

The Histopathological Features of Muscular Dystrophies

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ABSTRACT

Muscular dystrophies are degenerative muscle diseases due to mutations in proteins ranging in function such as sarcolemmal structure, nuclear envelope structure, or post-translational glycosylation. Each of them affects a specific group of skeletal muscles within the human body, suggesting that biological differences exist between individual muscles that predispose them to specific pathological etiologies.

Clinical manifestation of different muscular dystrophies is now well known and documented. Dystrophinopathies are X-linked recessive diseases and the most common form of muscular dystrophies with a relatively poor outcome. Other recognized varieties of muscular dystrophies are classified into different groups according to their clinical or genetic similarities. For example, limb girdle muscular dystrophy is an umbrella name for a group of diseases which exhibits proximal weakness of the shoulder and pelvic girdles. Similarly, the defining characteristic of congenital muscular dystrophies is presentation prior to 1 year of age.

Table 1: Genetic Features of Common Muscular Dystrophies (*).

Disease	Gen (protein)	Chromosome
X-LINKED MUSCULAR DYSTROPHIES		
Dystrophinopathies (Duchenne and Becker)	DMD (dystrophin)	Xp21
Emery-Dreifuss muscular dystrophy (EDMD)	EMD (emerin)	Xq28
AUTOSOMAL DOMINANT MUSCULAR DYSTROPHIES		
Facioscapulohumeral dystrophy (FSHD)	Complex genetic mechanism involving DUX4 (double homeobox 4)	Deletion of D4Z4 repeats at 4q35)
Myotonic dystrophy, type 1 (DM1)	DMPK (myotonic dystrophy protein kinase)	19q13
Myotonic dystrophy, type 2 (DM2)	CNBP (CCHC-type zinc finger nucleic acid-binding protein; formerly ZNF9, zinc finger protein 9)	3q21)
Oculopharyngeal muscular dystrophy	PABP2 (poly A binding protein, nuclear 1)	14q11
Limb-girdle muscular dystrophies (LGMD 1)		
LGMD 1A	MYOT (myotilin)	5q31
LGMD 1B (also dominant EDMD)	LMNA (laminA/C)	1q21
LGMD 1C	CAV3 (caveolin3)	3p25
LGMD 1D DNAJB6	(HSP-40 homologue, subfamily B, number 6)	7q36
LGMD 1E	DES (desmin)	2q35
LGMD 1F	TNPO3 (transportin 3)	7q32
AUTOSOMAL RECESSIVE MUSCULAR DYSTROPHIES		
Congenital muscular dystrophies (CMD)		
Merosin-deficient CMD (MDC1A)	LAMA2 (laminin 2)	6q22
Dystroglycanopathies –Collagen VI-related dystrophies (Ullrich CMD)	genetically and phenotypically heterogeneous muscular dystrophies; COL6A1, A2 or A3 (alpha1, alpha2, or alpha3 chains of type VI collagen)	21q22
		2q37
Limb-girdle muscular dystrophies (LGMD 2)		
LGMD 2A	CAPN3 (calpain-3)	15q15
LGMD 2B	DYSF (dysferlin)	2p12
LGMD 2C	SCGC (-sarcoglycan)	13q12
LGMD 2D	SCGA (-sarcoglycan)	17q21
LGMD 2E	SCGB (-sarcoglycan)	4q12
LGMD 2F	SCGD (-sarcoglycan)	5q33
LGMD 2G	TCAP (telethonin)	17q12
LGMD 2H	TRIM32 (tripartite motif-containing 32)	9q33
LGMD 2I	FKRP (fukutin-related protein)	19q13.3
LGMD 2J	TTN (titin)	2q31
LGMD 2K	POMT1 (protein-O-mannosyltransferase 1)	9q34
LGMD 2L	ANO5 (anoctamin 5)	11p14
LGMD 2M	FKTN (fukutin)	
LGMD 2N	POMT2 (protein-O-mannosyltransferase 2)	14q24
LGMD 2O	POMGnT1 (O-linked mannose beta1,2-N-acetylglucosaminyltransferase)	1p34
LGMD 2Q	PLEC1 (plectin 1)	8q24
(*) The table is copied from Sternberg'surgical Pathology, reference 2.		

Herein, it is aimed to display the complexity for the clinical, histopathological, and genetic characteristics of muscular dystrophies and also highlight the difficulties encountered during the process of their differential diagnosis. In conclusion, it must be kept on mind that the histopathological examination of muscle tissue is only one component of the total diagnostic effort and it mustn't be isolated from the patient's history, physical examination and relevant laboratory tests.

Keywords: Muscular dystrophy; Histopathological features; Genetic findings; Differential diagnosis

GENERAL ASPECTS OF MUSCULAR DYSTROPHIES

Muscular dystrophy (**MD**) is a group of primary hereditary myopathies with a chronic and unremitting progressive course [1]. The rather meaningless term dystrophy, which literally means “deficient nutrition,” was popularized toward the close of the nineteenth century, when the pathogenesis of the muscular dystrophies was totally mysterious [2]. With advances of molecular genetics, the pathogenesis of some of these conditions has become understood. It is now well known that all forms of muscular dystrophies are genetic; some are inherited, whereas others are de novo mutations (Table 1). These mutations are generally located in genes encoding proteins of the dystrophin- associated glycoprotein (**DAG**) complex at the sarcolemma and lead to its partial or complete absence [2]. These structural proteins render the support network that connects myofilament proteins within the cell to the basal lamina outside the cell. Without this complex to tether the actin cytoskeleton inside the muscle cell to the extracellular matrix, forces generated by the muscle fiber result in tears of the sarcolemma and lead to muscle damage. The regenerative capacity in muscle cannot compensate for increased susceptibility for structural damage. In addition, dystrophic muscle cannot adequately repair itself. The imbalance between muscle damage and muscle repair leads to a loss of muscle fibers and an increase in the amount of fibrosis over time until the functional capacity of the muscle diminishes to a point below the required force output [2,3]. Not only the defects of sarcolemmal proteins, but also the defects of nuclear envelope proteins or their post-translational glycosylation can cause of muscular dystrophies [1-5].

Within the group of muscular dystrophies (**MDs**), distinct entities are sorted by the different modes of inheritance, age of onset, clinical course and severity [1]. The knowledge of disease profile is very useful for differential diagnosis of MDs. In general, the initial symptoms are manifested during childhood or young adulthood; however, neonatal or late adult onset cases do occur [4]. Many MDs are characterized with an early onset and they must be considered for differential diagnosis with infantile denervation, metabolic or mitochondrial diseases. The cardinal symptom is muscular weakness that is steadily and unremittingly progressive, but severity is different [1-5]. Each MD affects a specific group of skeletal muscles within the body, suggesting that biological differences exist between individual muscles that predispose them to specific pathological

etiologies [5]. For example, limb girdle muscular dystrophy (**LGMD**) is an umbrella name for a group of diseases which exhibits proximal weakness of the shoulder and pelvic girdles. Similarly, the patients suffering from fascioscapulohumeral dystrophy (**FSHD**) present with progressive weakness involving the muscles of the face, shoulder and upper arms [1]. The presence of some specific symptoms such as myotonia, dysphagia or ptosis may support a specific diagnosis. Because the myotonic dystrophy is characterized with myotonia and the oculopharyngeal muscular dystrophy (**OPMD**) is characterized with the weakness of swallowing and extraorbital muscles. In addition, the course of disease is also useful in the differential diagnostic procedures of MDs. For example, a rapid onset of symptoms with very high CK levels is suggestive of an inflammatory myopathy, whereas insidious progression favors other noninflammatory myopathies (**NIMs**) such as metabolic myopathy, MD, and most of congenital myopathies [6-10].

Assessments of disease progression and response to therapies in patients with MDs remain challenging. Although several biomarkers have been currently identified, it is being studied to investigate new serum circulating proteins as biomarkers for muscle damage associated with muscular dystrophies [1,2,4]. The most famous marker is creatine kinase (**CK**), especially CK-M which is a muscle specific protein that reflects sarcolemma damage and it is currently used to screen for Duchenne muscular dystrophy (**DMD**) in newborns [11]. Although CK is a good marker to screen for suspected MDs, it is not suitable to monitor disease progression and response to therapy because it decreases sharply with age and its concentration is easily influenced by muscle trauma and exercise [11]. Carbonic anhydrase III (**CA-III**) and myoglobin are other two muscle specific proteins that were found elevated in blood of patients with muscular dystrophies. MMP9, TIMP1 and osteopontin are also associated with muscle inflammation and had altered levels in serum of patients relative to healthy controls. The CK levels in patients with MDs, especially in dystrophinopathies, are usually very high, 50- 100 times the normal level [4]. Levels of less than 10 times the normal are more likely to be associated with other forms of MDs [4,12].

Relatively little information about the prevalence of neuromuscular disorders (**NMDs**) has been published [7,9,13-19]. It was reported that the NMDs affect approximately one in 3500 children worldwide and X-linked dystrophinopathy has the highest incidence among them [1-5,13]. Knowledge of NMDs has expended dramatically during the last 4 decades thanks to modern pathological techniques and genetics. Currently the dystrophinopathies and most LGMDs can be diagnosed with immunohistochemical staining on the muscle tissues [1,2,4,7]. But it must be kept in mind that specific genetic diagnoses can be suggested by immunostaining, but definitive diagnoses rely on molecular genetic testing [2].

DISTINGUISHING CLINICAL FEATURES OF MUSCULAR DYSTROPHIES

The diagnosis of MD is based on the results of muscle biopsy, increased CK, electromyography, and genetic testing. A physical examination and the patient's medical history help to determine

the type of MD. Specific muscle groups are affected by different types of muscular dystrophy. Other tests that can be done for differential diagnosis are chest X-ray, echocardiogram, CT scan, and magnetic resonance image scan [1-5].

Dystrophinopathies, DMD and Becker muscular dystrophy (**BMD**) are caused by dystrophin deficiency. DMD is the most common form of the muscle disease, affecting 1 out 3500 newborn males [1-5]. Without the dystrophin complex to tether the actin cytoskeleton inside the muscle cell to the extracellular matrix, forces generated by the muscle fiber result in tears of the sarcolemma and lead to muscle damage. As an X-linked recessively inherited disorders, DMD mainly affects boys who are neurologically intact at birth. By the time the child attempts to stand or walk, the first signs of overt disease are noticeable. A subtle awkwardness gradually gives way to limb-girdle pattern weakness, sparing the muscles of facial expression and swallowing. A paradoxical enlargement of affected weak muscles is characteristic of DMD. This pseudohypertrophy, which is associated with fatty infiltration and reactive fibrosis, is especially apparent in the calves and buttocks. Mild intellectual disability that cannot be explained on the basis of physical incapacitation is considered intrinsic to the disease. Reduced expression of dystrophin isoforms normally found in brain may underlie the cognitive abnormalities. Death is often hastened by an insidious cardiomyopathy leading to sinus tachycardia, cardiac arrhythmias, and congestive heart failure. An extremely high level of serum creatine kinase is an early indicator of this form of dystrophy, and it may precede severe pathologic alterations in muscle [2]. BMD is the milder allelic form of dystrophinopathy seen in almost exclusively young men with the prevalence of at least 2.4/100000. The symptoms in BMD are less severe than in DMD, and the rate of progression is slower. BMD is mainly characterized by progressive skeletal muscle weakness. In BMD, the mutations allow for expression of truncated but functional dystrophin or a reduced amount of dystrophin protein. Therefore BMD patients may live until the fifth or sixth decade of life and cardiomyopathy represents the number one cause of death in these patients [2,11,12,19- 23].

LGMD is a collection of dystrophies which affects the proximal axial muscles. These dystrophies are genetically very heterogeneous with at least 6 autosomal dominant (LGMD type 1) and at least 15 autosomal recessive (LGMD type 2) forms (Table 1). The age of onset ranges from early childhood (overlapping with the congenital muscular dystrophies) to late adulthood (overlapping with BMD), and progression of disease varies widely. Many of the LGMD genes encode proteins associated with the sarcolemmal proteins critical for binding to extracellular matrix proteins. Other LGMD proteins are part of the sarcomeric apparatus, or nuclear envelope. Some of them have as yet uncertain localization and function. The prevalence of LGMD subtypes varies widely among ethnic groups and geographic regions. The most common sarcoglycanopathies are LGMD 2C, 2D, and 2E [2]. All LGMDs can affect both boys and girls. They show a similar distribution of muscle weakness, affecting both upper arms and legs. In LGMDs with autosomal recessive pattern of inheritance, patient receives two copies of the defective gene, one from each parent. The recessive LGMDs are more frequent than the dominant forms, and usually have childhood

or teenaged onset. The dominant LGMDs usually show adult onset. Some of the recessive forms have been associated with defects in proteins that make up the DAG. Though a person normally leads a normal life with some assistance, in some extreme cases, death from LGMD occurs due to cardiopulmonary complication [23-29].

Congenital muscular dystrophy (**CMD**) includes several disorders with a range of symptoms. The defining characteristic of CMDs is presentation prior to 1 year of age. Otherwise, clinical and pathologic phenotypes are exceedingly heterogeneous [2]. Age at onset is generally birth, the symptoms include general muscle weakness and possible joint deformities, disease progresses slowly, and lifespan is shortened. Muscle degeneration may be mild or severe. Problems may be restricted to skeletal muscle, or muscle degeneration may be paired with effects on the brain and other organ systems. Several forms of the CMD are caused by defects in proteins thought to have some relationship to the DAG and to the connections between muscle cells and their surrounding cellular structure. Some forms of them show severe brain malformations, such as lissencephaly and hydrocephalus [1- 5,10,23,29,30,31].

The patients with Emery-Dreifuss muscular dystrophy (**EDMD**) generally present in childhood and the early teenaged years with contractures. Clinical signs include muscle weakness and wasting, starting in the distal limb muscles and progressing to involve the limb-girdle muscles. Most patients also suffer from cardiac conduction defects and arrhythmias. The three subtypes of EDMD are distinguishable by their pattern of inheritance: X-linked, autosomal dominant and autosomal recessive. The X-linked form is the most common. Each type varies in prevalence and symptoms. The disease is caused by mutations in the LMNA gene, or more commonly, the EMD gene. Both genes encode for protein components of the nuclear envelope. However, how these mutations cause the pathogenesis is not well understood [1-5,32].

FSHD is a usually autosomal dominant inherited form of MD that initially affects the muscles of the face, shoulders, and upper arms with progressive weakness. Symptoms usually develop in early adulthood, affected individuals become severely disabled. The pattern of inheritance is generally autosomal dominant, though a number of spontaneous mutations occur. FSHD can occur both in males and females. FSHD is the third most common genetic disease of skeletal muscle with the prevalence as 4/100,000. Symptoms may develop in early childhood and are usually noticeable in the teenage years with 95% of affected individuals manifesting disease by age 20 years. A progressive skeletal muscle weakness usually develops in other areas of the body as well; often the weakness is asymmetrical. Life expectancy can be threatened by respiratory insufficiency and up to 20% of affected individuals become severely disabled requiring use of a wheel chair or mobility scooter. Non-muscular symptoms frequently associated with FSHD include subclinical sensorineural hearing loss and retinal telangiectasia. In more than 95% of known cases, the disease is associated with contraction of the D4Z4 repeat in the 4q35 subtelomeric region of Chromosome 4 [1- 5,33,34].

OPMD is a hereditary MD characterized by muscle weakness that begins in adulthood, typically after age 40. Symptoms affect muscles of eyelids, face, and throat followed by pelvic and shoulder muscle weakness. The first symptom in people with this disorder is usually ptosis, followed by difficulty swallowing. Dysphagia begin with food, but as the condition progresses, liquids can be difficult to swallow as well. Many people with this condition have atrophy of the tongue. These problems with food intake may cause malnutrition. Some affected individuals also have weakness in other facial muscles. Patients with OPMD also have weakness in the proximal muscles, particularly muscles in the upper legs and hips. The weakness progresses slowly over time, and people may need the aid of a cane or a walker. Rarely, affected individuals need wheelchair assistance. There are two types of OPMD, which are distinguished by their pattern of inheritance. They are known as the autosomal dominant and autosomal recessive types. OPMD has been attributed to a short repeat expansion in the genome which regulates the translation of some genes into functional proteins [1- 5,35-37].

Myotonic muscular dystrophy is an autosomal dominant condition that presents with myotonia, delayed relaxation of muscles, as well as muscle wasting and weakness. Myotonic MD varies in severity and manifestations and affects many body systems in addition to skeletal muscles, including the heart, endocrine organs, and eyes. Myotonic MD type 1 (**DM1**) is the most common adult form of muscular dystrophy. It results from the expansion of a short (**CTG**) repeat in the DNA sequence of the myotonic dystrophy protein kinase gene. Myotonic muscular dystrophy type 2 (**DM2**) is rarer and is a result of the expansion of the CCTG repeat in the zinc finger protein 9 gene [1-5,38-39].

EVALUATION OF MUSCLE BIOPSY IN PATIENTS WITH MUSCULAR DYSTROPHIES

With occasional exceptions, evaluation of muscle biopsy is an essential element in the assessment of a patients with suspected myopathy [1,2,9-16]. In theory, advances in molecular genetics diagnostic techniques should eliminate the need for muscle biopsies to diagnosis of muscle diseases. However, muscle biopsies continue to be done for several reasons. The most common histopathological features of muscular dystrophy are changes in fiber size and shape (Figure 1) muscle fiber necrosis with regeneration (Figure 2), and interstitial fibrosis (Figure 3). Although this trio, named as dystrophic feature, is the hallmark of dystrophies, the severity of dystrophic changes varies among genotypes, among allelic variants of the same genotype, among muscles within individual patients, within a single biopsy, and over time within individual patients. In addition, increased internally placed nuclei are generally striking in dystrophic muscle especially in myotonic dystrophy [1,2,9].

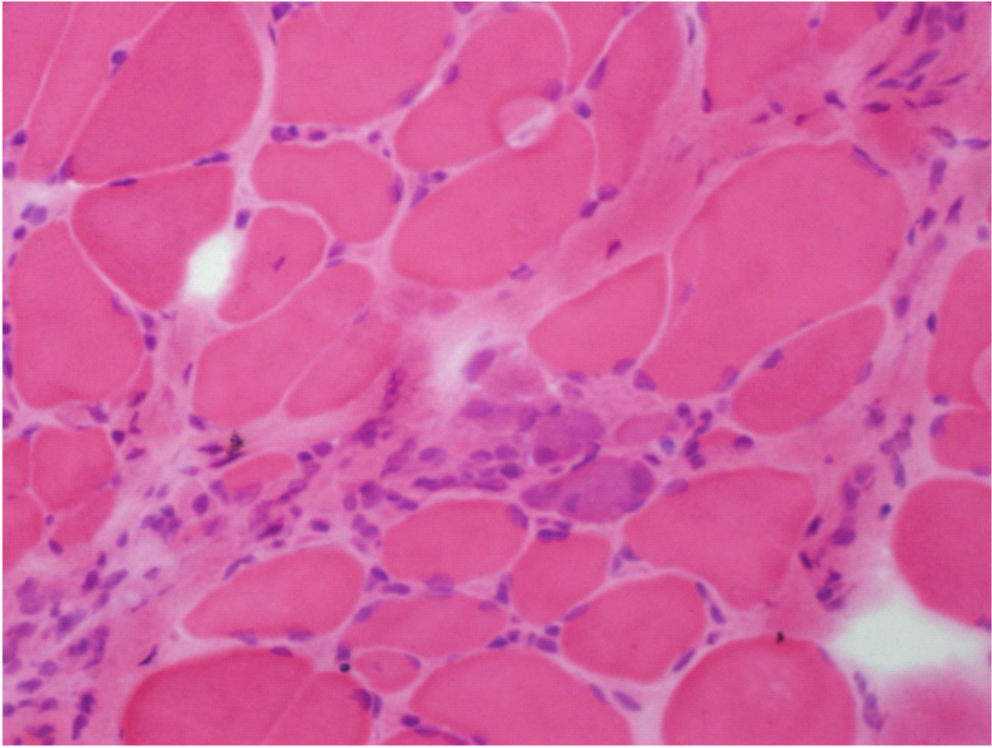


Figure 1: Marked changes in fiber size and regenerating fibers.

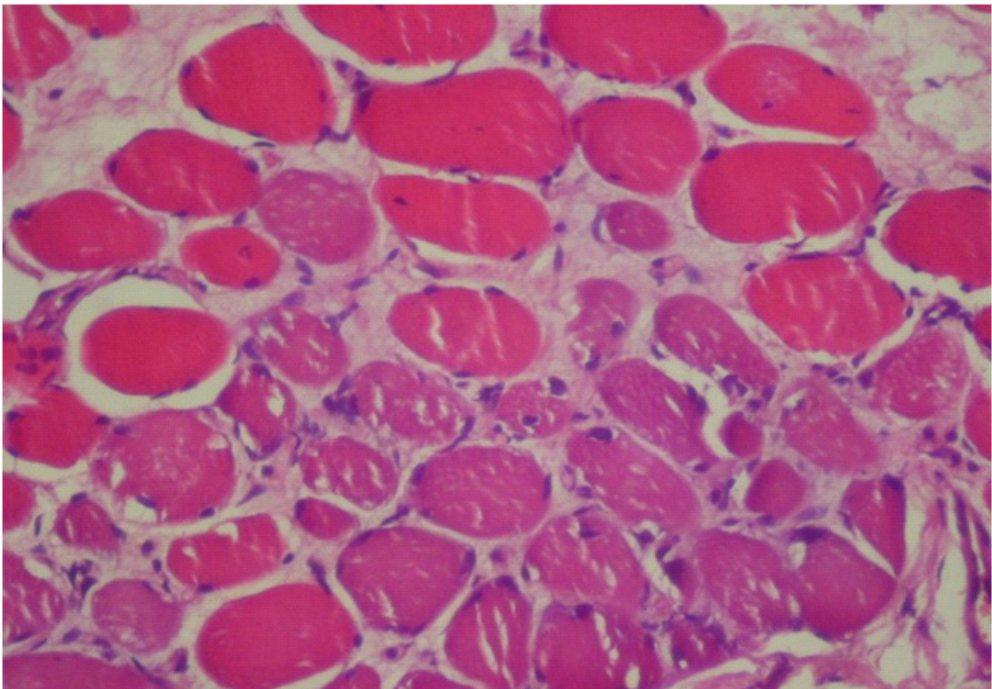


Figure 2: Fiber necrosis and regeneration.

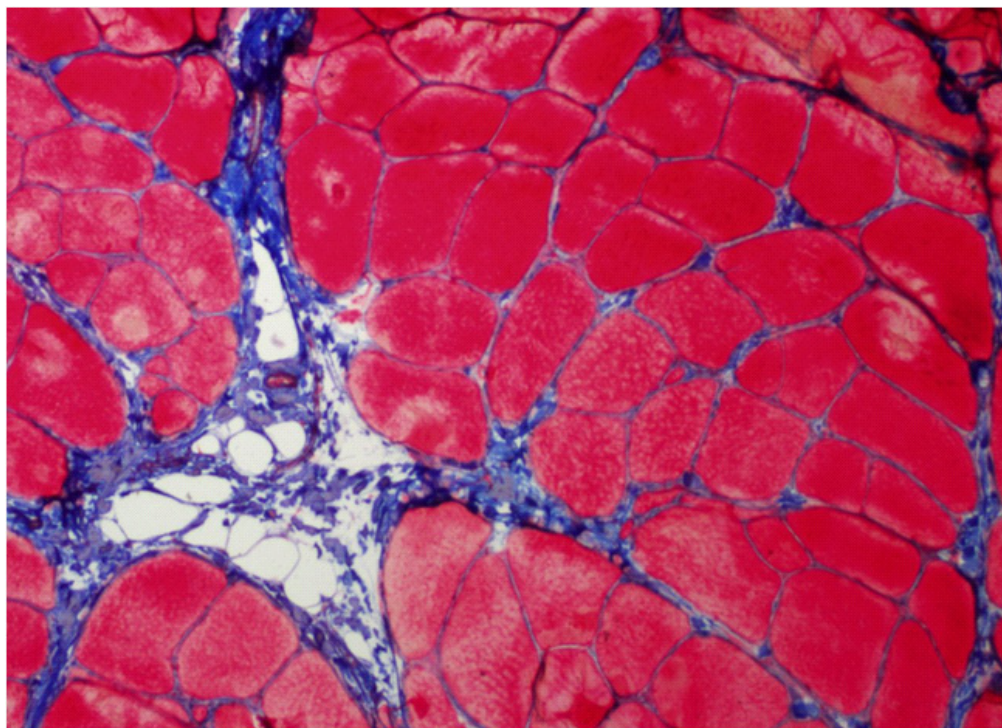


Figure 3: Interstitial fibrosis.

The fiber necrosis generally acts as a stimulus for subsequent regeneration. Therefore the presence of regenerating fibers in a biopsy specimen, even in the absence of necrotic fibers, is a likely indicator of previous necrosis. The regeneration of fibers is believed to arise primarily from the proliferation of satellite cells and generation and fusion of myoblasts [2]. It is a complex cellular process that restores injured muscle to a state that is morphologically and functionally similar to that of uninjured muscle. Regeneration of skeletal muscle occurs in two distinct phases: degeneration and regeneration [5]. The main characteristics of the degenerative phase involve myofiber sarcolemmal damage or myofiber necrosis, followed by an influx of mononucleated inflammatory cells and an increase in fibroblasts. Factors released from damaged myofibers initiate an inflammatory response that recruits neutrophils, macrophages, and activates fibro/adipogenic progenitors to facilitate the removal of cellular debris and regulate muscle repair. The basal lamina remains intact acting as a scaffold for the next phase, muscle regeneration. Several molecular signals, such as growth factors, chemokines, and cytokines, are released which activate satellite cells both locally and systemically within the first 24–48 h following injury. Myoblasts then terminally differentiate becoming post-mitotic myocytes, which then fuse with other myocytes or myofibers to regenerate or repair damaged myofibers [5,18]. Regenerating fibers are most readily visualized in hematoxylin and eosin (HE) sections by the basophilia of their sarcoplasm. The nuclei are typically increased in number, are larger than normal, have vesicular chromatin and prominent nucleoli, and often are internally placed. Ultrastructurally,

the regenerating fibers are replete with ribosome, which explains the sarcoplasmic basophilia at the light microscopic level [5,6].

In addition, the patient's history and the profile of the disease are also informative in the diagnostic procedures of NMDs [1]. Most of hereditary NMDs present an insidious progression, whereas the inflammatory myopathies (**IMs**) have acute fashions [7]. IMs are infectious in origin or immune mediated [2]. Recently the percent of inflammatory disorders which are diagnosed with muscle biopsies is decreased, because the laboratory findings aid in differential diagnosis. Therefore evaluation of muscle biopsy is rarely required. Interstitial inflammatory infiltrates are most frequently encountered in immunologically mediated or idiopathic inflammatory myopathies. Most important among these are polymyositis (**PM**), dermatomyositis (**DM**) and inclusion body myositis (**IBM**). Inflammation in PM, chiefly consists of mature lymphocytes, particularly activated CD8 cells, with few or no B cells, and these cells invade the endomysium, sometimes enveloping necrotic fibers (Figure 4). In DM, the immunophenotyping of the lymphocytes discloses a high percentage of B cells and T cells are mostly CD4 lymphocytes. They surround intramuscular blood vessels with minimal infiltration of the vascular walls and focally in the perimysium. On the other hand, inflammatory reaction is lacking except in the scattered necrotic fibers in MDs. These cells consist of mostly macrophages, scarce T lymphocytes, myoblasts, and mast cells [1,4]. In the IMs, deposits of immunoglobulins and complement also can be demonstrated within the intramuscular blood vessel walls, and they are presumably related to immunologically mediate capillary damage that results in a reduction in the size of the vascular bed [39-41]. Atrophic fibers at the periphery of fascicles, known as, perifascicular atrophy, is thought to be caused by ischemic process (**Figure 5**). The vasculitic changes are prominent in the childhood variant of DMs. There are also many necrotic and regenerating fibers histologically identical to MDs. However, crucial differences among location of these regenerating cells. For example, they are scattered within the fascicle in PMs and IBMs (Figure 6), while they are located in the periphery of the fascicles. In summary, it must be kept on mind that the presence of regenerating fibers and inflammatory infiltration is a hallmark of IMs [1-5].

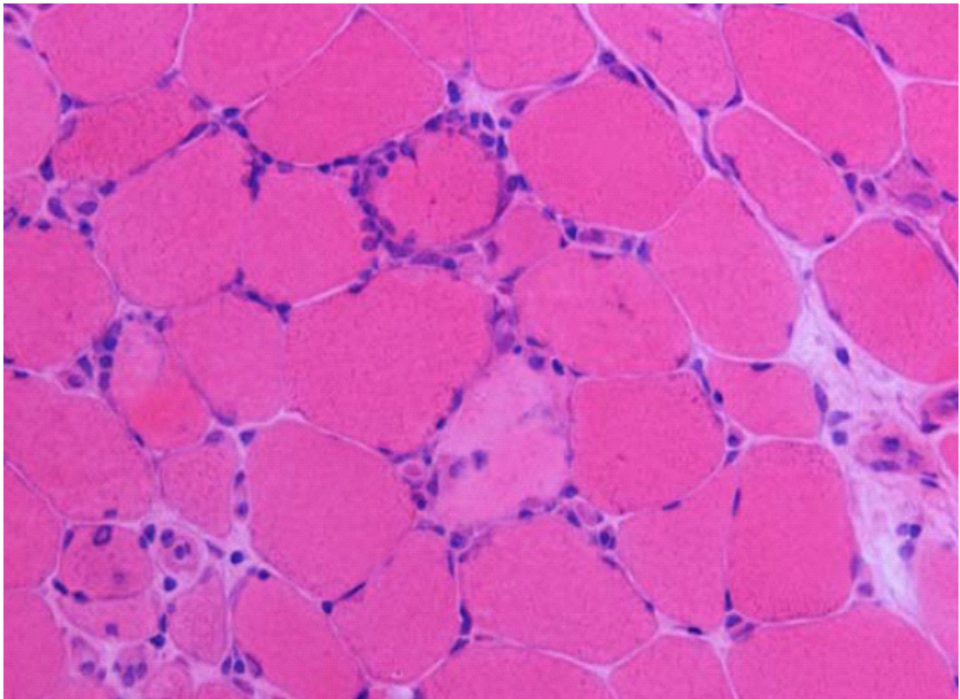


Figure 4: Interstitial lymphocytic infiltration in a polymyositis.

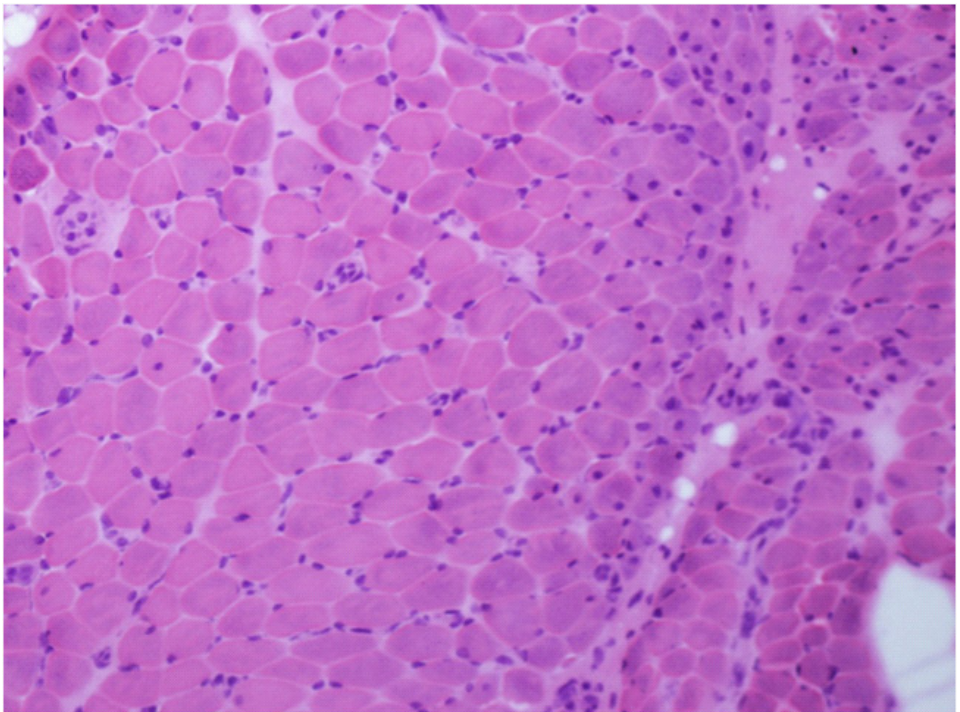


Figure 5: Perifascicular atrophy.

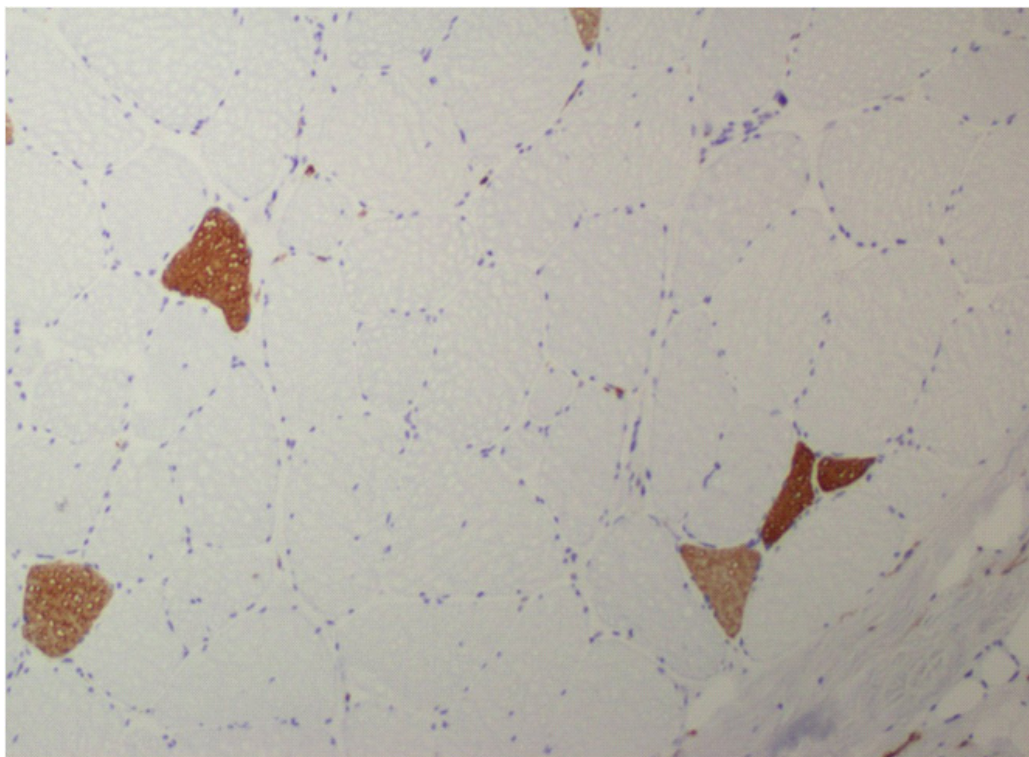


Figure 6: Scattered necrotic fibers with anti-neonatal myelin antibody in a patient with polymyositis.

Pathologic alterations in muscle fibers are conventionally classified as myopathic or neuropathic [1-6]. The disorders of neuromuscular junction are generally diagnosed with electrophysiological studies and muscle biopsy is not performed [14,17,42]. The distinction between neurological diseases and congenital muscular dystrophies may be difficult in infantile cases without histopathological examination [7]. Especially in the floppy infants who require persistent mechanical ventilator support, EMG couldn't be performed. Patient's history may not be suggestive, either. Because both the neurogenic processes and congenital myopathies have the similar insidious progression, muscle biopsy can help to determine whether a neonate has a neurogenic or myogenic disorder, which is a relatively common issue prompting performance of a biopsy [14,17,39-44]. Myopathic changes in muscles are generally characterized by a random atrophy of both fiber types. Contrary in neuropathies, a more specific atrophy patterns are determined. For example during early denervation mainly type II fibers atrophy occurs, then atrophic angulated fibers are seen and eventually large group atrophy is developed (Figures 7-10). Angulation, nuclear clumping and targetoid changes are also specific features of neuropathies. In spinal muscular atrophy, most of the larger fibers are type I and many of the atrophic fibers are type II (Figures 11 and 12).

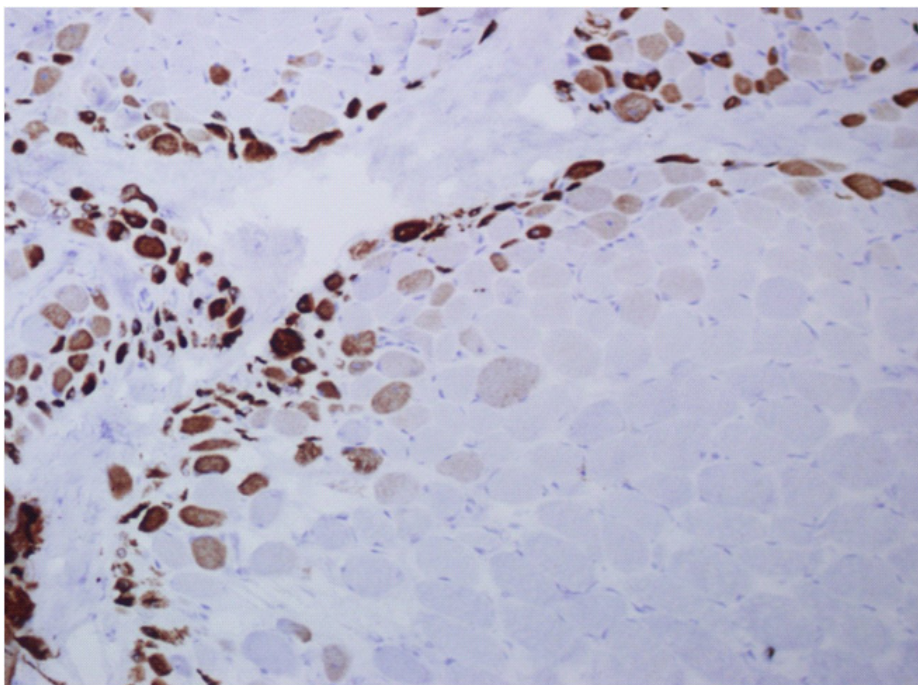


Figure 7: Perifascicularly arranged necrotic fibers with anti-neonatal myelin antibody. In a patient with dermatomyositis.

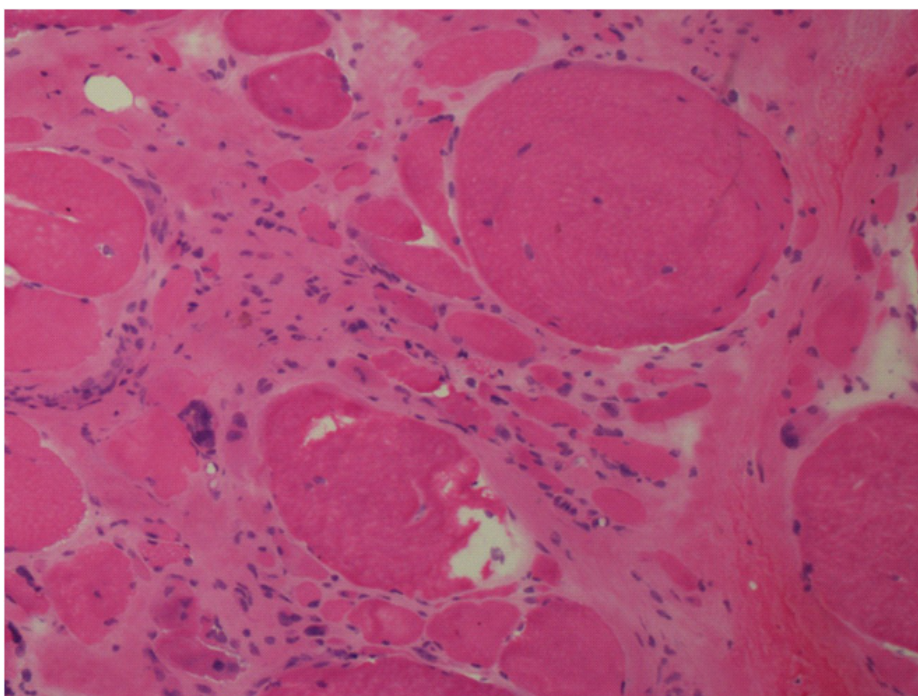


Figure 8: Large fibers are wrapped with atrophic angulated fibers nad nuclear clumping.

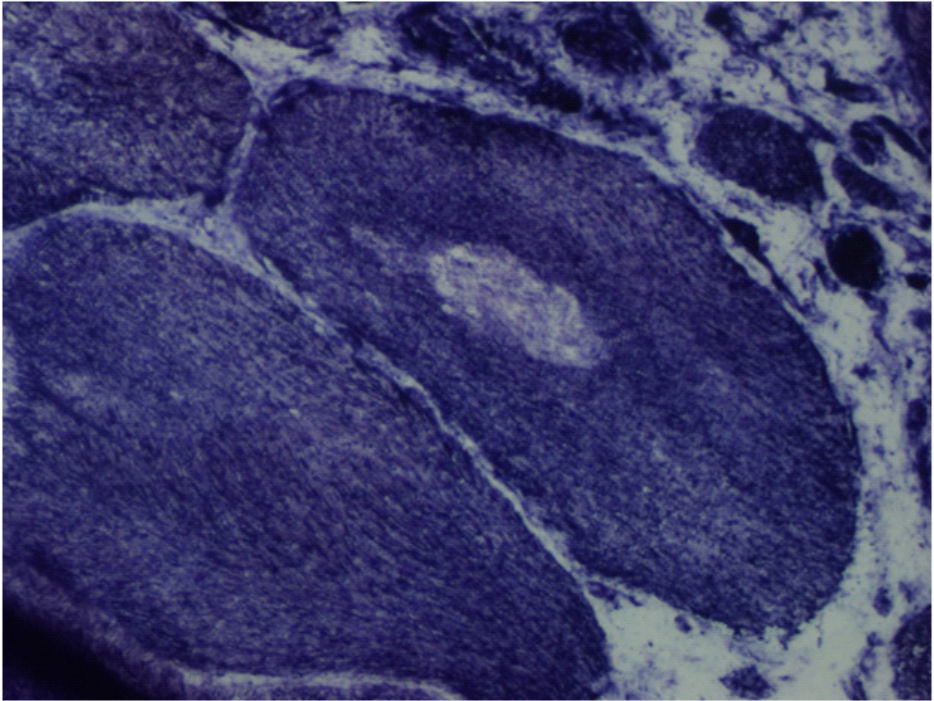


Figure 9: A target (core) fiber in neurogenic myopathy.

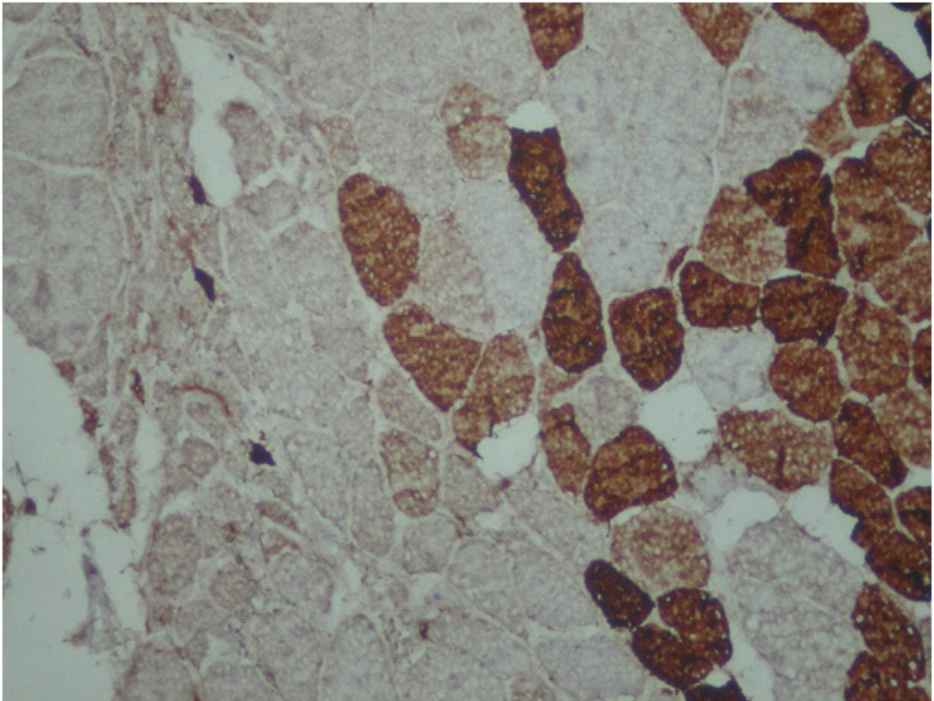


Figure 10: Large group atrophy with myosin heavy chain fast antibody.

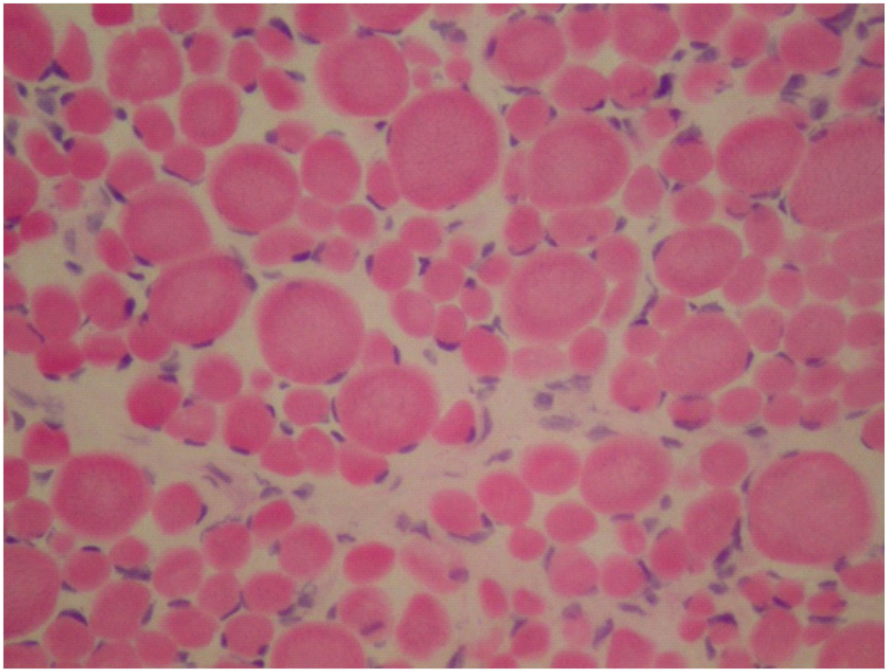


Figure 11: Infantile denervation is characterized with markedly atrophic fiber which have rounded contour.

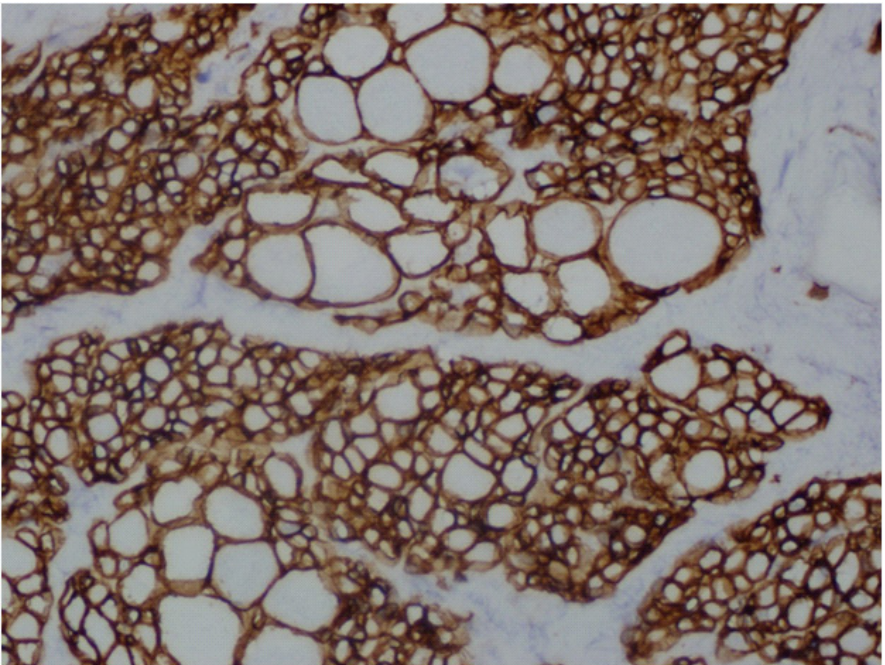


Figure 12: Mostly round type II myofiber and a few grouped type I fibers are specific for spinal muscular atrophy.

Endomysial fibrosis is a common pathologic component of both MDs and myositis. The fibrosis is simply a manifestation of inflammation and repair as can be seen in most tissues. However, the factors that provoke end-stage interstitial fibrosis and fatty replacement of muscle have not been adequately explained by research investigations even though they are the bequest of NMDs of both myopathic and neurogenic origin [1-4].

CLUES AND PITFALLS ON IMMUNOHISTOCHEMICAL EVALUATION OF MUSCULAR DYSTROPHIES

Immunohistochemical analysis of the sarcolemmal proteins such as dystrophin, SGCs, merosin, and dysferlin is an important part of the diagnostic evaluation of muscle biopsies in patients with muscular dystrophy. Reduced or absent sarcolemmal expression of one of the four SGCs can be found in patients with any LGMDs and also in patients with dystrophinopathies. It has been previously suggested that different patterns of SGC expression could predict the primary genetic defect, and that genetic analysis could be directed by these patterns. However Klinge et al. [45] reported that residual SGC expression could be highly variable and an accurate prediction of the genotype could not be achieved. Therefore they recommended using antibodies against all four SGCs for immunoanalysis of skeletal muscle sections. Similarly, a concomitant reduction of dystrophin and any one of SGCs may have a crucial importance in the differential diagnosis of dystrophinopathies for sarcoglycan deficient LGMD [1-5]. For this reason, it is not easy to decide whether the disease is a dystrophinopathy with defective expressions of SGCs or a LGMD with defective expression of dystrophin [25,26,45,46].

Patients with any LGMD may be clinically indistinguishable from those with primary dystrophinopathies. Probably, the diagnosis of LGMD has been underestimated and a number of male patients were diagnosed as DMD or BMD [19-29]. If a definitive diagnosis can be made based on appropriate immunohistochemical examinations and molecular analysis performed in those patients, a normal staining pattern of dystrophin and an autosomal recessive mode of inheritance can be determined. On the contrary, patients with dystrophinopathy may show variable findings from normal to regional absence or mosaic pattern of sarcolemmal staining with anti-SGCs antibodies which signify different presentation of abnormal organization of the cell membrane associated DAG. Therefore careful examination of immunohistochemical staining with genetic study is necessary to make an accurate diagnosis [25,26].

CONCLUSION

In summary, it must be kept on mind that the histopathologic examination of muscle tissue is only one component of the total diagnostic effort and it mustn't be isolated from the patient's history, physical examination and relevant laboratory tests. Paradoxically, in some disorders such as mitochondrial dysfunction, spinal muscular atrophy and most of the muscular dystrophies, differential diagnoses are very difficult without the evaluation of muscle biopsy specimens.

References

1. Ang LC. Skeletal Muscle. In Rosai and Ackerman's Surgical Pathology. New York: Mosby; 2004; 2: 2663- 2681.
2. Heffner RR, Moore SA. Muscle Biopsy in Neuromuscular Disease. In: Sternberg's Surgical Pathology. Ed: Mills SE, 6th ed, Philadelphia: Wolters Kluwer. 2015; 1: 113- 147.
3. Diniz G, Derici H, Kamer E, Unalp HR, Bozdog AD, et al. Effect of Sildenafil on the Skeletal Muscle Regeneration: An Experimental Study. Turkish Clinics J Med Sci. 2011; 31: 809- 815.
4. Dubowitz V, Sewry CA, Oldfords A. Muscle Biopsy: A Practical Approach, London: Elsevier. 2013.
5. Randolph ME, Pavlath GK. A muscle stem cell for every muscle: variability of satellite cell biology among different muscle groups. Front Aging Neurosci. 2015; 7: 190.
6. Carpenter S, Karpati G. Regeneration. Pathology of Skeletal Muscle. 2nd ed. New York: Oxford University Press; 2001: 83-99.
7. Diniz G, Tosun Yildirim H, Unalp A, Barutcuoglu M, et al. The evaluation of muscle biopsy findings in children with neuromuscular disorders. Izmir Dr. Behçet Uz Çocuk Hast Derg 2012; 2: 62-67.
8. Bove KE. Neuromuscular Diseases. In: Stocker JT, Dehner LP, Pediatric Pathology. Philadelphia, Lippincott Williams and Wilkins, 2002: 1272-1318.
9. Dubowitz V. Diagnosis and classification of the neuromuscular disorders. In: Muscle Disorders in Childhood, Dubowitz V. Philadelphia, WB. Saunders Co. 1995; 1-33.
10. Olukman O, Calkavur S, Diniz G, Unalp A, Atlihan F. Nemaline myopathy in a newborn infant: a rare muscle disorder. Neurol Neurochir Pol. 2013; 47: 493-498.
11. Zhang H, Zhu Y, Sun Y, Liang Y, Li Y. Serum creatinine level: a supplemental index to distinguish Duchenne muscular dystrophy from Becker muscular dystrophy. Dis Markers. 2015; 2015: 141856.
12. Hathout Y, Seol H, Han MH, Zhang A, Brown KJ. Clinical utility of serum biomarkers in Duchenne muscular dystrophy. Clin Proteomics. 2016; 13: 9.
13. Diniz G, Barutcuoglu M, Unalp A, Aktas S, Uran N, et al. Evaluation of the relationship between urogenital abnormalities and neuromuscular disorders. Eastern Medical Journal. 2008; 13: 19-24.
14. Dua T, Das M, Kabra M, Bhatia M, Sarkar C. Spectrum of floppy children in Indian scenario. Indian Pediatr. 2001; 38: 1236-1243.
15. Tangsrud SE, Halvorsen S. Child neuromuscular disease in southern Norway. Prevalence, age and distribution of diagnosis with special reference to "non-Duchenne muscular dystrophy". Clin Genet. 1988; 34: 145-152.
16. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. Neuromuscul Disord. 1991; 1: 19-29.
17. Darin N, Tulinius M. Neuromuscular disorders in childhood: a descriptive epidemiological study from western Sweden. Neuromuscul Disord. 2000; 10: 1-9.
18. Jiang C, Wen Y, Kuroda K, Hannon K, Rudnicki MA, et al. Notch signaling deficiency underlies age-dependent depletion of satellite cells in muscular dystrophy. Dis Model Mech. 2014; 7: 997-1004.
19. Stehle EM. Long-term management of children with neuromuscular disorders. Jornal de Pediatria. 2009; 85: 379-384.
20. Stark AE. Determinants of the incidence of Duchenne muscular dystrophy. Ann Transl Med. 2015; 3: 287.
21. Ho R, Nguyen ML, Mather P. Cardiomyopathy in becker muscular dystrophy: Overview. World J Cardiol. 2016; 8: 356-361.
22. Webster C, Blau HM. Accelerated age-related decline in replicative life-span of Duchenne muscular dystrophy myoblasts: implications for cell and gene therapy. Somat Cell Mol Genet. 1990; 16: 557-565.
23. Rae MG, O'Malley D. Cognitive dysfunction in Duchenne Muscular Dystrophy: a possible role for neuromodulatory immune molecules. J Neurophysiol. 2016.
24. Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ. Incidence, causes, and outcomes of dilated cardiomyopathy in children. JAMA. 2006; 296: 1867-1876.
25. Siciliano G, Simoncini C, Giannotti S, Zampa V, Angelini C. Muscle exercise in limb girdle muscular dystrophies: pitfall and advantages. Acta Myol. 2015; 34: 3-8.
26. Diniz G, Tosun Yildirim H, Akinci G, Hazan F, Ozturk A, Yazarbas K, Tukun A. Sarcolemmal alpha and gamma sarcoglycan protein deficiencies in Turkish siblings with a novel missense mutation in the alpha sarcoglycan gene. Pediatr Neurol 2014; 50: 640-647.
27. Diniz G, Hazan F, Yildirim HT, Unalp A, Polat M. Histopathological and genetic features of patients with limb girdle muscular dystrophy type 2C. Turk Patoloji Derg. 2014; 30: 111-117.

28. Cotta A, Carvalho E, da-Cunha-Júnior AL, Paim JF, Navarro MM4. Common recessive limb girdle muscular dystrophies differential diagnosis: why and how? *Arq Neuropsiquiatr*. 2014; 72: 721-734.
29. Diniz G, Tosun Yildirim H, Gokben S, Serdaroglu G, Hazan F, Yazarbas K, Tukun A. Concomitant alpha- and gamma-sarcoglycan deficiencies in a Turkish boy with a novel deletion in the alpha-sarcoglycan gene. *Case Rep Genet*. 2014; 2014: 248561.
30. Diniz G, Eryaşar G, Türe S, Akçay A, Ortaç R, Tekgöl H, Akhan G. A regional panorama of dysferlinopathies. *Turk Patoloji Derg*. 2012; 28: 259-265.
31. Rocha CT, Hoffman EP. Limb-girdle and congenital muscular dystrophies: current diagnostics, management, and emerging technologies. *Curr Neurol Neurosci Rep* 2010; 10: 267-276.
32. Cecil DM, Chaturvedi A, Kapoor D. Z-shaped brainstem and other magnetic resonance imaging findings in congenital muscular dystrophy. *Neurol India*. 2016; 64: 577-578.
33. Bonne G, Quijano-Roy S. Emery-Dreifuss muscular dystrophy, laminopathies, and other nuclear envelopopathies. *Handb Clin Neurol*. 2013; 113: 1367-1376.
34. Hauerslev S, Ørngreen MC, Hertz JM, Vissing J, Krag TO. Muscle regeneration and inflammation in patients with facioscapulohumeral muscular dystrophy. *Acta Neurol Scand*. 2013; 128: 194-201.
35. Statland JM, McDermott MP, Heatwole C, Martens WB, Pandya S, van der Kooi EL, Kissel JT, Wagner KR, Tawil R. Reevaluating measures of disease progression in facioscapulohumeral muscular dystrophy. *Neuromuscul Disord*. 2013; 23: 306-312.
36. Luigetti M, Lo Monaco M, Mirabella M, Primiano G, Lucchini M. Oculopharyngeal muscular dystrophy: Clinical and neurophysiological features. *Clin Neurophysiol*. 2015; 126: 2406-2408.
37. Harish P, Malerba A, Dickson G, Bachtarzi H. Progress on gene therapy, cell therapy, and pharmacological strategies toward the treatment of oculopharyngeal muscular dystrophy. *Hum Gene Ther*. 2015; 26: 286-292.
38. Werling S, Schrank B, Eckardt AJ, Hauburger A, Deschauer M. Oculopharyngeal muscular dystrophy as a rare cause of dysphagia. *Ann Gastroenterol*. 2015; 28: 291-293.
39. Bertrand JA, Jean S, Laberge L. Psychological characteristics of patients with myotonic dystrophy type 1. *Acta Neurol Scand*. 2015; 132: 49-58.
40. Leonardis L, Blagus R, Dolenc Groselj L. Sleep and breathing disorders in myotonic dystrophy type 2. *Acta Neurol Scand*. 2015; 132: 42-48.
41. Dalakas MC. Review: An update on inflammatory and autoimmune myopathies. *Neuropathol Appl Neurobiol*. 2011; 37: 226-242.
42. Pestronk A. Acquired immune and inflammatory myopathies: pathologic classification. *Curr Opin Rheumatol*. 2011; 23: 595-604.
43. Stenzel W, Goebel HH, Aronica E. Review: immune-mediated necrotizing myopathies—a heterogeneous group of diseases with specific myopathological features. *Neuropathol Appl Neurobiol* 2012;38:632–646.
44. Finnis MF, Jayawant S. Juvenile myasthenia gravis: a paediatric perspective. *Autoimmune Dis*. 2011; 2011: 404101.
45. Uusimaa J, Remes AM, Rantala H, Vainionpää L, Herva R, Vuopala K, Nuutinen M, Majamaa K, Hassinen IE. Childhood encephalopathies and myopathies: A prospective study in a defined population to assess the frequency of mitochondrial disease. *Pediatrics* 2000; 105: 598-603.
46. Klinge L, Dekomien G, Aboumoussa A, Charlton R, Epplen JT. Sarcoglycanopathies: can muscle immunoanalysis predict the genotype? *Neuromuscul Disord*. 2008; 18: 934-941.
47. Diniz G, Tekgul H, Hazan F, Yazarbas K, Tukun A. Sarcolemmal deficiency of sarcoglycan complex in an 18-month-old turkish boy with a large deletion in the beta sarcoglycan gene. *BJMG* 2015; 18: 71- 76.