

CENTRONUCLEAR MYOPATHY

HISTOPATHOLOGICAL ASPECTS IN TEN PATIENTS WITH CHILDHOOD ONSET

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ABSTRACT - Centronuclear myopathy is a rare congenital myopathy. According to the period of onset of signs and symptoms and the degree of muscular involvement three clinical forms are distinguished: severe neonatal; childhood onset; and adult onset. We describe herein the muscle biopsy findings of ten patients with the childhood onset form of the disease including three cases with ultrastructural study. The biopsies disclosed increased nuclear centralization that varied from 25 to 90% of the fibers, type I predominance, great variability in fiber diameters, involvement in the internal fiber's architecture, and focal areas of myofilament disorganization. The main histopathologic differential diagnoses included type I fiber predominance, congenital fiber type disproportion, and myotonic dystrophy. The histologic abnormalities in centronuclear myopathy may be due to an arrest of maturation on the fetal myotubular stage. The cause of this arrest remains elusive.

KEY WORDS: centronuclear myopathy, myotubular myopathy, histochemical, ultrastructure.

Miopatia centronuclear: aspectos histopatológicos em dez pacientes com a forma clínica de início na infância

RESUMO - A miopatia centronuclear (MCN) é uma forma rara de miopatia congênita. De acordo com a época do início dos sinais e sintomas e com o grau de envolvimento muscular são distinguidas três formas clínicas: forma neonatal severa; forma de início na infância; e de início na vida adulta. São apresentados neste estudo os achados histopatológicos de dez pacientes portadores da forma de início na infância da MCN. Os fragmentos musculares foram processados através de colorações de rotina e histoquímica, e em três casos foi realizado estudo ultraestrutural. Dentre os resultados obtidos, destacou-se o aumento da centralização nuclear na fibra muscular, que variou de 25 a 90%. Adicionalmente, foram observadas predominância de fibras do tipo I, variabilidade entre o diâmetro das fibras musculares, alterações da arquitetura interna das fibras musculares e presença de áreas focais de desorganização dos miofilamentos. Devido a estes aspectos, os principais diagnósticos diferenciais considerados foram as miopatias por predominância de fibras e por desproporção de fibras, e a distrofia miotônica. As anormalidades histológicas observadas na MCN podem ser devidas a uma parada no processo maturacional do músculo esquelético na fase miotubular fetal. A causa deste defeito ainda permanece sem explicação completa.

PALAVRAS-CHAVE: miopatia centronuclear, miopatia miotubular, histoquímica, ultra-estrutura.

Centronuclear myopathy (CNM) is a rare disease, classified in the congenital myopathy group, described in 1966 by Spiro, Shy and Gonatas³¹. It presents with diffuse involvement of skeletal muscles, including those innervated by the cranial nerves, with onset in early childhood and a slowly progressive evolution. The basic histological abnormality is the high percentage of centrally placed nuclei associated, in most cases, with a perinuclear halo without myofibrils and a high oxidative activity^{30,31}. Other

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histological findings include hypotrophy and predominance of type I fibers, variability in fiber size, and a slight increase in the connective tissue⁷. CNM presents a great clinical and genetic heterogeneity. According to the period of onset of signs and symptoms and degree of muscular involvement three clinical forms of CNM are distinguished: 1) severe neonatal; 2) childhood onset; 3) adult onset.

Due to the resemblance of the muscle fibers of these patients with those of the fetus in the myotubular stage of myogenesis, the disease was initially called myotubular myopathy³¹. However, this denomination is now likely to be restricted to the severe neonatal form, linked to chromosome Xq28³³, and whose histological alterations are more akin to fetal myotubes than are the other two forms^{7,27}.

The objective of this study is to present and discuss the histopathological findings in 10 patients with the childhood onset form of CNM.

MATERIAL AND METHODS

We present herein 10 patients, 7 females and 3 males, mean age 16.3 years (3-25), with clinical and histological diagnosis of CNM registered in the Department of Neurology, Universidade Federal de São Paulo (UNIFESP) - Escola Paulista de Medicina (EPM), from January 1984 to June 1996. Detailed clinical aspects of those patients are to be described elsewhere³⁵.

A muscle biopsy was performed in the deltoid muscle in all. Routine staining performed were hematoxylin and eosin (HE), modified Gomori trichrome (GT), periodic acid Schiff and oil red. Histochemical reactions for adenosine triphosphatase (ATPase) with preincubation at pH 9.4, 4.63 and 4.34, nicotinamide dehydrogenase tetrazolium reductase (NADH-tr), and succinate-dehydrogenase (SDH) were performed as previously described⁸.

Muscle fragments of three cases (Patients 2, 5 and 9) were submitted to an ultrastructural study. The muscle specimens were fixed in 2% glutaraldehyde in phosphate buffer. Ultrathin sections were double stained with uranyl acetate and lead citrate.

Muscle biopsy was repeated in Case 2 after nine years.

RESULTS

The percentage of nuclear centralization was assessed by counting the fibers with one or more centrally or paracentrally located nuclei in relation to a total of 200 fibers counted. Obviously, all muscle biopsies specimens revealed increased centrally placed nuclei, since this was the main

Table 1. Main histological abnormalities seen in the skeletal muscle (HE and Gomori) of ten patients with CNM.

Patients	1	2	3	4	5	6	7	8	9	10
Nuclear centralization	80%	70%	60%	60%	25%	40%	90%	90%	60%	85%
Hypotrophied fibers	+	+++	+++	-	++	+++	++	+++	+	+++
Hypertrophied fibers	-	+++	+++	-	-	+++	++	-	-	+
Variability among the size of the fibers	+	+++	+++	-	+	+++	++	+++	+	+++
Endomysial connective tissue	+	+	+	+	-	+	+	+++	+	+
Perimysial connective tissue	+	-	-	-	+	-	-	+++	-	-
Necrotic fibers	-	-	-	-	-	-	-	-	-	+
Inflammation	-	-	-	-	-	-	-	-	-	-

- absent, + light, ++ middle, +++ intense.

Table 2. Main histological abnormalities seen in skeletal muscle (ATPase, NADH-tr and SDH) of ten cases with CNM.

Patients	1	2	3	4	5	6	7	8	9	10
Type I fibers (ATPase)	99%	100%	100%	98%	100%	70%	90%	NA	100%	95%
Type II fibers (ATPase)	1%	0	0	2%	0	30%	10%	NA	0	5%
Internal architecture alterations of the muscle fibers (NADH-tr/SDH)	++	++	++	+++	+	+	++	++	++	++

NA no accomplished, + light, ++ middle, +++ intense.

criteria used for diagnosis of CNM (Table 1). A clear perinuclear halo was noted in most of the muscle fibers in Cases 1, 6, 7, 8, 9 and 10 (Fig 1a). In longitudinal sections, many of the nuclei were arranged in rows in all patients (Fig 1B).

We found fiber size variability in 90% of the biopsies (Table 1) (Fig 1c). Hypotrophied fibers were seen in 90% of patients, and hypertrophied in 50%.

Proliferation of endomysial connective tissue was observed in 90% of the biopsies, but this involvement was severe only in Case 8. The perimysial connective tissue was slightly increased only in cases 1 and 5 and severely increased in Case 8. Increased adipose tissue was not detected in the endomysium, but was found in the perifascicular spaces in all patients.

Rare necrotic fibers were observed in Case 10 with a mild macrophagic reaction. Inflammation was not observed in any patient. Nervous terminals, neuromuscular spindles and blood vessels, when present, were normal.

Differentiation of fiber type was poor on NADH-tr and SDH reactions (Table 2).

Internal architecture alterations of the muscle fibers on NADH-tr and SDH were seen in all cases. Those were characterized by either oxidative enzyme activity increase at the center of the fiber surrounded by a halo devoid of enzymatic activity, or by the absence of oxidative activity in the center of the fiber associated with a surrounding halo of higher activity with reduction of this activity in the most peripheral portions (Fig 2). A radial appearance of myofibrils surrounding the central portion was observed in many fibers, and areas with reduction of oxidative activity scattered throughout the muscle fiber was noted in Case 4.

On ATPase staining, fiber type distribution were counted out of a total of at least 200 fibers. In Case 8 that was impossible due to scarcity of material. In 4 patients (Cases 2, 3, 5 and 9) there was a type I fiber uniformity. In the cases in which differentiation was detectable, type I predominance and preservation of mosaic pattern were observed in all. Only in Case 7, type I fibers were hypotrophied and type II fibers hypertrophied. In the other cases, both type I and type II fibers were hypotrophied or hypertrophied. Nuclear centralization occurred in the two fiber types in all cases in which differentiation was observed. Clear areas in the central or paracentral portions, corresponding to absence of myofibrillar activity, were observed in all cases. It was not possible to differentiate the subtypes IIa, IIb and IIc in any case, due to the scarcity of type II fibers.

Muscle biopsy was repeated in Case 2 after nine years and no significant differences were found when compared with the first evaluation.

Ultrastructural findings in Patients 2, 5, and 9 confirmed the routine histological and histochemical studies. Centrally placed nuclei was surrounded by a halo without myofibrils where mitochondria accumulation and rare myelin figures were observed (Fig 3). The nucleus showed a normal internal structure and prominent nucleoli. The nucleous membrane presented indentations in all, with a multilobulated aspect in Case 2 (Fig 3). In Cases 2 and 9, areas with disorganized

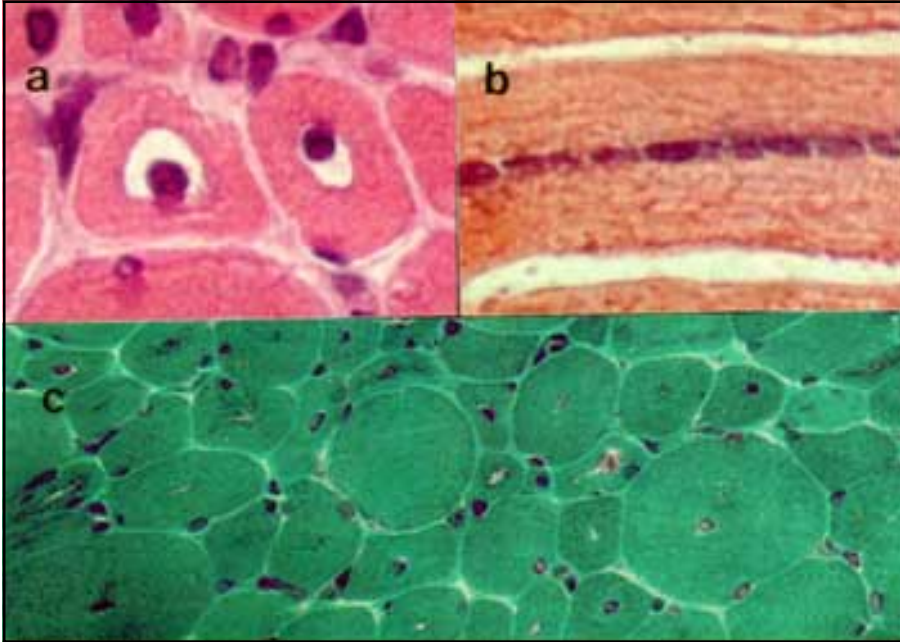


Fig 1. a) Hypotrophied muscle fibers with centrally placed nuclei surrounded by halo devoid of myofibrils, like fetal myotubes (Case 10) (HE X 450). b) In longitudinal section, the nuclei arranged in chains (Case 8) (HE X 450). c) Central nuclei through out the biopsy, without evidence of degeneration and connective tissue proliferation, and marked variability in muscle fibers size (Case 10) (TG X 200).

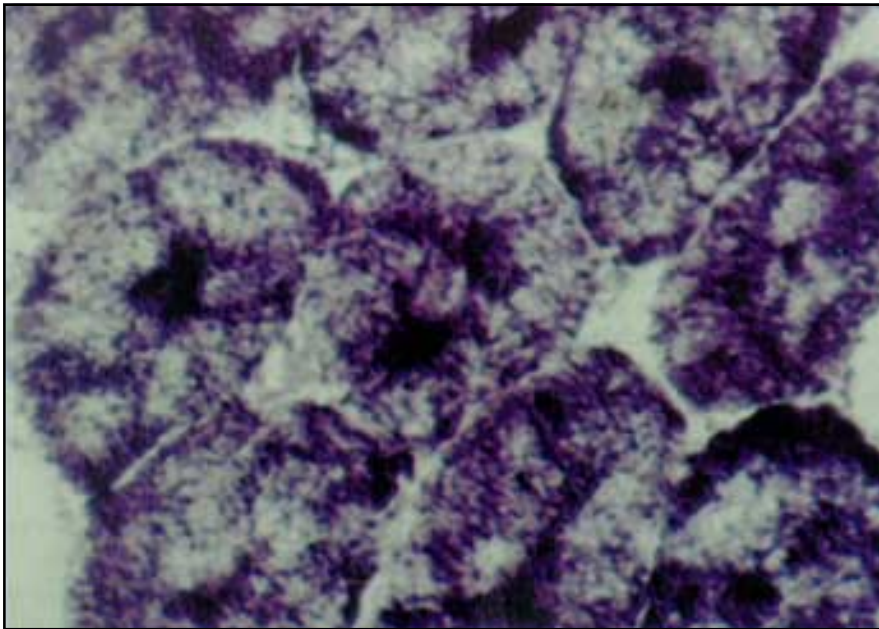


Fig 2. The internal myofibrillar network is disorganized, showing increased oxidative enzyme activity in the central and subsarcolemmal area of the muscle fibers and fibers with target aspect. (Case 4) (NADH-tr X 450).

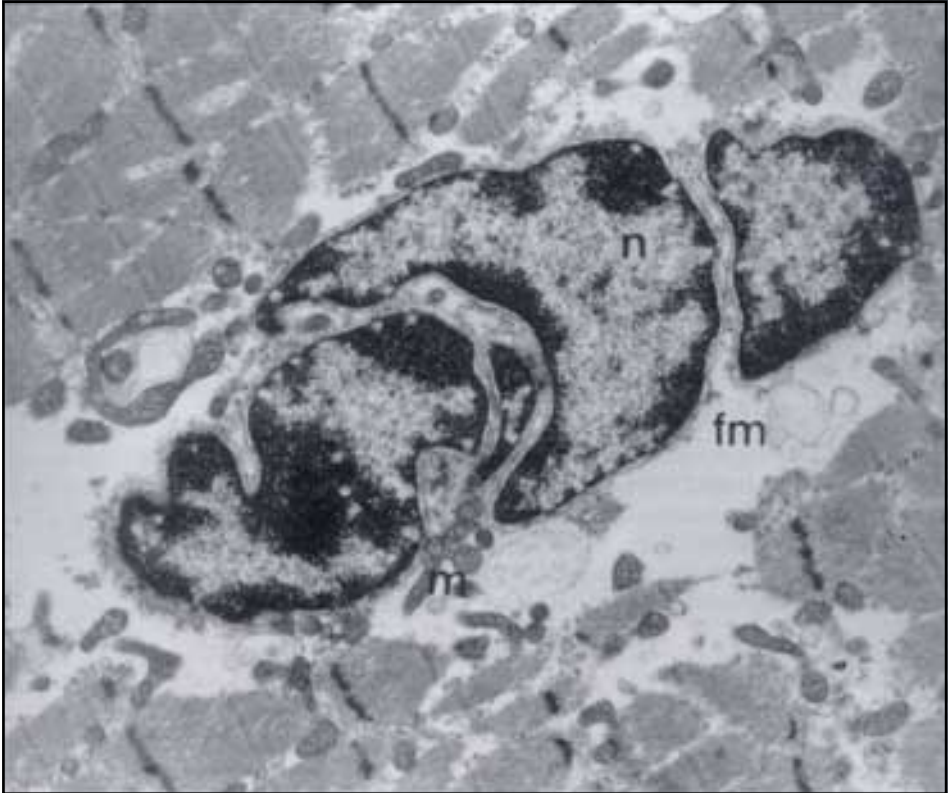


Fig 3. The central nucleus (n) with multilobulated aspect is surrounded by a halo containing mitochondria (m) and rare myelin figures (fm) (Case 2) (14100X).

myofibrils, fragmentation of the Z line and reduction of mitochondria were observed. Mitochondria and the sarcotubular system had a normal appearance. In the examined material it was not possible to analyze neuromuscular spindles and nervous terminals.

DISCUSSION

The main histological feature in CNM is the increase in the percentage of muscle fibers with a centralized nucleus. Many of these fibers are similar to fetal myotubes that are hypotrophic with a centrally placed nucleus and perinuclear halo devoid of myofibrils^{30,31}. In longitudinal sections the centralized nuclei are seen in rows and oriented longitudinally to the greater axis of the fiber^{5,30}. In our series of patients the percentage of nuclear centralization varied from 25% to 95%. This percentage may vary according to the muscle group examined, the type of sections (transversal or longitudinal) and the period in which the muscle biopsy is performed in a same patients^{12,14,20,26,30}.

Increase in the endomysial connective tissue is mild to moderate^{16,30,31}. Only one patient of our series had a severe increase and in the other patients it was absent or insignificant. Other authors have found an increase in the endomysial, or perifascicular adipose tissue^{2,3,4,14,30}. Only an increase in the perifascicular adipose tissue we have found in our patients.

On the NADH-tr and SDH stainings, there are findings characteristic of the myopathy. Frequently, there is an increase in oxidative activity in the central and subsarcolemmal portion of the muscle fiber due to excess of mitochondria and oxidative enzymes in the internuclear spaces. Other

fibers may show absence of activity in the center of the fiber surrounded by a halo with higher activity, more probably corresponding to a level of section on the centralized nucleus^{2,14,21}. Also, a radiated aspect of the myofibrils, of spoke-like appearance, may be observed surrounding the nucleus^{14,34}, and in some cases there are intense alterations in the internal architecture where areas with absence of oxidative activity can be observed throughout the whole muscle fiber^{4,20}. Such histochemical alterations were observed in our study but with variable intensity from case to case. Fiber type differentiation is very poor using these staining procedures^{4,14,20}, a fact we observed possibly due to internal architectural alterations.

Type I fiber predominance, one of the main findings in CNM^{3-5,11,12,15,16}, was seen in all our muscle samples. Four patients disclosed only type I fibers. Type I fiber predominance is nonspecific, and observed in many myopathies⁷. In some cases it is the only histological alteration found in muscle which makes some authors believe that it is an isolated form of congenital myopathy, named myopathy due to fiber predominance²². It is possible that in all these instances there is an inability of the muscle fibers to differentiate.

Congenital disproportion of fibers is a myopathy characterized by isolated type I fiber atrophy, and may be confused with CNM because in some cases a slight increase in nuclear centralization may occur¹⁹. The finding of classic pictures of CNM and those due to congenital disproportion of the fibers in the same family¹⁸ and in different phases in the same patients³⁴ suggests that myopathy by congenital disproportion of fibers may present a strong interrelationship with CNM, which might correspond to extremes of the same dysmaturational process²⁶ or even different phases of the same disease.

Another frequent finding in CNM is the increased variability in fiber size where there are hypotrophied fibers usually in a great number adjacent to normal size and hypertrophied ones^{4,5,12,14,15,16}. This variability occurred in 80% of our cases. Usually, hypotrophied fibers are type I while hypertrophied are type II^{12,14,15,16}, as observed in one of our cases. However, in some cases both type I and type II fibers may present hypotrophy or hypertrophy³⁶, as occurred in four of our cases.

Nuclear centralization occurs in both type I and type II fibers^{17,30}, although predominantly in the former^{6,18} as observed in all our patients. Engel et al.¹⁰ reported a patient with nuclear centralization almost exclusively in type I hypotrophied fibers. They considered it as another form of myopathy named "type I fiber hypotrophy and central nuclei". Similar findings were later published by other authors^{15,16}. However, the heterogeneity of clinical manifestations in these patients and the finding of this alteration in a same family of patients with classic histological pictures of CNM^{9,25} made the classification of these patients within an isolated entity of CNM impossible.

Other differential diagnosis may be considered, chiefly polymyositis and myotonic dystrophy. In polymyositis, in addition to muscle necrosis, there is an increase in the percentage of nuclear centralization due to the process of regeneration associated with the presence of inflammatory infiltrates. Muscle necrosis and inflammation are rarely described in CNM¹. In one of our cases, rare necrotic fibers with a surrounding macrophagic reaction and without evidence of inflammatory infiltrate were observed. Mild rhabdomyolysis secondary to excessive physical effort, viral infections or various systemic disorders might explain this finding²⁴.

The histological findings of myotonic dystrophy, mainly of the congenital form, are likely to be confused with those of CNM. The presence of hypotrophied fibers with an increase in the percentage of nuclear centralization could resemble myotubes¹³. In the congenital form of myotonic dystrophy, the child has hypotonic muscles at birth, respiratory failure and, in most cases, ophthalmoparesis^{13,28}. The myotonic phenomenon generally is not evident during the neonatal period^{13,28}. The presence of manifestations in the mother is, in many cases, the only way to differentiate congenital myotonic dystrophy from CNM²⁸. In the adult form of myotonic dystrophy, the finding of a great number of ring-like fibers, sarcoplasmic masses, and degenerative alterations of the muscle fibers help differentiate it from CNM¹³.

During the course of CNM, hypotrophy of type I fibers and the percentage of nuclear centralization may either persist^{11,12} or subside^{26,31}. Worsening of the histological findings has also been observed^{20,34}. Different findings in serial muscle biopsies, however, do not present a clear correlation with the evolution of the disease. A new muscle biopsy after 9 years of follow-up in one of our cases did not reveal significant histological differences in relation to the first examination.

In the severe neonatal form the fibers have an aspect similar to fetal myotubes. The fibers are intensively hypertrophied with increased percentage of nuclear centralization and there is an absence of myofibrils and a high concentration of oxidative activity in the center of these fibers^{7,27}. This myotube-like appearance is usually not as evident in the muscle biopsy of patients with other clinical forms of CNM.

Ultrastructural findings in our study did not add further information to routine histological and histochemical studies. In most of the muscle fibers, a centrally or paracentrally placed nucleus was observed surrounded by a halo without myofibrils and with mitochondria accumulation and rare myelin figures. Myofilaments could be better evaluated with the observation in two of our cases of fragmentation areas of the Z line, associated with reduction of mitochondria in these areas similar to minicores. The finding of minicores, also described in other cases^{11,12}, might indicate an association of CNM with minicore myopathy. On the other hand, that could be nonspecific since minicores are also described associated with other congenital myopathies⁷. Focal abnormalities of myofibrils are frequently described in CNM, usually as paracentronuclear, and are characterized by a tortuous Z line, fragmentation and disorganization of myofibrils and disarray of thin and thick filaments^{5,11,12,15,20,23,30,34}. The presence of nemaline bodies and central core in association with CNM reported by some authors²⁵ was not observed in our study. Although the internal structure of the nuclei was normal, indentations in the membrane and a prominent nucleolus were observed in our cases, as described in others^{4,14}.

Autopsy studies revealed that skeletal muscle involvement is diffuse including the extrinsic eye musculature, diaphragm and intercostal muscles^{10,21,27,29}. Smooth and cardiac muscles are spared^{5,21}. In these same studies, histological alterations in the central nervous system, spinal cord and nervous root were not detected. Abnormalities of peripheral nerves were rarely reported being characterized by axonal degeneration²⁹ and segmental demyelination^{12,32}.

The presence of a great number of muscle fibers similar to fetal myotubes led Spiro et al.³¹ to suggest that a complete fiber maturation arrest at the stage of myotubular myogenesis occurred. However that could not explain the clinical manifestation in late-onset patients. On the other hand, Sarnat²⁷ demonstrated a partial immaturity of the neonate muscle in patients with the severe neonatal form of CNM when compared to normal fetuses in the myotubular stage of embryogenesis. This could be related to an abnormal persistence of vimentin and desmin in the muscle fibers of CNM patients. The amount of those cytoskeletal proteins which decreases from the 15th gestational week on, would preserve the transitory central position of the nucleus. Congenital or acquired abnormalities in the vimentin and desmin genes would be the cause of the selective regression of development of muscle fibers in CNM. This explanation might account for the typical features that manifest later in life in some patients.

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