Original Article

Congenital muscular dystrophy with inflammation: Diagnostic considerations

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Abstract

Background and Purpose: Muscle biopsy features of congenital muscular dystrophies (CMD) vary from usual dystrophic picture to normal or nonspecific myopathic picture or prominent fibrosis or striking inflammatory infiltrate, which may lead to diagnostic errors. A series of patients of CMD with significant inflammatory infiltrates on muscle biopsy were correlated with laminin α 2 deficiency on immunohistochemistry (IHC). **Material and Methods:** Cryostat sections of muscle biopsies from the patients diagnosed as CMD on clinical and muscle biopsy features from 1996 to 2014 were reviewed with hematoxylin and eosin(H&E), enzyme and immunohistochemistry (IHC) with laminin α 2. Muscle biopsies with inflammatory infiltrate were correlated with laminin α 2 deficiency. **Results:** There were 65 patients of CMD, with inflammation on muscle biopsy in 16. IHC with laminin α 2 was available in nine patients, of which six showed complete absence along sarcolemma (five presented with floppy infant syndrome and one with delayed motor milestones) and three showed discontinuous, and less intense staining. **Conclusions:** CMD show variable degrees of inflammation on muscle biopsy. A diagnosis of laminin α 2 deficient CMD should be considered in patients of muscular dystrophy with inflammation, in children with hypotonia/delayed motor milestones.

Key Words

Congenital muscular dystrophies (CMDs), inflammation, laminin α^2 (LAMA2), merosin

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Introduction

Congenital muscular dystrophies (CMDs) are a clinically and genetically heterogeneous group of inherited muscle disorders, defined by a combination of early onset of hypotonia and weakness, contractures and variable progression, normal or elevated serum creatine kinase (CK), and myopathic changes on electromyography (EMG), and are usually associated with dystrophic muscle biopsy.[1]

These are the most common autosomal recessive neuromuscular disorders and are classified on the basis of the classes of proteins that are affected.[1] One of the most common forms of CMD is caused by the loss of function mutations of the laminin α2 (LAMA2) (merosin) gene, leading to a complete absence of the protein in the myofiber basal lamina.[2] This form of CMD is called LAMA2-related CMD and is defined in the genetic nomenclature as merosin-deficient congenital muscular dystrophy type 1A (MDC1A).[3]

Muscle biopsy features vary among the patients; it usually shows a dystrophic picture. However, muscle biopsy may be normal or may show nonspecific myopathic picture or

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prominent fibrosis.[4] In the early stages, a striking inflammatory infiltrate may be seen.[3] These variable histological features on muscle biopsy may cause diagnostic errors.[5]

In this paper, we present a series of patients with CMD with significant inflammatory infiltrates on muscle biopsy and correlate them with LAMA2 deficiency on immunohistochemistry (IHC).

Materials and Methods

All the patients diagnosed with CMD on clinical and muscle biopsy features from 1996 to 2014 were reviewed. The demographic, clinical, and laboratory findings were retrieved from hospital records. The muscle biopsies in all patients were done from left vastus lateralis. Cryostat sections of muscle biopsies were reviewed with hematoxylin and eosin (H&E), Masson's trichrome, modified Gomori trichrome (MGT), ATpase preincubated at ph 9.4 and ph 4.6, succinate dehydrogenase (SDH), nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR). Slides of immunohistochemistry (IHC) with LAMA2 (Novocastra, United Kingdom 1:50 dilution) were reviewed wherever available. Muscle biopsies of children above 3 years were subjected to IHC with dystrophin (Novocastra, United Kingdom 1:50 dilution) staining also.

The muscle histology was reviewed for architecture, fiber size variation, rounding, necrosis, degeneration, regeneration, presence of inflammation, and fibrosis. The type and location of inflammatory cells were noted. Immunohistochemistry for categorization of inflammatory cells and Collagen VI were not performed. Genetic tests were also not performed. Muscle biopsies with inflammatory infiltrate were analyzed, along with IHC for LAMA2.

Results

There were a total of 65 patients diagnosed with CMD during the study period. Inflammation was noted on muscle biopsy in 16 (24.6%) patients. IHC with LAMA2 was available in nine patients. These patients were analyzed further.

All the patients were children between 10 days and 11years with the male-to-female ratio of 5:4. There were six children below 3 years and 3 children above 3 years. The clinical presentations were floppy infant syndrome in five children, delayed motor mile stones in two children, and proximal muscle weakness and contractures in one child each. None of the patients had cutaneous manifestations or features of connective tissue disease (CTD). Creatine kinase (CK) was normal in four children and elevated in five children (297-10,400 IU/L).

Muscle biopsy showed a dystrophic picture in all biopsies with rounded atrophic fibers separated by dense fibrosis. Regenerating fibers were seen with few necrotic and degenerating fibers. Inflammatory infiltrate composed of lymphomononuclear cells was seen in all the biopsies. The lymphomononuclear infiltrate was diffuse in one biopsy and in remaining eight biopsies the infiltrate was in focal aggregates. The infiltrate was endomysial in three, perimysial in five, and diffuse in one. The infiltrate was moderate in degree [Figure 1].

None of the biopsies showed perifascicular atrophy, perivascular infiltrate, or fiber type grouping. There were no red ragged fibers and nemaline rods on MGT. There was no evidence of metabolic myopathy in the form of cytoplasmic vacuoles.

Immunohistochemistry with LAMA2 showed a complete absence along sarcolemma in six biopsies. The staining was discontinuous and less intense in three biopsies [Figure 2]. The patients with complete deficiency presented with floppy infant syndrome (five children) and delayed motor milestones (one child), and were below 3 years of age. IHC with dystrophin was positive along the sarcolemma in the three biopsies performed.

The demographic data and clinical and laboratory features are given in Table 1.

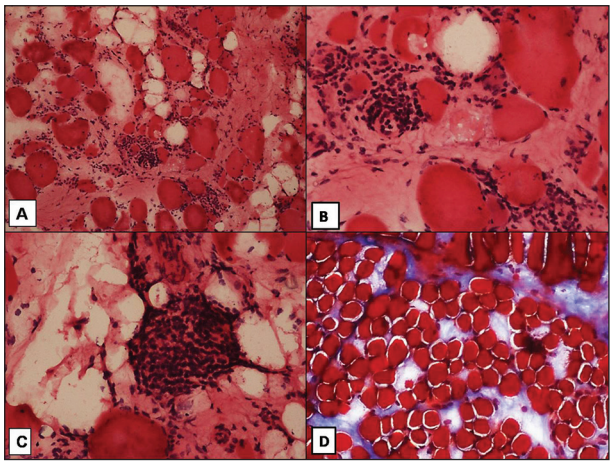

Figure 1: (A) Photomicrograph showing loss of architecture with fibrosis and adipose tissue infiltration (H&E ×10) (B) Rounded fibers and endomysial inflammation (H&E ×10) (C) Aggregate of lymphomononuclear cells in perimysium (H&E ×40) (D) Pericellular fibrosis (Masson's trichrome ×10)

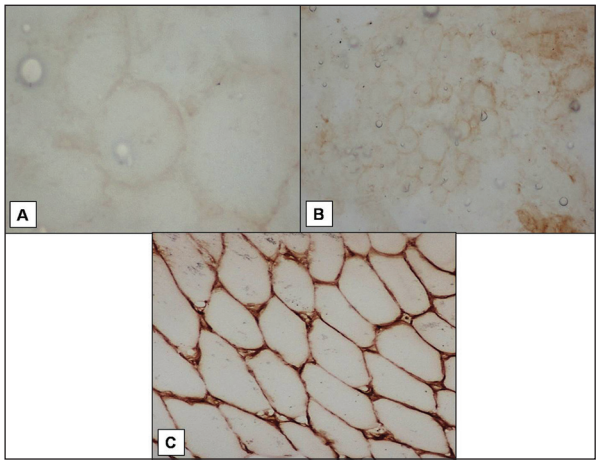

Figure 2: (A) Complete deficiency of laminin α2 (B) Partial deficiency of laminin α2 (C) Control immunohistochemistry with positive staining along the sarcolemma

Discussion

Muntoni and Voit reported that primary deficiency of LAMA2 accounted for 30-40% of all patients with CMD with regional variations.[6] Peat *et al*. in a large cohort of mixed ethnicity, using a combination of immunoflourescence, western blotting, and DNA sequencing reported that only 8% of CMDs were caused by LAMA2 deficiency.[7] In the present study, evaluation was done using IHC with LAMA2 antibodies. The majority of the patients present at birth and the pattern of inheritance are autosomal recessive.[8]

In the present study of 65 patients with CMD, prominent inflammation was seen in 16 (24.6%) patients. However, IHC with LAMA2 was available in nine patients only. Six of the nine patients whose IHC was available were less than 3 years. Pegora *et al*. observed that the inflammatory infiltrate was present in a biopsy taken at the age of 5 months and not in a subsequent biopsy taken after 9 months.[5] However, in our study, all nine patients with age ranging from 10 days to 11 years showed inflammation. Follow-up biopsies were not performed in any of the patients. In our study, patients with complete deficiency, especially children below 3 years, presented with hypotonia and delayed motor milestones. Children, with partial deficiency, above 3 years of age presented with proximal muscle weakness and contractures. A similar type of presentation was reported.[3,8] Children with complete deficiency do not achieve motor milestones beyond sitting and standing whereas children with partial deficiency have a milder phenotype with normal mental development and contractures.[3]

Table 1: Demographic, clinical laboratory, muscle histology, and laminin α2 status of congenital muscular dystrophy with inflammation (*n* = 9)

Parameter	Finding
Age (10 days-11 years)	<3 years: 6 cases
	>3 years: 3 cases
M:F	5:4
Clinical presentation	Floppy infant syndrome: 5
	Delayed milestones: 2
	Proximal muscle weakness: 1
	Contractures: 1
CK	Normal: 4
	Elevated: 5
Muscle histology	Dystrophy
Inflammation	Endomysial: 3
	Perimysial: 5
	Diffuse: 1
Laminin α2	Complete deficiency: 6
	Partial deficiency: 3

M = Male, F = Female, CK = Creatine kinase

Table 2: Congenital muscular dystrophy with inflammation: Comparison of the present study with a similar study.[5]

Parameter	Pegoraro *et al*.[5]	Present series
Number of patients	33	65
Number of patients with inflammation	10 (30.3%)	16 (24.6%)
Number of patients with inflammation and laminin α2 status	10	9
Method of laminin α2 evaluation	IF, immunoblotting	IHC
Age	10 months–11 years	10 days–11 years
Male:Female	1:1	5:4

IF = Immunoflourescence, IHC = Immunohistochemistry

Congenital muscular dystrophies differ from the more common juvenile or adult form of muscular dystrophy by early onset, less aggressive course, or static course.[4] The muscle biopsy also shows less active necrosis and more pronounced fibrosis. Muscle biopsy in all our patients showed a dystrophic picture with extensive pericellular fibrosis, fiber size variation, and rounding. There were variable degrees of lymphomononuclear infiltrates in all the biopsies. The inflammatory infiltrates were diffuse in one biopsy, endomysial in three biopsies, and permysial in five biopsies. Muscle biopsy from patients with muscular dystrophy shows necrotic fibers with a certain degree of inflammation. A few subtypes including dysferlinopathy, calpainopathy, and facioscapulo humeral muscular dystrophy show extensive inflammatory infiltrate and mimic inflammatory myopathy.[9-11] Appropriate clinical, immunohistochemical, and genetic studies help in differential diagnosis. Congenital muscular dystrophy with inflammation has not received much attention earlier. Pegoraro *et al*. studied 10 LAMA2-deficient patients identified by immunoflorescence and immunoblotting, and described focal infiltration of T cells and B cells in muscle biopsy in all their patients.[5] Three of the 10 biopsies were initially diagnosed as infantile polymyositis prior to immunostaining studies [Table 2].[5] To avoid diagnostic errors, they suggested that neonates with muscle histopathology showing inflammation should be considered to be with CMD.[5] Though childhood inflammatory myopathies are uncommon, juvenile polymyositis and overlap myositis are associated with high morbidity and mortality.[12] Jones *et al*. suggested that all dystrophic muscle biopsies should be subjected to immunostaining with antibodies to LAMA2 to increase the diagnostic yield of CMD, as the clinical phenotype is very varied.[13] Partial α2 deficiency can be seen in both primary LAMA2-related dystrophy and α-dystroglycan-related dystrophy. Review of clinical features, confirmation with a second LAMA2 antibody, immunoblot studies, and skin biopsy evaluation are necessary to differentiate the two entities.[3] All our patients with partial LAMA2 deficiency had milder phenotype and contractures. Inflammatory myopathy was not considered in the differential diagnosis in our patients.

LAMA2 is a major component of myofiber basal lamina, which mediates interactions between the basal lamina and extracellular matrix. Normally around the time of birth, there is a transition from α1 protein in the fetus to α2 protein in the neonate. In patients with LAMA2 mutations, there is a failure of this transition leading to immune-mediated attack on myofibers resulting in improper assembly of postnatal basal lamina. The persistence of laminin α4 and α5 protects muscle fiber from degeneration but fails in successful regeneration. Hence, the consequent regeneration is compromised and it results in muscle fiber.[8,14-16]

Conclusion

CMDs show variable degrees of inflammation on muscle biopsy. A diagnosis of LAMA2-deficient CMD should be considered in all patients of muscular dystrophy with inflammation, especially in patients presenting with hypotonia and muscle weakness at birth or in the first few months of life. The awareness of this entity is important to avoid misdiagnosis as infantile polymyositis, as the treatment and prognosis of these two entities are different.

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Conflicts of interest
There are no conflicts of interest.

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