An adult case of mitochondrial DNA depletion with associated IBM features

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Abstract

We describe a case of muscle mitochondrial DNA depletion and features of inclusion body myositis (IBM) in a young woman. The patient presented mild proximal muscle weakness, multiple small lesions of cerebral white matter, hypothalamic amenorrhea, minimal cognitive impairment, glucose intolerance, rise of creatinephosphokinase (CK) and family history positive for hyperckemia and endocrine disorders. Muscle tissue biopsy showed ragged red fibers (RRF) and severe cytochrome c oxidase (COX) deficiency plus typical IBM histopathological findings; Southern blot analysis of muscle mitochondrial DNA (mtDNA) demonstrated 80% depletion of mtDNA. Although mitochondrial histopathological and genetic alterations have been reported in association with IBM and other inflammatory myopathies, in our case the clinical multi-organ involvement, the severity of muscle mtDNA depletion and the familiarity for increased serum CK levels and endocrine dysfunction suggest a metabolic mitochondrial dysfunction with IBM features.

Key words: inclusion body myositis, mitochondrial DNA depletion, ragged red fibers, hyperckemia, endocrinopathy.

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Introduction

Sporadic IBM is the most common acquired myositis over age 50 years, men being affected more often than women. It is different from other inflammatory myopathies (e.g. poliomyelitis, dermatomyositis, myositis with collagenopathy) because of its peculiar clinical and histopathological pattern. Typically, it causes distal muscle involvement, especially foot extensor and deep finger flexor weakness. Early, at times asymmetric, affection of quadriceps, ileopsoas, triceps and finger flexors in the forearm is also characteristic. Dysphagia is common, occurring in up to 60% of the patients, especially late in the disease. Pathological features in IBM include necrosis of muscle fibers, sluggish phagocytes, cytoplasmic rimmed vacuoles and eosinophilic masses, possible RRF, nuclear abnormalities (15 nm filaments), amyloid-staining and ubiquitin-positive inclusions, lymphocytic endomysial infiltrates (CD4+/CD8+T cell), increased capillarity [19]. Familial IBM may be autosomal recessive or, rarely, dominant, and it is almost similar to the sporadic form even if it may present some specific features, e.g. early onset, quadriceps sparing with distal involvement, poor histopathological muscle inflammation signs [2, 18].

MtDNA depletion is a reduction of mtDNA copy number because of intergenomic (nuclear and mitochondrial) signaling abnormality [13]. It appears to be inherited as a Mendelian trait (autosomal recessive) and, recently, mutations in 2 genes involved in deoxyribonucleotide metabolism have been reported [11]. MtDNA depletion is associated with severe, almost deadly, congenital (83% to 98% depletion) or infantile-onset (66% to 83% depletion) mitochondrial syndrome (myopathy, lactic acidosis, seizures, nephropathy, hepatopathy). In these cases RRF were detected in skeletal muscles biopsies [13].

In the last years the clinical spectrum of manifestations associated with mtDNA depletion has been extended. Cases with later onset and/or different clinical pattern have been reported [5]. Indeed, Leigh syndrome cases have been also associated with mtDNA depletion [1]; in 1998 mtDNA depletion, associated with respiratory mitochondrial enzyme biochemical deficit, has been found in neonatal hypertrophic cardiomyopathy [15]. MtDNA depletion has been associated with a pure

mitochondrial myopathy and with 3 cases of Kearns Sayre [5]. In 2001, the Navajo neurohepatopathy (NNH), a juvenile neuropathy with hepatic dysfunction, has been suspected to be caused by mtDNA depletion [20]. MtDNA depletion may also be acquired by chronic exposure to zidovudine (AZT); the depletion in this settings is reversible when the AZT is discontinued [3].

Patient and methods

Case

A 26 years old woman was admitted to our Neurology Department because of mild proximal progressive muscle weakness and fatigability. She had a previous diagnosis of hypothalamic amenorrhea; her serum CK level was 1691 U/L (n.v. < 200 U/L). Family history was positive for hyperckemia (230 U/L in the mother, 282 U/L in the 28 y.o. sister and 238 U/L in the 18 y.o. brother), diabetes mellitus (mother) and endocrine dysfunction (the sister presented oligomenorrhea with hirsutism and weight gain). Patient neurological examination showed minimal neuropsychological impairment (MMSE = 26/30, IQ = 71 with n.v. > 69) deficit of ocular convergence and mild proximal limb weakness (MRC=4). Cerebral NMR showed dysomogeneous thalamic and left frontal T1 hyperintense lesions and multiple white matter T2 hyperintense lesions. Ocular examination demonstrated macular dystrophy in the right eye. Basal serum lactate was 28.2 mg% (n.v. 4.5-19.8 mg%). EMG and ENG disclosed myopathic signs associated with mild axonal neuropathy and still normal nerve conduction findings. Nerve conduction velocities were normal except for a slight reduction in right peroneal nerve.

A left biceps muscle biopsy was performed.

One year later the patient was readmitted to the department. Her clinical conditions were stable, but glucose intolerance was found. EMG performed in her brother and sister gave normal results. Patient's sister muscle biopsy was also normal.

Materials and methods

Left biceps muscle specimen was frozen in liquid nitrogen-cooled isopentane and studied with standard histologic and histochemical techniques, including Congo red stain [4]. Immunohistochemistry with antibodies against class I MHCP or HLA I (ABC), C5b-9 neoantigens (membrane attack complex or MAC), CD4, CD8, CD19, (from Dako) was performed as described [6].

Southern blot analysis of mtDNA and of mtDNA/nuclear DNA (28S), PCR of mtDNA and complete mtDNA sequence were performed following standardized methods [7, 17].

Results

Muscle biopsy showed marked fiber size variability and several necroses sometimes with surrounding cellular invasion; several COX-negative RRF (17% muscle fibers) and a few fibers with rimmed vacuoles (3%) were also present. Connective tissue was moderately, but diffusely increased whereas blood vessels showed no abnormalities.

Congo red positivity was evident in some vacuoles.

Immunohistochemistry with HLA antibody showed both sarcolemmal and cytoplasmic positivity in necrotic fibers. There were several CD4 and CD8 and rare CD19 positive cells in the described infiltrates.

Anti MAC Abs were negative.

Ultrastructural examination showed accumulation of abnormal mitochondria, sometimes with paracrystalline inclusions, in many observed muscle fibers.

Also, we observed intracytoplasmic or, more rarely, subsarcolemmal vacuoles packed with membranous structures, myeloid bodies and glycogen.

Southern blot analysis for both mitochondrial and nuclear DNA showed 80% reduction of mtDNA. Multiple mtDNA deletions were evident by PCR, but not at Southern Blot. Complete mtDNA sequence did not reveal any pathologic mutations.

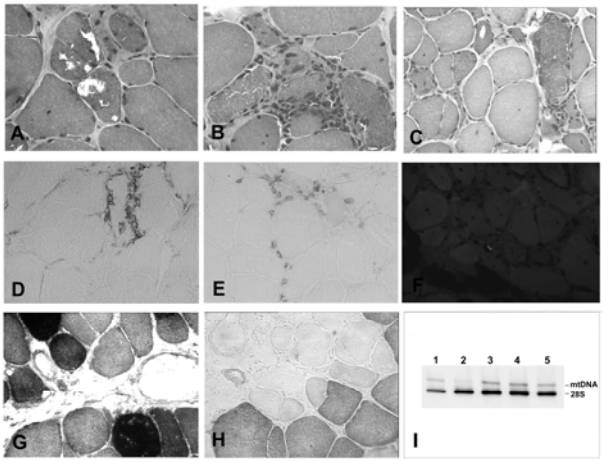

Fig. 1. A, B, C: Gomori Trichrome stain showing large rimmed vacuoles (A), necrotic degenerations with cellular invasion (B) and fiber size variability with muscle fiber degeneration and RRF (C); D, E: immunohistochemistry with anti-CD4 (D) and CD8 (E) antibodies showing positivity in some cellular infiltrates; F: Congo red positivity in intracytoplasmic vacuoles; G: strong SDH positivity in a few fibers (RRF); H: COX activity is absent in some muscle fibers; I: Southern blot analysis of mtDNA/nDNA (28S) shows 80% reduction of mtDNA in the patient (lane 2) compared with controls (lanes 3, 4 and 5); lane 1 is a positive control for mtDNA depletion.

Discussion

The patient had a multiorgan disease involving skeletal muscle (myopathy), central and peripheral nervous system (cerebral NMR alterations, axonal neuropathy), endocrine (amenorrhea, glucose intolerance) and visual abnormalities (macular dystrophy). This systemic in-

volvement suggests mitochondrial dysfunction. Proximal myopathic distribution, familial hyperckemia with endocrine dysfunction (diabetes mellitus, oligomenorrhea and hirsutism) and juvenile onset also indicate a possible mitochondrial pathogenesis. This possibility is further supported by the presence of mtDNA depletion, rise of lactic acid, presence of several RRF with COX deficiency (in about 17% of fibers) and accumulation of abnormal mitochondria with paracrystalline inclusions at EM. However, typical IBM changes (inflammatory infiltrates, vacuolated muscle fiber with positivity to Congo red, compatible ultrastructural changes) were also evident.

Mitochondrial alterations have been described in association with IBM and other inflammatory myopathies. These alterations include RRF, COX deficiency, mitochondria accumulation and, from a genetic point of view, mtDNA multiple deletions, point mutations and large deletions [8, 9, 10, 14, 15, 16]. These abnormalities are commonly explained as an unspecific muscle fiber sufferance secondary to predominant inflammatory myopathy and are not considered to be involved in the pathogenesis of the disease. In our case, however, features of mitochondrial disorders are overtly predominant and, also, there is familiarity for endocrine dysfunction (patient‘ s sister and mother) and increased serum CK without any muscle involvement typical of IBM (patient’ sister, brother and mother).

MtDNA Southern blot revealed 80% mtDNA depletion, a condition normally identified in infantile mtDNA depletion syndromes; this condition has never been reported in association with inflammatory myopathies. Considering that the clinical phenotype in mitochondrial disease often depends on the wild type/pathological mtDNA ratio [13], it is assumable that our patient has a mitochondrial dysfunction with associated IBM features.

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