A CLINICAL STUDY ON THE UTILITY OF MUSCLE BIOPSY IN PATIENTS WITH SUSPECTED MYOPATHY

THESIS

Submitted in partial fulfilment of the rules and regulations for the requirement of the degree of DM in Neurology

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DECLARATION

I, Dr Sudhakar K hereby declare that the thesis "A Clinical study on the utility of muscle biopsy in patients with suspected myopathy *"* was undertaken by me under the guidance and supervision of Dr Sanjeev .V. Thomas, Professor (Senior Grade) & Head, Department of Neurology at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram.

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CERTIFICATE

This is to certify that the thesis titled "A Clinical study on the utility of muscle biopsy in patients with suspected myopathy " is the bonafide work of Dr Sudhakar K, Senior Resident, DM Neurology and has been done under my direct guidance and supervision at the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram. He has shown keen interest in the research project and actively participated in all its phases.

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INTRODUCTION

INTRODUCTION

Myopathy is one of the common disorders in patients attending neuromuscular clinic. Systematic approach comprising a comprehensive clinical history, thorough neurological and systemic examination, nerve conduction studies, EMG and relevant biochemical tests should be undertaken in all cases. Muscle biopsy is one of the most frequently used diagnostic procedures in the evaluation of inherited and acquired myopathies. The yield of muscle biopsy result is dependent on number of factors, including appropriate selection of patients for biopsy, expertise of the laboratory, and techniques used in the analysis.

The history of muscle biopsy dates back to 1860 when Duchenne first performed a biopsy on a patient with symptoms of myopathy. Introduction of enzyme histochemical methods by Victor Dubowitz in 1970 revolutionised the role of muscle biopsy in the diagnosis of various primary and secondary muscle diseases(7). Diagnosis of various subtypes of dystrophies was further made easy with beginning of immunohistochemical methods in 1980s However, there are few modern reports documenting the diagnostic yield and clinical utility of open muscle biopsy.(1-5) Clinicians usually rely on personal experience to develop their own criteria for performing muscle biopsy. This may explain the variable diagnostic outcome of muscle biopsy found in previous cohorts which ranged from 13.2% to 59.9%.(8,9).

In most of the proximal myopathies and generalised/systemic diseases; vastus lateralis is the standard muscle biopsied by international consensus. (6) The other muscles that are good choices for biopsy are biceps and gastrocnemius. However, many forms of hereditary muscle disorders can now be diagnosed with molecular genetic testing, thereby eliminating the need for performing a muscle biopsy in every patient.

In this study, we evaluate the clinical utility of muscle biopsy in patients with suspected myopathy. The primary objective is to determine the diagnostic value of muscle biopsy, as measured by the probability of specific myopathy and depending on the available pre-procedure clinical and laboratory data.

AIMS AND OBJECTIVES

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PRIMARY OBJECTIVE

1. To study the utility of muscle biopsy in providing diagnostic, therapeutic or prognostic information that aid in clinical management of patients with suspected myopathies
2. To study the clinical and demographic profile of patients undergoing muscle biopsy

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Myopathy means muscle disease (Greek: myo- muscle + patheia -pathy: suffering). Myopathies are disorders affecting the channel, structure, or metabolism of skeletal muscle. Myopathies have distinctive clinical and laboratory features that can distinguish them from other disorders of the motor unit, including the neuromuscular junction, peripheral nerve, or motor neuron.

Once the lesion is localized to the muscle, the next step is to identify whether the myopathy is due to an abnormality in the muscle channel, an abnormality in the muscle structure, or a dysfunction in muscle metabolism.

Classification of myopathies (10)

Acquired

- Drug-induced myopathies
- Endocrine myopathies
- Inflammatory/immune myopathies
- Myopathies associated with other systemic illness
- Toxic myopathies

Hereditary

- Channelopathies
- Congenital myopathies
- Metabolic myopathies
- Mitochondrial myopathies
- Muscular dystrophies
- Myotonias

According to a study by Theadom et al (11) published in 2014 which was a systematic review of literature published in Neuroepidemiology journal a worldwide map of prevalence studies showed a deficiency of data from the Indian subcontinent.

As per this study the prevalence of muscular dystrophies as a group was found to be between 19.8 and 25.1 per 100,000 person years. Myotonic dystrophy (0.5–18.1 per 100,000), Duchenne muscular dystrophy (1.7–4.2 per 100,000) and facioscapulohumeral muscular dystrophy (3.2–4.6 per 100,000) were found to be the most common types of disorder.

Figure 1: Worldwide map showing the prevalence of muscular dystrophy(12)

Muscular dystrophy

Muscular dystrophies are inherited disorders caused by mutations in a number of genes. These genetic mutations cause either a dysfunction in, or lack of proteins that are essential for muscle cell stability, leading to progressive destruction and weakness in the muscles.(13) The term muscular dystrophy encompasses a range of disorders including Duchenne, Becker, congenital, myotonic, Emery-Dreifuss, facioscapulohumeral, oculopharyngeal, and limb-girdle muscular dystrophies. Each disorder varies in severity, age of onset, pattern of inheritance, and affected muscle groups and other organs.(14)

Diagnosis of muscular dystrophies requires a proper medical history, noting the distribution of weakness, age of onset, family history, and disease specific features. A physical examination needs to document the distribution of weakness (face, distal, or proximal or specific muscle groups), change in muscle bulk and the presence of contractures and other specific features such as myotonia. These findings together with investigations such as serum creatinine phosphokinase, electromyography, and muscle biopsy may direct testing toward a specific genetic diagnosis.

Dystrophinopathies

The Dystrophinopathies cover a spectrum of X-linked muscle disease ranging from mild to severe that includes Duchenne muscular dystrophy, Becker muscular dystrophy, and DMD-associated dilated cardiomyopathy (DCM). (15) The mild end of the spectrum includes the phenotypes of asymptomatic increase in serum concentration of creatine phosphokinase (CK) and muscle cramps with myoglobinuria. The severe end of the spectrum includes progressive muscle diseases that are classified as Duchenne/Becker muscular dystrophy when skeletal muscle is primarily affected and as *DMD*-associated dilated cardiomyopathy (DCM) when the heart is primarily affected.

Mapping data and molecular genetics study indicate that both forms are the result of mutation in gene that encodes for the protein dystrophin. Multiplex polymerase chain reaction (MPCR) is one of the cost-effective and highly specific techniques for the detection of large gene deletions. Recently, multiplex ligation-dependent probe amplification (MLPA) method is also used to detect deletion and duplication as well as distribution and extent of deletion and duplication in the DMD gene.(16)

Limb girdle muscular dystrophy

Limb-girdle muscular dystrophies have an estimated incidence of 1 to 6 out of 100,000. The limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of genetic disorders generally characterized by weakness of the shoulder and the pelvic girdle muscles. Based on the inheritance patterns, autosomal dominant (LGMD type 1), autosomal recessive (LGMD type 2), and X-linked forms of LGMD have been described. Even with multigene panel testing, a significant proportion of LGMD cases (up to 25% in some instances) may remain undiagnosed.(17) At present, more than 25 different genes causing LGMD have been

identified, and nomenclature of different LGMDs in an alphabetical manner (Table 1) has been done according to the chronology of identification of the genetic locus.

Table 1: LGMD types and common features

Type	Feature
LGMD1A (myotilinopathy)	Upper extremities distal weakness and contractures, dysarthria, palatal hypophonia, footdrop, and areflexia
LGMD1B (laminopathy)	Contractures and cardiac conduction defects
LGMD1C (caveolinopathy)	Rippling muscles and muscle hypertrophy
LGMD1E (desminopathy)	Facial weakness and cardiac conduction defect
LGMD1F (transportinopathy)	Early respiratory muscle involvement
LGMD1G (HNRPDL proteinopathy)	Finger and toe flexion limitation
LGMD2A (calpainopathy)	Contractures and atrophy of shoulder and pelvic girdle muscles
LGMD2B (dysferlinopathy)	Medial gastrocnemius atrophy
LGMD2C, LGMD2D, LGMD2F (sarcoglycanopathies)	Tongue hypertrophy
LGMD2H (sarcotubular myopathy, TRIM32 proteinopathy)	Scapular winging and calf hypertrophy
LGMD2I (fukutinopathy)	Tongue and calf hypertrophy, cardiac and respiratory muscle involvement
LGMD2L (anoctaminopathy)	Asymmetric atrophy of muscles, mandibular dysplasia
LGMD2R (desminopathy)	Facial weakness, respiratory muscle involvement, high-arched palate
LGMD2S (TRAPPC11 proteinopathy)	Scapular winging, hyperkinetic movements
LGMD2V (acid maltase deficiency)	Proximal weakness and respiratory insufficiency
LGMD2W (LIMS2 proteinopathy)	Calf and tongue hypertrophy and triangular tongue
Dystrophinopathy	Calf hypertrophy, distal contractures, preserved ankle reflex despite the weakness

In a paper published in 2015 from India dyferlinopathy was found to be the most common, followed by alpha-dystroglycanopathies. Sarcoglycanopathies were seen in 14% and calpainopathies were less common, at 10% of the cohort.(18)

In a study from North India, Pathak et al.(19) found calpainopathies to be the most common type, almost accounting for half of the studied samples. In the authors work from the West India, calpainopathies seem to form about one-third of the diagnosed LGMDs. GNE myopathy is common in South India and also in parts of Rajasthan, Gujarat, and Madhya Pradesh.

Myotonic dystrophy

Myotonic dystrophy type 1 is linked to chromosome 19q13.3, and myotonic dystrophy type 2 is linked to chromosome 3q. Both are repeat disorders. Myotonic dystrophy type 1 is due to expansions of more than 50 repeats of the CTG trinucleotide on the DMPK gene on chromosome 19q. Myotonic dystrophy type 2 is due to expansions, ranging from 75 to 11,000 repeats, of a CCTG tetranucleotide on the CNBP (previously gene ZNF9) gene on chromosome 3q. The general consensus is that larger repeats are associated with more severe phenotypes. This is clearly represented by the phenomenon of anticipation.

Table 2: Types of myotonic dystrophy

Clinical Features	Myotonic Dystrophy Type 1	Myotonic Dystrophy Type 2 (Proximal Myotonic Myopathy Phenotype)	Myotonic Dystrophy Type 2 (Proximal Myotonic Dystrophy Phenotype)
Genetics	Autosomal dominant	Autosomal dominant	Autosomal dominant
Age at onset	All ages	Third to fourth decades	Sixth to seventh decades
Main clinical findings	Facial weakness, ptosis, distal muscle atrophy, and weakness; absent tendon reflexes	Mild to moderate proximal lower limb muscle weakness with muscles of normal bulk and brisk tendon reflexes; mild neck flexor weakness	Severe proximal lower limb muscle atrophy and weakness; mild to moderate neck flexor weakness
Myotonia	Grip myotonia, percussion myotonia; warmup phenomenon; no fluctuation of symptoms	Mild grip myotonia, very variable, much fluctuation of symptoms	No clinical myotonia
Triggers	Cold exposure	Cold exposure, menses, but fluctuations occur independently	Unknown

Fascioscapulohumeral muscular dystrophy

FSHD is a slowly progressive dystrophic myopathy with predominant involvement of facial and shoulder girdle musculature. The condition has autosomal dominant inheritance with linkage to the chromosome 4q35 locus. It is the second most common inherited muscular dystrophy in the adult population, with a prevalence estimate of 1 to 5/100,000.(20)

Overall, FSHD is the third most common of the dystrophies, behind DMD and myotonic muscular dystrophy. Presentation ranges from congenital to late in life but typical age of presentation is generally before age 20. Initially, 85% of patients show predominant involvement of facial and shoulder girdle musculature with facial weakness commonly being the initial manifestation. Facial weakness typically involves the orbicularis oris, zygomaticus, and orbicularis oculi.

Oculopharyngeal muscular dystrophy

OPMD is a late-onset, dominantly inherited disorder of the muscle. It is associated with progressive eyelid ptosis, dysarthria, dysphagia and proximal weakness. The cardinal pathologic findings of OPMD are the presence of distinctive muscle fibres containing rimmed vacuoles under a light microscope (LM) and of unique myocyte nuclei containing tubulofilamentous inclusion under an electron microscope (EM).Treatment largely remains supportive as in other muscular dystrophies.

Emery–Dreifuss muscular dystrophy

Emery–Dreifuss muscular dystrophy (EDMD) is an uncommon muscular dystrophy with a characteristic phenotype, which consists of a triad of early contractures, slowly progressive muscle weakness which begins in humero-peroneal distribution and cardiac involvement in the form of conduction defects, arrhythmias and dilated cardiomyopathy. Limited data exists regarding prevalence of EDMD. The prevalence of XL-EDMD has been estimated to be 1:100,000.

Distal myopathies

Distal myopathy is characterized by progressive muscular weakness and atrophy beginning in the distal parts of upper and lower limbs with an extremely variable age of onset. Distal myopathy is a genetically heterogeneous group (currently comprising more than 20 genetic types) which affects different regions of the distal extremities and is classified according to clinical features, inheritance pattern, histopathological criteria, and molecular genetics.

The main autosomal dominant forms of distal myopathy are Welander distal myopathy (weakness in the distal upper extremities which later progresses to distal lower extremity), tibial muscular dystrophy (mainly affects the front of the lower leg), distal myotilinopathy (progressive distal muscle weakness and peripheral neuropathy with hyporeflexia), late-onset distal myopathy, Markesbery-Griggs type, Laing early-onset distal myopathy, and adult-onset distal myopathy due to VCP mutation.

The genetically confirmed autosomal recessive forms are distal myopathy, Nonaka type (GNE-myopathy, weakness in the anterior distal legs), Miyoshi myopathy (weakness in the distal lower extremity posterior compartment), nebulin-related early-onset distal myopathy and distal anoctaminopathy.

The age at onset is extremely variable and for recessive varieties of distal myopathy, symptoms usually develop in early adult life whereas in the dominant Welander and tibial muscular dystrophy, the onset is usually later. However, in some disorders (i.e. Laing early-onset distal myopathy and nebulin-related early-onset distal myopathy) the onset may be during childhood, and even in infancy.

Metabolic myopathy

Metabolic myopathies are genetic disorders that impair intermediary metabolism in skeletal muscle. Impairments in glycolysis/glycogenolysis (glycogen-storage disease), fatty acid transport and oxidation (fatty acid oxidation defects), and the mitochondrial respiratory chain (mitochondrial myopathies) represent the majority of known defects.

The metabolic myopathies may present in the neonate or during infancy as part of more systemic involvement with hypotonia, hypoglycemia, and encephalopathy; however, most cases present in childhood or adulthood with exercise intolerance (often with rhabdomyolysis) and weakness. The glycogen-storage diseases present during brief bouts of high-intensity exercise, whereas fatty acid oxidation defects and mitochondrial myopathies present during a long-duration/low-intensity endurance-type activity or during fasting or another metabolically stressful event (eg, surgery, fever).

The clinical examination is often normal between acute events, and evaluation involves exercise testing, blood testing (creatine kinase, acylcarnitine profile, lactate, amino acids), urine organic acids (ketones, dicarboxylic acids, 3-methylglutaconic acid), muscle biopsy (histology, ultrastructure, enzyme testing), MRI/spectroscopy, and targeted or untargeted genetic testing.

Mitochondrial myopathy

Mitochondrial myopathies are a diverse group of disorders characterized by morphological abnormalities of muscle mitochondria. Mitochondrial disorders are the most common form of inherited metabolic disorders with an incidence of 1 in 4000. Patients with this disorder have a wide spectrum of symptoms due to varied genotype penetrance and disease severity.(21) Patients may present with fibromyalgia, skeletal muscle weakness, ptosis, pain, fatigue, and exercise intolerance that progressively worsens over time.(22) In addition, patients may present with slowly progressive peripheral muscle weakness, multisystem organ failure, or respiratory insufficiency requiring mechanical ventilation. There are no specific therapeutic strategies for mitochondrial myopathies.

Certain histopathological features are specific indicators of mitochondrial dysfunction. For example, the presence of multiple ragged-red fibres with modified Gomori trichrome stain indicates abnormalities of mitochondrial function.

Electron microscopy may show abnormal mitochondria with increased size and abnormal cristae . Further, a histologically and ultra-structurally normal muscle biopsy does not exclude a mitochondrial disease since the biopsied muscle may not be involved in the disease process.

Inflammatory myopathies

The inflammatory myopathies are simply those disorders in which the primary pathological process is inflammation within muscle. Patients with inflammatory myopathy may present to, and be managed within, one of several specialties (for example, dermatology, rheumatology, neurology, general medicine).

Bohan and Peter Diagnostic Criteria for Inflammatory Myopathy

1. Symmetrical proximal muscle weakness with or without dysphagia and respiratory muscle weakness
2. Elevation of the serum enzymes, especially the CPK, but also the transaminases, the LDH and the aldolase
3. The electromyographic triad of
   a. small amplitude, short-duration, polyphasic motor unit potentials
   b. fibrillations, positive sharp waves, increased insertional irritability, or
   c. spontaneous bizarre high frequency discharges
4. Muscle biopsy abnormalities of degeneration, regeneration, necrosis, phagocytosis, and an interstitial mononuclear infiltrate
5. The typical skin rash of dematomyositis

CPK, creatine phosphokinase; LDH, lactic dehydrogenase.

Dermatomyositis (DM) is the most common form of classical inflammatory myopathy. Isolated polymyositis (PM) is rare, but a frequent misdiagnosis. But PM is relatively frequently associated with various manifestations of connective tissue disease, a situation for which many use the term “overlap syndrome”.

DM is a humorally mediated autoimmune disorder. Complement dependent attack leads to destruction of capillaries in muscle and other tissues. In muscle, the resulting microangiopathy leads to the characteristic pathological features of infarction and perifascicular atrophy.

PM is caused by a cell mediated immune phenomenon. Autoinvasive CD8+ T cells, recognising an unknown muscle antigen, invade non-necrotic muscle fibres expressing class 1 major histocompatibility complex antigen (MHC-1) and lead to their destruction.

In IBM, although there is some similarity with the immunocytological findings seen in PM, there is evidence that the fundamental pathological process is different and that at least some of the inflammatory and immune changes seen in IBM may be epiphenomena.

A consistent finding in all three disorders is expression of MHC-1 antigen (which is not constitutively expressed) on the surface of undamaged muscle fibres, and indeed this may be used as a pointer to the diagnosis of an idiopathic inflammatory myopathy even in the absence of inflammatory infiltrates.

Endocrine myopathy

Major categories of endocrine myopathy include those associated with

1. Adrenal dysfunction (as steroid myopathy);
2. Thyroid dysfunction (as in myxedema coma or thyrotoxic myopathy);
3. Parathyroid dysfunction (as in multiple endocrine neoplasia);
4. Pituitary dysfunction; and
5. Islands of langerhans dysfunction (as in diabetic myopathy from ischemic infarction of the femoral muscles).

Steroid myopathy is the most common endocrine myopathy. Endocrine myopathies have been underreported in neurological practice.

There are only few studies on endocrine myopathy from India. As per a study published by Sharma et al (23) Out of the 37 patients who were diagnosed with endocrine myopathies, thyroid dysfunction was the most common cause (17 cases), followed by vitamin D deficiency in nine, adrenal dysfunction in six, parathyroid dysfunction in three, and pituitary dysfunction in two

Congenital myopathy

Congenital myopathies are rare. In a large series of 10,332 muscle biopsies, in 597 (5.8%) biopsies the diagnosis was congenital myopathy. The true incidence of congenital myopathy is difficult to estimate. Nonaka, (24) estimated the incidence of congenital myopathy at 6/100,000 live births or one-tenth of all neuromuscular disorders.

The congenital myopathies are a group of genetic muscle disorders characterised clinically by hypotonia and weakness, usually from birth, and a static or slowly progressive clinical course. Hypotonia is the clinical hallmark of most of the CMs and present in early life with hypotonia, hyporeflexia and generalized weakness that is more often proximal. Dysmorphic facies, external opthalmoplegia, contractures are other features. Scoliosis and contractures are seen in childhood onset form, whereas muscle weakness is the usual presentation during adult hood.

Historically the congenital myopathies have been classified on the basis of the major morphological features seen on muscle biopsy – e.g., rods (nemaline myopathy), cores (central

core disease and multiminicore disease), central nuclei (centronuclear/myotubular myopathy) and selective hypotrophy of type1 fibres (congenital fibre type disproportion).

There can be significant clinical overlap between congenital myopathies and other neuromuscular disorders including the congenital muscular dystrophies (CMD),congenital myotonic dystrophy, congenital myasthenic syndromes (CMS), metabolic myopathies including Pompe disease, spinal muscular atrophy (SMA), as well as Prader–Willi syndrome, which can all present in the new born period with marked weakness and/or hypotonia ('floppy infant').(25)

In India, there are limited reports on CMs, mostly case reports and very few large series published by Jain D et al (26) in 2008,Thaha et al (27) in 2011 and the largest series of 50 cases of biopsy confirmed CMs, seen over a period of 12 years in a tertiary care University Teaching Hospital at Hyderabad.(28)

Investigations other than muscle biopsy are rarely specific for congenital myopathies, but are widely used to exclude other possible diagnoses. Serum creatine kinase is usually normal or mildly elevated and if raised more than five times normal should prompt consideration of a muscular dystrophy. Genetic testing for many congenital myopathies is relatively new. Therefore, many tests may result in variants of uncertain significance.

Approach to muscle disorders

Table 3: Diagnostic clues based on pattern of involvement

S.no.	Pattern of involvement	Diagnosis
1.	Limb girdle	Most myopathies –hereditary and acquired
2.	Distal	Distal myopathies
3.	Proximal arm / distal leg "scapuloperoneal"	FSH, Emery-Dreifuss, acid maltase deficiency, congenital scapuloperoneal

4.	Distal arm / proximal leg	IBM Myotonic dystrophy
5.	Ptosis / Ophthalmoplegia	Oculopharyngeal muscular dystrophy, myotonic dystrophy, mitochondrial myopathy
6.	Neck – extensor	Isolated neck extensor myopathy
7.	Bulbar (tongue, pharyngeal, diaphragm)	Oculopharyngeal muscular dystrophy
8.	Episodic weakness/ Pain/rhabdo + trigger	McArdle's, drugs, toxins
9.	Episodic weakness Delayed or unrelated to exercise	Primary periodic paralysis Channelopathies: Na+ Ca++ Secondary periodic paralysis
10.	Stiffness/ Inability to relax	Myotonic dystrophy, channelopathies

Creatine kinase

Most laboratories use the central 95% of observations in people as a reference range for serum CK, assuming that levels have a gaussian (bell-shaped) distribution, which is usually about 0 to 200 IU/L. Using these parameters, an abnormal CK level was observed in 19% of men and 5% of women in a study of nearly 1,000 healthy young people,(29) leading to overdiagnosis. The European Federation of Neurological Societies suggests redefining elevated CK as values 1.5 times beyond the upper limit of normal.

Role of electrodiagnostic studies in the diagnosis of myopathies

Exclude neuromuscular conditions that may mimic a myopathy

- Motor neuron disease
- Motor neuropathies
- Neuromuscular junction disorders

Provide EMG evidence of the presence of a myopathy (although EMG may be normal in the presence of selected myopathic processes).
Characterize the myopathy

- Location (proximal, distal, symmetric, or asymmetric)
- Presence/absence of abnormal spontaneous activity
- Severity

Identify target muscles for biopsy

Electrodiagnostic (EDX) studies are an extension of the physical examination and may help establish the diagnosis of myopathy. EDX studies, however, are not always needed to diagnose a myopathy. This is particularly true in the paediatric and, occasionally, the adult population. Often patients with inherited myopathies present with characteristic phenotypes, and, possibly, a positive family history. In these cases, it is reasonable to proceed directly to genetic testing. In addition, at times, the diagnosis ultimately requires a muscle biopsy, regardless of the EDX study results.

Therefore, if clinical suspicion for a myopathy is high, generally corroborated by elevated creatine kinase (CK) levels, it is often reasonable to skip or limit the extent of the EDX studies. Finally, EDX studies may be normal in selected muscle diseases (certain endocrine, metabolic, congenital, and mitochondrial myopathies). Thus, in the appropriate clinical context, normal EDX studies do not necessarily rule out the presence of a myopathy.

EMG

The analysis of spontaneous activity is helpful in narrowing down the differential diagnosis. Muscle membrane irritability, in the form of increased insertional activity, fibrillation potentials, and positive sharp waves (PSWs), is characteristic of certain myopathies (inflammatory and toxic/necrotic processes, muscular dystrophies, and selected congenital and metabolic disorders) but not others. Occasionally, in chronic myopathies, complex repetitive discharges (CRDs) may be seen. Myotonic discharges, similarly to fibrillations, PSWs and CRDs, are generated at the muscle fiber level. Although their morphology is similar to fibrillations and PSWs, they characteristically wax and wane in both frequency and amplitude. They are typically seen in myotonic disorders, such as DM1 and DM2, myotonia congenita.

The normal insertional activity generated by needle insertion is decreased in chronic end-stage myopathies.

Analysis of motor unit action potential (MUAP) morphology and recruitment pattern is the key element of needle EMG that helps establish the diagnosis of a myopathy. In myopathic processes, there is dropout or dysfunction of individual muscle fibers. Thus, the size of the motor unit decreases. This results in the emergence of short, small, polyphasic MUAPs. In myopathies, MUAP duration and amplitude both decrease, whereas the number of phases increases. Acoustically, this corresponds to a crisp, high-pitch sound. In a myopathy, these short, small, polyphasic, high-pitch MUAPs display a characteristic early recruitment pattern. The only exception to the pattern of short, small, polyphasic MUAPs with early recruitment is cases of end-stage muscle that may occasionally be seen in severe chronic myopathies. If all the muscle fibers of an individual motor unit are lost, there is a reduction in the number of available motor units resulting in reduced recruitment. Some reinnervation and motor unit remodelling may also occur overtime and a mixed population of short and long duration MUAPs may occasionally be seen in severe chronic myopathies.

Muscle biopsy

Muscle biopsy is one of the most frequently used diagnostic procedures in the evaluation of inherited and acquired myopathies. Alongside the clinical examination, electrodiagnostic, laboratory and molecular genetic testing, muscle biopsy has a critical role, providing diagnostic evidence that either establishes a disease etiology or focuses the differential diagnosis.

General Indications of muscle biopsy

- Presence of evidence of **muscle disease** characterised by muscle symptoms, elevated creatine kinase (CK), myopathic EMG
- Presence of a **systemic disorder like vasculitis, sarcoidosis** that may have silent manifestations in muscle

Selection Of Muscle for Biopsy

While performing muscle biopsy the following factors should be looked upon,

- Chronic disease: Muscle with moderate, but not severe, weakness
- Acute disease: Muscle with severe or moderate weakness
- Ideal power – MRC – 4/ 5

The muscles where biopsy is usually performed are biceps, deltoid, triceps in upper extremity and quadriceps in lower extremity. To minimize artefacts sites of EMG, injections, trauma should be avoided as they may exhibit local destruction of fibers and inflammation. Muscle with heavy workload like gastrocnemius and muscle which are usually involved in disease processes like radiculopathy should be avoided.

Technique

Muscle biopsy may be done by open biopsy method or needle biopsy. Open biopsy method is the standard procedure. Ideal measure of biopsy specimen in open biopsy method is ~0.5 X 0.5 cm in cross-section and 1 cm in length along the longitudinal axis of the muscle fibers. The advantages include getting adequate sample and useful in patchy conditions. However the disadvantages are that they are time consuming and results in scarring. Needle biopsy methods are minimally traumatic and less time consuming. Deeper muscle could be obtained by needle biopsy technique with the help of MRI.

Staining

Staining is a rational and step wise process. The staining techniques used in muscle biopsy are,

A. Routine stains - H&E (Fig 2)
B. Histochemical stains – NADH, TR, SDH, MGT, PAS, Lipid stains/Acid phosphatase/Enzymes
C. Immunohistochemistry - Dystrophin, Sarcoglycans, Dysferlin

Table 4:Routine stains used for muscle biopsy analysis

Class of stain	Stain	Use
Morphology	Hematoxylin and eosin (H&E)	General morphology including fiber size, split fibers, location of nucleii, regenerating and degenerating fibers, connective tissue, inflammatory cells, inclusions and storage material,
	Modified Gomori Trichrome	Mitochondrial abnormalities, inclusion bodies, nemalin rods, and connective tissue
	Verhoeff van Gieson (VvG)	Connective tissue and elastin in vessels
Fiber Type Enzymes	Adenosine triphosphatase (ATPase) pH 9.4 – pale type I/dark type II pH 4.6 - Sub-typing type II pH 4.3 - pale type II/dark type I	Performed at different pH's to visualize different fiber types. Shows fiber type grouping and fiber type predominance.
Oxidative Enzymes	Nicotinamide adenine dehydrogenase (NADH)	Intracellular structures and myofibrillar organization
	Succinate dehydrogenase (SDH)	Mitochondrial pathology
	Cytochrome oxidase (COX)	Mitochondrial pathology
Hydrolytic Enzymes	Esterase	Denervated fibers, lysosomes, macrophages
	Acid phosphatase	Lysosomes, macrophages, vacuoles
	Alkaline phosphatase	Increased perimyseal staining in inflammatory myopathies
Storage material	Periodic Acid Schiff (PAS)	Glycogen, presence of ring fibers
	Oil red O or Sudan Black	Lipids

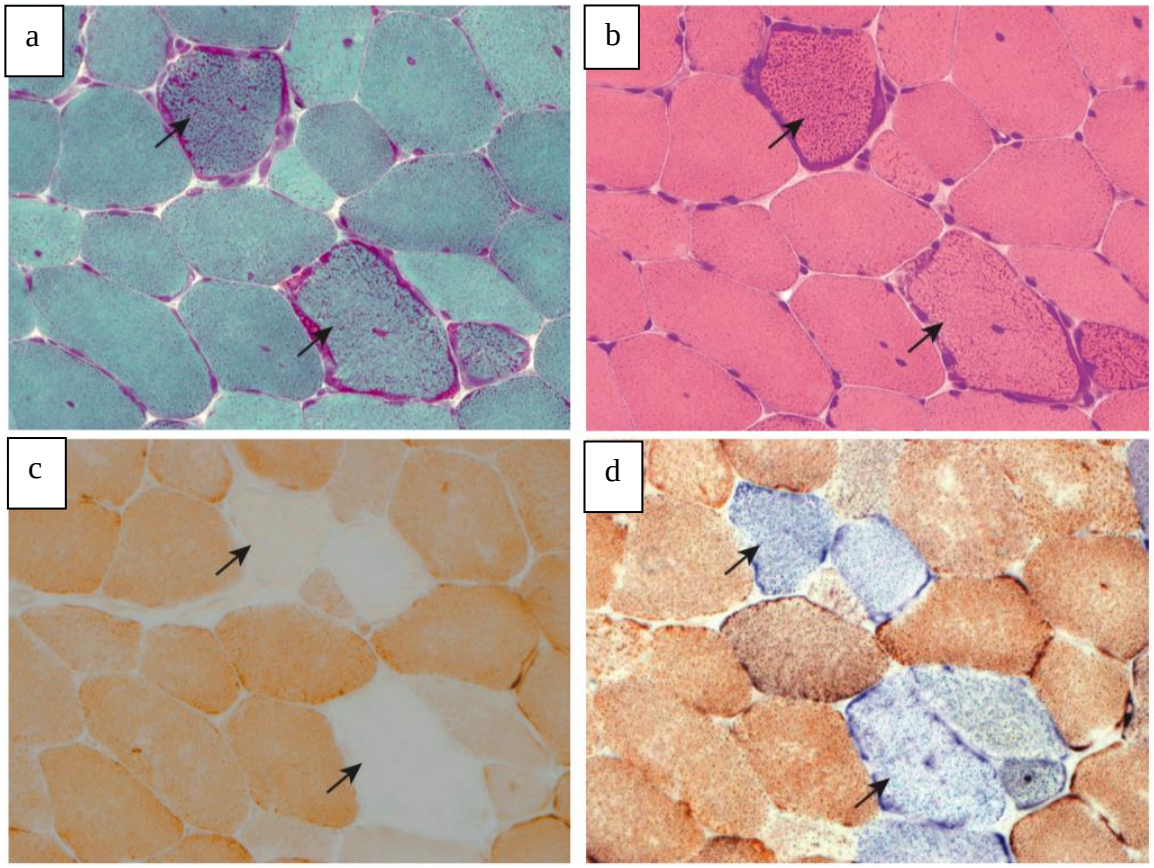

Figure 2: Biopsy from the quadriceps femoris muscle in a patient with Kearns–Sayre syndrome due to a large-scale mtDNA deletion. Serial sections demonstrate the appearance of COX deficient fibres, some of which are ragged-red fibres (arrows). (a)Gomori trichrome; (b)H&E; (c)COX; (d)COX/SDH double staining

Immunohistochemistry

Immunohistochemistry is the science of antigen localization and visualization in tissues by specific antibodies. It is an indirect technique in which a secondary antibody directed against the primary antibody is conjugated to a visual marker/enzyme/metallic complex. The method can be applied at light or electron microscope. Few commonly studied protein groups (Table 5, Fig 3) in immunohistochemistry include,

Table 5: Commonly studied protein groups in immunohistochemistry

Extracellular protein	- Laminin - Collagen VI - Perlecan
Sarcolemma-related protein	- Dystrophins - Sarcoglycans - Dystroglycan - Dysferlin - Caveolin-3
Cytoplasmic protein	- Actin - Desmin - Calpain-3 - Titin - Myosin
Nuclear protein	- Emerin

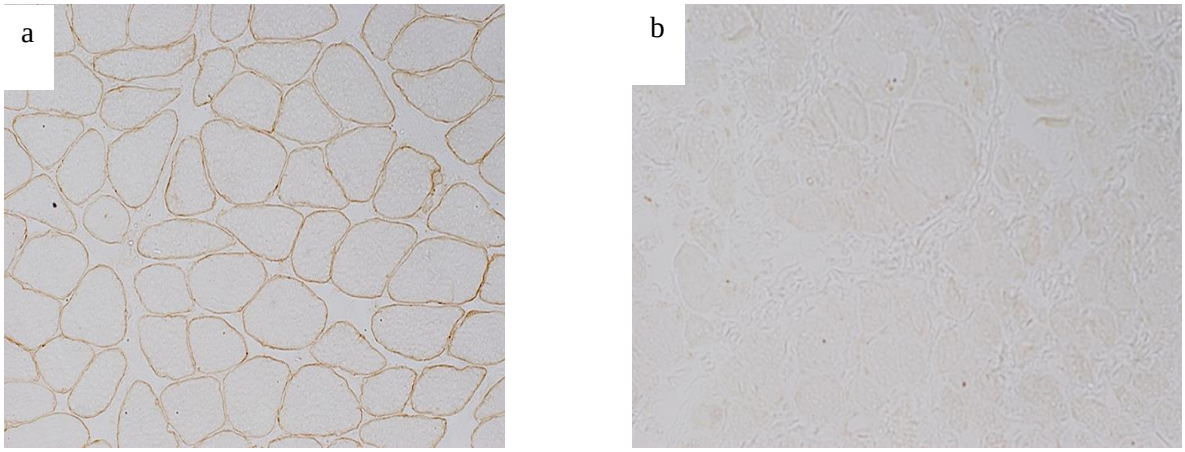

Figure 3: Immunohistochemistry (a) Dystrophin IHC: normal, (b) Dystrophin IHC: DMD

Pathologic findings

Myopathic changes, if early in the disease may cause focal myofiber damage as in mitochodrial disorders, segmental damage as may occur in the dystrophies, or multifocal damage as occurs in the inflammatory myopathies.(30) Common myopathic features include fiber size variation with both atrophied and hypertrophied muscle fibers. The atrophied fibers are often rounded, as opposed to the sharply angulated atrophic fibers observed in neurogenic atrophy. The hypertrophied fibers, as they enlarge, may eventually divide into two fibers and are referred to as split fibers. Degenerating and regenerating fibers are scattered throughout myopathic muscle.

In polymyositis muscle biopsy shows endomysial mononuclear cells and myonecrosis. Polymyositis is a cell-mediated autoimmune disorder in which cytotoxic (CD8-positive) lymphocytes and macrophages invade and destroy myofibers expressing MHC-I antigens. The inflammatory cells are in the endomysium (between and around individual myofibers.) Inflammation may be focal and the MRI is useful in identifying affected areas for biopsy.

The pathology of dermatomyositis includes inflammation, vasculitis, and perifascicular atrophy. The inflammatory cells are predominantly B-cells. The key pathological change of dermatomyositis is a vasculitis, which involves endomysial and perimysial capillaries and arterioles.

The pathology of inclusion body myositis combines inflammation and myofiber degeneration. The inflammation is similar to polymyositis with cytotoxic (CD8-positive) lymphocytes invading and destroying myofibers. The degenerative changes consist of accumulation of myelinoid bodies and amyloid deposition.

Chronic myopathy may be identified by the increase of internalized nuclei which are common in many myopathies. An extreme example of this phenomenon is observed in centronuclear myopathy, where 20-100% of fibers demonstrate this abnormality. Internal nuclei may also be significantly increased in myotonic muscular dystrophy and has been previously reported to affect approximately15% of fibers. Endomysial and perimysial thickening occurs with chronic progression of most myopathic diseases.

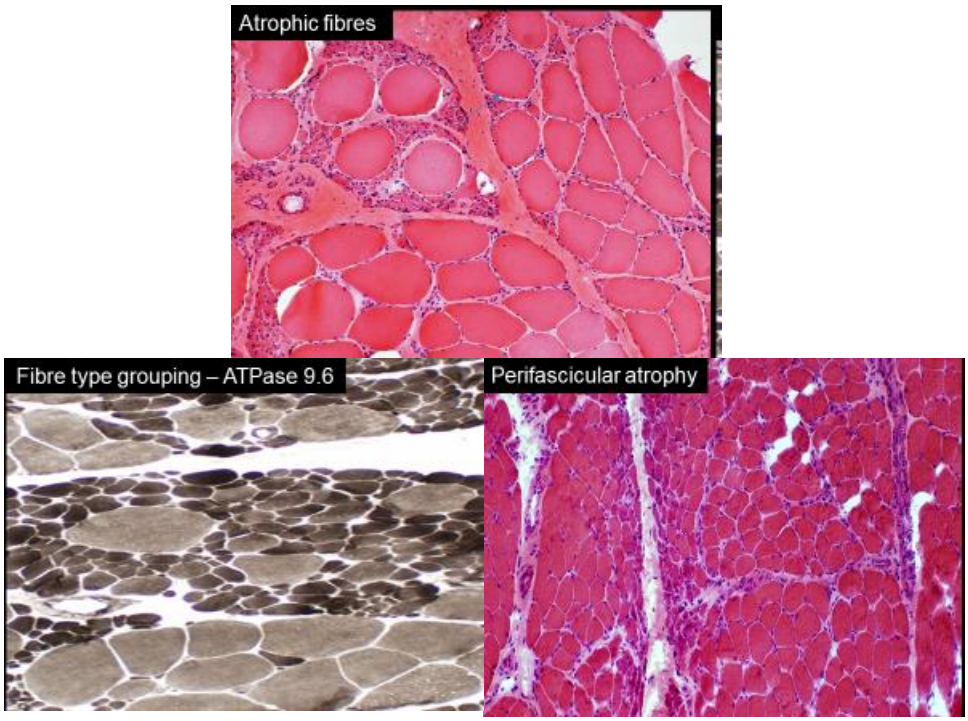

Figure 4: Pathologic findings

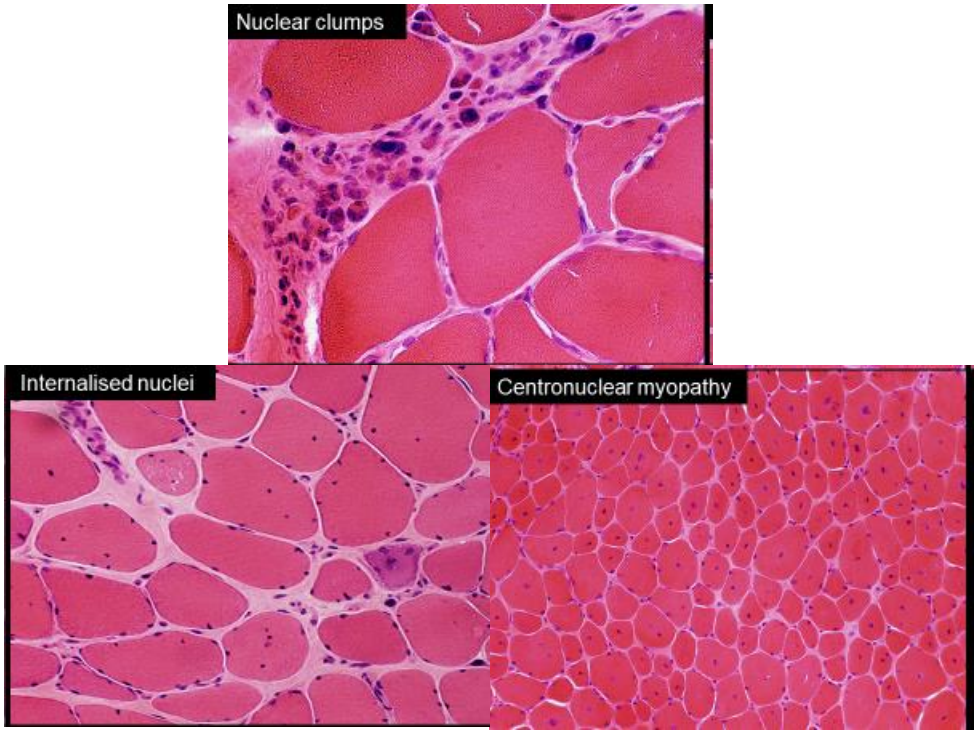

Figure 5: Pathologic findings

While most diseases of muscle have some or all of the above findings, a few will appear normal on muscle biopsy with only minimal hints of disease. This may be because the disease is patchy and the tissue sampled during biopsy missed pathologic muscle or the disease does not typically cause structural abnormalities. Examples – metabolic. Others myopathies will have histologic abnormalities considered strongly indicative for a certain disease such as; ragged red fibers, which are common in mitochondrial disease central cores, found in central core disease; and rimmed vacuoles, found in inclusion body myositis and some distal myopathies. However, each of these histopathologic findings may be seen in other myopathies, adding to the complexity of reading a muscle biopsy and reaching a conclusion.

Immunohistochemical staining, biochemical testing and electron microscopy can add to the diagnostic yield of routine muscle histology staining when indicated; however, the tissue samples must be handled and processed appropriately for each specialty technique to ensure optimal results.

Muscle biopsy : Current scenario

There are few modern reports documenting the diagnostic yield and clinical utility of open muscle biopsy. A review of paediatric patients undergoing muscle biopsy in 1999 found that a specific diagnosis was provided in 36% of cases.(31) A report from Singapore in 1986 about adult and paediatric patients noted a diagnostic yield of 69%. (32) Open muscle biopsy is an invasive and time consuming procedure. The dawn of molecular genetics in the 1990s and, more recently, the increasing application of next-generation massive parallel-sequencing technologies have resulted in a fundamental change in how these disorders are defined, identified, diagnosed, and managed. These modern molecular genetic tests have improved the diagnostic accuracy in many condition. Therefore, the diagnostic yield and clinical utility of muscle biopsy deserves investigation.

METHODOLOGY

METHODOLOGY

Case selection

The study is a retrospective and prospective hospital based observational study. The subjects will be recruited on admission into Neuromedical ward in Sree Chitra Thirunal Institute for Medical Sciences and Technology. The retrospective arm of the study will include patients fulfilling the eligibility criteria from April 2017 to the date of IEC approval and the prospective arm will include patients consenting for the study from the date of IEC approval to April 2019.

Inclusion criteria

1. Patients with suspected myopathies who underwent muscle biopsy

Exclusion criteria

1. Patient or parents (in minors) not consenting for the study
2. Biopsy sample inadequate or improper for review and comment by pathologist

Clinical examination and follow up

In the retrospective arm of the study, Patients with suspected myopathies who underwent muscle biopsy during the study period will be identified from the medical records. The demographic data, clinical features at admission, electrophysiological data, and blood investigations including serum CPK, will be extracted from the patient records using a structured proforma. The patients in the prospective arm will be identified and followed by the Principal Investigator by regular survey of the Neurology ward . This is strictly an observational study and the investigator will not influence investigation or treatment decisions.

A complete neurological examination (cranial nerve examination, muscle power charting, reflexes, and sensory examination at admission) is available routinely for all patients at admission. Complete blood count, renal function tests (blood urea and serum creatinine), serum electrolytes (sodium and potassium) and liver function tests and serum CPK are also routinely

done at admission which will be collected. Electrophysiological data (NCV/EMG) will also be collected.

Pre- biopsy clinical diagnoses will be categorized into 6 groups:

i. Inflammatory myopathies
ii. Muscular dystrophies
iii. Congenital myopathies
iv. Mitochondrial myopathies
v. Metabolic or toxic myopathies
vi. Indeterminate or non-specific

Pre-biopsy treatments will be classified into three types:

(i) no treatment
(ii) immune-modulating therapies
(iii) Other therapies – Includes Vitamin E supplementation , Carnitine supplementation , mitochondrial cocktail therapy

The muscle biopsy reports of all the patients will be reviewed by the pathologist and the principal investigator and will be categorized into any of these 7 groups:

i. Inflammatory myopathies
ii. Muscular dystrophies
iii. Congenital myopathies
iv. Mitochondrial myopathies
v. Metabolic or toxic myopathies
vi. Indeterminate or non-specific
vii. Normal findings

Inflammatory myopathies include polymyositis, dermatomyositis, and inclusion body myositis. Dystrophic myopathies if dystrophic changes predominated the pathological picture regardless whether a specific type was determined by further studies. Mitochondrial myopathies if abnormal numbers of ragged red or blue fibres were noted under light or electron microscope.

Nonspecific myopathies were indicated by the presence of myopathic changes (variation of fibre shape and size, rounded fibres, split fibres, moth-eaten fibres, basophilic fibres, increased internal nuclei, necrotic fibres, and phagocytic fibres) with insufficient evidence to classify them as a specific myopathy.

Post-biopsy treatments were classified into three types:

(i) no treatment
(ii) immune-modulating therapies
(iii) Other therapies – Includes Vitamin E supplementation , Carnitine supplementation , mitochondrial cocktail therapy

Muscle biopsy is defined as clinically useful if the biopsy changed the diagnosis, changed treatment, or a specific diagnosis was found.

Utility of muscle biopsy

The utility of muscle biopsy was assessed based on a scoring system. If the biopsy results were indeterminate or non-specific score 0 was given. If the biopsy has helped to reach at a diagnostic group or a specific diagnosis ,score 1 was given. Along with it if the biopsy has changed the pre biopsy diagnosis, score 2 was given. If the biopsy has helped to reach at a specific diagnosis/diagnostic group and has changed the prebiopsy diagnosis and also has changed the pre biopsy treatment, a score of 3 is given.

Ethical considerations

The study will be initiated only after approval from the Institutional Ethics Committee. A written informed consent will be obtained from all patients or parents (in case of minors) at the time of entry into the study.

Statistical analysis

The demographic details and outcomes of the study population was entered in Microsoft Excel sheet, and descriptive analysis will be done. Quantitative data was expressed in mean and standard deviation. Qualitative data were expressed in proportions. Change in prebiopsy and

post biopsy results were analysed using McNemar test. A p value < 0.05 was considered as statistically significant. Data analysis was performed using SPSS version 16.0.

RESULTS

RESULTS

In the period from April 2017 to April 2019, 70 patients who underwent muscle biopsy were included for analysis.

Demographic details

Among the 70 patients included in the study 39 (55.7%) were males and 31 (44.3%) were females (Figure 6). Mean age of the study population was 23.4 (+-SD) years. The youngest patient was 4 years and the oldest was 65 years old. The age distribution of patients is shown in Figure 7.

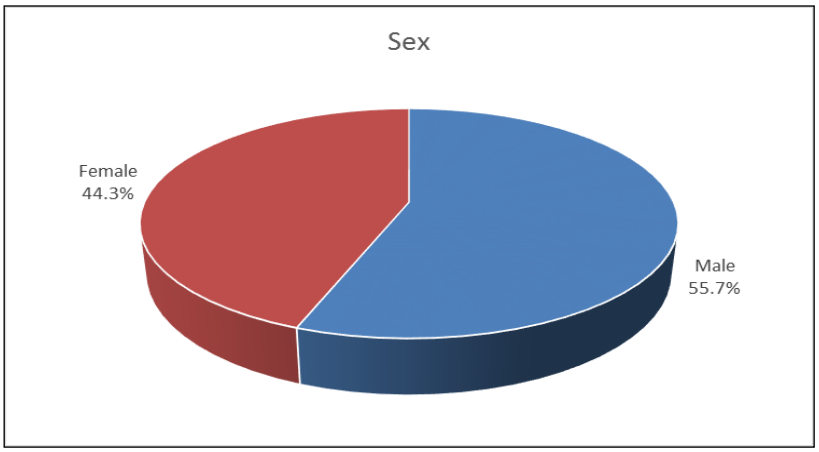

Figure 6: Gender distribution of the patients

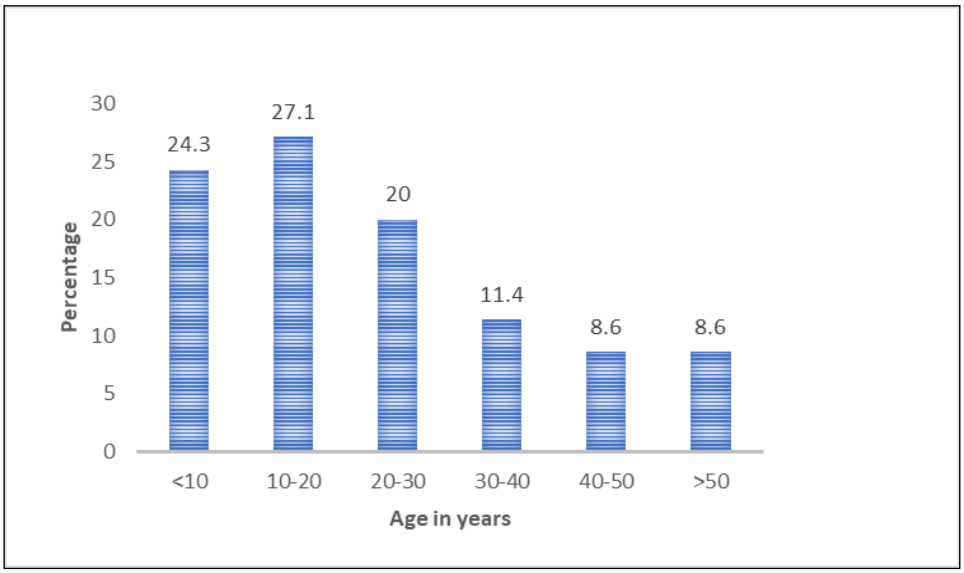

Figure 7:Age distribution of the patients

Majority of patients were in the age group 10-20 years. Pediatric population defined as those aged below 18 years constituted 47.14% (33/70) of the study population (Figure 8). In this group 24 were males and 9 were females (Figure 9). Mean age in pediatric population was 9.83 (±4.1) years.

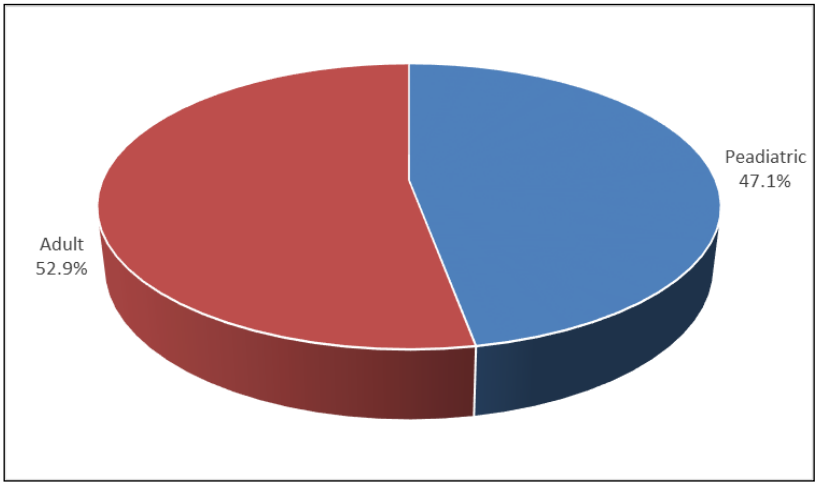

Figure 8: Proportion of pediatric population in total study group

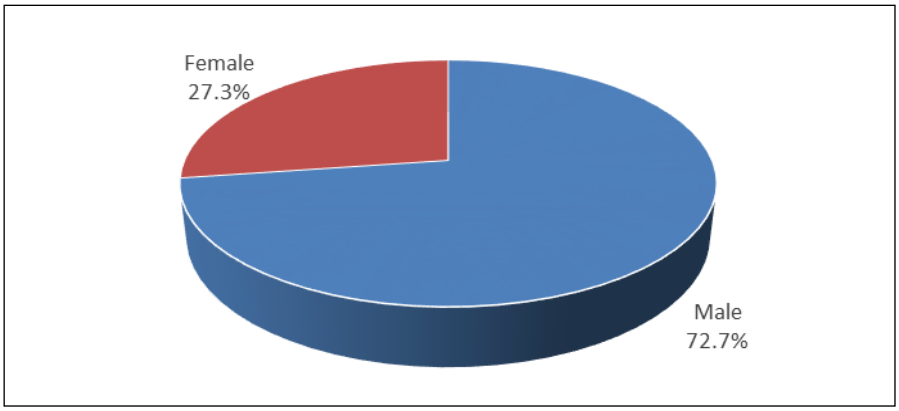

Figure 9: Gender distribution among pediatric population

Clinical profile of the patients

At the time of biopsy, 47 patients (67.1%) had proximo - distal weakness, 21 (30%) patients had predominant proximal muscle weakness either upper limbs or lower limbs or both and 2 (2.8%) had only distal weakness. Gait difficulty was reported by 27 (38.5%) of patients among whom 25 had waddling gait.

Neck weakness was noted in 45 (64.28%) of patients. Weakness of cranial musculature was seen in 16 (22.8%). Bifacial weakness was the most common noted in 10 (14.28%) of patients

followed by external ophthalmoplegia in 3, and palatal and tongue weakness in one patient each. Six (8.5%) patients had features suggestive of respiratory muscle weakness. Family history was positive in 14 (20%) cases. Consanguinity was noted in 20 (28.6%) of cases.

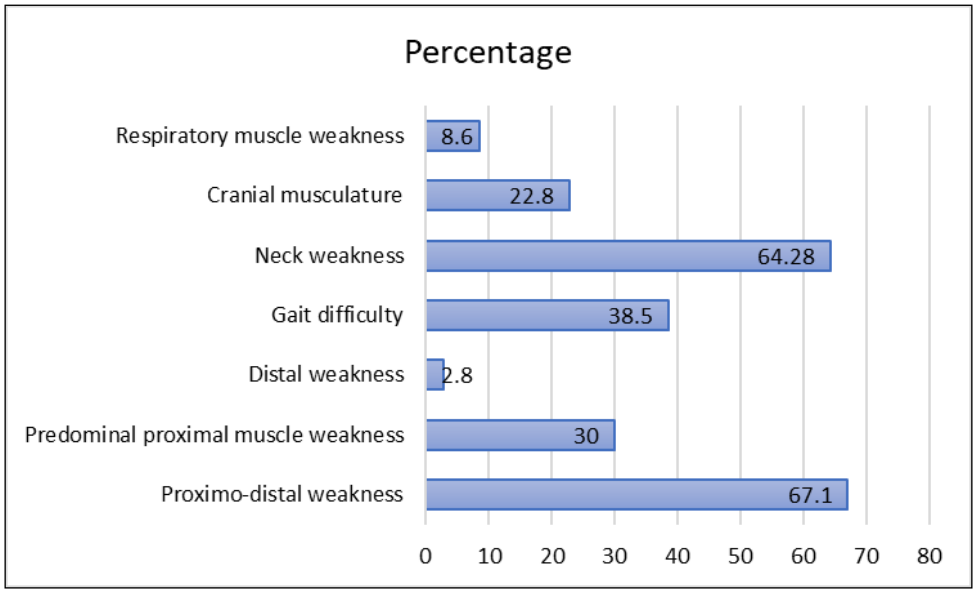

Figure 10: Frequency of clinical presentation

In the pediatric population, developmental delay was noted in 25 (75.8%) patients, of which 36% (9/25) had global developmental delay, 60% (15/25) had isolated motor delay, and 4% (1/25) had regression of milestones.

Examination

Examination findings are summarized in Table 6. The most common finding was proximal muscle weakness in lower limbs (81.5%) followed by proximal muscle weakness in upper limbs (72.8%). Majority had symmetrical weakness of limbs with only 4/70 patients (5.7%) showing asymmetric weakness.

Table 6: Frequency of various examination findings noted

S.no	Examination findings	Frequency n=70
1.	Motor weakness in lower limbs	Proximal: 57(81.5%) Distal: 34(48.6%)
2.	Motor weakness in upper limbs	Proximal:51(72.8%) Distal: 29(41.4%)
3.	Neck weakness	45(64.2%)
4.	Trunk weakness	Beevors sign positive 34(48.6%) Beevors sign negative 36(51.4%)
5.	Craniobulbar symptoms	14(20%)
6.	Ptosis	4(5.71%)
7.	EOM Weakness	3(4.28%)
8.	Facial paresis	10 (14.28%)
9.	Bulbar weakness	1(1.43%)
10.	Bulk	Atrophy 18(25.7%) Hypertrophy 22 (31.4%) Calf muscle)
11.	Pattern of weakness	Symmetric 66 (94.3%) Asymmetric 4 (5.7%)
12.	Other signs	Gowers sign Valley sign Myotonia Polyhill sign : 1
13.	Deformities	Contractures : TA 7 (10%) Lordosis : 11(15.7%)
14.	mRS at presentation	1- 8 (11.4%) 2- 38 (54.3%) 3- 14(20.0%) 4- 10(14.3%)

Electrophysiological study

68/70 patients underwent electromyography and nerve conduction studies, Two pediatric patients did not undergo EMG study. Forty nine patients 49/68(72%) showed myopathic process among which 14 had irritable myopathic process. Two patients showed neurogenic process. Electrophysiological study was found to be normal in 18 (26.5%) patients. Myotonia was elicited in 1 patient.

Table 7: Frequency of various electrophysiological findings noted

	Number (n =68)	**Percentage**
Myopathic process	49	72%
Irritable	14	20.6%
Neurogenic	2	2.9%
Normal	18	26.5%
Myotonia	1	1.5%

In the electrophysiological study of pediatric population, 25/31 patients (80.6%) showed myopathic process, one patient showed neurogenic process. Electrophysiological study was normal in 5/31 patients (16.1%).

Laboratory investigations

In our laboratory the normal range of creatinine kinase was taken as 0 to 200. Creatinine kinase (CK) was elevated in 49 (70%) patients and was within normal range in 21(30%) patients (Table 8).

Table 8: Frequency of CPK values noted

Creatinine kinase levels	**Frequency**	**Percent**
<= 200	21	30%
200-1000	18	25.7%
1000-5000	17	24.3%
>5000	14	20%
Total	70	100%

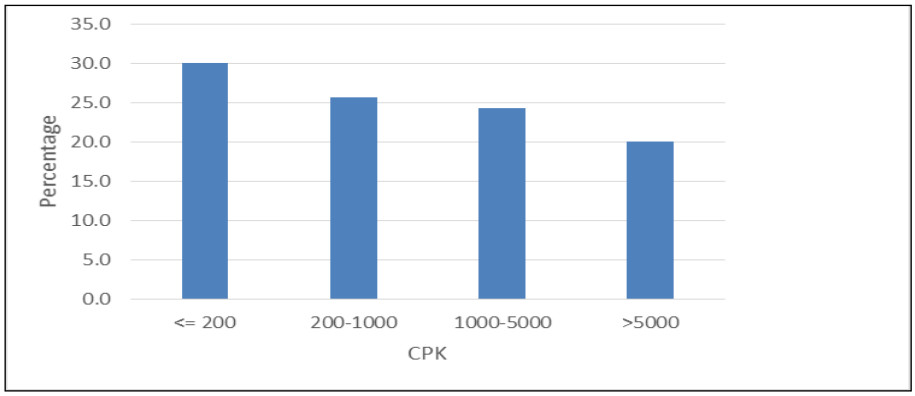

Figure 11: Frequency of CPK values noted

Echocardiogram was normal in 63/70 (90.0%) patients and had abnormal findings in 2 patients. One patient had dilated cardiomyopathy and another had mitral regurgitation. Hypothyroidism was detected in 3 patients.

Prebiopsy diagnosis and treatment

Prebiopsy diagnosis was made based on clinical profile, electrophysiological study and lab parameters. Patients were grouped under following diagnostic groups,

1. Inflammatory myopathies
2. Muscular dystrophies
3. Congenital myopathies
4. Mitochondrial myopathies
5. Metabolic or toxic myopathies
6. Indeterminate or non-specific

Nine (12.8%) patients had a pre-biopsy diagnosis of inflammatory myopathies, 30 (42.8%) muscular dystrophies, 3 (4.3%) congenital myopathies, 11(15.7%) mitochondrial myopathies, and 2 (2.8%) metabolic or toxic myopathies. Fifteen (21.5%) patients were categorised under indeterminate or non-specific group.

In the paediatric population 2/33 (6%) were diagnosed to have Inflammatory myopathies 17/33(51.5%) were diagnosed to have muscular dystrophies 2/33 (6.1%) were diagnosed to have congenital myopathies 7/33 (21.2%) were diagnosed to have mitochondrial myopathies

1/33 (3.03%) were diagnosed to have metabolic or toxic myopathies, 4/33 (12.12%) were categorised under indeterminate or non-specific.

No treatment was given in 23 (32.9%) patients. Among the 47 patients who received treatment, 13 (27.7%) were administered immunosuppressive therapy; steroid in 12 and intravenous immunoglobulin in one. The rest of the 34 patients had non-specific therapies in the form of vitamin E supplementation, mitochondrial cocktail or carnitine supplementation.

Post-biopsy diagnosis and treatment

The muscle biopsy reports of all the patients were reviewed by the pathologist and the principal investigator and was categorized into any of these 6 groups.

1. Inflammatory myopathies
2. Muscular dystrophies
3. Congenital myopathies
4. Mitochondrial myopathies
5. Metabolic or toxic myopathies
6. Indeterminate or non-specific

In this study of 70 patients, muscle biopsy was found to be useful in 45 (64.3%) of patients. 42 (60%) patients could be classified under a specific diagnostic group of muscle disorders. 7 (10%) were diagnosed to have inflammatory myopathies, 27(38.6%) muscular dystrophies, 7 (10%) congenital myopathies, and 2 mitochondrial myopathies. Biopsy results were indeterminate or non-specific in 25 (35.7%) patients (Table 9). Two patients were diagnosed to have spinal muscular atrophy.

In the paediatric population, based on biopsy results 3 (9.09%) were diagnosed to have inflammatory myopathies, 17 (51.5%) muscular dystrophies, and 2 (6.06%) congenital myopathies. Biopsy study was indeterminate or non-specific in 10 paediatric patients (30.3%). (Fig 13).One patient was diagnosed with spinal muscular atrophy. No cases were diagnosed as mitochondrial and metabolic myopathies in paediatric population based on muscle biopsy.

Table 9: Comparison of pre and post biopsy diagnosis

Diagnostic groups	Prebiopsy diagnosis (n=70)	Post biopsy diagnosis (n=70)
Inflammatory myopathies	9 (12.8%)	7(10%)
Muscular dystrophies	30 (42.8%)	27 (38.6%)
Congenital myopathies	3 (4.3%)	7 (10%)
Mitochondrial myopathies	11(15.7%)	2 (2.8%%)
Metabolic or toxic myopathies	2 (2.8%)	0
Indeterminate or non-specific	15 (21.5%)	25 (35.7%)

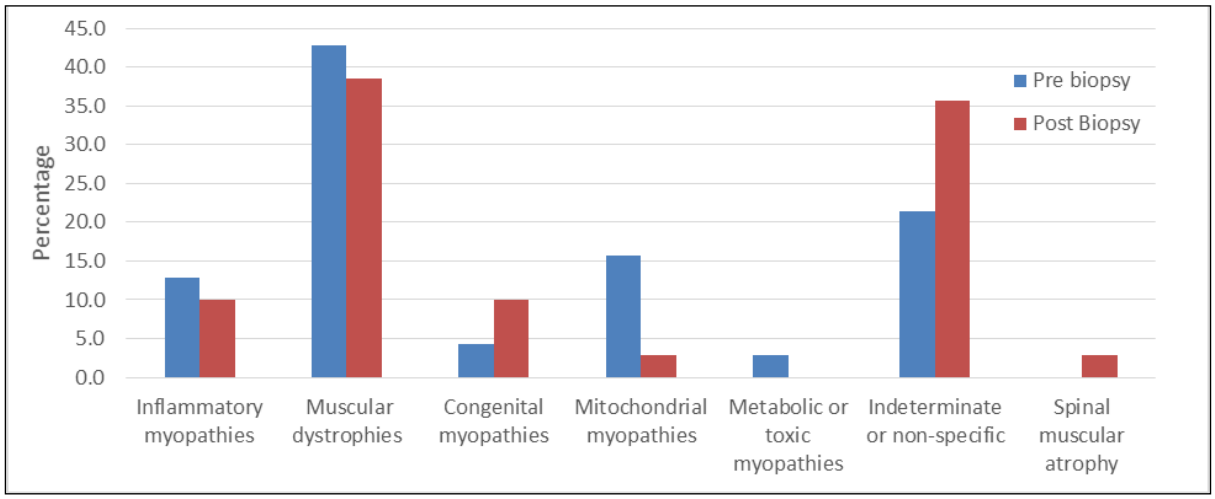

Figure 12: Comparison of pre and post biopsy diagnosis

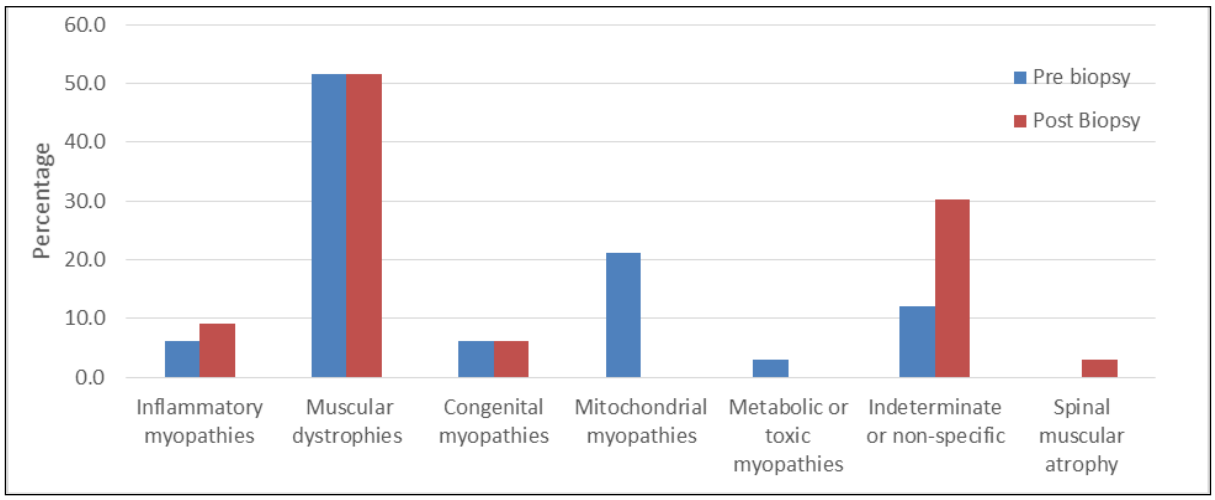

Figure 13: Comparison of pre and post biopsy diagnosis of pediatric patients

Among the 70 patients, 42 (60%) could be classified under a specific diagnostic group and among the 33 paediatric patients, 22 (66.67%) could be classified under specific diagnostic group after muscle biopsy. Specific diagnosis based on muscle biopsy study is shown in table 10.

Table 10: Specific diagnosis and diagnostic categories based on muscle biopsy

	Total	**Paediatric group**
Inflammatory myopathy	7/70	3/33
Polymyositis	3/7	0 /3
Dermatomyositis	2/7	1 /3
Not definite	2/7	2/3
Muscular dystrophy	27/70	17/33
Dystrophinopathy	8/27 (Becker 2)	8/17 (Becker 2)
Dysferlinopathy	3/27	0/17
Sarcoglycanopathy	2/27	1/17
CMD	1/27	1/17
Not Definite	13/27	7/17
Congenital myopathy	7/70	2/33
Desminopathy	1/70	
Mitochondrial myopathies	2/70	0/33
Metabolic or toxic myopathy	0/70	0/33
Indeterminate or non-specific	25/70	10/33
SMA	2/70	1/ 33

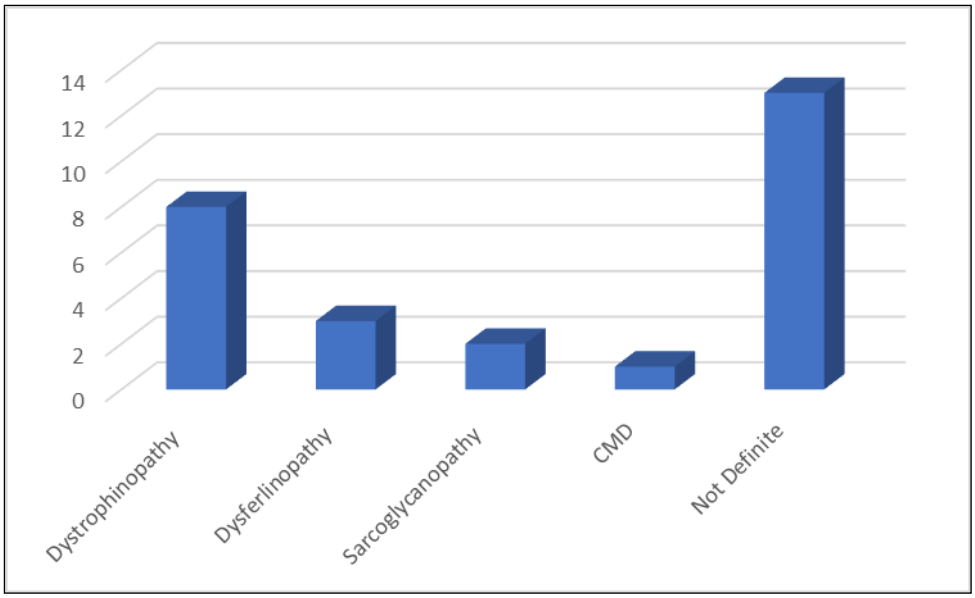

Figure 14: Specific diagnosis under muscular dystrophy group

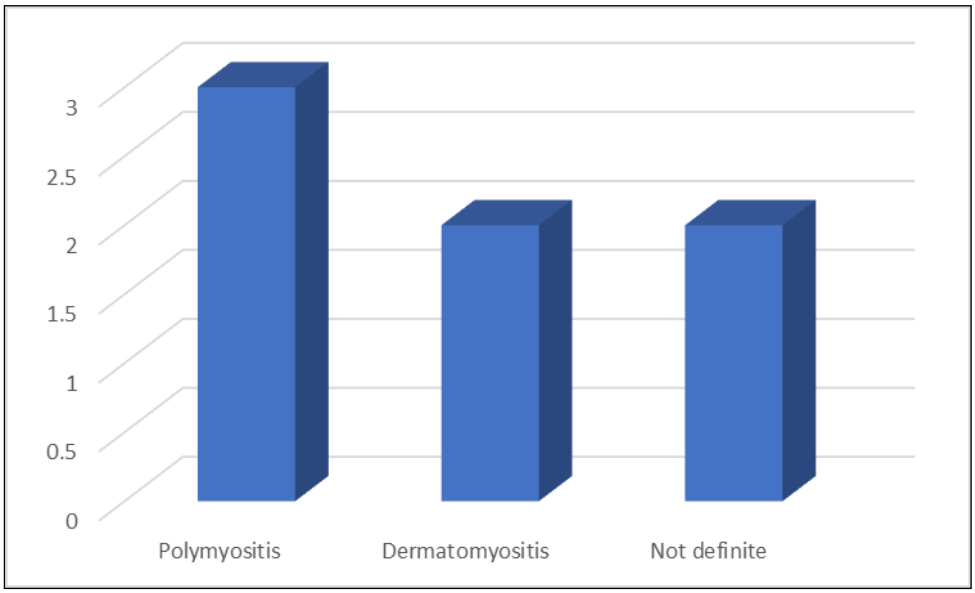

Figure 15: Specific diagnosis under inflammatory myopathy group

Thirty-two patients received no treatment after biopsy. Out of the remaining 38 patients, 6 received immunosuppressive therapy and the remaining 32 patients had other therapies in form of vitamin E supplementation, carnitine supplementation. And mitochondrial cocktail therapy. Among them 14 patients received mitochondrial cocktail therapy. Prior to biopsy, 13 patients were given immunosuppressive therapy. After biopsy only 3 among the 13 continued to receive immunosuppressive therapy(Fig 16). In addition one patient was initiated on

immunosuppressive therapy after biopsy, which means only 4 patients were on immunosuppressive therapy post-biopsy.

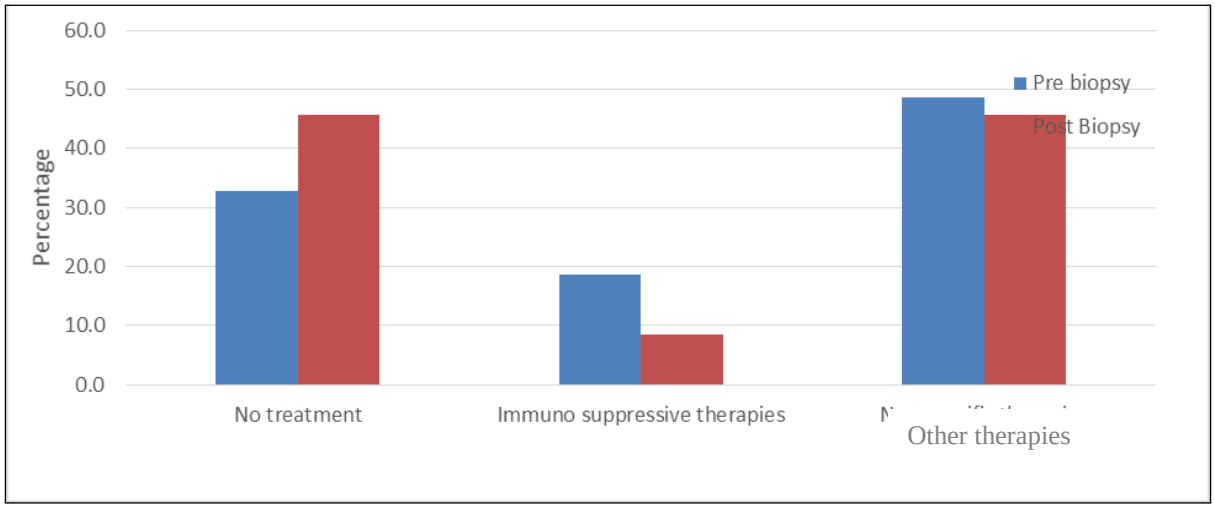

Figure 16: Comparison of pre and post biopsy treatment of total patients

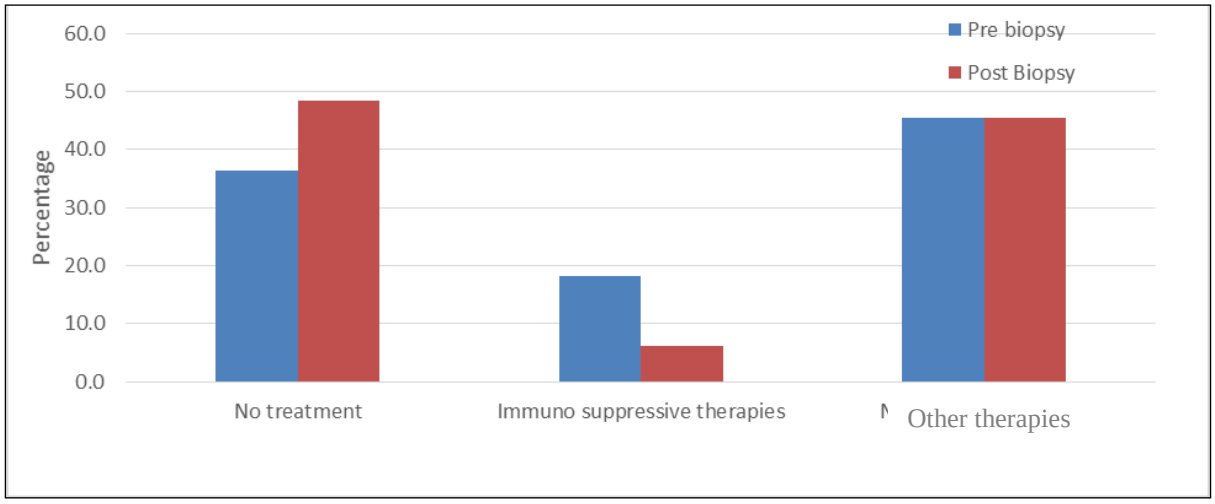

Figure 17: Comparison of pre and post biopsy treatment of pediatric patients

Utility of muscle biopsy

In this study the utility of muscle biopsy was assessed based on a scoring system. If the biopsy results were indeterminate or non-specific score 0 was given. If the biopsy has helped to reach at a diagnostic group or a specific diagnosis, score 1 was given. Along with it if the biopsy has changed the pre biopsy diagnosis, score 2 was given. If the biopsy has helped to reach at a specific diagnosis/diagnostic group and has changed the prebiopsy diagnosis and also has changed the pre biopsy treatment, a score of 3 is given. On assessing the muscle biopsy of total

70 patients 25 (35.7%) had an indeterminate or nonspecific biopsy results with score 0. 25/70 (35.7%) had a score 1. 14/70 (20 %) had a score 2 and 6/70 (8%) had score 3 (Fig 18).

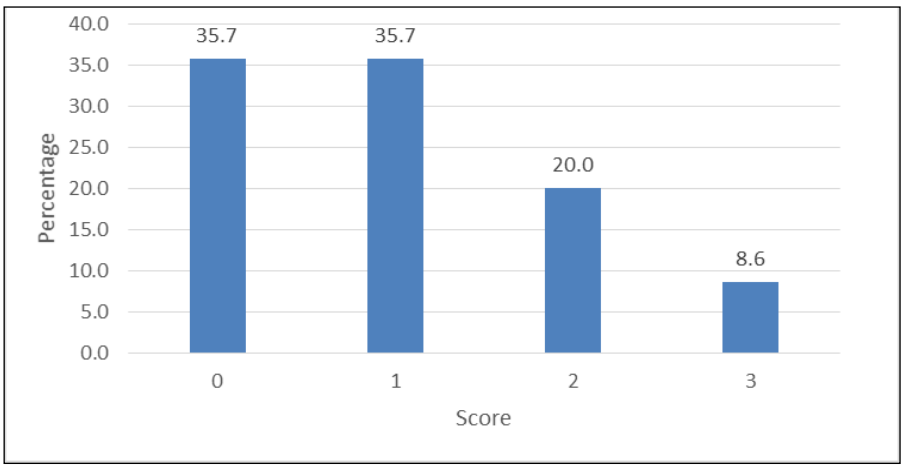

Figure 18: Biopsy utility score in total patients

Similar scoring system was applied in pediatric population and the results are shown in the bar diagram (Fig 19).

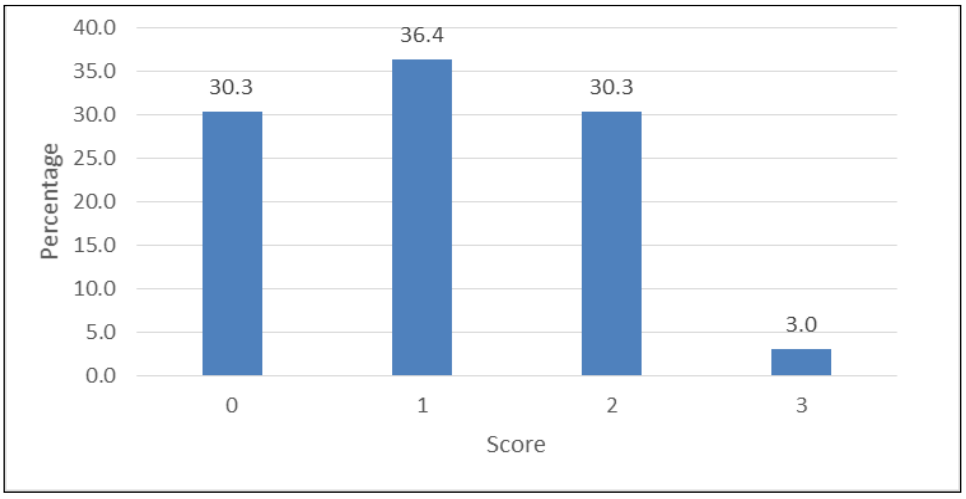

Figure 19: Biopsy utility score in pediatric patients

Statistical analysis

Change in prebiopsy and post biopsy results were analysed using McNemar test. p value < 0.05 was considered as statistically significant. Prior to biopsy 11(15.7%) patients were diagnosed with mitochondrial myopathies. Post biopsy diagnosis of mitochondrial myopathy was made in 2 (2.9%) patients. The change of diagnosis of mitochondrial myopathy based on muscle

biopsy was statistically significant (McNemar Test p 0.012). Similarly the change made by muscle biopsy on immunomodulatory therapies was statistically significant (McNemar Test p 0.012)

Table 11: Statistical analysis of change in prebiopsy and post biopsy results using McNemar test

	Total	**Paediatrics**
	McNemar Test p	McNemar Test p
Inflammatory myopathies	0.688	1.000
Muscular dystrophies	0.581	1.000
Congenital myopathies	0.219	1.000
Mitochondrial myopathies	0.012	-
Indeterminate or non-specific	0.087	0.146
No treatment	0.122	0.388
Immune-modulating therapies	0.012	0.125
Other therapies	0.000	0.065

Note : p value < 0.05 considered as statistically significant

Table 12: Statistical analysis of change in pre and post biopsy diagnosis of mitochondrial myopathy using McNemar test

Mitochondrial myopathies		**Post biopsy**		**Total**	**McNemar Test p**
		Diagnosed	**Not diagnosed**		
Prebiopsy	Diagnosed	1	10	11	0.012
	Not diagnosed	1	58	59	
Total		2	68	70	

Note : p value < 0.05 considered as statistically significant

Table 13: Statistical analysis of change in pre and post biopsy immune modulatory therapies using McNemar test

Immune-modulating therapies		Post biopsy		Total	McNemar Test p
		Yes	No		
Prebiopsy	Yes	3	10	13	0.012
	No	1	56	57	
Total		4	66	70	

Note : p value < 0.05 considered as statistically significant

Atlas of pathological findings seen in patients included in the study

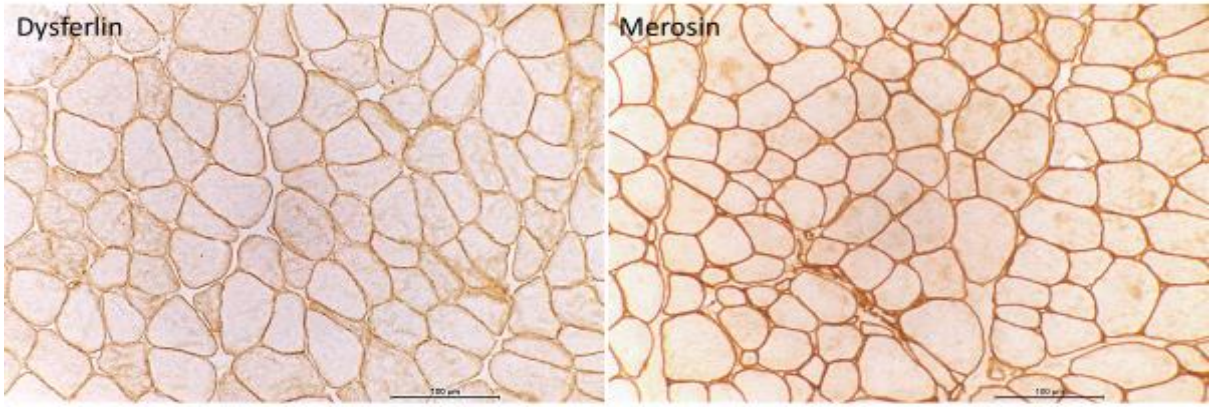

Figure 20:Immunohistochemistry of patient A showing normal labelling for dysferlin and merosin

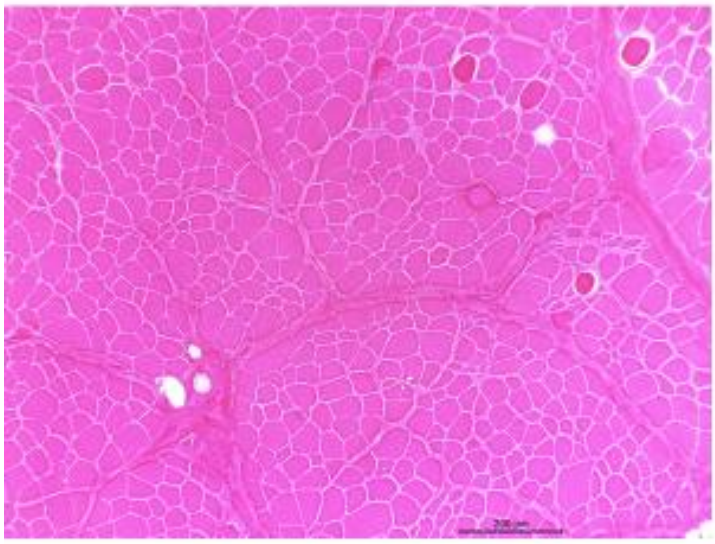

Figure 21: Haematoxylin and eosin images of patient B showing partially effaced architecture with fibre size variation and increased endomysial connective tissue suggestive of muscular dystrophy

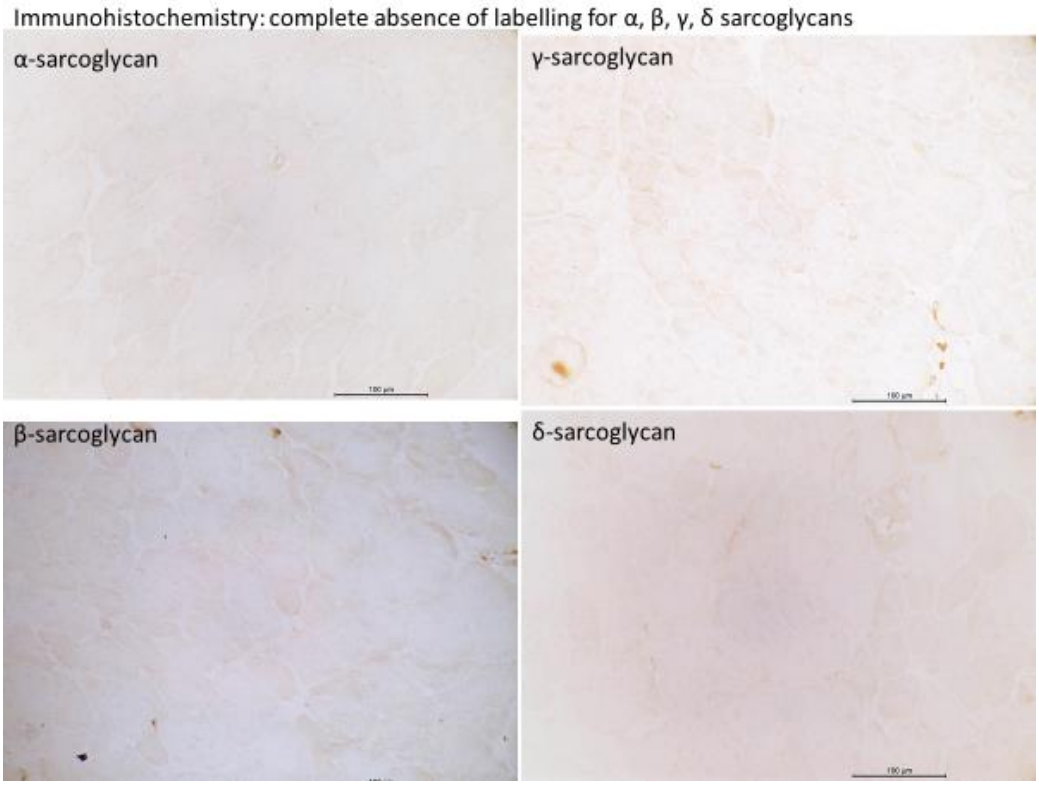

Figure 22: Immunohistochemistry of patient B showing complete absence of alpha, beta, gamma, delta sarcoglycans consistent with sarcoglyconopathy

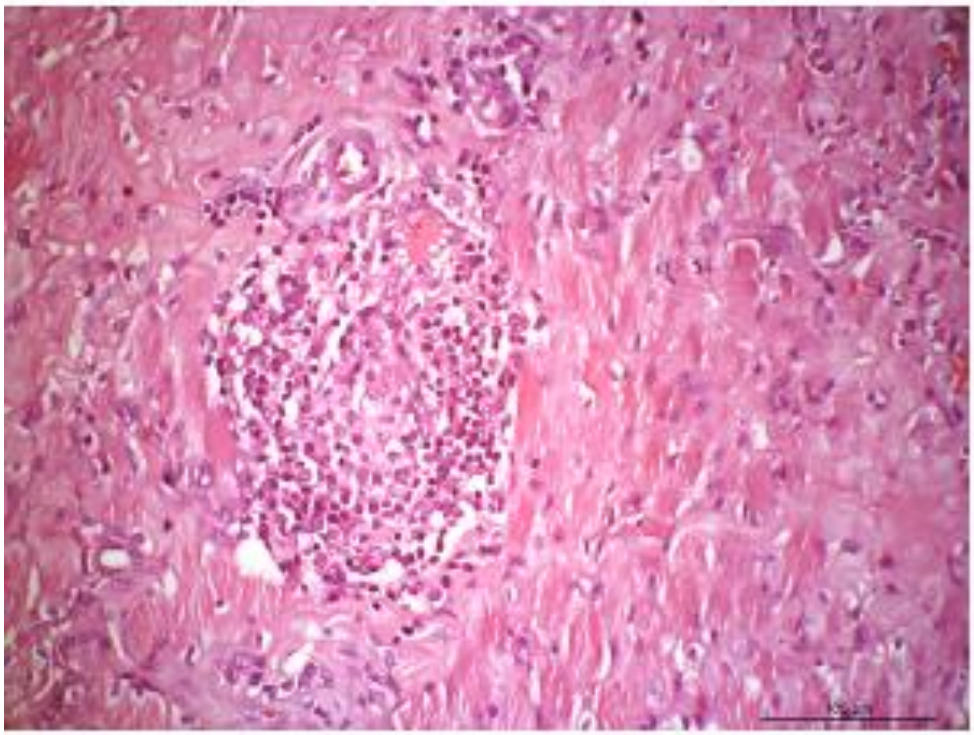

Figure 23: Haematoxylin and eosin images of patient C showing dense endomysial inflammation consistent with polymyositis

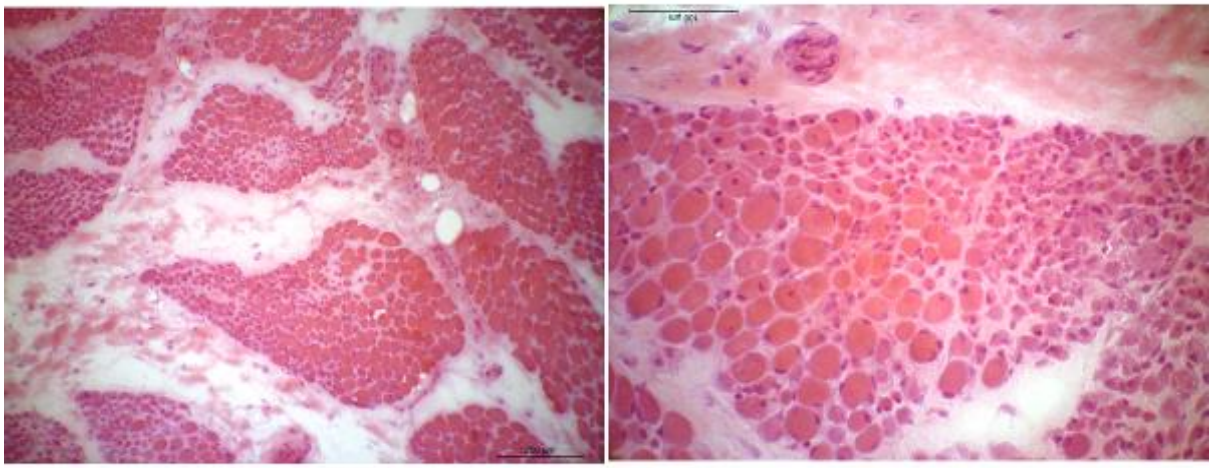

Figure 24: Haematoxylin and eosin images of patient D showing perifascicular atrophy consistent with dermatomyositis

DISCUSSION

DISCUSSION

In the evaluation of patients with muscular disorders muscle biopsy is an important diagnostic tool. Muscle biopsy is an invasive procedure and cumbersome especially in pediatric patients. In a review of pediatric patients undergoing muscle biopsy in 1999, 36% of cases had specific diagnosis (1). With the advent of newer genetic techniques in the current era the diagnostic utility of muscle biopsy needs to be reassessed.

In this study the diagnostic utility of muscle biopsy was assessed. This was done objectively by analyzing the prebiopsy diagnosis, post biopsy diagnosis, change in prebiopsy and post biopsy diagnosis. In addition the direct implications of muscle biopsy on treatment plans were analyzed.

Muscle biopsies of 70 patients done between the period April 2017 – April 2019 in our institute were studied. Pediatric population constitute 47.14% (33/70) of the total population. Majority of the study population were males (55.7%).Majority of the patients were in age group of 10 – 20 years (27.1%) (Fig 7). The most common presenting symptom among the study population was weakness with proximo – distal weakness noted in 67.1% and predominant proximal weakness noted in 30% (Fig 10). Majority of patients (42.8%) had a pre-biopsy diagnosis of muscular dystrophy.

Based on number of patients having definitive diagnosis and based on the number of patients having change in diagnosis the diagnostic utility of muscle biopsy was analysed. In this study of 70 patients, muscle biopsy was found to be useful in 64.3% (45/70) of patients . In a similar study by C.-H. Lai et al (33), 74% of muscle biopsies were considered useful in the diagnosis and management of patients with muscular diseases.

Among the 33 pediatric patients muscle biopsy was found to be useful in 66.67% of patients. Four hundred and nineteen biopsies were analyzed in a similar pediatric study by Cuisset JM et al. (34) .44% of the muscle biopsies revealed specific histopathological anomalies based on which a specific diagnosis could be made. In our study 30.3% of (10/33) pediatric patients s had nonspecific or inconclusive biopsy findings. This is similar to the results observed in an another pediatric study by Chang XZ et al. (35), where 30.5% of the patients had unspecific

alterations at muscle biopsy. These observations from various studies show that muscle biopsy is often indispensable to establish the etiological diagnosis in children with myopathy.

In our study the most common pathologic finding noted was muscular dystrophies noted in 38.6%. Inflammatory myopathies and congenital myopathies were noted in 10%. 2.8% had pathologic findings of mitochondrial myopathies (Table 9,fig 12). In contrast the most common pathological finding observed in the study by C.-H. Lai et al. (33) was inflammatory myopathies (22.5%). In our study there were no patients with pathologic finding of metabolic myopathy. In the study by C.-H. Lai et al (33) metabolic myopathies were noted in 14.2% of cases. The reason for this could be explained by the regional differences in prevalence of metabolic myopathies or a referral bias.

In our study among the pediatric population the most common pathologic findings was muscular dystrophies noted in 51.5% of the pediatric population followed by inflammatory myopathies noted in 9% of the pediatric population(Fig 13). In the pediatric study of eighty-two children by Chang XZ et al(35), muscular dystrophies were noted in 39.4%, inflammatory myopathies were noted in 12.12%, congenital myopathies were noted in 6%, mitochondrial myopathy were noted in 1% and metabolic myopathy was noted in 6%.In another pediatric study of four hundred and nineteen biopsies by Cuisset JM et al (34), one hundred and ninety-three were compatible with congenital myopathies (39.38%), ninety-two revealed muscle dystrophy (18.77%), nineteen mitochondrial myopathies, thirteen metabolic myopathies and eleven inflammatory myopathies.

In our study the prebiopsy and post biopsy diagnosis remained same in 35.7% of patients. Change in diagnosis after biopsy was noted in 20% of patients. Change in treatment was noted in 8.6% of patients. In another study on two hundred and fifty-eight patients by C.-H. Lai et al(33), change in diagnosis was noted in 47% of the cases and a change in treatment in 33% of the subjects. In our study prior to biopsy 11 patients were diagnose as mitochondrial myopathy. However after biopsy only 2 patients were diagnosed as mitochondrial myopathy based on biopsy findings. The change of diagnosis with regard to mitochondrial myopathy was statistically significant (McNemar Test P = 0.012). This would reflect the trend of over diagnosing mitochondrial myopathies based on clinical grounds. With regard to treatment ,the change in immunosuppressive therapy with biopsy was statistically significant (McNemar Test

P = 0.012) (Table 11). Prior to biopsy 13 patients were receiving immunomodulatory therapies. However after biopsy only 4 patients received immunomodulatory therapies.

Although muscle biopsy was found to be useful in 64.3% of patients in our study the degree of usefulness would vary with each patient in practical scenario. Hence the utility of muscle biopsy was assessed based on a scoring system. If the biopsy results were indeterminate or non-specific score 0 was given. If the biopsy has helped to reach at a diagnostic group or a specific diagnosis ,score 1 was given. Along with it if the biopsy has changed the pre biopsy diagnosis, score 2 was given. If the biopsy has helped to reach at a specific diagnosis/diagnostic group and has changed the prebiopsy diagnosis and also has changed the pre biopsy treatment, a score of 3 is given. On assessing the muscle biopsy of total 70 patients 25 (35.7%) had an indeterminate or non-specific biopsy results with score 0. 25/70 (35.7%) had a score 1. 14/70 (20%) had a score 2 and 6/70 (8%) had score 3.This shows that among the patients benefitted the utility of muscle biopsy was not uniform.

The importance of this study comes from its clinical applicability for helping clinicians to decide if a muscle biopsy is indicated and to discuss its yield with their patients depending on pretest variables. All EMGs, physical exams, and muscle biopsy interpretations were performed by the same qualified neuromuscular specialist, which helped decrease measurement error.

Limitations of our study

1. The study procedures were performed in a tertiary neuromuscular center and may not precisely reflect the muscle biopsy yield in different situations and for different populations (community clinics or general neurology offices)
2. This is a retrospective study, and data were not collected specifically for this study
3. Limited panel of immunohistochemistry antibodies: immunostains for caveolin, collagen VI and C5b-9 could not be performed. Western blot for calpain could not be performed
4. Only very few patients had genetic study done. Hence a direct correlation analysis of the diagnostic outcomes of muscle biopsy and genetic studies could not be done

CONCLUSION

CONCLUSION

- The overall diagnostic yield of muscle biopsy found in this study was 64.3%
- In pediatric patients muscle biopsy was found to be useful in 66.67% of patients. Muscle biopsy is often valuable to establish the etiological diagnosis in children with myopathy
- 35.7% patients had specific diagnosis or diagnostic group detected. 20% patients showed a change in pre-biopsy diagnosis
- Muscle biopsy significantly influenced the decision of continuation or stoppage of immunomodulatory therapies
- Among the patients benefitted the utility of muscle biopsy was not uniform
- Muscle biopsy still plays an valuable role in myopathy evaluation. However further larger studies which directly correlates and analyses the diagnostic outcomes of muscle biopsy and genetic studies are required in the modern genetic era

REFERENCES

REFERENCES

1. Reynolds EM, Thompson IM, Nigro MA, Kupsky WJ, Klein MD. Muscle and nerve biopsy in the evaluation of neuromuscular disorders: the surgeons perspective. J Pediatr Surg 1999; 34: 588–590
2. Lee YS. The role of muscle biopsy in the diagnosis of neuromuscular disorders. Ann Acad Med Singap 1989; 18: 410–415
3. Lee YS. Muscle diseases in Singapore. Pathology 1986; 18: 35–40
4. Lockett LJ. Hawaii_s Neuromuscular Disease Biopsy Registry. A quarter-century compilation of muscle biopsy diagnoses in Hawaii. Hawaii Med J 2002; 61: 142–147
5. Laguno M, Miro O, Perea M, Picon M, Urbano-Marquez A, Grau JM. Muscle diseases in elders: a 10-year retrospective study. J Gerontol A Biol Sci Med Sci 2002; 57:M378–384
6. Kakulas BA, Adams RD. Diseases of muscle. Pathological foundations of clinical myology. 4th ed. Philadelphia: Harper and Row, 1985
7. Dubowitz C, Sewry CA. In: Dubowitz C, Sewry CA (eds.) Muscle biopsy. A practical approach. Philadelphia, USA: Elsevier, Saunders, 2007
8. Lockett LJ. Hawaii's Neuromuscular Disease Biopsy Registry. A quarter-century compilation of muscle biopsy diagnoses in Hawaii. Hawaii Med J 2002;61:142–147
9. Chi JG, Koo HS, Roh JK. Histopathologic study on muscle disease among Koreans (274 muscle biopsy analysis). J Korean Med Sci 1989; 4:55–61
10. Barohn RJ. General approach to muscle diseases. In: Goldman L, Bennett JC, eds.Cecil textbook of medicine. 23rd edition Philadelphia, PA: Saunders, 2008
11. Theadom A, Rodrigues M, Roxburgh R, Balalla S, Higgins C, Bhattacharjee R,Jones K, Krishnamurthi R, Feigin V. Prevalence of muscular dystrophies: a systematic literature review. Neuroepidemiology. 2014;43(3-4):259-68
12. Fujimura-Kiyono C, Racz GZ, Nishino I. Myotubular/centronuclear myopathy and central core disease. Neurol India 2008;56:325-32
13. Mercuri E, Muntoni F: Muscular dystrophies. Lancet 2013; 381: 845–860
14. Mercuri E, Muntoni F: Muscular dystrophy: new challenges and review of the current clinical trials. Curr Opin Pediatr 2013;25:701– 707
15. Darras BT, Urion DK, Ghosh PS. Dystrophinopathies. 2000 Sep 5 [Updated 2018 Apr 26]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet].

Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1119/

16. Lai KK, Lo IF, Tong TM, Cheng LY, Lam ST. Detecting exon deletions and duplications of DMD gene using multiple ligation dependent probe amplification (MLPA). Clin Biochem 2006;13:367-72
17. Ankala A, da Silva C, Gualandi F, et al. A comprehensive genomic approach for neuromuscular diseases gives a high diagnostic yield. Ann Neurol 2015;77(2): 206-14
18. Khadilkar SV. Limb girdle muscular dystrophies in India. Neurol India 2015;63:495-6
19. Pathak P, Sharma MC, Sarkar C, Jha P, Suri V, Mohd H, et al. Limb girdle muscular dystrophy type 2A in India: A study based on semi-quantitative protein analysis, with clinical and histopathological correlation. Neurol India 2010;58:549-54
20. Emery AH. Population Frequencies of Inherited Neuromuscular Diseases—A World Survey. Neuromuscul Disord. 1991; 1:19
21. Milone M, Wong LJ. Diagnosis of mitochondrial myopathies. Mol Genet Metab 2013; 110:35–41
22. Tarnopolsky MA, Raha S. Mitochondrial myopathies: diagnosis, exercise intolerance, and treatment options. Med Sci Sports Exerc 2005; 37:2086–2093
23. Sharma V, Borah P, Basumatary LJ, Das M, Goswami M, Kayal AK. Myopathies of endocrine disorders: A prospective clinical and biochemical study. Annals of Indian Academy of Neurology. 2014 Jul;17(3):298
24. Nonaka I. Clinical and pathologic aspects of congenital myopathies. Neurol J Southeast Asia 2001;6:99-106
25. North KN, Wang CH, Clarke N, Jungbluth H, Vainzof M, Dowling JJ, Amburgey K, Quijano-Roy S, Beggs AH, Sewry C, Laing NG. Approach to the diagnosis of congenital myopathies. Neuromuscular Disorders. 2014 Feb 1;24(2):97-116
26. Jain D, Sharma MC, Sarkar C, Gulati S, Kalra V, Singh S, et al. Congenital myopathies: A clinicopathological study of 25 cases. Indian J Pathol Microbiol 2008;51:474-80
27. Thaha F, Gayathri N, Nalini A. Congenital myopathies: Clinical and immunohistochemical study. Neurol India 2011;59:879-83
28. Uppin MS, Meena AK, Sundaram C. Spectrum of congenital myopathies: A single centre experience. Neurol India 2013;61:254-9
29. Lev EI, Tur-Kaspa I, Ashkenazy I, et al. Distribution of serum creatine kinase activity in young healthy persons. Clin Chim Acta. 1999;279:107–115

30. Dalakas MC. Muscle biopsy findings in inflammatory myopathies. Rheum Dis Clin North Am. 2002 Nov; 28(4):779–98
31. Reynolds EM, Thompson IM, Nigro MA, Kupsky WJ,Klein MD. Muscle and nerve biopsy in the evaluation of neuromuscular disorders: the surgeons perspective. J Pediatr Surg 1999; 34: 588–590
32. Lee YS. The role of muscle biopsy in the diagnosis of neuromuscular disorders. Ann Acad Med Singap 1989; 18:410–415
33. Lai C-H, Melli G, Chang Y-J, Skolasky RL, et al. Open muscle biopsy in suspected myopathy: diagnostic yield and clinical utility. European Journal of Neurology 2010; 17: 136-142
34. Cuisset JM, Maurage CA, Carpentier A, Briand G, et al. Muscle biopsy in children: Usefulness in 2012. Revue neurologique 2013; 169: 632-639
35. Chang XZ, Zhou JY, Yuan Y, Wu Y et al. Diagnostic value of muscle, sural nerve and skin biopsies in childhood neuromuscular disorders. Zhonghua Er Ke Za Zhi 2006; 44(12): 909-12

ANNEXURES

ANNEXURE 1

LIST OF ABBREVIATIONS

ATPase	Adenosine triphosphatase
CM	Congenital myopathy
CMD	Congenital muscular dystrophy
CMS	Congenital myasthenic syndromes
COX	Cyclooxygenase
CPK	Creatinine phosphokinase
DCM	Dilated cardiomyopathy
DMD	Duchenne muscular dystrophy
EM	Electron microscope
EMG	Electromyography
ESR	Erythrocyte sedimentation rate
FSHD	Fascioscapulohumeral muscular dystrophy
H&E	Haematoxylin and eosin
LGMD	Limb girdle muscular dystrophy
LM	Light microscope
MGT	Modified gomori trichrome
MHC	Major histocompatibility complex antigen
MLPA	Multiplex ligation-dependent probe amplification
MPCR	Multiplex polymerase chain reaction
MUAP	Motor unit action potential

NADPH	Nicotinamide adenine dinucleotide phosphate
NCS	Nerve conduction study
OPMD	Oculopharyngeal muscular dystrophy
PAS	Periodic acid - schiff
SDH	Succinate dehydrogenase
SMA	Spinal muscular atrophy
EDX	Electrodiagnostic
CRD	Complex repetitive discharges
PSW	Positive sharp waves
MRC	Medical research council

ANNEXURE 2

mRS score

0	No symptoms
1	No significant disability, despite symptoms; able to perform all usual duties and activities
2	Slight disability; unable to perform all previous activities but able to look after own affairs without assistance
3	Moderate disability; requires some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, and requires constant nursing care and attention
6	Death

ANNEXURE 3

MRC (Medical research council) Muscle power scale

Score	Description
0	No contraction
1	Flicker or trace of contraction
2	Active movement, with gravity eliminated
3	Active movement against gravity
4	Active movement against gravity and resistance
5	Normal power

ANNEXURE 4

PROFORMA

A CLINICAL STUDY ON THE UTILITY OF MUSCLE BIOPSY IN PATIENTS WITH SUSPECTED MYOPATHY

1. Identification information

1.1 Serial number ----------

1.2 Unique identification number ----------

2. Demographic data

2.1 Age ---------- years

2.2 Sex ---------- 1. Male 2. Female

2.3 Occupation ----------

2.4 Year of registration ----------

3. History(1 = Yes, 0 = No)

3.1 Age of onset ----------

3.2 Duration of illness ----------

3.3 Developmental delay ----------

3.3.1 If yes, specify----------

3.4 Poor athletic performance ----------

3.5 Distal lower limb weakness ---------- Duration ----------

3.6 Proximal lower limb weakness --------- Duration ----------

3.7 Distal upper limb weakness ---------- Duration ----------

3.8 Proximal lower limb weakness --------- Duration ----------

3.9 Gait difficulty --------- Duration ----------

3.10 Falls---------- Duration ----------

3.11 Neck weakness ----------

3.12 Trunk weakness ----------

3.13 Respiratory difficulty ----------

3.14 Cranial nerve involvement ----------

3.14.1 I yes, specify ----------

3.15 Sensory symptoms ---------- Duration ----------

3.15.1 If yes, specify ----------

3.16 Pain ----------

3.17 Cramps----------

3.18 Deformities----------

3.19 Functional status ----------

(0 = normal, 1 = mild difficulty, independent, 2= moderate difficulty, independent,

3 = partly dependent, 4 = bed/ wheel chair bound)

4.Other history (1= Yes, 0 = No)

4.1 Other illnesses ----------

4.1.1 If yes, details ----------

4.2 Family history ----------

4.2.1 If yes, details ----------

4.3 Consanguinity ----------

4.3.1 If yes, details ----------

5.Physical signs (1 = Yes, 0 = No)

5.1 Systemic signs ---------- Specify ----------

5.2 Cranial neuropathy ---------- Specify ----------

5.3 Contractures ---------- Specify ----------

5.4 Atrophy---------- Specify ----------

5.5 Hypertrophy---------- Specify ----------,

5.6 Power of lower limbs (Right/ Left)

5.6.1 Toe flexors ----------

5.6.2 Toe extensors ----------

5.6.3 Ankle dorsiflexion ----------

5.6.4 Ankle plantar flexion ----------

5.6.5 Knee flexion ----------

5.6.6 Knee extension ----------

5.6.7 Hip abduction ----------

5.6.8 Hip adduction ----------

5.6.9 Hip flexion ----------

5.6.10 Hip extension ----------

5.7 Power of upper limbs (Right/ Left)

5.7.1 Intrinsic muscles ----------

5.7.2 Hand grip ----------

5.7.3 Wrist flexion ----------

5.7.4 Wrist extension ----------

5.7.5 Elbow flexion ----------

5.7.6 Elbow extension ----------

5.7.7 Shoulder abduction ----------

5.7.8 Shoulder adduction ----------

5.7.9 Shoulder flexion ----------

5.7.10 Shoulder extension ----------

5.8 Axial muscles

5.8.1 Neck flexion ----------

5.8.2 Neck extension ----------

5.8.3 Trunk ----------

5.9 Reflexes

5.9.1 Biceps jerk ----------

5.9.2 Supinator jerk ----------

5.9.3 Triceps jerk ----------

5.9.4 Knee jerk ----------

5.9.5 Ankle jerk ----------

5.9.6 Abdominal reflex ----------

5.9.7 Plantar response ----------

5.10 Sensory involvement ----------

5.11 Gait (describe) ----------

5.12 Myotonia

5.13 Other signs (specify) ----------

6. Electrophysiological study

6.1. NCS done yes/no

6.2. Findings

6.3. EMG done yes/no

6.4. Muscles sampled

6.5. Insertional activity

6.6. Spontaneous activity

6.7. MUAP duration

6.8. MUAP amplitude

6.9. MUAP phases

6.10. Recruitment pattern

Final diagnosis:

7. Laboratory parameters

7.1 Hemoglobin, ESR, Total leucocyte count, Differential leucocyte count

7.2 CPK

7.3 Serum lactate

7.4 Others

8. Pre biopsy diagnosis

8.1 Inflammatory myopathies

8.2 Muscular dystrophies

8.3 Congenital myopathies

8.4 Mitochondrial myopathies

8.5 Metabolic or toxic myopathies

8.6 Indeterminate or non-specific

9.Pre-biopsy treatments

9.1 no treatment

9.2 immune-modulating therapies

9.3 non-specific therapies

10. Biopsy stains

10.1. H&E

10.2. MGT

10.3. Oxidative

NAPDH

SDH

COX

ATPase

10.4. PAS

10.5. Oil red O

10.6. Others

10.7. Immunohistochemistry

10.8. Details

10.9. Electron microscopy

11. Post Biopsy diagnosis

11.1 Inflammatory myopathies

11.2 Muscular dystrophies

11.3 Congenital myopathies

11.4 Mitochondrial myopathies

11.5 Metabolic or toxic myopathies

11.6 Indeterminate or non-specific

11.7 Normal findings

12. Specific diagnosis (If any) :

13. Post –biopsy treatments

13.1 no treatment

13.2 immune-modulating therapies

13.3 non-specific therapies

14. Genetic study (If any)

Utility of muscle biopsy :

Score 0

Score 1

Score 2

Score 3

Note : If the biopsy results were indeterminate or non-specific score 0 was given. If the biopsy has helped to reach at a diagnostic group or a specific diagnosis, score 1 was given. Along with it if the biopsy has changed the pre biopsy diagnosis, score 2 was given. If the biopsy has helped to reach at a specific diagnosis/diagnostic group and has changed the prebiopsy diagnosis and also has changed the pre biopsy treatment, a score of 3 is given.

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
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Institutional Ethics Committee

(IEC Regn No. ECR/189/Inst/KL/2013/RR-16)

SCT/IEC/1246/AUGUST-2018 30.10.2018

Dr. Sudhakar. K
Senior Resident
Department of Neurology
SCTIMST, Thiruvananthapuram

Dear Dr Sudhakar,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "A CLINICAL STUDY ON THE UTILITY OF MUSCLE BIOPSY IN PATIENTS WITH SUSPECTED MYOPATHY (IEC/1246)" on 31st August, 2018.

The following documents were reviewed:

Original submission

1. *Covering Letter addressed to the Chairperson, IEC, SCTIMST dated 16.07.2018 with checklist .*
2. *TAC Approval Letter*
3. *IEC Application Form*
4. *Project Proposal*
5. *Proforma*
6. *Patient Information Sheet and Consent Form in English and Malayalam*
7. *CV of Principal Investigator and Co-Principal Investigators*

Revised submission

1. *Covering Letter addressed to the Chairperson, IEC, SCTIMST dated 11.10.2018 with checklist*
2. *TAC Approval Letter*
3. *IEC Application Form*
4. *Project Proposal*
5. *Proforma*
6. *Patient Information Sheet and Consent Form in English and Malayalam*
7. *CV of Principal Investigator and Co-Principal Investigators*

The following members of the Ethics Committee were present at the meeting held on 31st August, 2018 at G. Parthasarathi Board Room, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. R V G Menon	M Tech, PhD	Male	Lay Person (Chairman)	No
2.	Dr. Rema M. N	MD	Female	Basic Medical Scientist	No
3.	Dr. K R S Krishnan	M.E., Ph.D.	Male	Medical Technology	Yes
4.	Dr. S S Giri Sankar	LL.M. Ph.D.	Male	Legal Expert	No
5.	Dr. Aneesh V Pillai	BA. LLB (Hons.), LLM, Ph. D, SET (Law)	Male	Legal Expert	No
6.	Mr. Satheesh Chandran	MSW, PGDPM	Male	Lay person/ NGO/ Social Scientist	No
7.	Dr. Harikrishna Varma PR	Ph.D(Materials Science)	Male	Medical Technology	Yes
8.	Dr. P. Manickam	BSMS, MSc (Epid).,PhD	Male	Health Science Expert/ Social Scientist	No
9.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
10.	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
11.	Dr. Anand Kumar A	MD, DM	Male	Clinician	No
12.	Dr. V. Raman Kutty	M D, M Phil, M P H	Male	Health Sciences Expert/Clinician	Yes
13.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

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