Necklace fibers as histopathological marker in a patient with severe form of X-linked myotubular myopathy

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Abstract

X-linked myotubular myopathy due to mutations in the MTM1 gene is classically characterized by a severe neonatal phenotype and a typical muscle biopsy presenting globular and centrally located nuclei in muscle myofibers. Recently, four patients with mild late-onset form have been described, a male with a hemizygous mutation and three females with heterozygous mutations in the MTM1 gene. The muscle biopsies were performed at 13–35 years of age and a new histological marker, the necklace fibers, was described. Here, we report two siblings with the pathogenic c.664 C > T mutation in the MTM1 gene, presenting a severe muscle weakness and respiratory impairment requiring ventilatory support since the first months of life until death, at the age of 36 months and 5 months. In the older brother the muscle biopsy, performed at the age of 30 months, showed almost 100% of necklace fibers, which were not present in the younger one submitted to muscle biopsy at 5 months of age. Our findings confirm the necklace fibers can be a histopathological finding of MTM1 myopathies, even in the severe neonatal form, and suggest that the necklace fibers appear or increase in number over time.

Keywords: X-linked myotubular myopathy; Necklace fibers; MTM1 gene; Severe neonatal phenotype

1. Introduction

Centronuclear myopathies (CNM) are a group of inherited muscular diseases with a common histopathological feature characterized by centrally placed nuclei in muscle fibers. Different forms of CNM are recognized based on the clinical severity and the pattern of inheritance [1]. The X-linked recessive myotubular myopathy (XLMTM) is a severe form of disease presenting from birth with respiratory failure, ophthalmoplegia and muscle weakness. This form is caused by mutations in the MTM1 gene which encodes the 3-phosphoinositides phosphatase myotubulin. Mutations in the DNM2 gene were described in mildly affected patients and an autosomal dominant inheritance, while a small number of patients with autosomal recessive pedigree have been related to the mutations in the BIN1 or RYR1 genes [1,2].

Affected males with MTM1 mutations present classically with a severe muscle weakness, ventilatory failure and external ophthalmoplegia since birth. The majority of these patients has a poor prognosis and dies during the first months of life. Long-term survival is related to medical intervention and ventilation support. Analysis of muscle biopsy shows prominent central nuclei in most muscle fibers, resembling immature myotubes associated with type 1 fiber predominance in ATPase reactions. Histochmical reactions for oxidative enzymes identify the
aggregation mitochondrias in the central region of muscle fibres, indicating a lack of ATPase activity. Additionally, a perinuclear halo devoid of myofilaments and occupied by mitochondrial and glycogen aggregates can be observed [1,2].

Some atypical forms of milder XLMTM have been recognized in females and males [1,2]. In 2009, Bevilacqua et al. described the presence of necklace fibers as a histopathological marker of this late-onset MTM1-related form. They found necklace fibers in four patients, three women and one man, who presented with myopathy early in childhood and a slow progressive course. Necklace fibers are formed by a peculiar myofibrillar structure, presenting as a basophilic ring with hematoxylin and eosin staining. Ultrastructurally, these necklaces consist of myofibrils of smaller diameter, in oblique orientation, surrounded by mitochondria, sarcoplasmic reticulum and glycogen granules. The internally located nuclei are usually aligned with the necklace [3].

The aim of the study is to describe two brothers with the classical neonatal severe form of XLMTM, in which almost 100% of necklace fibers were detected in the muscle biopsy of the older brother, but were not present in the younger one (biopsy performed at 30 months and 5 months of age, respectively). These findings suggest that necklace fibers could be a histological marker of MTM1 myopathy, including the severe neonatal form.

1.1. Case report

1.1.1. Case 1

The index case of this family was first evaluated when he was 30-month-old. He was abandoned and lived at the unit care until his death at 36 months of age with pneumonia complicated with generalized infection. He presented with a birth asphyxia requiring continuous ventilator support and did not show any visual contact. At clinical examination he was macroscopic and showed a severe muscle weakness and global hypotonia, ophthalmoplegia, and abolished tendon reflexes. Cranial computed tomography showed cerebral atrophy. Serum creatine kinase level was normal. The muscle biopsy was performed at that time and analysis with hematoxylin and eosin staining showed a basophilic ring located underneath the sarcolemma following the contour of muscle fibers. This pathological finding has been recently described as necklace fiber [3]. At the Gomori’s trichromic staining, the ring was also clearly defined as well in the oxidative stains. At the ATPase reaction, there was an almost total type 1 fiber predominance. The necklace was observed in almost 100% of the fibers and the myonuclei were adjacent to the ring region, without any central nuclei (Fig. 1). There were scattered atrophic fibers with central nuclei but without a necklace area, resembling myotubes. Immunohistochemical analysis showed positive labeling in the rings for desmin, actin and alpha-actinin 3 (Fig. 2). No muscle tissue was available for ultrastructural analysis.

1.1.2. Case 2

The patient is a younger brother of the index case. He presented with a severe neonatal weakness, global hypotonia and abolished tendon reflexes. He needed ventilatory support from the age of two months. The transfontanellar ultrasound and the serum creatine kinase level were normal. A muscle biopsy was performed at five months of age. There was a prominent nucleus placed centrally in several muscle fibers with hematoxylin and eosin staining and with Gomori staining. Using the oxidative enzymes reactions the fibers

![Fig. 1. Muscle biopsy of the case 1 showing the necklace fibers (HE, Gomori and COX). At the ATPase 9,4 reaction there is a type 1 fiber predominance (almost total).][1]

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showed at the central region an increased enzyme activity surrounded by a pale peripheral halo. The ATPase stain showed a type 1 fiber predominance, with a reduced reaction in the central region of the fibers. No necklace fibers were identified (Fig. 3). The patient died at 5 months of age due to respiratory complications.

1.2. Molecular analysis

DNA from both patients was extracted from muscle tissue using the DNA Extraction KIT (Promega, Southampton, England). The complete sequencing of the MTM1 gene was done using primers and conditions as described by Laporte et al. [4], and the mutation c.664C > T (p.R222X) was identified in each brother.

2. Discussion

In this work we identified the presence of the necklace structures, with adjacent myonuclei, in almost 100% of the muscle fibers in a child with the severe neonatal form of XLMTM confirmed by the DNA analysis.

The ring or necklace fibers were first described in 2009, by Bevilacqua et al. in four patients, one male and three women, with a mild late onset MTM1 form, with the onset of symptoms during childhood and a slowly progressive course. Ultrastructural analysis characterized these structures as small myofibrils obliquely oriented, surrounded by mitochondria, sarcoplasmic reticulum and glycogen granules. The authors concluded that the necklace fibers were a marker of the late onset MTM1 myopathy [3].

Up to this moment, there is no description of the presence of necklace fibers in muscle biopsy of patients with a severe XLMTM. However, candidate patients for MTM1 mutations are usually diagnosed at a premature age because of the severity of disease and eminent risk of death. In fact, the youngest brother of our family had his muscle biopsy performed at 5 months of age and the histopathological pattern was typical of XLMTM, without any necklace fibers. On the other hand, the older brother was biopsied at the age of 30 months, in a very late stage of the disease. Therefore, the formation of the necklace structures could be related to time and progression of the disease, and this would be the cause of their first identification in mildly affected patients.

In 2007, Pierson et al. published a careful histopathological description in 15 XLMTM patients. The age at muscle biopsy ranged from 8 days to 12 months. They found 19.2% of myofibers with central nuclei in patients with missense mutations and 11.1% in those with truncation or deletion mutations. They also showed that the proportion of fibers with central nuclei increased with the age of the patient. However, they did not refer to any abnormality similar to necklace fibers in this study [5]. These results suggest progressive alterations in fiber architecture with time.

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In XLMTM. Consequently, the absence of necklace fibers in this study could be related to the early stage at which the muscle biopsy was performed.

Increase in specific pathological findings over time in other forms of congenital myopathies has been described previously. In nemaline myopathy, for example, an increase over time in the amount of rods and in the proportion of type I fibers can be observed. Also, the spatial arrangement of rods inside fibers can change with age within the same patient [6]. Changes in histopathological features has also been recognized in Central Core Myopathy (CCD). Cores, the hallmark of this disease, can be absent in the muscle biopsy when performed at a younger age, but cores are present in the muscle, when a muscle biopsy is repeated in a later stage of the disease [7]. An illustrative case was described by Jungbluth et al. in 2007. In a 16-year-old girl with a phenotype of centronuclear myopathy (congenital myopathy and external ophthalmoplegia), a first muscle biopsy showed multiple central nuclei affecting up to 50% of fibers. In a second biopsy done 8 years later, however, some core-like areas appeared, which suggested and afterward confirmed the presence of a mutation in the RYRI gene [7].

In the original description of necklace fibers by Bevilacqua et al. a comparative study was done in one patient submitted to muscle biopsy at 29 and 43 years of age. They concluded that the necklace fibers could be related to specific MTM1 mutations. Both brothers in the present study showed the c.664 C>T nonsense mutation, which introduces a premature stop codon in exon 8 of the gene. This could cause the loss of the putative catalytic site and the SET-interacting domain of myotubulin, codified by exons 11, 12 and 13. Up to the present date, this mutation has been described in four unrelated patients. The first one, reported in 1997 by Laporte et al. was apparently mildly affected, since he remained alive up to the age of 14 years (although no detailed clinical description was presented) [8]. The three other cases showed a typical severe phenotype, similar to that observed in the family here reported [9,10]. Truncation mutations in the MTM1 gene are frequently associated with the severe phenotype and poor prognosis [11]. On the other hand, Bevilacqua et al. described patients with milder and later onset myopathy associated with two missense mutations, one frameshift deletion causing a premature stop codon at position 69 and another mutation in exon 11 leading to an abnormal splicing and deletion of nine aminoacids in the catalytic domain of the myotubularin protein. All these nonsense and missense mutations, frameshift deletion were associated with the presence of necklace fibers. An additional important point is the quantity of necklace fibers. Our older brother presented almost 100% of fibers with necklace, differently from the patients described by Bevilacqua et al. in whom the necklace proportion ranged from 3% to 10% of fibers. So, we can suggest that the presence and the amount of necklace fibers could be related to the severity phenotype and probably to specific molecular defect.

In 2010, Liewluck et al. described a patient with a para-vertebral muscles hypertrophy and mild neutropenia, associated with the heterozygous c.1269C>T (p.Arg369Trp) mutation in DNM2 gene. The muscle biopsy revealed the CNM typical pattern with scattered necklace fibers [12]. However, the necklace fibers identified in this patient showed some differences which were pointed out by Romero in 2011, who emphasized that the myonuclei were not adjacent to the “necklace” area, but located in the central part of the muscle fiber [13]. In our patient, we clearly observed the myonuclei in a position adjacent to the necklace region.

In conclusion, based on the data here presented, we can confirm the necklace fiber as a marker of MTM1-related centronuclear myopathy. However, it is not an exclusive feature of the milder and late-onset forms but it could also be observed in the severe neonatal form.

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