Case Report

Nemaline rod myopathy: A rare form of myopathy


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Nemaline rod myopathy (NM) is a rare form of congenital myopathy characterized by slowly progressive or nonprogressive muscle weakness and pathognomonic rod-like structures within the muscle fibers. To the best of our knowledge, this is first documentation of the clinicopathological features of this rare entity from India. All cases of NM diagnosed in our laboratory were retrieved. Clinical and pathological features were reviewed. During a period of 1.5 years (Jan 2004 to June 2005), we received 750 muscle biopsies for various reasons. Of which, 15 were diagnosed as congenital myopathies and four as nemaline rod myopathies. Thus, NM comprises 0.53% of all muscle diseases and 22.6% of all congenital myopathies. All of them presented in childhood (first five years of life) with generalized hypotonia, feeding problems, repeated respiratory infections and muscle weakness. Both males and females were equally affected. The CPK levels were normal and EMG was myopathic. Microscopic examination revealed minimal changes but characteristic red-colored material was seen on modified Gomori trichrome staining which was immunopositive to alpha actinin. Ultrastructural examination confirmed this material to be nemaline rods. NM, although a rare form of congenital myopathies, should be suspected in children who present with generalized hypotonia, repeated chest infections and slowly progressive muscle weakness. This report highlights the importance of histochemistry and ultrastructural examination in the diagnosis of this entity, in the absence of the availability of methodology for genetic studies.

Key words: Congenital myopathies, histochemistry, muscle diseases, nemaline rod myopathy, ultrastructure

Introduction

Congenital myopathies are a heterogeneous group of rare neuromuscular disorders characterized by nonprogressive or slowly progressive muscle weakness and often are associated with structural abnormalities in the muscle fibers. Nemaline myopathy (NM) is a rare form of congenital myopathy characterized by slowly progressive muscle weakness, mainly of facial, neck flexors and proximal muscles of the limbs.\(^1,2\) The characteristic pathognomic feature of NM is the presence of rod-like structures or nemaline rods seen on light microscopy with modified Gomori trichrome (MGT) stain, and electron dense nemaline bodies of Z-band origin on ultrastructural examination. We report the clinicopathological features of four cases of NM because of its rarity and to the best of our knowledge this is the first report from India.

Case Reports

Clinical features are summarized in Table 1

Case 1

This 1.5-months-old female child, the only product of a nonconsanguineous marriage, was born by lower segment caesarian section and had feeble cry at birth. There was history of decreased fetal movements in the third trimester, repeated episodes of cyanosis and excessive secretion of saliva since birth. Tube feeding was started because of poor suck and swallowing problems. She had a frog-like posture due to profound hypotonia. Examination showed retrognathia, low set ears, high arched palate with small tongue, long slender fingers and bilateral simian crease. No cardiac abnormality was detected. Family history was noncontributory. Muscle biopsy was done with a clinical diagnosis of spinal muscular atrophy Type I. She died at the age of four months.

Case 2

This four-year-old female child presented with history of progressively increasing difficulty in walking, running, climbing stairs, getting up from floor, chewing and swallowing since one year of age. There was history of frequent falls, diplopia and breathlessness. Examination showed generalized hypotonia, calf hypertrophy,
myopathic facies, high arched palate, weakness of face and neck muscles. The CPK level was 218 U/L and electromyographic (EMG) showed myopathic changes. None of the other two siblings had similar problems.

**Case 3**

A five-year-old male child presented with difficulty in walking, running, climbing stairs and frequent falls for the last one and a half years. There was history of difficulty in chewing and swallowing in addition to diplopia and breathlessness. The ante-natal and perinatal periods were uneventful. Two of his brothers and one sister were normal but one younger sister had similar problems. Examination revealed waddling gait, generalized hypotonia of all four limbs and calf hypertrophy. The CPK level was 75 U/L and EMG revealed myopathic changes. Echocardiography showed no abnormality. Muscle biopsy was done with a clinical diagnosis of congenital myopathy.

**Case 4**

A seven-year-old male child, product of a nonconsanguineous marriage, presented with difficulty in getting up from the sitting position and standing. There was history of repeated chest infections. He was detected to be floppy at birth. Examination showed generalized hypotonia and proximal muscle weakness. The CPK level was 200 U/L and EMG showed myopathic changes. Muscle biopsy was done with a clinical diagnosis of congenital myopathy.

In all patients muscle biopsy specimens were taken from the vastus lateralis of left quadriceps. Each biopsy was received in fresh state without any fixative or additive and divided into three pieces. One piece was fixed in 10% neutral buffered formalin, which was routinely processed and paraffin-embedded. Five-micron-thick sections were cut and hematoxylin and eosin-stained. The second piece was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer saline for electron microscopy, postfixed with 1% buffered osmic acid and embedded in epoxy resin. Ultra-thin sections were double-stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (TEM, Morgagni 268, Holland). The third bigger piece was immediately snap frozen in isopentane precooled in liquid nitrogen at –80°C. Six to 8 mm thick cryostat sections were cut and stained with hematoxylin and eosin (H and E), MGT, periodic acid Schiff, oil red O, ATPase at pH 9.6, 4.6 and 4.3, nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), succinic dehydrogenase (SDH) alone as well as combined with cytochrome oxidase (Cox with SDH), myophosphorylase, amylopectinate phosphofructokinase and adenylate deaminase. Immunohistochemical staining was done by streptavidin-biotin immunoperoxidase complex method using antibody against α-2 actinin.

**Results**

During a period of one-and-half year (January 2004 to June 2005), we received 750 muscle biopsies in the Department of Pathology of the All India Institute of Medical Sciences, New Delhi, India, which is a tertiary hospital catering both to pediatric and adult patients. Of which, 15 were diagnosed as congenital myopathies and four of them were nemaline rod myopathies. Thus, NM comprises (0.53%) of all muscle diseases and 22.6% of all congenital myopathies.

Muscle biopsies in all four patients showed similar changes. Microscopic examination revealed normal fascicular architecture with no endomyositis or perimysial fibrosis and adipose tissue infiltration. There was mild variation in fiber size without central localization of nuclei. Subsarcolemmal accumulation of eosinophilic material was noted in some of the fibers [Figure 1a]. At places they were giving the appearance of group atrophy or hypotrophy especially in Case 1 in which it was looking like neurogenic atrophy [Figure 1b]. The atrophic fibers were rounded but not angulated. Oxidative stains revealed Type I predominance which was further confirmed by ATPase staining at pH 9.4. Atrophied fibers were only of Type I, thus excluding the possibility of neurogenic atrophy. MGT stain showed characteristic greenish granular material to red-colored rods in the subsarcolemmal region, which were more prominent in Type I fibers [Figure 2a]. This material was immunopositive to alpha actinin [Figure 2b]. This material was also evident in routinely formalin-fixed and paraffin-embedded sections in Case 2. No central cores, multiminicores or ragged red fibers were identified. Degenerative or regenerative fibers were not seen. Myophagocytosis was absent.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Age of onset</th>
<th>Signs and symptoms</th>
<th>Family history</th>
<th>CPK</th>
<th>electromyographic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 m/f</td>
<td>Since birth</td>
<td>Excessive secretions, episodes of cyanosis hypotonia</td>
<td>Not done</td>
<td>218 IU/L</td>
<td>Myopathic</td>
</tr>
<tr>
<td>4/f</td>
<td>2 years</td>
<td>Delayed developmental milestones, difficulty in walking myopathic facies, Gower sign</td>
<td>Not done</td>
<td>250 IU/L</td>
<td>Myopathic</td>
</tr>
<tr>
<td>5/m</td>
<td>3½ years</td>
<td>Difficulty in walking, frequent falls, thinning of limbs, generalised hypotonia</td>
<td>Not done</td>
<td>200 IU/L</td>
<td>Myopathic</td>
</tr>
<tr>
<td>7/m</td>
<td>Since birth</td>
<td>Floppy since birth, repeated chest infections, difficulty in getting up from sitting position</td>
<td>Not done</td>
<td></td>
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**Table 1: Clinical features of nemaline rod myopathy**

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Figure 1: (a) Photomicrophotograph of case 2 showing mild variation of fibre size without any myopathic changes. In some of the fibres eosinophilic material is seen (a, H/E, 200x). (b) In case 1 fibre appears to be atrophic like neurogenic atrophy (b, H/E, 40x)

Figure 2: (a) MGT stain demonstrating greenish to reddish material in the subsarcolemmal location (a, 200x). (b) This material is immunopositive to alpha2 actinin (b, 100x)

Figure 3: (a) Electron microphotograph of case 2 showing numerous nemaline rods in the subsarcolemmal location (a, 6250x original magnification). (b) On higher resolution these rods appear to be lattice like similar to Z-band (b, 12000x original magnification)

**Ultrastructural examination**

Electron microscopic examination revealed characteristic rod-like structures predominantly in the subsarcolemmal location but in places were within the myofibers [Figure 3a]. Their number varied from single rod to large aggregates. On higher magnification these rods appeared as lattice-like structures similar to Z-band material [Figure 3b]. Immunolabeling with gold particles was done in one case and these rods were labeled with alpha 2-actinin. No intranuclear rods were seen in any case.

Based on the above features the diagnosis of nemaline rod myopathy was made in all cases.
Discussion

NM is characterized by rod-like structures in the muscle fibers. The term nemaline was first coined by Shy and colleagues in 1963[3] who reported a new type of nonprogressive myopathy characterized by numerous thread-like structures within the muscle fibers. In the same year Conen et al[4] described the same phenomenon in the muscle fibers and named them as “Myogranules”. However, the first description of this entity was probably given by Reye in 1958[5] who described some inclusions in formalin-fixed, routinely processed, paraffin-embedded tissue sections. Genetic analysis of the same patient done later on in life showed ACTA1 mutations.

Nemaline myopathy is supposedly a relatively rare form of congenital myopathy but is probably not that rare as evident from this report. In the last one and a half year period we received 750 muscle biopsies for various reasons in our laboratory, of which 15 were congenital myopathies and four were diagnosed as NM, constituting 0.53% of all muscle diseases and 22.6% of all congenital myopathies.

NM are, genetically as well as phenotypically, a heterogeneous group of congenital myopathies, however, genophenotypic correlation has been suggested. Based on the age of onset and severity of motor and respiratory involvement, NMs are classified as severe congenital, intermediate congenital, typical congenital, childhood and adult onset forms.[6] Severe congenital form presents at birth with severe hypotonia and muscle weakness, difficulties with sucking, swallowing and respiratory insufficiency. Most of these deaths occur in utero. Sometimes may be associated with dilated cardiomyopathy and arthrogryposis. Most of the children die due to respiratory insufficiency or aspiration pneumonia. Our first patient under discussion belongs to this subtype of NM. Intermediate congenital form has independent respiration at birth but fails to achieve motor milestones, develops joint contractures and requires respiratory support or is wheel-chair-bound by 11 years of age. Typical congenital form presents at neonatal period or first year of life with hypotonia, weakness, feeding difficulty and respiratory infections. A minority of the patients in this group present after the first year of life with delayed motor milestones, waddling gait, bulbar weakness and swallowing difficulties. Weakness is proximal and cardiac involvement is rare. Most of these patients are able to lead an independent life. Children should be classified as typical congenital NM, if they crawl before 12 months and walk before 18 months of age. All our patients except Case 1 belong to this category as all can walk but had delayed developmental milestones, proximal muscle weakness and hypotonia.

Childhood onset form of NM presents at the second or third decade of life with symmetrical weakness of ankle dorsiflexors and foot drop akin to peripheral neuropathy. Weakness eventually progress to proximal muscles and they are wheel-chair-bound by 40 years of age. Adult or late onset form of NM is heterogeneous in clinical presentation and progression. Weakness starts between 20-50 years of life without any family history. Myalgia may be prominent and weakness is progressive. Respiratory and cardiac involvement is rare.

Although cardiac involvement is rare it is a definite associated feature of NM. Most of these patients present with dilated cardiomyopathy[7] or hypertrophic cardiomyopathy in children[8] and may even require cardiac transplantation.[9] These rod-like structures had been demonstrated in the cardiac muscles. None of our patients had associated cardiac involvement.

Genetically, causative mutations have been described in five genes encoding different skeletal muscles thin filaments namely sarcomeric actin (ACTA 1), α-tropomyosin (TPM3), beta tropomyosin (TPM2), troponin T1 (TNNT1) and nebulin (NEB). Genes for these proteins have been localized and mutations are fully characterized.[2,9] Nebulin gene mutations are seen most commonly in autosomal recessive cases and these patients present as typical congenital NM.[10] Some authors had shown by linkage studies that nebuline gene mutations at 2q21.2-q22 are responsible for 50% cases of NMs.[10] Troponin 3 gene mutations (TPM3) have been described in rare cases of dominant and recessive cases of NMs[11] and rarely TPM2, TNNT1 are also implicated. Recently, mutations in ACTA1 gene have been described in NM[12-14] similar to actinopathy, a form of protein surplus myopathy. Nowak et al first described mutations of ACTA1 gene at 1q42 in 10% cases of NMs. Over 60 mutations have been described. Most of the reported mutations in NMs are sporadic but familial autosomal dominant forms are also recorded. ACTA1 mutations are more common in severe cases of NM[13] but have been described in recessive form also. Most mutations of ACTA1 are de novo and missense. ACTA1 gene mutations are associated with heterogeneous clinical, pathological and genetic findings.

Therefore, this study highlights the importance of histochemistry and ultrastructural examination for the diagnosis of this rare form of congenital myopathy.

References

5. Schell C, Kan A, North KN. An artefact gone awry: Identification of the first case of nemaline myopathy by Dr R.D.K. Reye...


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