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MULTI-MINICORE DISEASE REVISITED

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ABSTRACT - Multi-minicore disease (MmD) is an infrequent congenital myopathy, defined by structural changes in optic and electron microscopy, namely, multiple small areas lacking oxidative enzyme activity and focal disorganization of contractile proteins involving at most a few sarcomeres. The classical form of the disease manifests as more or less severe hypotonia and generalized weakness with predominance in axial and proximal limb muscles. Clinical variants also exist. Usually MmD is inherited as an autosomal recessive trait. Genetic heterogeneity is recognized and up to now mutations in the genes of RYR1 and SEPN1 have been detected. We record three unrelated cases of MmD. Case 1, with the classical benign form, was followed-up for 15 years. Case 2, presenting pharyngolaryngeal involvement and severe delay of head control, improved gradually, until independent gait was acquired at age of six years. A moderate restriction of daily life activities remains. Case 3, of antenatal-onset, was expressed by arthrogryposis of hands, predominance of scapular girdle deficit and a stable course after ten years on physiotherapy. All cases were selected by the characteristic morphological abnormalities in *biceps brachii* samples, including electron microscopy. Emphasis is given to case 2 due to type 1 fiber uniformity and mild endomysial fibrosis, posing a difficult differential diagnosis with congenital muscular dystrophy were it not for the significant number of multi-minicores.

KEY WORDS: congenital myopathy, multi-minicore disease, phenotype, histochemistry, electron microscopy.

Miopatia dos multi-minifocos revisitada

RESUMO - A miopatia dos múltiplos minifocos (MM) é doença congênita rara, definida por alterações estruturais observadas ao microscópio óptico e eletrônico: múltiplas e pequenas áreas sem atividade enzimática oxidativa e desorganização focal das proteínas contráteis envolvendo poucos sarcômeros. A forma clássica da doença se manifesta com hipotonia mais ou menos grave e fraqueza generalizada, predominante em músculos axiais e proximais em membros. Entretanto, variantes clínicas existem. A MM é usualmente herdada como traço autossômico recessivo. Heterogeneidade genética tem sido reconhecida e até o momento mutações nos genes RYR1 e SEPN1 foram detectadas. Relatamos três casos de MM. Caso 1, que tem a forma clássica e benigna da doença, assim permaneceu ao longo de 15 anos. Caso 2 apresentou envolvimento faringo-laríngeo e grave atraso no controle cefálico que melhorou gradualmente, até que a deambulação plena foi adquirida aos seis anos; permanece com moderada limitação das atividades da vida diária. Caso 3 teve início pré-natal, expresso através de artrogripose das mãos. Havia predominância de déficit em cintura escapular e o curso tem sido estável, com fisioterapia, por 10 anos. Os casos foram selecionados pelas características morfológicas na biópsia do biceps braquial que incluiu microscopia eletrônica. Enfatizamos, no caso 2, a uniformidade das fibras do tipo 1 e a leve fibrose do endomísio, tendo sido necessário o diagnóstico diferencial com distrofia muscular congênita.

PALAVRAS-CHAVE: miopatia congênita, miopatia dos multi-minifocos, fenótipo, histoquímica, microscopia eletrônica.

Multi-minicore disease (MmD) is a rare myopathy defined through muscle biopsy (MB)[1]. Cores are well-demarcated, non membrane bound mitochondria-free foci in muscle fibers, associated with disorganized myofibrils. They may be single, central or eccentric in transverse sections. In longitudinal sections they stretch for several sarcomeres or up to the whole length of the fiber, as classically seen in central core disease (CCD)[2,3], in which they involve almost always type 1 fibers. When multiple (designated multicores by Engel et al.[4]) and small (named minicores by Ricoy et al.[5]), cores may affect both fiber types, according to the International Consensus on MmD[1].

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A "congenital nonprogressive myopathy with multifocal degeneration of muscle fibers" was briefly described in 1966 and designated multicore myopathy in 1971 by Engel et al.[4]. Since then, at least 70 cases were published[1]. The largest multi-institutional series presented 38 patients, leading to identification of four phenotypically homogeneous subgroups[6].

MmD is transmitted as a recessive trait, although sporadic cases are more frequent in practice[7]. Recently, studies provided evidence for genetic heterogeneity of MmD[8-11]. Mutation in the skeletal muscle ryanodine receptor (RYR-1) gene (locus 19q13)[8,9,11], well recognized in CCD, has been found in cases of MmD, thus linking the two diseases at least at the genetic level. On the other hand, classical cases of MmD harbor a mutation in the selenoprotein N (SEPN-1) gene (locus 1p36)[10], which may also be found in congenital muscular dystrophy with rigid spine syndrome.

We describe three new sporadic cases of MmD with distinct clinical phenotypes and course, followed-up for 10 to 15 years. Recent insights about MmD are also discussed.

METHOD

Cases were selected among those referred to UNICAMP Neuromuscular Clinic from 1982 to 2000 and which fulfilled the following criteria: 1. clinical evidence of congenital myopathy; 2. creatine kinase (CK) analysis performed at least once; 3. normal sensory and motor nerve conduction velocities (NCV); 4. electromyographic examination (EMG); 5. MB showing mini-multifocal areas devoid of oxidative enzyme activity in a significant number of muscle fibers and short length disorganization of myofibrils observed ultrastructurally.

MB specimens (left *biceps brachii*) were frozen in n-hexane previously cooled in liquid nitrogen and sectioned at 4 to 8 μm in a cryostat. Serial sections were stained with hematoxylin and eosin (H&E), modified Gomori trichrome (TRI), oil red O (ORO). Histochemistry for myofibrillar adenosine triphosphatase (ATPase), reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-TR) and succinate dehydrogenase (SDH) was performed. A muscle sample was fixed in cacodylate-buffered glutaraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Araldite®. Transverse and longitudinal sections 1 μm thick were stained with toluidine blue. Ultrathin sections were analyzed in a Zeiss-10 electron microscope.

CASES

Case 1. A male child was born in September 1981 by cesarean section due to pelvic presentation. The parents were healthy and non consanguineous and denied neuromuscular disease in their families. Floppiness was detected early and a mild delay in motor milestones was observed by his pediatrician. In contrast with good psychological development, running was accompanied by frequent falls. He had to help himself with his hands in order to stand up or climb steps. When examined at 5 years and 10 months he was smart and collaborative, with moderate diffuse hypotonia, flat feet and high arched palate. Muscles were slender. Cervical flexion and scapulo-humeral muscle forces were graded 4+. Pelvic girdle deficit was evident by Gowers sign and a slightly clumsy gait. Muscle stretch reflexes were present. No facial muscle deficit, ophthalmoparesis or other neurological abnormal function were detected. NCV examined in upper and lower limbs were normal as well as EMG. A serum CK sample revealed twice the upper normal limit. Another sample, taken a year earlier, had been normal. MB was done at the age of 6 years when the diagnosis of MmD was confirmed. Hydrotherapy and physiotherapy were recommended and weight gain was discouraged. Prognathism was detected at age 12 and corrected by conservative orthodontic methods, remaining mild. At age 19 and height of 1.90 m the same motor disabilities were observed. No significant vertebral column abnormsality developed, but a mild thoracolumbar scoliosis, slight flat thorax and mild bilateral elbow contractures were seen.

Case 2. A female neonate was normally delivered in September 1988 after a term gestation that was remarkable for poor fetal movements. She weighed 2,460 g and measured 50 cm; head circumference was 38 cm. She was the first child of young, healthy and non consanguineous parents that informed to be unaware of muscle disease in their families. Her younger sister is healthy. Severe hypotonia, weak cry and bilateral clubfoot were noted at birth. Despite difficulty for breast feeding, the child gradually acquired weight and height over the 50 percentile and could stay seated at the age of 15 months. Neuromuscular consultation at two years and six months was motivated by poor head control and marked delay in motor milestones, including inability to walk. Physical examination disclosed generalized hypotonia, diffuse amyotrophy, absent muscle stretch reflexes and muscle weakness (proximal exceeding distal). Prominent cervical deficit led to abnormal head posture. High arched palate, elongated hands and bilateral clubfoot were noted. CK samples and NCV were normal. EMG of right *biceps brachii* disclosed 100% of short duration polyphasic motor units, with low amplitudes. She was submitted to MB in May 1991. The patient was fitted with a molded ankle-foot orthosis and with a specially designed orthosis that provided head support. A regular follow-up over 12 years revealed normal cognition, persistent diffuse amyotrophy and arreflexia; development of moderate kyphoscoliosis and mild elbow contractures [Fig 1 a-c]. Normal head control was gradually acquired.

She walked with aid at about 5 years of age and autonomously at 6. Routine exams, EKG, thorax radiographs and cranial CT scans were non contributory. A moderate restriction of motor performance persists.

Case 3. A male child was born in September 1981 after normal pregnancy and cesarean delivery weighing 3,120 g and measuring 52 cm. Parents informed general good health, non consanguinity and absence of known neuromuscular problems in their families. Since birth, digits of both hands were kept in permanent flexion and he had great difficulty in opening them. He walked at about 18 months and had frequent falls specially when running. Cerebral palsy was suspected, but his mother refused to accept it. He was able to follow school though hampered by his hand deformity. Only physical therapy was recommended. He was first seen at our service in March 1993. On examination, there was motor deficiency of shoulder girdle with winging of scapulae and difficulty in digital extension. A mildly clumsy gait and slight general hypotonia were detected. Muscle stretch reflexes were normal. NCV and two CK samples proved normal and EMG was myopathic. MB was done at age 11 leading to diagnosis of MmD. The condition has kept stable up to present [Fig 2 a-c].

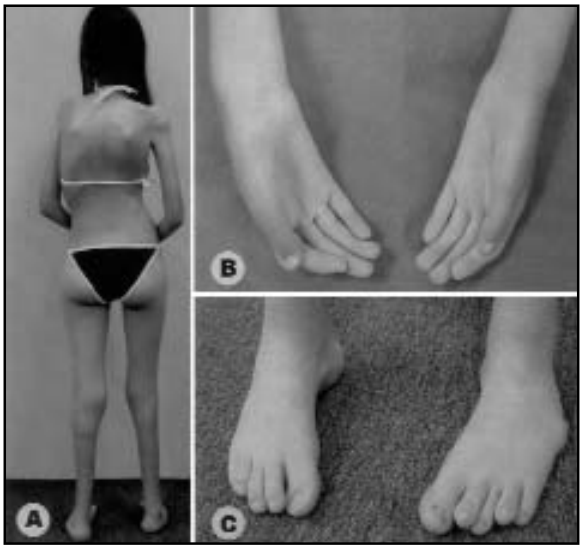

Fig 1. Case 2. Recent picture showing diffuse muscle hypotrophy and kyphoscoliosis (a); abnormal hands(b) and feet (c).

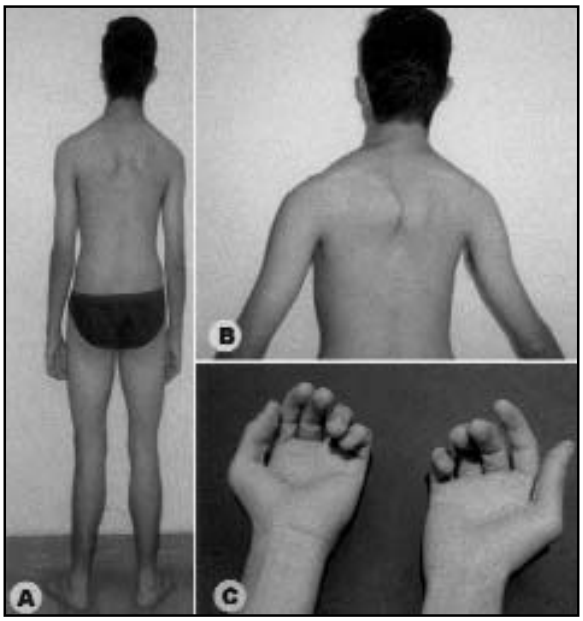

Fig 2. Case 3. Winging of scapulae and wasting of shoulder girdle (a and b). In contrast, note good trophic appearance of pelvic girdle and congenital deformity of hands with failure to completely extend the fingers on voluntary attempt (c).

DISCUSSION

MmD usually presents as congenital myopathy[2-7] rarely as adult-onset disease[12]. As regards the Brazilian literature, Werneck's[13] series of 1500 MB included 47 cases of congenital neuromuscular disorders, of which four were MmD. Another congenital case was published by Tsanaclis and Levi[14]. Our three cases are congenital, though they were first examined by us at 5 years and 10 months, 3 years and 6 months and 11 years and 6 months, respectively. MB was performed shortly thereafter.

Our patients have had a long follow-up that allowed us to recognize the benign natural history of the disease, as have others[2,4,5]. In spite of slow progression in most instances, fatal cases of MmD have been described, generally associated with cardiomyopathy[15]. Worse prognosis has also been associated with respiratory insufficiency due to weakness of diaphragm and accessory respiratory muscles and/or thoracic deformities[6]. Respiratory failure occurred in half of the patients of Jungbluth et al.[7] aged over 10 years and correlated with the degree of scoliosis.

Clinical manifestations vary greatly in MmD. Ferreiro et al.[6] could identify four clinical phenotypes: 1. Classical; 2. Severe form with pharyngolaryngeal involvement and lack of head control; 3. Antenatal-onset with arthrogryposis; 4. Progressive form with hand amyotrophy. Our case 1 is an example of classical and benign MmD. Case 2 seemed to fit into the severe form with pharyngolaryngeal involvement and lack of head control during the first two years of life, but marked improvement of motor function was observed afterwards. Ambulation occurred late resembling the cases of Heffner et al.[16] and Penegyres and Kakulas[17]. Case 3 had ante-

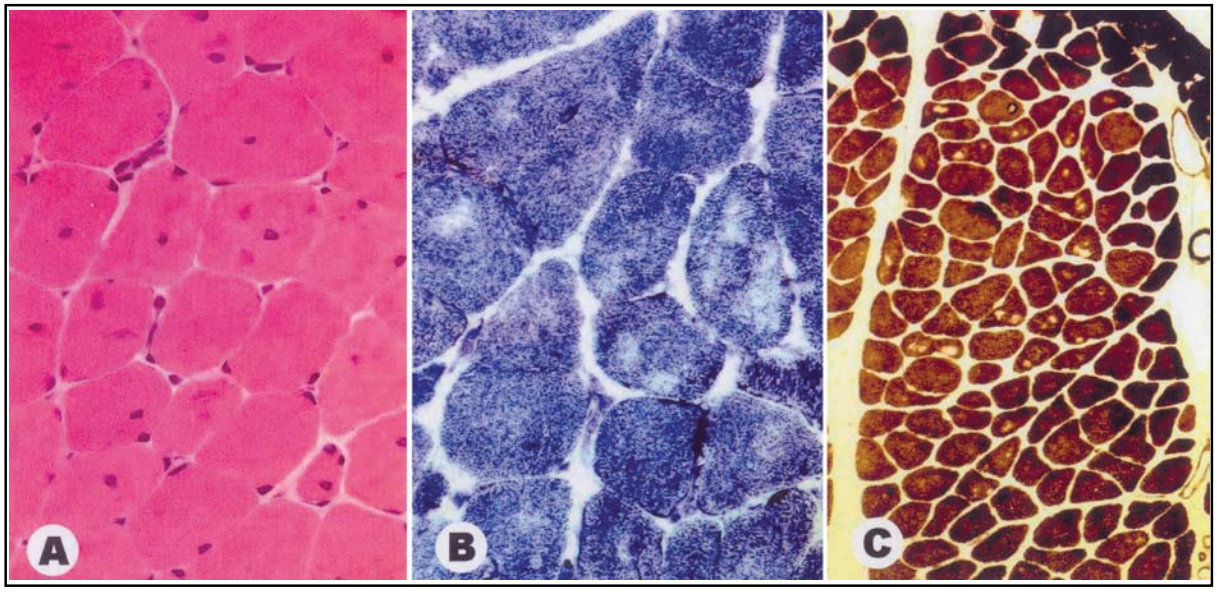

Fig 3. (a) Case 1. H&E. Variation in fiber size, internal nuclei, irregular basophilic areas, multiple in some fibers. (b) Case 1. NADH-TR. Multiple and variably sized foci of decreased enzyme activity. (c) Case 2. ATPase pH 4.3. Type 1 fiber uniformity. Many fibers show foci of decreased or absent activity which vary in size and number.

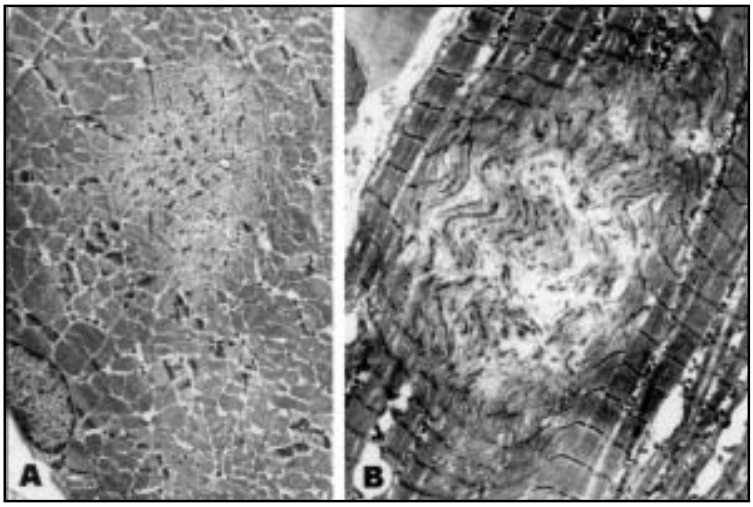

Fig 4. (a) Case 1. Electronmicrograph of transversely sectioned muscle fiber. Small area of disorganization of myofibrillar structure with Z line fragmentation and absence of mitochondria (minicore). (b) Case 2. Similar structure in a longitudinally sectioned muscle fiber. Cases 1-3 had the same ultrastructural appearance.

natal-onset manifested by mild arthrogryposis of hands. Weakness predominated in the shoulder girdle, similar that observed by Jungbluth et al.[7] in five of their 19 cases. The myopathy had a benign and stable course.

Engel et al.[4] described mild ptosis in one of their two patients. Another ocular feature of the disease is external ophthalmoplegia[18-20] noted in the two most severely affected patients of Jungbluth et al's.[7] series of 19. A challenging case of epilepsy, complex encephalopathy and minicore myopathy was recorded by Avoni et al.[21]. Multi-minicores have been seen also in Marfan's syndrome[22], short chain acyl-CoA dehydrogenase deficiency[20], type III glycogenosis[23] and anhidrotic ectodermal dysplasia[24].

Our three cases were selected by the characteristic optic and electron microscopic abnormalities on MB, as recommended[1]. In case 1 a routine H&E

showed slight variation of fiber diameter, numerous fibers with central nuclei, almost all exhibiting small and multiple basophilic areas (Fig 3 a). With myofibrillar ATPase both fiber types were present in similar numbers and both had multi-minicores. These foci were negative for oxidative enzymes [NADH-TR (Fig 3 b) and SDH]. Type 1 fiber uniformity was a peculiarity of case 2 (Fig 3 c) and a moderate type 1 predominance was seen in case 3. Cases 2 and 3 also had significant numbers of central nuclei and multi-minicores. In case 2 some restricted areas had mild perimysial and endomysial fibrosis and a more prominent variation in fiber size, leading initially to the diagnosis of congenital muscular dystrophy. Electron microscopy (Fig 4 a and b) and re-evaluation of the frozen sections defined the diagnosis of MmD. This case was exclued from our series of congenital muscle dystrophy patients[25]. We are thus in accordance with the guidelines of MmD consortium[1] that recommended electron microscopy as one of the criteria for diagnosis. Immunohistochemistry may help by demonstrating filamin C and alpha-B-crystallin within the minicores, where they react stronger than desmin[26].

Clinical genetics and DNA studies have shown autosomal recessive inheritance in MmD. Rare autosomal dominant cases diagnosed as MmD[27,28] have not yet been submitted to genetic molecular analysis. Molecular heterogeneity was confirmed because mutations in RYR1 as well as SEPN1 genes have been identified.

Apart from an anecdotal case with reaction to anesthesia[29] no cases of malignant hyperthermia have been documented in MmD. More than half of the patients of Jungbluth et al.[7] have been under general anesthesia uneventfully at least once. In Ferreiro et al.[6] series of 38 cases, 19 surgical procedures with general anesthesia were performed in 16 patients, with no abnormal reactions or malignant hyperthermia being recorded in patients or their relatives. In our patients, MB (cases 1 and 2) and cerebral CT scan (case 2) were performed under propofol or isoflurane without problems. However, considering that multi-minicores may be a transient phenotype of CCD[9], susceptibility of patients with this alteration to malignant hyperthermia should always be kept in mind. Therefore, a careful monitoring of the patient by the anesthesiologist and ready availability of dantrolene in the operative room are mandatory.

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