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Neuromuscular Disorders 26 (2016) 880–884

www.elsevier.com/locate/nmd

Case report

Tubular aggregates in autoimmune Lambert–Eaton myasthenic syndrome

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Received 22 May 2016; received in revised form 31 August 2016; accepted 12 September 2016

Abstract

Tubular aggregates are accumulations of densely packed tubules in muscle fibers, occurring in distinct hereditary and acquired disorders. We present a patient with tubular aggregates and autoimmune Lambert–Eaton myasthenic syndrome. Initially, he showed mild proximal weakness, borderline decrement on 3 Hz stimulation, and slightly elevated creatine kinase. Muscle biopsy revealed tubular aggregates in type II fibers. Due to a good response to pyridostigmine, a limb-girdle myasthenia with tubular aggregates was suspected, but genetic analyses of *GFPT1*, *DPGAT1*, and *ALG2* were normal. Two years later, the patient presented with progressive weakness and autonomic dysfunction. 17% decrement on 3 Hz stimulation and 100% increment after brief exercise were revealed. Autoantibodies to voltage-gated calcium-channels confirmed the diagnosis of Lambert–Eaton myasthenic syndrome. Steroids, azathioprine, and 3,4-diaminopyridine significantly improved symptoms. No tumor was found during follow-up. This is the first report about tubular aggregates associated with an acquired myasthenic syndrome. Our findings are important because of the therapeutic implications.

Keywords: Lambert–Eaton myasthenic syndrome; Muscle biopsy; Acquired; Tubular aggregates

1. Introduction

Tubular aggregates are accumulations of densely packed membranous tubules within skeletal muscle fibers that are most probably derived from the sarcoplasmic reticulum [1]. They are the predominant histopathological finding in tubular aggregates myopathies, for which causative mutations in *STIM1* or *ORAI1* have recently been identified [2]. However, tubular aggregates can also occur in several hereditary disorders including congenital myasthenic syndromes (CMS) and in a variety of acquired conditions (Table 1). CMS associated with tubular aggregates include hereditary limb-girdle myasthenia with tubular aggregates caused by mutations in *GFPT1*, *DPGAT1,* or *ALG2* [3].

Lambert–Eaton myasthenic syndrome is a rare acquired autoimmune myasthenic disorder caused by antibodies directed against presynaptic P/Q-type voltage-gated calcium channels (VGCC), leading to an impaired transmission across the neuromuscular junction. The condition can occur as a paraneoplastic syndrome, mostly associated with small cell lung carcinoma, or as an autoimmune disease without underlying tumor. Clinical characteristics are proximal muscle weakness and autonomic dysfunction. Repetitive nerve stimulation (RNS) in Lambert–Eaton myasthenic syndrome usually shows a low compound muscle action potential (CMAP), a decrement >10% with stimulation at low frequency (1–5 Hz), and an increment >100% after maximum voluntary contraction or with high stimulation frequencies (50 Hz) [19].

Here, we present for the first time the occurrence of tubular aggregates in the muscle of a patient with an *acquired* myasthenic syndrome. We highlight the diagnostic difficulties associated with the presence of tubular aggregates in the muscle biopsy.

2. Case report

A 36-year-old Portuguese man presented with a five-month history of non-fluctuating exercise-induced proximal weakness of both upper and lower limbs. He complained of difficulties to climb stairs. Myalgia, cramps, or stiffness as well as sensory, bulbar, or autonomic symptoms were denied. Due to struma nodosa, he underwent a thyroidectomy at the age of 34 years and has since then been treated with L-thyroxine. Family history for neurologic and neuromuscular disorders was negative. The endurance test in

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http://dx.doi.org/10.1016/j.nmd.2016.09.011
0960-8966/

Table 1
Disorders associated with tubular aggregates in human muscle biopsies (literature review) and investigations to exclude the respective condition in our patient.

Conditions associated with tubular aggregates	Diagnostic assessment in our patient
Tubular aggregate myopathy [2]	Genetic analysis normal (*STIM1*, *ORAI1*)
Limb-girdle myasthenia with tubular aggregates [3]	Genetic analysis normal (*GFPT1*, *DPAGT1*, *ALG2*)
Gyrate atrophy of choroid and retina [4]	Clinical: normal vision
Periodic paralysis [1,5]	Genetic analysis normal (*CACNA1S*, *KCNE3*, *KCNJ2*, *KCNJ5*, *KJNJ18*, *SCN4A*)
	Clinical: no paralytic attacks
Malignant hyperthermia [5]	Genetic analysis normal (*RYR1*, *SCN4A*, *CACNA1S*)
	Clinical: no incidents with previous anesthesias
Myotonic myopathy [5], myotonic dystrophy [6]	Genetic analysis normal (*CLCN1*)
	Clinical and EMG: no myotonia, no typical features
Glycogen storage diseases and other metabolic myopathies [5]	Alpha-glucosidase activity in blood normal
	Genetic analysis normal (*PGAM2*)
	Muscle biopsy: no glycogen or lipid accumulations
CADASIL [5]	Brain MRI normal
	Clinical: no strokes, no headache
Morbus Fabry [5]	Genetic analysis normal (*GLA*)
Mitochondrial disorders [5,7]	Muscle biopsy: no mitochondrial abnormalities
Polyneuropathy [1,5,8]	NCV normal
	Clinical: no abnormalities suggestive for polyneuropathy
Alcoholic myopathy [5]	No alcohol abuse
Toxic/drug-induced myopathy [1,5]	No relevant toxins/drugs
Diabetic amyotrophy [5]	Blood analysis: HbA1c normal
Polymyositis, dermatomyositis [5]	Muscle biopsy: no inflammatory infiltrates, no necroses, no perifascicular atrophy
	Blood analysis: no considerably increased CK, no myositis-associated/-specific antibodies
Porphyria cutanea tarda [1]	Clinical: no skin changes
Whipple's disease [9]	Clinical: no gastrointestinal symptoms
Hyperaldosteronism [10]	Blood analysis: no elevated aldosterone
Amyotrophic lateral sclerosis [8]	Clinical: no upper and/or lower motor neuron signs, no progressive disease
Facioscapulohumeral muscular dystrophy [11]	Clinical: no manifestation in face and shoulder region, no typical features
Lupus erythematosus [8]	Clinical: no abnormalities of skin or internal organs
	Blood analysis: no inflammation parameters, ANA negative, no cytopenia
Muscle infarction [12]	Clinical: no acute episode with pain and swelling
Fukuyama congenital muscular dystrophy [13]	Clinical: no congenital onset, no typical features
Osteomalacic myopathy due to anticonvulsant drugs [14]	No anticonvulsant drugs
After adrenalectomy [15]	No adrenalectomy
Neoplastic disease [16]	No tumor
Zidovudine therapy for AIDS [17]	No zidovudine
Steroid response myopathy [18]	Tubular aggregates documented in our patient prior to the intake of steroids

EMG: electromyography, CADASIL: cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, MRI: magnetic resonance imaging, NCV: nerve conduction velocity, HbA1c: hemoglobin A1c, CK: creatine kinase, ANA: antinuclear antibody, AIDS: acquired immune deficiency syndrome.

which the lower limbs are maintained above the bed plane while lying on the back was abnormal (60 s, normal 75 s). Further neurological examination was normal, including muscle strength and tone, deep tendon reflexes, and sensory testing. In particular, there was no evidence for extraocular muscle dysfunction or bulbofacial weakness.

Serum analysis showed slightly elevated creatine kinase levels (281 U/l, normal < 190). Antibodies against acetylcholine receptor and muscle-specific receptor tyrosine kinase in serum were both negative. Nerve conduction velocity studies and electromyography were normal. 3 Hz RNS of the ulnar and accessory nerves, recorded at the abductor digiti minimi and trapezius muscle, induced a 9% and 11% borderline decrease of CMAP, respectively. The amplitudes for resting CMAP were normal and no increment after 10 s of maximum voluntary muscle contraction was detected. A chest computed tomography scan as well as magnetic resonance imaging of the brain and spinal cord revealed no abnormalities.

Due to the inconclusive findings, an open muscle biopsy of the right vastus lateralis muscle was performed and processed according to standard procedures [20], after obtaining the patient's written informed consent. Light microscopy revealed the presence of inclusions that stained dark blue on nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), red on modified Gomori's trichrome (GT), and blue on adenosine monophosphate deaminase (AMPDA) stains, compatible with tubular aggregates (Fig. 1). The aggregates appeared basophilic on hematoxylin and eosin (HE) and red-brown on esterase stains, and showed a positive reaction for periodic acid Schiff and acid phosphatase (Fig. 1). Although the inclusions stained dark blue on succinate dehydrogenase (SDH), they only partially reacted on cytochrome-c-oxidase (COX) (Fig. 1G–H). Adenosine triphosphatase (ATPase) staining at pH 4.2 and 9.4 confirmed that the aggregates were present exclusively in type 2 muscle fibers. Tubular aggregates were distributed unevenly across the biopsy and were present in approximately 4% of the muscle fibers. The biopsy showed a slightly increased variation in fiber size diameter. The number of internalized nuclei was not increased. Fibrotic, necrotic, neurogenic, or inflammatory