neurons, unusual subcortical dendritic pTDP-43 pathology, and prominent glial involvement [7]. In a recent review, Tada and Kakita referred to another group of patients with atypical ALS who showed a rapid clinical course associated with cognitive impairment and the predominant involvement of upper motor neurons with relative preservation of lower neurons [4]. Some of the clinical and pathological features that were described in these articles overlap with those of the present patient. However, whereas their patients had dementia and FTLD was pathologically demonstrated [4], in our patient the cytoplasmic aggregation of pTDP-43 was mostly restricted to the motor neuron system. Cognitive impairment in our patient was considered to have been caused by concomitant Alzheimer pathology and argyrophilic grain pathology of the limbic area. Whereas degeneration of the substantia nigra was noted in their patients [4], it was mild in our patient. The appearance of a large number of SLIs was not described in their patients [4, 7, 8].

The presence of an abnormally large number of SLIs was an interesting finding in our patient. At the level in which the largest number of SLIs was found (L5), 40 SLIs were present in the bilateral anterior horns on a single section. SLI is a fibrillary cytoplasmic inclusion characteristically observed in anterior horn cells of sporadic ALS [11-14]. Based on the immunoreactivity for pTDP-43 [1, 15], the formation of SLI is considered an important event in the pathogenesis of ALS [1, 2, 15].

Studies including quantitative analyses of SLIs in the spinal cord of ALS are sparse. Leigh et al. examined 2,057 anterior horn cells and found 92 and 40 cells containing SLIs and round inclusions, respectively [13]. They also found 185 anterior horn cells (17%) with ubiquitin-positive inclusion bodies (including both SLIs and round inclusions) among 1,111 cells in the sacral cord [13]. Schiffer et al. found a higher number of inclusion-bearing neurons in patients with a short clinical course, indicating that the inclusions were formed in early stages of the disease progression [16]. The number of SLIs in the present patient was unusually large, and it could not be simply ascribed to the relative preservation of anterior horn cells. It is unknown why so many SLIs were formed in the present case but this may be related to the short clinical course. Nakano et al. suggested, based on an ultrastructural study, that at least some SLIs finally underwent degradation by an autophagic process [17]. The presence of a large number of SLIs might indicate that the autophagic process to dispose SLIs has been interrupted in some way. To our knowledge, the interrelationship between the number of SLIs and the clinico-pathological features of various subtypes of sporadic ALS has not been investigated.

Mori et al. studied the maturation process of pTDP-43-positive inclusions in ALS [18]. In addition to SLIs, round inclusions, and dot-like inclusions, they found small "linear wisps" of pTDP-43-positive material and postulated that, whereas SLIs were formed by the aggregation of "linear wisps", round inclusions arose from dot-like inclusions. SLIs and round inclusions did not coexist. The absence of round inclusions in our patient is consistent with their suggestion that the formation of SLIs and round inclusions are distinct processes [18].

Finally, the presence of pTDP-43-positive fine granules that were aligned in a bead-like fashion in axons of anterior horn cells was another intriguing finding. The intra-axonal location of pTDP-43-positive fine granules was documented in a few previous reports [9, 19, 20]. It might represent one of the routes of pTDP-43 propagation, which has recently been postulated to show prion-like properties and be transmitted across synapses [9, 21]. Onozato et al. demonstrated intra-axonal pTDP-43-positive aggregates in ALS patients with a relatively short clinical course [20].

Disclosure of conflict of interest

None.

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