Review Article

Muscle Biopsy

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ABSTRACT

Muscle biopsy has been used for a long time for diagnosis of muscular, nerurogenic and systemic disorders with muscle involvement, because only very few of these disorders show sufficient specific clinical features for definite diagnosis. Since the presence of difficulties in the screening of numerous genes, muscle biopsy could be a time and cost effective procedure for solving the diagnostic problems. The aim of this article is to mention the importance of muscle tissue in the evaluation of primary and secondary muscle diseases, special consideration of how to biopsy, handling the specimen and performing the special staining, and the microscopic findings in order to have better interpretation results.

Keywords: Muscle, Biopsy, Pathology

Introduction

The removal of a small piece (10-15mg) of muscle tissue for examination is called "muscle biopsy" (1).

It is usually performed when the patient is awake and feels little or no discomfort. There are two essential ways for biopsy:

1-Needle biopsy is using a needle which inserts into muscle for taking the specimen, it is less time- consuming, more cost- effective with fewer complications than an open biopsy but obtaining a small and often traumatized sample (2,3) so could be preserved when peripheral nerve sampling is not required and when large tissue samples are not needed for extensive biochemical analyses (4).

2-Open biopsy is performed after a small cut in the skin to take samples and is preferred because the physician (surgeon, pathologist or neurologist) is able to inspect the tissue (especially important in patchy involvements of muscle) and then take the sample. Studying the muscle tissues is a golden tool in diagnosis of muscle disease, some of the primary neurogenic disorders, some of the systemic diseases with muscle involvement and also differentiation and categorization of the primary muscle disorders (5).

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General Considerations

First of all, the physician or the teamwork should be aware about the indications and contraindications of biopsy.

Indications

1- Evidences of muscle disease: weakness and discomfort, muscle cramps, post exercise early fatiguibility (non-myasthenic)

2- Increase in SCK (Serum Certain Kinase) (6)

3- Myopathic pattern in electromyography (EMG)

4- Systemic disease which could have muscular involvements (7)

Contraindications

1- The diseases which could be diagnosed by electric diagnostic tools such as myasthenia gravis (MG), myotonia and CMS (congenital myasthenic syndrome) and also could be confirmed by mutation detection methods (8).

2- Traumatized or injected muscle or the muscle which has undergone electromyography till minimally one month after trauma or the procedure.

3- Muscle with severe weakness (which may show severe necrotic features in biopsy) (9).

Muscle of Choice

The muscle of choice for biopsy is characterized by physical exam or imaging techniques such as Magnetic Resonance Imaging (MRI). MRI also could reveal the pattern of muscle involvement and facilitates the differential diagnosis (10, 11).

The best muscle for biopsy is a moderately involved muscle (12). Deltoid, biceps and quadriceps muscle are preferred, however in special instances, special muscles such as, orbicularis oculi is recommended for the diagnosis of mitochondrial myopathies, using samples which are taken during belfaroplasty of ptosis surgery in order to reduce morbidity and cost (13).

Gastrocneumous should be avoided because of the increase in connective tissue & internal nuclei which could be misdiagnosed by muscular dystrophies. In some instances gastrocneumous is chosen for biopsy in association with sural nerve biopsy for the diagnosis of vasculitis. Intercostals and anconeus are preserved for microelectrode studies & electron microscopy of the end plate because of rich sources of neuromuscular junctions. Fascia could be biopsied at the time of open muscle biopsy for ruling out of fasciatis.

Since the therapeutic intervention may have altered the pathologic findings, biopsy should be taken from muscle samples without a history of muscle treatment (5).

Procedures

Such as other tissue samples, there should be a completed requisition form composed of patient's name, identification number, age, sex, date of biopsy, precise biopsy site, clinical history, first diagnosis and differential diagnoses. The requisition form should also include radiographic, laboratory and treatment history.

Muscle biopsy preparations

Many of the muscle morphological abnormalities such as regeneration, fibrosis, fatty infiltration and vaculated fibers are evident in hematoxylin & eosin stained sections, but none of the muscle disorders especially muscular dystrophies could be distinguished on the basis of muscle histology (14).

For biopsies which are intended to investigate inflammatory diseases (dermatomyositis, polymyositis, inflammatory myopathy) the tissue should be divided to frozen for enzyme histochemistry, formalin-fixed for hematoxylin & eosin (H&E) staining, and

glutaradheyde- fixed for electron microcopy (EM).

For investigation of muscular dystrophies (Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Limb-Girdle Muscular Dystrophy (LGMD), Fascio-scapulo-Humeral Dystrophy (FSHD) and Myotonic Dystrophy (MD) the tissue should be divided to formalin fixed for H&E, frozen for enzyme histochemistry & immunohistochemistry.

If the biopsy is intended for evaluation of a metabolic abnormality of mitochondrial myopathy (MELAS, Kearn-Sayre syndrome Lipid storage disease) the tissue should be divided to frozen for enzyme histochemistry, and histochemistry and glutaraldeheyde-fixed for EM.

Specimen preparation

***Fresh tissue*:**

1-The muscle sample should be divided minimally to three parts (as will be described below) and transferred to the laboratory within 30 minutes.

2- The 1st sample (only 2mm in diameter) is clamped and is fixed in glutaraldyhyde for electron microscopy. The 2nd sample, for histochemistry and light microscopy is oriented lougitudinally and placed on saline-moinsted paper or gauze for transferring to the pathology laboratory where it is frozen or fixed in formalin. The 3rd sample is also placed on saline-soaked paper and reserved for biochemical studies in the cases they may be indicated (15).

Frozen Tissue:

The best results for enzyme histochemistry & immunohistochemistry studies are obtained when tissues are frozen rapidly and kept in -80ºC until sectioned. Any thawing and re-freezing leads to ice crystal formation with loss of morphologic details and cell membrane integrity (surface antigens). Enzymatic activity can also be lost during thaw& freeze (16).

Freezing Methods

1-Liquid nitrogen: tissue for biochemical studies.

2- Isopentane /liquid nitrogen: tissue for enzyme histochemistry and immunohistochemistry studies.

Residual tissue, with preserved orientation, may be immersed in formalin for routine histology. 10% natural buffered formalin (NBF) is suitable with minimum 4 hours fixation time. Muscle may be fixed while still clamped.

Findings

After studying the muscle, the pathologist could be able to answer the following issues (17, 18):

- Size, shape, type of muscle fibers, internal architectures and storage materials.
- Myopathic or neurogenic pattern of involvement (round: myopathic & angular: neurogenic).
- Distribution of atrophic fibers (if present): grouped or scattered.
- Fiber type smallness:

Small type1: Hereditary myopathy

Small type 2: Congenital myasthenia gravis

- Acute or chronic inflammatory process (if seen):

Acute: -myopathy: muscle fiber degeneration & regeneration

- neuropathy: Small angular fibers

Chronic: -myopathy: fiber hypertrophy & increase in endomysial connective tissue

- Neuropathy: fiber type grouping, pyknotic nuclear chromatin

Abnormalities in :

- Vessels
- Connective tissue: endo & perimysial
- Intramuscular nerves

Histochemistry stains

The golden data could be extracted from different methods of staining; each could be applied for a special goal or to differentiate a few disorders which could not be clinically differentiated or categorized. The most common histochemistry stains are as the Table 1(17).

Table 1- Muscle biopsy histochemical stains

Category	Method	Utility
Morphology	Hematoxylin and eosin	Muscle fiber phathology; Nuclei
	Verhof van Giesson (VvG)	Connective tissue; vessel structure intramuscular nerve
	Gomori Trichrome	Connective tissue; Nemaline rods
Fiber Type Enzyme	Myofibrillar ATPase	Muscle fiber type grouping or atrophy
	ATPase pH 9.4	Myosin loss; type 1 or 2 fiber atrophy
	ATPase pH 4.6	Type 2B muscle fibers
	ATPase pH 4.3	Type 2C (Immature)
Oxidative Enzymes	NADH-TR	Muscle fiber internal architecture; tubular aggregates; cores
	Succinate dehydrogenase	Mitochondrial pathology
	Cytochrome oxidase	Mitochondrial pathology Mitochondrial DNA encoded protein
Glycolytic Enzymes	Phosphorylase	Phosphorylase deficiency
	Phosphofructokinase (PFK)	PFK deficiency
Hydrolytic Enzymes	Acid phosphatase	Macrophages; Llysosomes; lipofuscin
	Non-specific esterase	Macrophages; lysosomes; Neuromuscular & myotendinous junctions Denervated (small angular) muscle fibers
	Acetylcholimesterase	Neuromuscular & myotendinous junctions
	Alkaline phosphatase	Regenerating muscle fibers; Immune disease of connective tissue
Storage material	PAS	Glycogen & Carbohydrate disorders
	Alcian blue	Mucopolysaccharide
	Sudan black B	Lipid storage
	Oil red O	Lipid storage
Other	Congo red	Amyloid; Inflammation; Vacuoles
	Myoadenylate deaminase	AMPDA deficiency
	Methyl green pyronine	RNA
	Acridine orange	RNA
	Von Kossa	Calcium
	Alizarin red	Calcium
Fixed muscle	Toluidine blue	Muscle fibers; Capillaries

Protein Analysis

Histological and histochemical analysis should be completed with protein analysis. Protein analysis could be assessed through immunohistochemistry and western blot analysis (19).

Immunohistochemistry

There are some immune stains which are very important in differentiation of some hereditary muscle disorders such as muscular dystrophies. For example Dystrophin analysis by immunohistochemistry is a very sensitive method for diagnosis of DMD and BMD (20, 21).

The immune stains reveal expressed or absent protein in the muscle tissue as the result of one or more mutations in the corresponding genes. This process is a golden method which the pathologist could follow & confirm the mutant gene products and could be performed with flurochrome (immunoflurescence) or peroxidase (22).

In some instances, secondary protein changes(e.g. Utrophin expression) in the absence of another type of protein (e.g. Dystrophin) as we see severely in DMD and mildly in most cases of BMD could be assayed using immunohistochemistry in two different tissue sections (23). Utrophin is a dystrophin homologue which is upregulated in the absence or reduction of dystrophin; it should be also noted that the intensity of utrophin expression isn't related to disease severity (24). Symptomatic mother carriers of mutated dystrophin gene (either with clinical symptoms and /or increased SCK levels) could be examined by utrophin which may result in on/off expression pattern of protein (25-28). Reciprocal expression between dystrophin & urtophin is also evident in these cases (28). Table 2 shows some of the immunostains and their potential diagnostic roles.

Table 2- Commonly used antibodies as immuno stains in protein analysis method

Disease	Antibody	Protein-based diagnosis
Dystrophinopathy (DMD and BMD)	Dystrophin	Immunohistochemistry Western blot analysis
Calpainopathy LGMD-2A	Calpain	Western Blot analysis not for immunohistochemistry
Dysferlinopathy LGMD-2B	Dysferlin	Immunohistochemistry Western blot analysis
Sarcoglycanopathy LGMD2C-F	Sarcoglycan α,β,γ,δ	Immunohistochemistry Western blot analysis
Telethoinopathy LGMD-2G	Telethoin	Immunohistochemistry Western blot analysis
Congenital muscular dystrophy	Merosin (Laminin α2)	Immunohistochemistry Western blot analysis

Immunoblotting

The quality and quantity of the target protein could also be assessed by western blot analysis using muscle extract. This procedure could offer the clues of the mutation type (11, 28, 29).

Immunoblotting is a suitable method for detecting the missed or reduced protein in muscle tissue, and could be performed as multiplex western blot analysis (30, 31).This procedure is preferred by some centers instead of tissue immunostaining in all affected males suspected for DMD (1).

Genetic studies

As mentioned above, muscle biopsy could provide the material for immunoblotting in order to detect abnormal or absent proteins, however mutation detection techniques are also available using blood samples (32). If the mutation could not be detected with general methods, another techniques such as Single-Strand-Conformation-Polymorphism (SSCP), Polymerase Chain Reaction(PCR) and Southern blot analysis are recommended (1).

Conclusion

The precise study of muscle tissue is a valuable method for detecting muscle disorders, primary and also secondary, if it follows standard protocols. Routine histology could not be used for diagnosis of muscular dystrophies and in most instances methods of protein analysis recommended.

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References

1. Beggs AH, Kunkel LM. Improved diagnosis of Duchenne/Becker muscular dystrophy. J Clin Invest 1990;85(3):613-9.

2. 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(1):41-5.

3. Edwards RH, Round JM, Jones DA. Needle biopsy of skeletal muscle: a review of 10 years experience. Muscle Nerve 1983;6(9):676-83.

4. Derry KL, Nicolle MN, Keith-Rokosh JA, Hammond RR. Percutaneous muscle biopsies: review of 900 consecutive cases at London Health Sciences Centre. Can J Neurol Sci 2009;36(2):201-6.

5. Seidman RJ. Muscle biopsy and clinical and laboratory features of neuromuscular disease. Available from :Htpp://emedicine.medscape.com/article/184877-overview.

6. Gasper MC, Gilchrist JM. Creatine kinase: a review of its use in the diagnosis of muscle disease. Med Health R I 2005;88(11):398.

7. Keyhani E. An Introduction to Muscle Histo-pathology.Tehran:University of Social Welfare & Rehabilitation Sciences Press;2010.

8. Kahrizi K, Keyhani E, Hantai D ,Urtizbrea JA , Najmabadi H . Report of the first mutation of ƐAchR in an Iranian patient with congenital myastenic syndrome. Genetics in the 3rd Millenium 2004;2(4):422-6.

9. Tulio E Bertorini . Clinical Evaluation and Diagnostic Tests for Neuromuscular Disorders . New York:Butterworth-Heinmann;2006.

10. Sewry CA. Muscular dystrophies: an update on pathology and diagnosis. Acta Neuropathol 2010;120(3):343-58.

11. Barresi R. From proteins to genes: immuno-analysis in the diagnosis of muscular dystrophies. Skelet Muscle 2011;1(1):24.

12. Carpenter S,Karpati G. Pathology of Skeletal Muscle. 2nd ed. New York; Oxford University Press;2001.

13. Almousa R, Charlton A, Rajesh ST, Sundar G, Amrith S. Optimizing muscle biopsy for the diagnosis of mitochondrial myopathy. Ophthal Plast Reconstr Surg 2009;25(5):366-70.

14. Sewry C, Dubowits V. Muscle Biopsy; A Practical Approach. 3rd St .Louis: Saunders; 2007.

15. Royden JH, De Vivo Daryl C, Darras Basil T. Neuromuscular Disorders of Infancy, Childhood and Adolescence. New York:Butterworth-Heinemann;2007.
16. Biopsy-General Instruction. Available from : http://www.medicine.uiowa.edu/path_handbook/Appendix/ex_muscle_biopsy.html.
17. Muscle Biopsy. Available from: http://neuromuscular.http://wustl.edu/lab/mbiopsy/html.
18. Engel AG, Franzini-Armstrong C. Myology. 2nd ed. New York:McGraw-Hill;1994.
19. Bornemann A, Anderson LV. Diagnostic protein expression in human muscle biopsies. Brain Pathol 2000;10(2):193-214.
20. Freund AA, Scola RH, Arndt RC, Lorenzoni PJ, Kay CK, Werneck LC. Duchenne and Becker muscular dystrophy: a molecular and immunohistochemical approach. Arq Neuropsiquiatr 2007;65(1):73-6.
21. Muntoni F. Is a muscle biopsy in Duchenne dystrophy really necessary? Neurology 2001 Aug 28;57(4):574-5.
22. Dubowitz V . Muscle Disorders in Childhood. 2nd ed. Philadelphia:WB Saunders;1995.
23. Keyhani E, Gharesouran J, Kahrizi K, Shafeghati Y, Najmabadi H, Banan M, *et al.* The Diagnostic Value of Utrophin in Mild forms of Dystrophinopathies. Iran Pathol 2010;5(2):65-71.
24. Taylor J, Muntoni F, Dubowitz V, Sewry CA. The abnormal expression of utrophin in Duchenne and Becker muscular dystrophy is age related. Neuropathol Appl Neurobiol 1997;23(5):399-405.
25. Arahata K, Ishihara T, Kamakura K, Tsukahara T, Ishiura S, Baba C, *et al.* Mosaic expression of dystrophin in symptomatic carriers of Duchenne's muscular dystrophy. N Engl J Med 1989;320(3):138-42.
26. Clerk A, Rodillo E, Heckmatt JZ, Dubowitz V, Strong PN, Sewry CA. Characterisation of dystrophin in carriers of Duchenne muscular dystrophy. J Neurol Sci 1991;102(2):197-205.
27. Sewry CA, Matsumura K, Campbell KP, Dubowitz V. Expression of dystrophin-associated glycoproteins and utrophin in carriers of Duchenne muscular dystrophy. Neuromuscul Disord 1994;4(5-6):401-9.
28. Mizuno Y, Nonaka I, Hirai S, Ozawa E. Reciprocal expression of dystrophin and utrophin in muscles of Duchenne muscular dystrophy patients, female DMD-carriers and control subjects. J Neurol Sci 1993;119(1):43-52.
29. Sewry CA. Immunocytochemical analysis of human muscular dystrophy. Microsc Res Tech 2000;48(3-4):142-54.
30. Anderson LV, Davison K. Multiplex Western blotting system for the analysis of muscular dystrophy proteins. Am J Pathol 1999;154(4):1017-22.
31. Sadjadi R, Rose MR. What determines quality of life in inclusion body myositis? J Neurol Neurosurg Psychiatry 2010;81(10):1164-6.
32. Tuffery-Giraud S, Saquet C, Chambert S, Echenne B, Marie CJ, Rivier F, *et al.* The role of muscle biopsy in analysis of the dystrophin gene in Duchenne muscular dystrophy: experience of a national referral centre. Neuromuscul Disord 2004;14(10):650-8.