Original Article

Muscle histopathology in today's era of molecular genetics: Role and limitations

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Abstract

Introduction: Past few decades have seen an increasing application of techniques like electron microscopy, western blotting, and molecular genetics in the evaluation of muscle diseases. However, due to their limited availability, histopathological interpretation of muscle biopsies still remains an important component of diagnostic approach to muscle diseases. A systematic methodology is required in the evaluation and interpretation of muscle biopsies. This study was undertaken to analyze the histopathological spectrum of 164 muscle biopsies and to assess the diagnostic yield of basic histopathological procedures in the work up of muscle biopsy. **Materials and Methods:** Retrospective analysis was done for 164 cases of muscle biopsies. Step-wise approach was adopted to assess the efficacy of routine stains, enzyme histochemistry, and immunohistochemistry. Based on hematoxylin and rosin (H and E) appearance, biopsies were broadly categorized into destructive, nondestructive but myopathic, and inflammatory morphology. Role of special stains, enzyme, and immunohistochemical stains in each category was then evaluated. **Results:** On the basis of histopathological features, 164 muscle biopsies were broadly categorized into biopsies with abnormal histopathological features (115) and biopsies with normal histopathology (49). Abnormal muscle biopsies were further categorized into destructive morphology (56.5%), nondestructive but myopathic morphology (30.5%), and inflammatory pathology (13%). A near definitive diagnosis could be made in 115 cases out of 164 muscle biopsies on the basis of routine histopathology, enzyme histochemistry, and immunohistochemistry. **Conclusion:** Though advanced techniques like electron microscopy, western blotting, and molecular genetics are essential for confirmatory diagnosis, a substantive diagnostic yield can be offered with the basic armamentarium of routine (frozen) stains, enzyme histochemistry, and immunohistochemistry.

Key Words

Diagnostic yield, histopathology, muscle

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Ann Indian Acad Neurol 2015;18:398-402

Introduction

Muscle disorders are known to be phenotypically and genetically heterogeneous. Muscle biopsy in these disorders is an important and at times an indispensible diagnostic tool for diagnosing or corroborating the clinical impressions.[1-3] Rapid advances have been seen in the techniques applied for diagnosing muscle diseases. Application of molecular genetic testing and electron microscopy offer the prospect of an accurate diagnosis which forms the basis of patient management and, also, family counseling. However, not all centers are equipped with these advanced diagnostic modalities and not all patients can afford these. Mainstay of diagnosis, in such instances, continues to remain histopathology, enzyme, and immunohistochemistry. There is a paucity of literature, however, that specifically assesses the diagnostic yield of the basic armamentarium of routine and special stains, enzyme histochemistry, and immunohistochemistry.[4]

This study was undertaken to analyze the histopathological spectrum of 164 muscle biopsies received in our department over the last 5 years. The aim was to observe the histopathological spectrum of changes seen in muscle diseases and assess the diagnostic yield of basic procedures like routine stains on frozen sections, enzyme histochemistry, and immunohistochemistry in the work up of muscle biopsy.

Access this article online	
Quick Response Code:	Website: www.annalsofian.org
	DOI: 10.4103/0972-2327.165455

Materials and Methods

A retrospective analysis of the histopathology records and clinical case files was done in 164 cases of muscle biopsies received over a period of 5 years in our department. Following histopathological features were analyzed in detail: Fascicular architecture, variation in fiber size, nuclear features, fibers showing abnormal features like necrosis, splitting, basophilia,

phagocytosis, cellular reactions, and endomysial as well as perimysial fibrosis. In addition, findings pertaining enzyme histochemistry and immunohistochemistry were also analyzed.

Step-wise approach was adopted to assess the efficacy of routine stains (on frozen sections), enzyme histochemistry, and immunohistochemistry. Based on hematoxylin and eosin (H and E) appearance, biopsies were broadly categorized into: Muscle biopsies with destructive morphology, muscle biopsies with nondestructive morphology, muscle biopsies with inflammatory pathology, and normal muscle biopsies.

Role of special stains, enzyme histochemistry, and immunohistochemistry was further evaluated in each category.

Results

Based on the histopathology, 164 muscle biopsies [results summarized in Figure 1] were broadly categorized into abnormal (115) and normal (49) biopsies. After analyzing the histopathological features on hematoxylin and eosin stain, the abnormal biopsies were further categorized into biopsies showing destructive morphology, biopsies showing nondestructive but myopathic features, and biopsies showing inflammatory morphology [Table 1]. Biopsies with destructive morphology predominantly showed effaced architecture, marked variation in fiber size, excessive internal nuclei and nuclear clumps, fibers showing necrosis, myophagocytosis, splitting, and basophilia. Endomysial as well as perimysial fibrosis was variable [Figure 2]. Biopsies with nondestructive but myopathic morphology predominantly showed relatively preserved architecture. Myopathic features like mild-to-moderate variation in fiber size, angulated atrophic fibers, and internalization of nuclei in few/fair number of fibers. Centrally placed nuclei were observed in a single case. Endomysial as well as perimysial fibrosis was mild [Figure 3]. Biopsies with inflammatory morphology predominantly showed relatively preserved architecture, mild variation in fiber size, internalization of nuclei in few fibers, marked inflammatory infiltrate, necrotic fibers, prominent myophagocytosis, fiber splitting, and basophilic fibers. Perifascicular atrophy was evident in one case [Figure 4 and Table 2].

Modified Gomori trichrome (MGT) stain did not add much to the biopsies with destructive pattern. In contrast amongst nondestructive but myopathic muscle biopsies, MGT showed ragged red fibers (RRFs) in four cases [Figure 5a] suggesting mitochondrial myopathy, red staining rods in a single case [Figure 5b] suggesting nemaline myopathy, and darkly stained areas with red stained cytoplasmic bodies in one case suggesting myofibrillar myopathy [Figure 5c]. Biopsies with inflammatory pathology showed fibers with vacuoles rimmed by red granular material in three cases that suggested inclusion body myopathy (IBM) [Figure 5d and Table 3].

Table 1: Table showing broad categorization of muscle biopsies

Total muscle biopsies (*n* = 164)		
Abnormal (115)	Normal (49)	
Destructive morphology (65)	Nondestructive but myopathic morphology (35)	Inflammatory (15)

Table 2: Table showing histopathological features for various categories of muscle biopsies on H and E stain

Destructive morphology (65)	**Nondestructive but myopathic morphology (35)**	**Inflammatory morphology (15)**
Effaced architecture (46)	Relatively preserved architecture (31)	Relatively preserved architecture (14)
Marked variation in fiber size (52)	Mild (17)/moderate (8) variation in fiber size	Mild variation in fiber size (6)
Excessive internal nuclei (33), nuclear clumps (14)	Angulated atrophic fibers (9)	Internalization of nuclei (9)
Necrotic fibers (21)	Internalization of nuclei in few (11)/fair (6) number of fibers	Inflammatory infiltrate (12)
Myophagocytosis (32), basophilic fibers (22)	Centrally-placed nuclei (1)	Necrotic fibers (10), fiber splitting (5), basophilic fibers (8)
Splitting (48)	Mild fibrosis (10)	Myophagocytosis (8)
Variable amount of fibrosis (55)		Perifascicular atrophy (1)

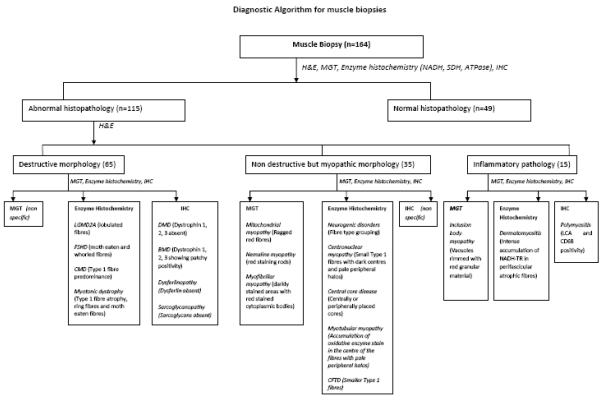

Figure 1: Diagnostic algorithm for the muscle biopsies. H and E = Hematoxylin and eosin, MGT = modified Gomori trichrome, NADH = nicotinamide adenine dinucleotide reductase, SDH = succinate dehydrogenase, ATPase = adenosine triphosphatase, IHC = immunohistochemistry

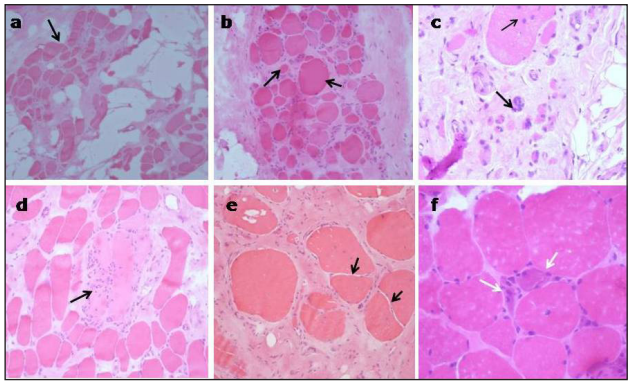

Figure 2: H and E staining showing (a) totally effaced architecture with marked endomysial and perimysial fibrosis, (b) marked variation in fiber size, (c) internalization of nuclei with nuclear clumps, (d) necrotic fibers undergoing myophagocytosis, (e) fiber splitting, and (f) basophilic fibers

Muscle biopsies with destructive morphology with enzyme histochemistry revealed lobulated fibers in 15 cases suggesting calpainopathy (limb girdle muscular dystrophy (LGMD) 2A) [Figure 6]; moth eaten [Figure 7a] and whorled fibers, small type 1 fibers in one case [Figure 7b] suggesting facioscapulohumeral dystrophy (FSHD), type 1 fiber predominance in a single case [Figure 7c] suggesting congenital muscular dystrophy (CMD); and type 1 fiber atrophy, and ring fibers, moth eaten fibers in one case that suggested myotonic dystrophy. Biopsies showing nondestructive but myopathic morphology with enzyme histochemistry revealed large group of atrophic fibers [Figure 8a], fiber type grouping [Figure 8b], large type 1 fibers with ATPase (pH 9.4) in 11 cases suggesting neurogenic disorders; small type 1 fibers with dark centers, and pale peripheral halos with NADH in one case suggesting centronuclear myopathy; and centrally or peripherally placed cores with NADH in three cases suggesting central core disease [Figure 9a], accumulation of oxidative enzyme stain in the center of fibers and pale peripheral halos with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) in two cases suggesting myotubular myopathy, type 1 fibers smaller than type 2 fibers with ATPase (pH 9.4) in 12 cases suggesting congenital fiber type disproportion [Figure 8c]. Biopsies in the category of inflammatory morphology revealed intense and aggregated NADH-TR activity in perifascicular fibers in three cases that suggested the diagnosis of dermatomyositis [Figure 9b].

A definitive diagnosis was rendered by immunohistochemistry among biopsies showing destructive morphology and biopsies with inflammatory pathology. Dystrophin 1, 2, and 3 showed complete membranous immunonegativity in most of the fibers in 18 cases [Figure 10c], thus providing a definite diagnosis of Duchenne muscular dystrophy (DMD). Uneven, patchy labeling with reduced intensity on most of the fibers in eight cases confirmed these cases as Becker muscular dystrophy (BMD) [Figure 10b]. Complete membranous immunonegativity for dysferlin in 13 cases confirmed the diagnosis of dysferlinopathy (LGMD 2B). A definitive diagnosis of sarcoglycanopathies (LGMD 2C, 2D, 2E, 2F) was made in all eight cases where complete membranous immunonegativity for α, β, γ, and δ sarcoglycans was observed. Inflammatory cells showing immunopositivity for LCA and CD68 among the biopsies showing inflammatory morphology confirmed all the nine cases as polymyositis.

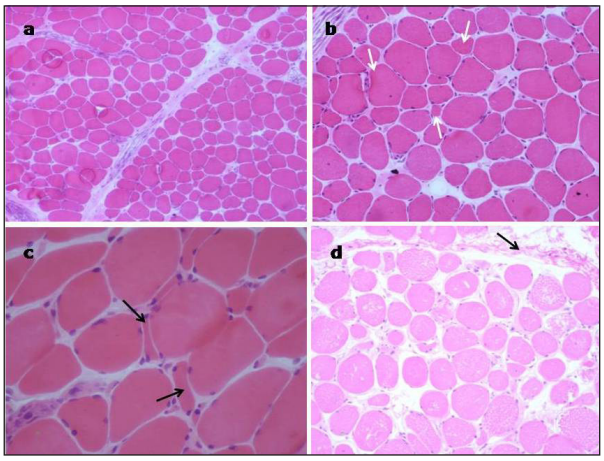

Figure 3: H and E staining showing (a) relatively preserved architecture, (b) mild-to-moderate variation in fiber size, (c) angulated fibers, and (d) mild endomysial and perimysial fibrosis

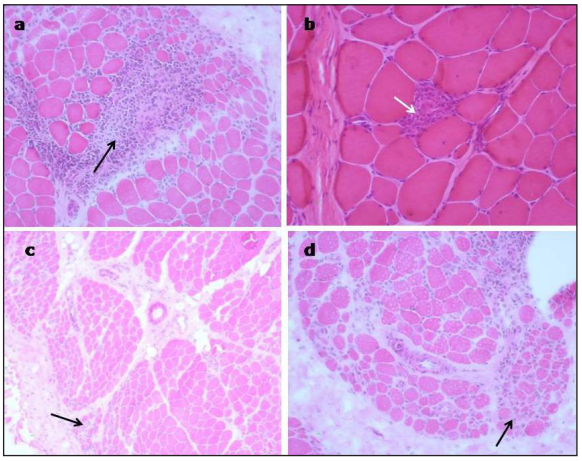

Figure 4: H and E staining showing (a) marked inflammatory infiltrate, (b) myophagocytosis, and (c and d) perifascicular atrophy

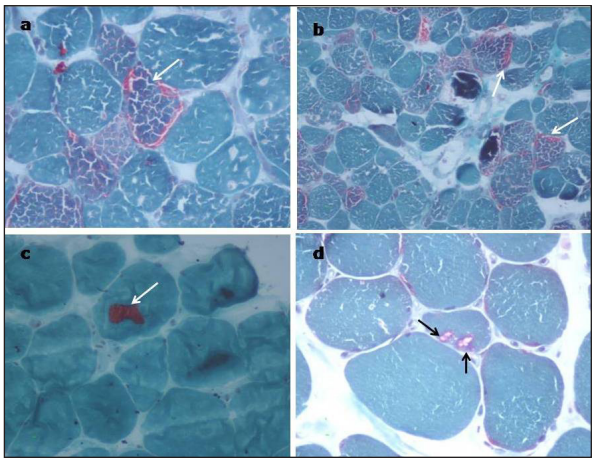

Figure 5: MGT showing (a) ragged red fibers, (b) red staining rods, (c) red staining cytoplasmic bodies, and (d) vacuoles rimmed by red granular material

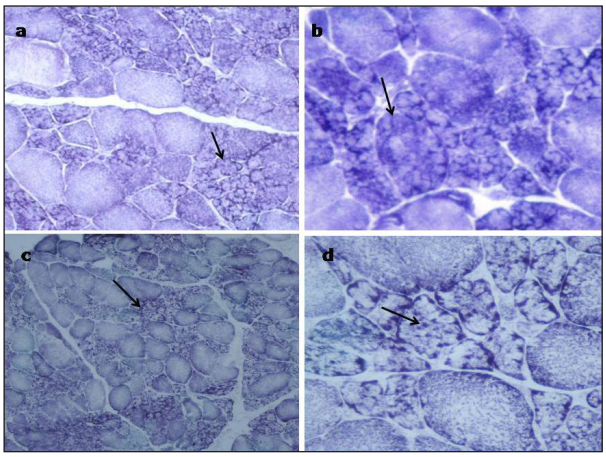

Figure 6: Nicotinamide adenine dinucleotide tetrazolium reductase showing (a and b) large number of lobulated fibers, and SDH showing (c and d) extensive lobulation in fair number of muscle fibers

Table 3: Table showing histopathological features for various categories of muscle biopsies on MGT stain, enzyme histochemistry and immunohistochemistry

Histopathology parameters	Destructive morphology (65)	Nondestructive but myopathic morphology (35)	Inflammatory pathology (15)
Modified Gomori trichrome (MGT)	Nonspecific	Ragged red fibers (4; mitochondrial myopathy), red staining rods (1; nemaline myopathy), darkly stained areas with red stained cytoplasmic bodies (1; myofibrillar myopathy)	Fibers with vacuoles rimmed by red granular material (3) (Inclusion body myopathy)
Enzyme histochemistry	Lobulated fibers (15; LGMD2A), moth eaten and whorled fibers, small type 1 fibers (1; FSHD), type 1 fiber predominance (1; CMD), type 1 fiber atrophy, ring fibers and moth eaten fibers (1; myotonic dystrophy)	Large group of atrophic fibers, fiber type grouping, large fibers type 1: ATPase 9.4 (11; neurogenic disorders), small type 1 fibers with dark centers and pale peripheral halos: NADH (1; centronuclear myopathy), centrally or peripherally placed cores: NADH (3; central core disease), accumulation of oxidative enzyme stain in the center of fibers and pale peripheral halos: NADH-TR) (2; myotubular myopathy), type 1 fibers smaller than type 2 fibers: ATPase (12; CFTD)	Intense and aggregated NADH-TR activity in perifascicular fibers (3; dermatomyositis)
Immunohistochemistry (IHC)	Dystrophin 1, 2, and 3 absent in the muscle fibers (18; DMD), uneven patchy labeling with reduced intensity in most of the fibers (8; BMD), dysferlin absent in the muscle fibers (13; LGMD2B), α, β, γ, and δ sarcoglycans absent in the muscle fibers (8; LGMD2C, 2D, 2E, and 2F)	Nonspecific	LCA and CD68 positive inflammatory cells (9; polymyositis)

LGMD = Limb girdle muscular dystrophy, FSHD = facioscapulohumeral dystrophy, CMD = congenital muscular dystrophy, BMD = Becker muscular dystrophy, DMD = Duchenne muscular dystrophy, ATPase = adenosine triphosphatase, NADH-TR = nicotinamide adenine dinucleotide tetrazolium reductase, CFTD = Congenital fibre type disproportion, EM = Electron microscopy

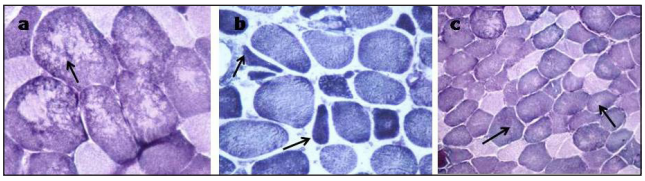

Figure 7: NADH showing (a) moth eaten fibers, (b) small type 1 fibers, and (c) type 1 fiber predominance

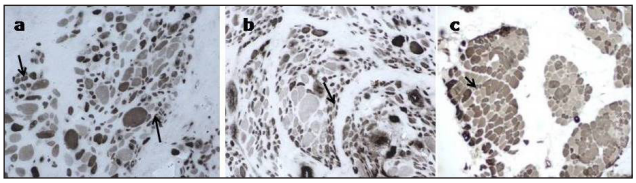

Figure 8: ATPase at pH9.4 showing (a) large group of atrophic fibers, (b) fiber type grouping, and (c) smaller type 1 fibers

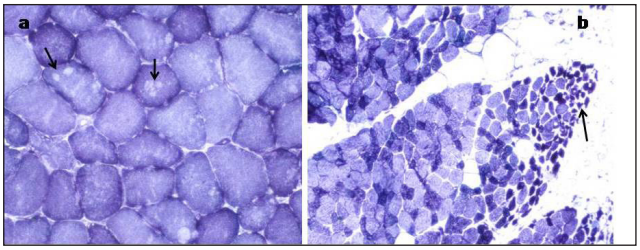

Figure 9: NADH showing (a) centrally and peripherally placed cores, (b) intense perifascicular NADH activity

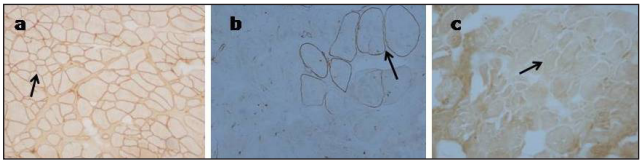

Figure 10: Immunohistochemistry showing complete membranous immunopositivity for (a) dystrophin 1, 2, and 3; (b) patchy membranous positivity for dystrophin 1, 2, and 3; and (c) complete membranous immunonegativity for dystrophin 1, 2, and 3

Diagnostic yield (based on H and E, MGT, enzyme histochemistry, and IHC) was variable among the three categories. The broad categorization was based on H and E features dividing the muscle biopsies (n = 164) into normal (49), destructive (65), nondestructive but myopathic (35), and inflammatory (15) morphology. For the biopsies showing destructive and inflammatory pattern IHC was the most specific diagnostic tool through which among the destructive morphology we could provide a definite diagnosis in 47 (72.3%) cases; whereas with enzyme histochemistry, a probable diagnosis was provided in 18 (27.7%) cases; however, for inflammatory morphology a definite diagnosis was provided in nine (60%) cases, while MGT provided a probable diagnosis in three cases (20%) and similarly a probable diagnosis was provided by enzyme histochemistry in three (20%) cases. In contrast, among the biopsies showing nondestructive, but myopathic pattern a probable diagnosis was given in 29 cases (82.8%) by enzyme histochemistry, while with MGT a probable diagnosis was given in six (17.2%) cases [Table 4].

Among the muscle biopsies showing abnormal histopathology, histopathological diagnosis showed concordance with the clinical diagnosis in 72.2% of cases (83/115). Discordance was observed in 25.2% of cases (29/115). In three (2.6%) cases no provisional diagnosis was mentioned in the requisition forms.

Table 4: Table showing diagnostic yield with H and E, MGT, enzyme histochemistry and immunohistochemistry

Diagnostic yield with	Destructive morphology (65)	Nondestructive but myopathic morphology (35)	Inflammatory pathology (15)
Routine stains (H and E)	Destructive pattern (65)	Nondestructive pattern (35)	Inflammatory pattern (15)
Modified Gomori trichrome (MGT)	Unremarkable	Probable diagnosis (6)	Probable diagnosis (3)
Enzyme histochemistry	Probable diagnosis (18)	Probable diagnosis (29)	Probable diagnosis (3)
Immunohistochemistry	Definite diagnosis (47)	Unremarkable	Definite diagnosis (9)

H and E = Hematoxylin and eosin

Discussion

Tremendous advances in our understanding of the molecular basis of muscle diseases over the past few years have led to several conceptual shifts in our approach to clinicopathologic diagnosis of muscle biopsy specimens. However, one cannot get far in the discussion of muscle diseases before entering the somewhat arcane world of specimen processing, histochemical staining, immunohistochemical staining, and electron microscopy.[5] Unfortunately though, availability of these diagnostic tools is still limited in majority of the laboratory settings in our country. Under such circumstances, interpretation of muscle biopsy histopathology in the perspective of clinical details can serve to narrow down to probable diagnoses. An algorithmic approach to muscle biopsy was adopted in our department to maximize diagnostic yield. The techniques available were routine histopathology and some special stains on frozen and paraffin embedded sections, enzyme histochemistry (NADH, SDH, and ATPase), and immunohistochemistry (dystrophin 1, 2, and 3; merosin; dysferlin; and α, β, γ, and δ sarcoglycans). With this basic and essential panel for muscle biopsy workup, we could offer a reasonable possible diagnosis in 47/65 (72.3%) muscle biopsy cases showing destructive morphology and in 9/15 (60%) muscle biopsies showing inflammatory morphology. However, among the biopsies showing nondestructive but myopathic morphology with this basic panel, a probable diagnosis was provided in all 35 cases with the help of MGT and enzyme histochemistry. Definite diagnosis could not be rendered in this category as molecular genetic analysis and electron microscopy (EM) is required for providing a definite diagnosis as described by Rollins *et al*., in their study assessing the diagnostic yield of muscle biopsy in patients with clinical evidence of mitochondrial cytopathy.[6]

The limited diagnostic yield within the category of muscle biopsies showing destructive morphology could be attributed to the lack of diagnostic modalities like immunoblot and molecular genetic analysis at our center as LGMD 2A require immunoblot,[7] while LGMD2B and FSHD need molecular genetic analysis for definite diagnosis[8] as described by Upadhyaya and Cooper[9] in their study elaborating molecular genetics of FSHD. Similarly the unsatisfactory diagnostic yield within the category of muscle biopsies showing nondestructive but myopathic morphology could be attributed to the lacking diagnostic modalities including molecular analysis and EM as molecular analysis is required for confirmation of neurogenic disorders, diminishing the role of muscle biopsy in neurogenic disorders as emphasized by Echaniz-Laguna *et al*.[10] Similarly in all congenital myopathies, EM is essential for the diagnosis and for directing molecular analysis. To maximize the diagnostic yield for the third category of muscle biopsies showing inflammatory pathology again molecular genetic analysis is required specifically for definitive diagnosis in cases of IBM as described by Nonaka *et al*.[11]

Conclusion

To conclude, the authors would highlight that though tools like electron microscopy, western blotting, and molecular genetics are available at apex centers, at most places pathology diagnosis still rests on routine stains on frozen sections, enzyme histochemistry, and immunohistochemistry. Though advanced techniques like electron microscopy, western blotting, and molecular genetics are essential for confirmatory diagnosis; a substantive diagnostic yield can be offered with the basic armamentarium of routine and special stains, enzyme histochemistry, and immunohistochemistry. In our experience, this basic laboratory support helps the clinicians in planning further management and guidance to the patient and family in majority of the cases.

References

1. Mitchell JA, Waclawik AJ. Muscle biopsy in diagnosis of neuromuscular disorders: The technical aspects, clinical utility and recent advances. J Histotechnol 2007;30:257-69.
2. Prayson RA. Diagnostic yield associated with multiple simultaneous skeletal muscle biopsies. Am J Clin Pathol 2006;126:843-8.
3. Rendt K. Inflammatory myopathies: Narrowing the differential diagnosis. Cleve Clin J Med 2001;68:505, 509-14, 517-9.
4. Skram MK, Gulati S, Larsson E, Lindal S, Torp SH. Muscle biopsies in children- an evaluation of histopathology and clinical value during a 5-year period. Ups J Med Sci 2009;114:41-5.
5. Miles JD, Cohen ML. Skeletal Muscle and Peripheral Nerve Disorders. In: Neuropathology by Richard A Prayson. 2nd edition, Elsevier Saunders; 2012.
6. Rollins S, Prayson RA, Mcmahon JT, Cohen BH. Diagnostic yield of muscle biopsy in patients with clinical evidence of mitochondrial cytopathy. Am J Clin Pathol 2001;116:326-30.
7. Anderson LV, Davison K. Multiplex Western blotting system for the analysis of muscular dystrophy proteins. Am J Pathol 1999;154:1017-22.
8. Vainzof M, Anderson LV, McNally EM, Davis DB, Faulkner G, Valle G, *et al*. Dysferlin protein analysis in limb girdle muscular dystrophies. J Mol Neurosci 2001;17:71-80.
9. Upadhyaya M, Cooper ND (eds). Introduction and Overview of FSHD. In: FSHD Fascioscapulohumeral muscular dystrophy: clinical medicine and molecular cell biology. BIOS Scientific Publishers, London Taylor and Francis, Oxford; 2004, p. 1-16.
10. Echaniz-Laguna A, Guiraud Chaumeil C, Trachant C, Reeber A, Melki J, Warter JM. Homozygous exon 7 deletion of the SMN centromeric gene (SMN2): A potential susceptibility factor for adult onset lower motor neuron disease. J Neurol 2002;249:290-3.
11. Nonaka I, Noguchi S, Nishino I. Distal myopathy with rimmed vacuoles and hereditary inclusion body myopathy. Curr Neurol Neurosci Rep 2005;5:61-5.

How to cite this article: Pant I, Chaturvedi S, Bala K, Kushwaha S. Muscle histopathology in today's era of molecular genetics: Role and limitations. Ann Indian Acad Neurol 2015;18:398-402.
Received: 17-01-15, **Revised:** 04-04-15, **Accepted:** 28-04-15
Source of Support: Nil, **Conflicts of Interest:** None declared.