CASE REPORT
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Basophilic peripheral nerve inclusions in a patient with L144F *SOD1* amyotrophic lateral sclerosis

Bazofilne inkluzije u perifernom nervu kod bolesnika sa L144F *SOD1* amiotrofičnom lateralnom sklerozom

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Abstract

Introduction. Histopathological findings of various inclusions were reported in the central nervous system of amyotrophic lateral sclerosis (ALS) patients but not in the peripheral nerves. **Case report.** We present a 66-year-old man with lower limb weakness, with later development of weakness in the upper limbs and loss of sphincter control. Neurological examination showed the affection of both upper and lower motor neurons. He had paresthesia on the left side of his body and socks-distribution numbness. Histopathology of the sural nerve and genetic report showed basophilic periodic acid-Schiff (PAS)-positive intra-axonal inclusions and heterozygous L144F mutation in the exon 5 of the *SOD1* gene. **Conclusion.** It seems that the presence of the basophilic peripheral nerve inclusions may suggest a diagnosis of *SOD1*-positive ALS.

Key words:
amyotrophic lateral sclerosis; diagnosis; genes; histological techniques; mutation; staining and labeling; superoxide dismutase.

Apstrakt

Uvod. Postoje izveštaji o histopatološkim nalazima različitih inkluzija u centralnom nervnom sistemu kod bolesnika sa amiotrofičnom lateralnom sklerozom (ALS), ali ne i u perifernim nervima. **Prikaz bolesnika.** Prikazan je bolesnik star 66 godina sa slabošću donjih ekstremiteta, kasnijim razvojem slabosti i gornjih ekstremiteta i gubitkom kontrole sfinktera. Neurološkim pregledom utvrđeni su znakovi oštećenja i gornjeg i donjeg motornog neurona. Pored parestezija leve strane tela, bolesnik je osećao i utrnulost sa distribucijom po tipu „čarapa". Histopatološkom analizom suralnog nerva i genetskim analizama utvrđene su bazofilne *periodic acid-Schiff* (PAS)-pozitivne inkluzije unutar aksona i heterozigotna mutacija L144F u egzonu 5 *SOD1* gena. **Zaključak.** Prisustvo bazofilnih inkluzija u perifernom nervu može ukazati na dijagnozu *SOD1*-pozitivne ALS.

Ključne reči:
amiotrofijska lateralna skleroza; dijagnoza; geni; histološke tehnike; mutacija; bojenje preparata; superoksid dismutaza.

Introduction

Around 5–10% of patients with amyotrophic lateral sclerosis (ALS) have a genetic basis for the disease. Mutations in the *SOD1* gene are one of the most common causes of ALS, accounting for around 23% of familial ALS and about 7% of apparently sporadic ALS in all studies. Over 185 disease-associated variations in *SOD1* have been identified so far [1].

From 5% to 10% of ALS individuals have at least one additional family member affected by ALS (so-called familial ALS – fALS), but the majority of ALS cases (90–95%) occur in people with no prior family history (so-called sporadic ALS – sALS) [2]. In both fALS and sALS, genetic causes of the disease may be established, which is marked as hereditary ALS. Various histopathological changes have been demonstrated in *SOD1* fALS patients, both in the central nervous system (CNS) and peripheral nervous system (PNS) [2, 3]. Spheroid shape and

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eosinophilic inclusions containing neurofilaments in the anterior horn cells and Betz cells were observed in fALS patients with the I113T *SOD1* mutation [4]. There are numerous indicators of PNS involvement in fALS, especially in patients with the *SOD1* mutations [2]. Examinations of the sural nerve showed an axonal loss, *tomacula*-like myelin thickenings, and inflammatory infiltrate [5]. In a patient with the D90A *SOD1* mutation, a sural nerve biopsy showed loss of large myelinated fibers and degenerative changes of myelinated and unmyelinated fibers [6].

To the best of our knowledge, we present a unique case of apparently sALS patient with the L144F mutation in the *SOD1* gene who had intra-axonal basophilic periodic acid-Schiff (PAS)-positive inclusions.

Case report

A 66-year-old male presented with a five-year history of progressive weakness with atrophy of distal muscles, especially of both legs, as well as a slight weakness and distal muscle atrophy in both arms, rare fasciculations, and loss of sphincter control. On admission, he was not able to walk.

A neurological examination of the patient revealed bilateral distal muscle atrophy and rare fasciculations with generalized mild weakness in the upper limbs. In the lower limbs, the patient had bilateral distal muscle atrophy and provoked fasciculations with severe generalized weakness. The tendon reflexes were brisk, except for ankle jerks which were diminished, and the presence of bilateral Babinski sign was noticed. Standing and walking were impossible. He had socks-distribution paresthesia. The urinary catheter was placed due to incontinence. The Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-r) was 38/48. The mini-mental status examination (MMSE) score was normal. Electromyography showed neurogenic changes with actual denervation activity at rest in the cervical and lumbosacral regions. A nerve conduction study showed axonal sensory and motor neuropathy, which was more pronounced in the lower limbs. Laboratory and cerebrospinal fluid (CSF) studies were normal. Magnetic resonance imaging of the brain and spinal cord were unremarkable. The conclusion was that the patient fulfilled El Escorial revised criteria for probable ALS, with atypical clinical features such as axonal neuropathy and bladder dysfunction. In the next three months, nerve and muscle biopsies and genetic examinations were performed. The patient died five months later at the age of 67 due to cardiopulmonary arrest.

A biopsy of the right gastrocnemius muscle revealed a neurogenic lesion, rare and atrophic end-stage muscle fibers, and a dominant presence of fatty and connective tissue. Sural nerve biopsy showed the following: fatty and connective tissue infiltration (Figure 1A); intense reduction of small and large myelinated fibers in number; secondary remyelination

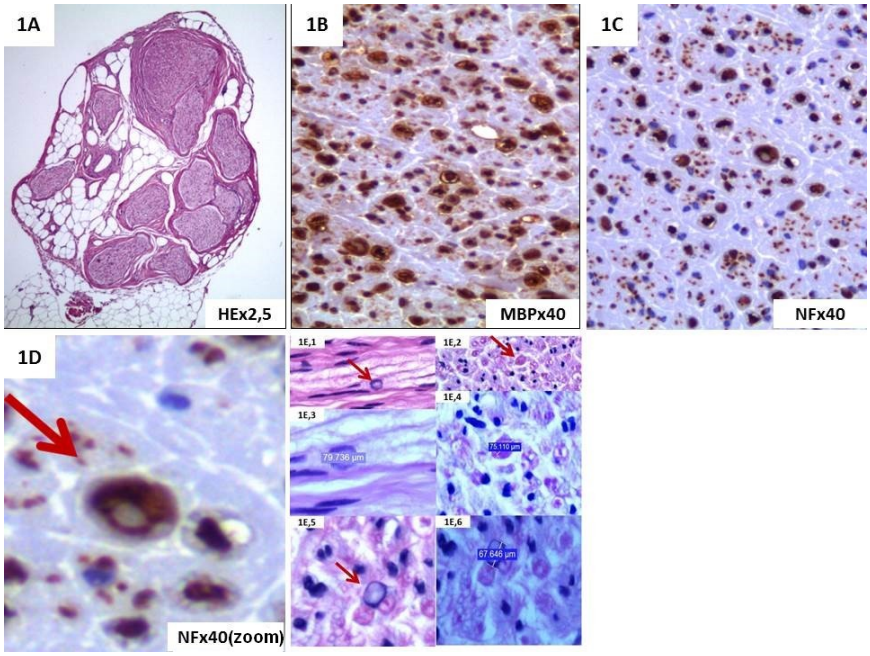

Fig. 1 – The sural nerve biopsy: A) Interfascicular infiltration with fat and connective tissue (HE, ×2,5); B) Intense reduction in the number of small and large myelin fibers, secondary remyelination and regeneration clusters (MBP, ×40); C) Axonal degeneration (NF, ×40); D) A cross-section of a fiber stained for the NF (×40-zoom) shows an empty space at the inclusion site (red arrow); E) (1–6): Oval inclusions of variable diameters, visible on transverse and longitudinal sections (×40): 1E1) Basophilic inclusions (red arrow) on a longitudinal section (HE); 1E2) PAS-positive inclusions (red arrow); 1E3) PAS-positive inclusions of diameter 79 µm (intra-axonally) on a longitudinal section; 1E4) PAS-positive inclusions of diameter 75 µm (intra-axonally) on a cross-section; 1E5) The inclusion (red arrow) fills the space of the axon (PAS); 1E6) PAS-positive inclusions of diameter 67 µm (intra-axonally) on a cross-section.
HE – hematoxylin and eosin staining; MBP – myelin basic protein; NF – neurofilament; PAS – Periodic acid-Schiff.

and regeneration (Figure 1B), together with axonal degeneration (Figure 1C). Basophilic PAS-positive inclusions, 14–79 μm in diameter, were observed intra-axonally in several fascicles. These inclusions were PAS-positive (Figures 1D and 1E). The inclusions were described as polyglucosan body-like alterations. Genetic analysis showed a pathogenic heterozygous missense L144F mutation in the exon 5 of the S*OD1* gene, which led to the final diagnosis of genetic ALS.

Discussion

We presented a patient with the L144F mutation of the *SOD1* gene and atypical presentation of ALS. Polyglucosan body-like inclusions were seen on the peripheral nerve biopsy. Polyglucosan bodies are pathologic hallmarks of a very rare neurometabolic disorder, i.e., adult polyglucosan body disease (APBD) with peripheral neuropathy, upper motor neuron signs, neurogenic bladder, and dementia. Many patients lack one or more of these features. The diagnosis is confirmed based on the activity of the branched enzyme and genetic analysis. White matter involvement in APBD may be extensive [4]. Very rare manifestations include ALS-like features registered in only six patients [7]. Clinical presentation of APBD and *SOD1* L144F ALS may be similar. Patients with L144F have a slowly progressive disease with lower limb onset, combined upper and lower motor neuron signs, and atypical clinical features, e.g., sphincter and sensory disturbances [8].

APBD may be diagnosed incorrectly based only on the presence of polyglucosan bodies, which is a nonspecific finding [7]. We registered a reduction of small and large myelinated fibers in number, axonal degeneration, as well as remyelination and regeneration that have been previously described in ALS patients [5, 6] with PAS-positive basophilic inclusions in the sural nerve specimens that have not been described in *SOD1* patients so far. PAS-positive inclusions resembled findings in APBD, so only genetic analyses were able to distinguish between ABPD and ALS [9]. Based on the clinical presentation, needle electromyography, and even nerve biopsy, it is not always possible to distinguish with certainty between APBD and L144F *SOD1* ALS, so it is necessary to perform genetic analyses that will confirm APBD or ALS.

It is well known that neuronal inclusions containing aggregated *SOD1* are the pathological hallmark of *SOD1* ALS seen both in patients and in transgenic animal models overexpressing mutant *SOD1* protein. Some *SOD1* ALS patients, including those with L144F mutation, have larger skein-like and Lewy body-like inclusions, in addition to smaller misfolding *SOD1* inclusions [10]. We were not able to perform additional enzymatic and genetic analyses for adult polyglucosan body disease, as well as immunohistochemical studies covering epitopes across the entire *SOD1* protein to see if the inclusions seen in our patients contain *SOD1* aggregates.

Conclusion

Basophilic peripheral nerve inclusions may be associated with *SOD1*-positive ALS.

Conflict of interest

The authors report no conflict of interest.

Acknowledgment

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