Original Article

Coexistence of central nucleus, cores, and rods: Diagnostic relevance

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Abstract

Background: Congenital myopathies (CMs) though considered distinct disorders, simultaneous occurrence of central nucleus, nemaline rods, and cores in the same biopsy are scarcely reported. **Objective:** A retrospective reassessment of cases diagnosed as CMs to look for multiple pathologies missed, if any, during the initial diagnosis. **Materials and Methods:** Enzyme histochemical, and immunohistochemical-stained slides from 125 cases diagnosed as congenital myopathy were reassessed. **Results:** The study revealed 15 cases (12%) of congenital myopathy with more than one morphological feature. Central nucleus with cores (n = 11), central nucleus, nemaline rods and cores (n = 3), and nemaline rods with cores (n = 1). 4/11 cases were diagnosed as centronuclear myopathy (CNM) in the first instance; in addition, cores were revealed on reassessment. **Discussion:** The prevalence of CMs of all neuromuscular disorders is approximately 6 in 100,000 live births, with regional variations. Three main defined CMs include centro nuclear myopathy (CNM), nemaline rod myopathy (NRM), and central core disease (CCD). However, they are more diverse with overlapping clinical and histopathological features, thus broadening the spectra within each category of congenital myopathy. **Conclusion:** Identification of cases with overlap of pathological features has diagnostic relevance.

Key Words

Congenital myopathies (CMs), cores, nemaline rods

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Ann Indian Acad Neurol 2016;19:201-204

Introduction

Congenital myopathies (CMs) are clinically, genetically, and pathologically a heterogeneous group of neuromuscular disorders with unique structural abnormalities.[1] Though considered distinct disorders, that simultaneous occurrence of the central nucleus, nemaline rods, and cores in the same biopsy have been reported.[2,3] In the recent years, linkage analysis has demonstrated specific genetic defects to be associated with mixed pathologies (central nucleus, cores, and rods), in particular, mutation in RYR1 gene susceptible to malignant hyperthermia and its clinical implication.[4,5] In view of this, a retrospective analysis of cases morphologically diagnosed as CMs were reassessed to look for multiple pathologies missed, if any, during the initial diagnosis.

Access this article online	
Quick Response Code:	Website: www.annalsofian.org
	DOI: 10.4103/0972-2327.176861

Materials and Methods

Cases diagnosed as CMs were extracted from the muscle biopsy data and clinical details noted from the case sheets were maintained in the department. Among 15,578 muscle biopsies received (1983-2013), 5,955 biopsied at a tertiary referral hospital for neuromuscular disorders were subjected to enzyme histochemical staining and electron microscopy while the rest (9,623), referred from the neighboring institutes and from all over the country, were fixed in formalin and/or glutaraldehyde and a few were received fresh. Hence, the data

How to cite this article: Dhinakaran S, Kumar RS, Thakkar R, Narayanappa G. Coexistence of central nucleus, cores, and rods: Diagnostic relevance. Ann Indian Acad Neurol 2016;19:201-4.
Received: 11-08-15, **Revised:** 25-10-15, **Accepted:** 11-11-15

of CMs presented includes cases biopsied at a tertiary referral hospital alone.

A total of 125 cases were diagnosed as CMs. Paraffin sections stained for hematoxylin and eosin (H&E) and Masson's trichrome (MAT) and cryosections stained for H&E, modified Gomori trichrome (MGT), enzyme histochemical stains nicotinamide adenine dinucleotide tetrazolium reductase (NADH-Tr), succinic dehydrogenase (SDH), adenosine triphosphatase (ATPase-pH9.4 and 4.6) and immunostained slides to monoclonal antibodies against desmin, vimentin, and α-actinin were reassessed independently by two of the authors and the findings were noted. Details of ultrastructural findings were simultaneously assessed and where necessary the stored grids were retrieved and scanned under Tecnai G^2 Spirit Biotwin, FEI, The Netherlands[2] transmission electron microscope.

Results

There were 15 cases of congenital myopathy with more than one morphological feature.

Clinical

Clinical features in three groups are summarized in Table 1.

Morphological features

Routine histology revealed myopathic pattern with mild to moderate fibrosis and adipose tissue infiltration. Fifteen cases with more than one structural change seen include: Central nucleus with cores (n = 11), central nucleus, rods and cores (n = 3), and nemaline rods with cores (n = 1).

Central nucleus with cores (n = 11)

Myofibers with central nuclei (geographic center) ranged from 15% to 70%. The central nuclei are predominantly seen in the smaller diameter type I fibers. Central intense staining and radiating spoke-like appearance were observed on oxidative stains (NADH-Tr/SDH) in 3-5% of the fibers and predominance of type I fiber in all 11 cases. In addition, single, central, or eccentric cores (5-73% of fibers) and multiple cores (7-32% of fibers) were noted. Rubbed-out appearance was seen in one of the cases.

Desmin staining was variable with streaks of positive labeling,

Table 1: Clinical features

Parameters	Central nucleus with cores (n = 11)	Central nucleus, nemaline rods and cores (n = 3)	Nemaline rods with cores (n = 1)
Age range at presentation (yr)	3-41	11-19	16
Age range at onset	Birth- 5yrs	Birth	Birth
M/F	6:5	3:0	1:0
Consanguity	2	–	–
Positive family history	2	–	–
Motor delay	8	3	1
Elongated facies	5	2	1
Tented upper lip	1	–	1
Large ears	3	–	–
Low set ears	1	–	–
Pescavus	2	–	–
Pectus excavatum	2	–	–
Scoliosis	4	1	1
Kyphosis	–	2	1
High arched palate	6	3	–
Ptosis	6	2	1
Opthalomopheresis	3	1	–
Facial weakness	5	1	1
Hyperextensibility of wrist	2	–	–
Prominent calcaneum	2	–	–
Contractures	6	2	1
Proximal	10	3	1
Distal	1	–	–
Neck weakness	2	1	–
Tongue atrophy	1	1	–
Macroglossia	–	1	–
Calf hypertrophy	2	–	–
Mental retardation	–	1	–
Creatine kinase (range) (normal70-170IU/L)	60-1102 IU/L	110 -406 IU/L	339 IU/L
EMG	Myopathic	Myopathic	Myopathic
NCV	Normal	Normal	Normal
ECG	Normal	Normal	Normal
MRI Brain	Normal(2)	Normal(1)	Normal (1)

intense labeling in the core region, or labeling along the margin of core. Immnunostaining to α-actinin failed to demonstrate rod bodies in all 11 cases.

Electron microscopically, myofibrillar disorganization, streaming of Z band, loss of mitochondria, and displaced tubular system represented the cores. Central nucleus with myofibrils organized in a radiating pattern was evident. There were no rod bodies in any of the cases. 4/11 cases were diagnosed as centronuclear myopathy (CNM) in the first instance; in addition, cores were revealed on reassessment by electron microscope.

Central nucleus, cores, and rod bodies (n = 3)

Three cases with coexistence of central nucleus, cores, and rods with fiber size disproportion were encountered. The percentage of fibers with the central nucleus was 25% with single, central/eccentric and/or multicores in 10-50% of the fibers. Radiating spoke-like pattern was seen in two cases. In addition, rod bodies were noted in 75% of the fibers. Type I fiber hypoplasia and predominance was seen in all. Immunostaining to α-actinin labeled the rod bodies. Desmin immunoreaction was seen bordering the cores in most fibers while its concentration in the core was seen in a few. Ultrastructural analysis confirmed the presence of rod bodies and cores. The rods were multiple, seen in the subsarcolemmal regions and other regions of the fiber. In addition, distinct core area with disorganized filamentous pattern and Z band streaming was observed. Interestingly, small rod bodies were noted within the cores as also surrounding the core region, which was appreciated on α-actinin [Figure 1].

Rods and cores (n = 1)

Numerous rods and small multiple cores seen were confirmed electron microscopically. Immunostaining to α-actinin labeled the rod bodies. Desmin immunoreaction was variable.

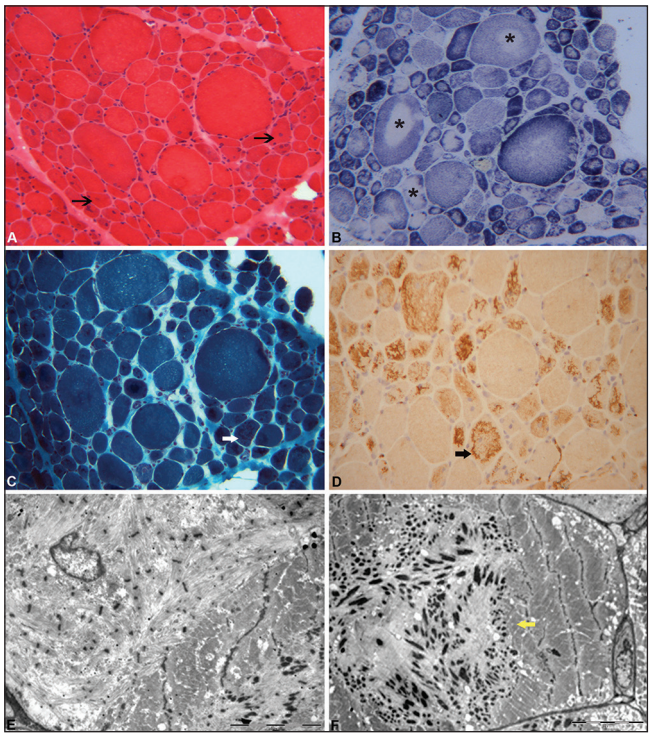

Figure 1: Transversely cut skeletal muscle tissue showing (a) fiber size disproportion, central nucleus in smaller diameter fibers (→) HE ×400 (b) Distinct cores (*) SDH ×400 (c) Numerous rod bodies (white arrow) MGT X400 (d) Immunostaining to α-actinin highlighting rod bodies (→) ×400 (e) Electron micrograph showing central nucleus, core area ×4800 (f) Small rods bodies within the cores, as also surrounding the core region (yellow arrow) ×1900

Discussion

CMs constitute 1/10th of all neuromuscular disorders affecting approximately six in 100,000 live births, with regional variations.[6] Three main defined CMs include centro nuclear myopathy (CNM), nemaline rod myopathy (NRM), and central core disease (CCD). However, they are more diverse with overlapping clinical and histopathological features, thus broadening the spectra within each category of CM. The present study revealed 15/125 (12%) cases of CMs with more than one pathological feature. Central nucleus with core morphology was commonest (73%) followed by three cases (20%) with rods, cores, and central nuclei and one case with nemaline rods and cores. None of our cases had intranuclear rod bodies. The most common association of central nuclei and cores seen in the present study was similar to the frequency of occurrence reported in the literature.[7]

Classical cores are either central or eccentric, single or multiple. However, cores are not always appreciated and are missed particularly when there are areas with subtle uneven staining on oxidative stains or multiple small unstained areas similar to minicores. Electron microscopic observation revealed small and large areas of myofibrillar disorganization with loss of sarcomeric pattern and Z band streaming confirming the presence of cores. Four cases were diagnosed as CNM in the first instance; in addition, cores were revealed on reassessment by electron microscope.

The occurrence of the central nuclei and multicores,[7] cores, and nemaline rods[8,9] in the same muscle biopsy and the presence of different morphologies in the same family[10] has been reported from the 1960s to the 1980s (premolecular era), raising the possibility of either dual pathology or variable morphological expression of the same genetic defect. With the advent of molecular genetics, mutation in ryanodine receptor (RYR1) gene was identified[11] and its association with malignant hyperthermia in cases diagnosed with CCD was established.[12] Five genes have been associated with nemaline rod myopathies: α-actin (ACTA1), α-tropomyosin (TPM3), nebulin (NEB), β-tropomyosin (TPM2), and troponin T (TNNT1) while dynamin 2(DNM2) and amphiphysin 2(BIN1) have been assigned for autosomal dominant and recessive CNM, respectively.[13]

Attempts to establish genotype-phenotype correlation in CMs have been inconclusive since mutations associated in classical CMs are scattered across different histological types. In a correlation between the phenotype, genotype, and associated histological types, RYR1 mutation has been implicated in cases with coexistence of cores and the central nucleus, cores, and rods[14] and in mixed core, the central nucleus, and fiber size

disproportion and grouped under RYR1-related CMs. Similarly, NEB, an actin-binding protein that regulates thin filament length associated with autosomal recessive NRM is also known to be mutated in cases with coexistence of cores and rods and hence, is designated as NEB-associated myopathies. Recently, two genes, cofilin 2(CFL2), α tropomoysin-binding protein, and (KBTBD 13), a member of BTB/kelch family (bric-a-brac tramtrack broad complex protein) involved in the regulation of cytoskeletal remodeling, gene transcription, and myofiber assembly are known to cause overlap between cores and rods.[15]

The diagnosis of CMs is based on morphological criteria as genetic studies reveal overlap within and among various forms. Muscle biopsy, with a battery of histochemical stains supplemented by electron microscopy and immunohistochemistry is the gold standard for the diagnosis of CMs and for directing molecular analysis.

Diagnostic relevance

There is no effective treatment for CMs. A multidisciplinary approach to the management of affected individuals improves quality and longevity of the patients. Respiratory insufficiency is the major contributor to morbidity and mortality in cases with rods and hence pulmonary management and support are critical. The precise association between cores and malignant hyperthermia is not clear. However, all patients with cores are considered at risk and a word of caution is warranted so that they are not deprived of potential valuable anesthesia.

Cases with overlap of pathological features particularly with evidence of cores must be evaluated for RYR1 mutation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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