Myopathology, a 14-Year Siriraj Experience: What have we learned?

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Abstract : **Neuromuscular laboratory, Department of Pathology Siriraj Hospital was established in 1989. Within fourteen years, 315 specimens from 304 patients were received. The patients' age ranged from 22-week gestation to 76 years old. The ratio of males to females was 1.11:1. Muscular dystrophies comprised 26.63%, non- specific changes 25.66%, primary myopathies, including unclassified myopathy 13.82%, neurogenic muscle diseases 12.82%, dysimmune and infectious myopathies 8.89%, mitochondrial myopathy 4.94% and others 7.24%. Summaries of our patients were described.**

Key words : Muscular dystrophy, myopathy, neurogenic muscle diseases, Thai.

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ห้องปฏิบัติการพยาธิวิทยาสำหรับระบบกล้ามเนื้อและเส้นประสาท โรงพยาบาลศิริราช ได้เริ่มขึ้นในปี พ.ศ. ๒๕๓๒ โดยเริ่มพัฒนาการตรวจกล้ามเนื้อให้ได้เทียบเท่ามาตรฐานสากล ในระยะเวลา ๑๔ ปี มีชิ้นเนื้อ ๓๑๕ ตัวอย่าง จากผู้ป่วย ๓๐๔ ราย ผู้ป่วยอายุต่ำสุด คือตัวอ่อนอายุ ๒๒ สัปดาห์ สูงสุดคือ ๗๖ ปี อัตราส่วนของ เพศชายต่อหญิงเป็น ๑.๑๑ ต่อ ๑ กลุ่มโรคสำคัญที่คิดเป็นร้อยละ ได้แก่ Muscular dystrophies 26.63%, non- specific changes 25.66%, primary myopathies, including unclassified myopathy 13.82%, neurogenic muscle diseases 12.82%, dysimmune and infectious myopathies 8.89%, mitochondrial myopathy 4.94% และอื่นๆ 7.24%.

INTRODUCTION

Myopathology is a distinctive subspecialty of pathology for which a special laboratory is essential. Routine histological stains on formalin-fixed, paraffin-embedded tissue offer very limited information and are now out of date for myopathology. Many years ago, enzyme histochemical method was a research tools for specialists. Now, it is a world-wide routine procedure for laboratories providing muscle biopsy services[1]. Basically, fresh muscle tissue should be frozen at minus 160 degree Celsius as soon as possible to preserve its enzyme activities. A battery of histochemistry, enzyme histochemistry, immunohistochemistry, morphometric studies and electron microscopic studies (EM) are performed, depending on the facilities of individual laboratories[2]. In addition, modern molecular pathology yields extensive knowledge of muscle diseases that will provide new treatment for previously incurable diseases.

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The Department of Pathology at Siriraj Hospital established a neuromuscular laboratory unit in 1989. Here, the freezing of muscle in isopentane at liquid nitrogen temperature (-160°C) (snap frozen technique) was first developed. Then, cryostat sections with special stainings were performed. The initial stainings were Hematoxylin & Eosin (H&E), periodic acid-Shift (PAS), oil-red-O (ORO), modified Gomori trichrome (mGT). Two enzyme histochemistry were nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) and myosin adenosine triphosphatase (ATPase) pH 9.4. Electron microscopic study was an option. The addition of ATPase pH 4.3 and 4.6 and succinate dehydrogenase (SDH) was developed in 1993, followed by cytochrome oxidase (COX), and acid phosphatase in 2000 and 2001, respectively. The congo-red and, on occasion, Verhoeff-van Gieson (VVG) were performed. In 2002, immunohistochemistry using dystrophin, sarcoglycan, desmin, and spectrin was applied. Prior to the year 2001, all frozen samples were transfered into formalin and were processed as a routine histopathology. The collection of frozen muscles in -70°C started since then.

MATERIALS AND METHODS

Records of muscle biopsy or necropsy from the department files from January 1989 to June 2003 were reviewed. The samples include all surgical, autopsy, and consultation cases from outside hospital that were processed following muscle biopsy protocol of the department. Formalin fixed muscle samples and samples for particular research were excluded. Some patients had double biopsies. A non-diagnostic one or a second diagnostic biopsy of the same patient was deleted. The patient's age, sex, and pathological diagnoses were analyzed. The pathological diagnoses were classified on the molecular basis rather than a traditional morphological one, according to the recent text "Structural and Molecular Basis of Skeletal Muscle Diseases"[3]. By following this reference, some difficulties eventually occur, since our diagnoses are largely based on morphology with only a little aid from immunohistochemistry or genetic study. For this reason, seven additional extracategories were defined as follows:

Normal muscle : A morphologically normal biopsy taken from a patient who has no clinical relevant to neuromuscular diseases.

Non-specific changes : A biopsy taken from a patient query neuromuscular diseases but morphologically normal or minimal changes.

Unclassified muscular dystrophies : A biopsy displaying dystrophic features but unable to be classified histologically or clinically into any muscular dystrophies.

Unclassified myopathies : An abnormal muscle biopsy of unclassified etiology, excluding dystrophy or neurogenic muscle diseases.

End stage muscle diseases : A severely affected muscle nearly or totally replaced by fibroadipose tissue.

Unsatisfactory specimens : A biopsy having very few muscle fibers or bearing remarkable artefacts that cannot be evaluated.

Miscellaneous : A biopsy with a definite clinical or pathological diagnosis that does not fit in any of the above categories.

RESULTS

There were 315 specimens (282 biopsies, 18 necropsies and 15 consultation cases). Eleven patients had two samples, either from repeated biopsy or subsequent autopsy. One record from patients who had two samples was discarded according to the above criteria. Therefore, a total of 304 patients were included in this study. Table 1 shows the number of specimens and percentage received per year. Age group and sex were shown in Table 2. Table 3. shows pathological diagnoses under disease categories and number of patients.

DISCUSSION

According to the age group, 163 patients (53.61%) were children at the age of ten or younger. Regardless of the age group, nonspecific biopsies represent about one-fourth of all cases (25.66%). Basically, muscle diseases can be simplified into three main groups: muscular dystrophies, primary myopathies, and neurogenic muscle diseases. Our data showed that muscular dystrophies comprise 26.63%.

Table 1. Number and type of specimens per year from 1989 to 2003*

Year	Number of specimen			Total	%
	Surgical specimen	Consulted specimen	Autopsy specimen		
1989	1	0	0	1	0.3
1990	7	1	0	8	2.5
1991	19	0	1	20	6.3
1992	23	1	1	25	8.0
1993	26	1	0	27	8.6
1994	48	0	0	48	15.2
1995	21	0	0	21	6.7
1996	19	0	0	19	6.0
1997	33	0	0	33	10.5
1998	18	0	0	18	5.7
1999	12	3	4	19	6.0
2000	18	5	2	25	8.0
2001	10	0	0	10	3.2
2002	14	1	8	23	7.3
2003*	13	3	2	18	5.7
	282	***15***	***18***	***315***	***100***

* January to June 2003

Table 2. Age group and sex of patients.

Age (year)	Male	%	Female	%	Total	%
< 1	10	3.3	13	4.28	23	7.58
≥ 1 - ≤ 10	87	28.62	53	17.43	140	46.05
> 10 - ≤ 20	26	8.55	25	8.22	51	16.77
> 20	37	12.17	53	17.43	90	29.60
	160	***52.64***	***144***	***47.36***	***304***	***100***

Primary myopathies including a variety of diseases represent 27.98%. Neurogenic muscle diseases accounted for only 12.82%. Analysis of the pathological diagnoses found that they were matched with ten categories of the reference[3]. As mentioned, our diagnoses were made on a morphological basis. It is worth clarifying features or diagnostic criteria of our patients that are compatible with each category. General features of each disease are described elsewhere[1,3-6]. Details of extracategories are discussed afterward.

1. Diseases associated with sarcolemmal and extracellular matrix defects. This group includes well-known dystrophinopathies, (Duchenne and Becker muscular dystrophy, DMD and BMD), and sarcoglycanopathies. There were 54 patients in this study. The diagnosis was made by clinically DMD and was histologically compatible with DMD. Characteristic features of DMD include very high serum creatine kinase (CK), hypercontracted fibers and endomysial fibrosis even in the early course of disease (Figure1). Forty-nine male children younger

Table 3. Number of patients in each disease categories.

Disease categories	No. of case	%
Diseases associated with sarcolemmal & extracellular matrix defects	***54***	***17.76***
Duchenne muscular dystrophy	49	
Becker muscular dystrophy	5	
Diseases associated with myonuclear abnormalities	***2***	***0.66***
Centronuclear myopathy	2	
Myofibrillar and internal cytoskeletal proteins	***4***	***1.31***
Nemaline myopathy	2	
Central core disease	2	
Myopathy based on complxe molecular defects	***6***	***1.97***
Myotonic dystrophy	1	
Facioscapulohumeral muscular dystrophy	5	
Developmental disorders of skeletal muscle	***2***	***0.66***
Myotubular myopathy	1	
Congenital fiber type disproportion	1	
Disorders of catabolic mechanisms	***2***	***0.66***
Pompe's disease	2	
Myopathies affecting fuel and energy metabolism (Mitochondrial encephalo-myopathies)	***15***	***4.94***
Kearns-Sayre Syndrome	5	
Chronic progressive external ophthalmoplegia	3	
Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episode	2	
Seizure	2	
Weakness	2	
Leigh's syndrome	1	
Dysimmune and infectious myopathies	***27***	***8.89***
Polymyositis	7	
Dermatomyositis	10	
Other dysimmune myositis	7	
Infectious myositis	3	
Toxic and iatrogenic disorders	***1***	***0.33***
Drug induced rhabdomyolysis	1	
Effects of chronic denervation and disuse on muscle	***39***	***12.82***
Spinal muscular atrophy	21	
Other neurogenic muscle diseases	18	
Normal	***1***	***0.33***
Non-specific change	***78***	***25.66***
Unclassified muscular dystrophies	***21***	***6.9***
Limb girdle muscular dystrophy	5	
Congenital muscular dystrophy	2	
Unclassified muscular dysrtophies	14	
Unclassified myopathies	***32***	***10.53***
End stage muscle diseases	***5***	***1.64***
Unsatisfactory samples	***12***	***3.95***
Miscellaneous	***3***	***0.99***
Total	***304***	***100***

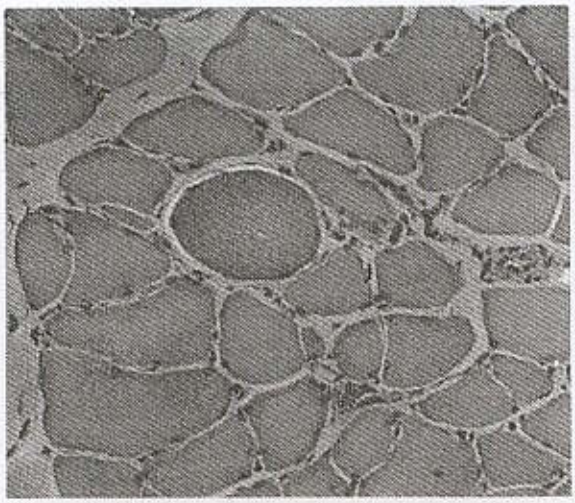

Figure 1. Duchenne muscular dystrophy demonstrates hypercontracted fiber in the center with adjacent muscle necrosis, regeneration and endomysial fibrosis. HE. 200x.

than 10 years of age were found to be DMD. Only two patients were confirmed by dystrophin staining. One was an aborted fetus at 22-week gestation. The other was a boy who had no family history of muscle disease and no deletion detected by multiplex deletion technique. In this circumstance, a simple muscle biopsy can provide definite diagnosis.

Among five cases of BMD, four patients were older than 20 years. One interesting patient presented with isolated bilateral quadriceps atrophy for 4 years. Dystrophin staining was incomplete and patchy pattern, compatible with BMD[7]. One problematic case was a boy who was noticed walking on his toes at the age 5. The biopsy was done at the age of 10. It showed mild dystrophic features, more pronounced variations in fiber size, less necrosis and fibrosis. The diagnosis of DMD or BMD was debated. DMD and BMD are disease-continuum concepts. In such cases, cryostat section with dystrophin immunostaining is a diagnostic tool.

2. Diseases associated with myonuclear abnormalities. Two siblings, one male one female, had childhood-onset with autosomal recessive inheritance. Both had slow progressive muscle weakness and deformities . Limb girdle muscular dystrophy (LGMD) was our first impression. Their unique histopathology showed numerous centrally-placed nuclei, type I predominance and relatively small type I fibers, compatible with centronuclear myopathy. Without clinical history and physical examination, the biopsy may mimic myotonic dystrophy.

3. Myofibrillar and internal cytoskeletal proteins. Nemaline myopathy and central core disease are in this category but are grouped under congenital myopathies in other textbooks[1,6].

3.1 *Nemaline myopathy*. Two patients had different manifestations, severe infantile form and an adult onset. The former was a 4-year-old girl who suffered from pneumonia and required ventilator support since birth. She also had a typical myopathic face, i.e. high arch palate. The latter was a 52-year-old female diagnosed with inclusion body myositis. Both biopsies exhibited numerous rods structures. The rods were bright red on mGT. and electron dense needle shaped on EM (Figure 2).

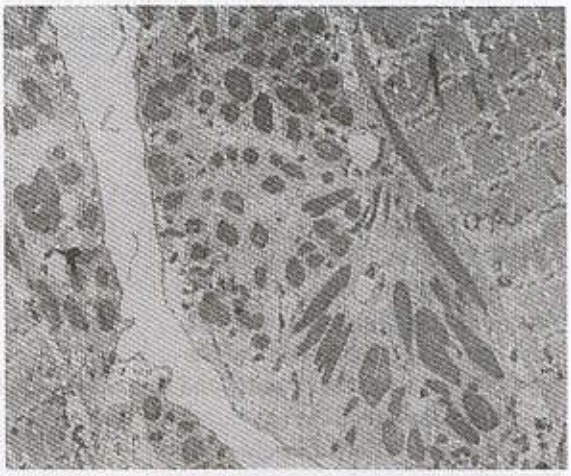

Figure 2. Nemaline myopathy. Muscle fibers contain numerous subsarcolemmal rods structures on EM. 12320x

3.2 *Central core disease*. One patient was an 11 year-old girl and the other was 14 years old. The record of the former query mitochondrial disease while the latter had muscle weakness and stiffness of the joints since a very young age. The

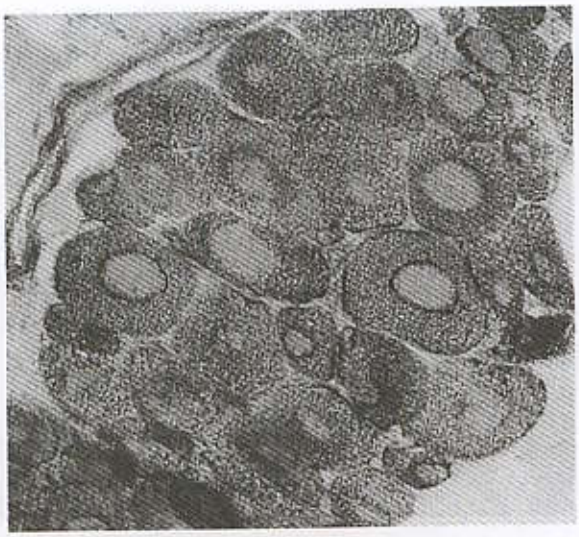

Figure 3. Central core disease. The cores devoided of oxidative enzyme activities with intense rimming. NADH-TR. 400x.

biopsies revealed predominance minicores in the former and central cores in the later (Figure 3). The cores of both types devoided oxidative enzyme activities with or without intense rimming.

4. Myopathies based on complex molecular defects. This group includes myotonic dystrophy, facioscapulohumeral dystrophy, and oculopharyngeal muscular dystrophy.

4.1 *Myotonic dystrophy*. One patient was clinically diagnosed as myotonic dystrophy. The biopsy exhibited variable fiber size, profuse internal nuclei, type I predominance, and type I atrophy (Figure 4). Such findings could be seen in myotonic dystrophy as well as in childhood or adult onset centronuclear myopathy. Although some architectural changes, such as sarcoplasmic masses and ring fibers, distinguish myotonic dystrophy from centronuclear myopathy, clinical correlation with myotonia is more useful.

4.2 *Facioscapulohumeral dystrophy (FSHD)*. Two patients were clinically and pathologically compatible with FSHD. Another three patients from a family of Leber's hereditary optic neuropathy (LHON) had clinical characteristics of FSHD. One biopsy showed variable in fiber size, occasional small angulate fibers, clusters of lymphocytes and fiber type II predominance, consistent with FSHD. The other two were non-specific myopathy and endstage muscle disease.

There were three patients suspected for oculopharyngeal muscular dystrophy. None of them were confirmed by muscle biopsies.

5. Developmental disorders of skeletal muscle.

5.1 *Myotubular myopathy*. A floppy male infant who was first diagnosed as infantile spinal muscular atrophy (SMA), but subsequent SMA gene analysis was unremarkable. A muscle biopsy was performed, which showed typically abnormal small cross sectional diameter with large centrally-placed nuclei, enhanced oxidative enzyme activities (Figure 5) and PAS positive in the center of fibers. The patient died soon after, and the autopsy confirmed this findings.

5.2 *Congenital fiber type disproportion. (CFTD)*. A male neonate presented with persistent hypoglycemia, cardiomegaly and hypotonia since birth. Muscle biopsy revealed fiber size in bimodal pattern. Atrophic fibers and on occasion hypertrophic fibers were predominant type I. Excess glycogen or lipid was not found. Type one hypotrophy is the cardinal feature of CFTD (Figure 6) but also is a common finding in any congenital myopathy. We diagnosed CFTD by the early onset of the patient and had no other specific structural defects or accumulation, other than fiber type abnormality.

6. Disorders of catabolic mechanism.

Acid maltase deficiency (glycogenoses type II) is a disordered lysosomal catabolism of glycogen. Three main phenotypes are infantile (Pompe's disease), juvenile and adult myopathies.

Two patients were an infantile type. One was a female infant who had hypotonia, hepato- cardiomegaly. The muscle fibers contained large amount of glycogen (PAS positive, diastase labile material) and numerous glycogen granules on electron micrographs. The other was a 25-week gestation fetus. The autopsy found skeletal muscle, cardiac muscle and liver fully packed with glycogen.

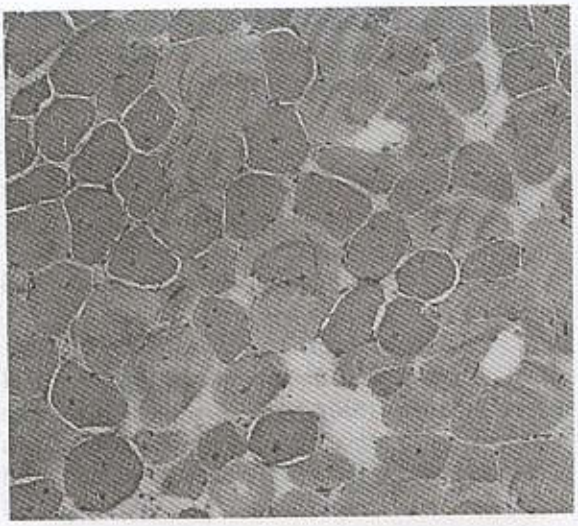

Figure 4. Myotonic dystrophy. Numerous central nuclei and whorl fibers. HE. 200x.

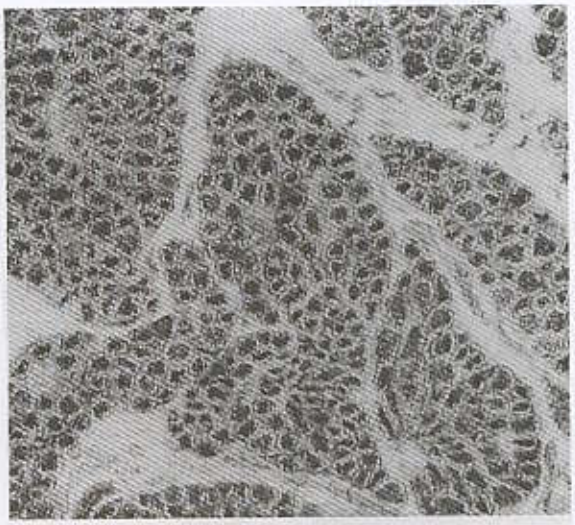

Figure 5. Myotubular myopathy. Abnormal small fibers, enhanced oxidative enzyme activities with centrally- placed nuclei. NADH-TR 400x.

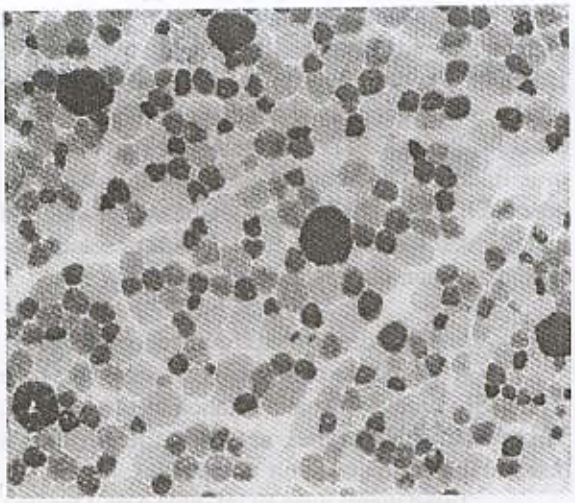

Figure 6. Congenital fiber type disproportion. Most type 1 fibers are smaller than type 2. ATPase 4.6 400x.

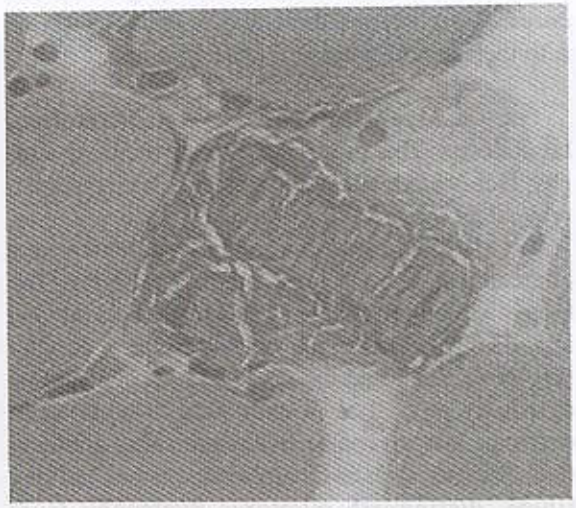

Figure 7. Mitochondrial myopathy. Subsarcolemmal granular deposit forming ragged red fiber. mGT.400x.

7. Myopathies affecting fuel and energy metabolism.

Mitochondrial encephalomyopathies are heterogeneous disorders caused by defective mitochondrial oxidative phosphorylation (OXPHOS). Clinical syndromes associated with gene defects have been described[8]. Virtually all tissue has been shown to be involved, but none is more appropriate for diagnosis in most cases than skeletal muscle. Histopathology of mitochondrial myopathy including ragged red fiber (RRF) (Figure 7), strong subsarcolemmal SDH activity (so-called ragged blue fiber), COX negative fiber, and abnormally shaped enlarged mitochondria, with or without inclusions. On EM(Figure 8). These findings are recognized in certain mitochondrial diseases, particularly mitochodrial DNA deletions or t RNA mutation. These result in defective mitochondrial protein synthesis; for example, myoclonus epilepsy with ragged red fibers (MERRF), Kearns-Sayre Syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), and mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). With exception, COX positive fiber and strong SDH activity in intramuscular vessels (SSV) are characteristics of MELAS. On the other hand, in Leigh syndrome, Leber's hereditary optic neuropathy (LHON) and most nuclear DNA defects, the myopathology is not diagnostic. Hence, biochemical and genetic diagnosis are more important[9].

Fifteen patients had mitochondrial diseases. Some of them were reported[10,11]. The patients' phenotype was demonstrated in Table 3. Thirteen patients were confirmed by histopathology. Two patients did not have specific changes on the biopsies. One was a boy with seizures. He was proven by mitochondrial deletion on PCR. The other patient, a 10-year-old girl, had Leigh syndrome and, as expected, a muscle biopsy was normal. Two patients were clinical MELAS. One biopsy had characteristics of MELAS including COX positive fibers and SSV. The other one displayed numerous COX negative fibers without RRF. It raised the possibility of Complex IV deficiency in this case. In addition, there were three patients from the same family who had LHON (G11778A mutation) and FSHD phenotype. They were classified under the FSHD in this study.

8. Dysimmune and infectious myopathy.

Twenty-seven patients were in this category.

8.1 *Dysimmune myopathies.*

Polymyositis and dermatomyositis. Polymyositis is a T-cell mediated disorder affecting muscle. Seven patients were clinically diagnosed with polymyositis. The biopsies showed lymphocytes and mononuclear cells infiltration in the endomysium rather than the perimysium (Figure 9). Lymphocytes invading muscle fiber were a key feature of polymyositis. Some patients were previously treated with steroids and inflammatory cells were sparse. Without clinical information, the diagnosis was doubtful.

Unlike polymyositis, dermatomyositis is an antibody mediated vascular disorder. Vascular injury resulting in perifascicular atrophy of muscle fascicles is a common finding (Figure 10). There were ten patients whose biopsies showed characteristic perifascicular atrophy, with or without muscle necrosis and/or regeneration. Perivascular lymphocytic infiltration in perimysium or endomysium varied from case to case.

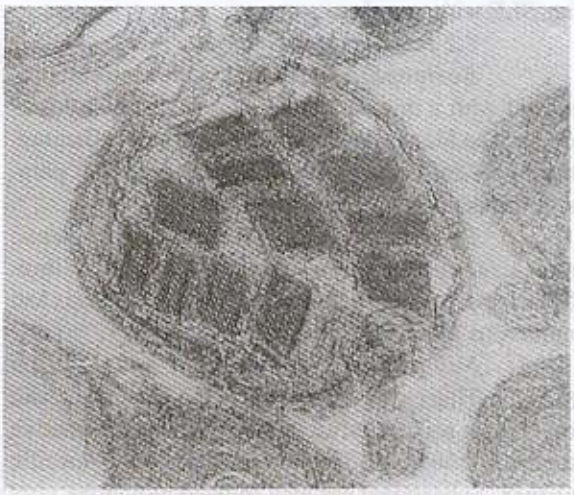

Figure 8. Mitochodrial myopathy. Parking lot inclusions in abnormal mitochondria on EM. 154000x

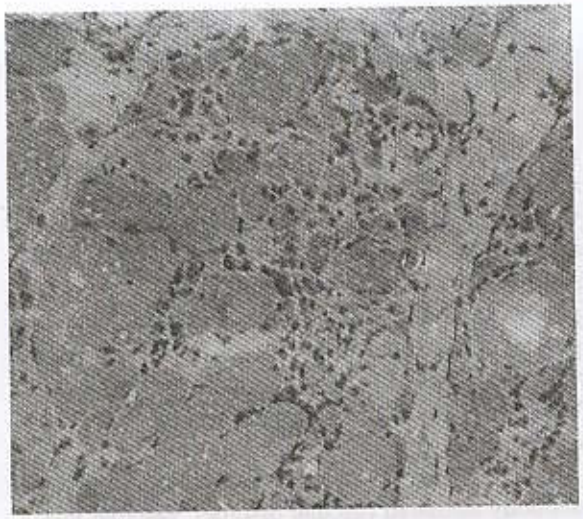

Figure 9. Polymyositis. Intense endomysial lymphocytic infiltration and necrosis of muscle. HE.200x.

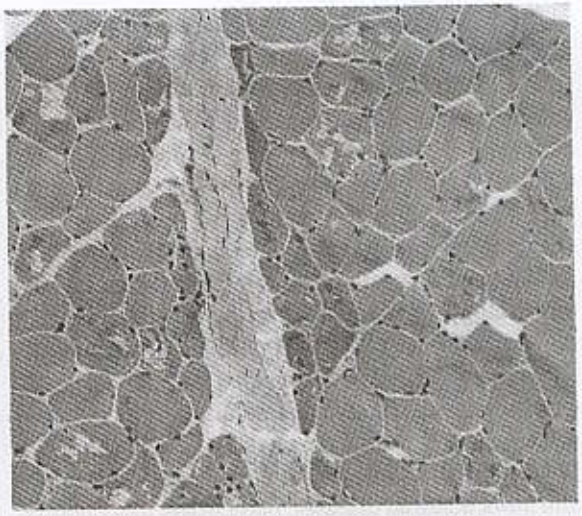

Figure 10. Dermatomyositis. Perifascicular atrophy. HE.200x.

Other dysimmune myopathies. Seven patients showed perimysial and perivascular lymphocytic infiltration similar to those seen in dermatomyositis without perifascicular lesion or vasculitis. Some of them had other connective tissue disease, i.e. SLE with muscle symptoms.

8.2 *Infectious myopathy.*

Eosinophilic myositis. One patient presented with a muscle mass, queried rhabdomyosarcoma. The biopsy demonstrated localized inflammation with eosinophilic infiltration, suggesting parasitic infestation.

Bacterial myositis. One patient had septic shock, acute rhabdomyolysis and renal failure. Muscle necropsy revealed extensive myonecrosis and numerous gram positive cocci.

Subacute sclerosing pan encephalitis (SSPE). There was a case of SSPE presented with myoclonic seizure. Muscle biopsy was performed in the early course of disease and showed no specific change. Subsequent autopsy showed numerous viral inclusions in the brain and spinal cord.

9. Toxic and iatrogenic disorders.

Certain drugs may cause muscle injury with several types of clinical presentation, ranging from mild myalgia to fatal rhabdomyolysis. One patient in this study had acute rhabdomyolysis without inflammatory reaction (Figure 11). He had HMG-CoA reductase inhibitor (statin) and Lipoprotein lipase activator (gemfibrozil) intake for hyperlipidemia. Statin is one of the common drug known to have myotoxicity[12] and was thought to be the cause of rhabdomyolysis in this patient.

10. Effects of chronic denervation and disuse on muscle.

Denervated muscles are altered muscles secondary to interruption of motor axon from any cause. The striking morphological changes of denervated muscle are shrinkage in cross-sectional areas and small or large group atrophy (Figure 12), depending on numbers of disrupted axons. Fiber type grouping is evidence of reinnervation. It is usually not possible to pinpoint the etiology of the denervating disease from a study of the muscle biopsy[5].

Twenty-one patients were clinically and histopathologically spinal muscular atrophy (SMA). The patient's age ranged from 5 months to 25 years. Eighteen patients (85%) were under 10 years. SMA is the most common cause of denervation in infancy. Most of our young patients presented with floppy

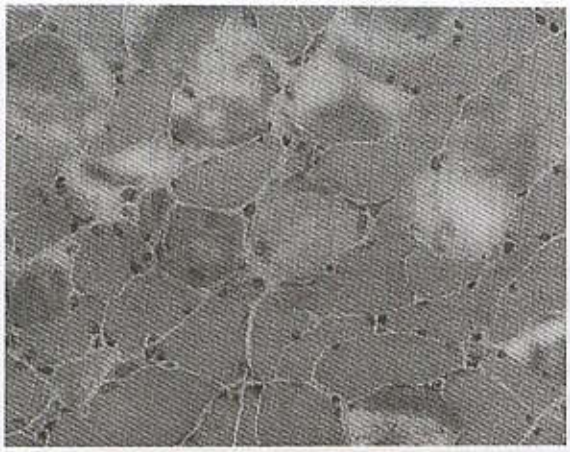

Figure 11. Drug-induced myopathy. Extensive muscle necrosis without inflammation. HE.400x.

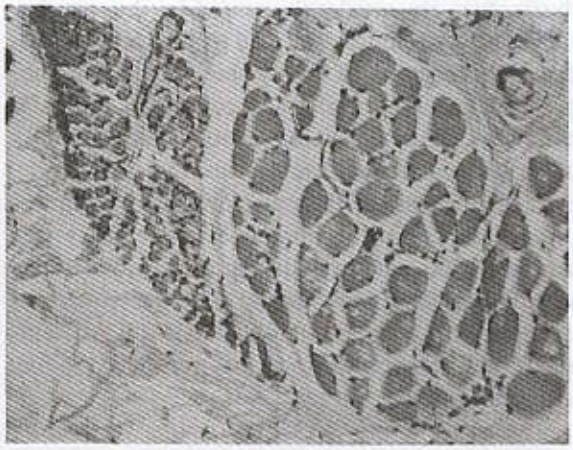

Figure 12. Spinal muscular atrophy. Large group atrophy. HE.400x.

infant, hypotonia, arthrogryposis and were already diagnosed by clinical. The milder form was more easily confused with muscular dystrophy. Some muscular dystrophy may exhibit neurogenic features on biopsy.

Eighteen patients were neurogenic muscle diseases of unspecified etiology. One patient had post-polio syndrome and displayed chronic long-standing denervation/reinnervation features. One patient was clinically amyotrophic lateral sclerosis (ALS). Another 18 year-old female had muscle weakness and high CK level, which suggested muscular dystrophy, in particular Duchenne's carrier. Her biopsy showed group atrophy and fiber type grouping. In addition, dystrophin immunostainings were normal.

There were a number of patients that we could not diagnose for various reasons. They were discussed under extracategories proposed exclusively in this study.

11. Normal muscle. There was only one normal muscle for control. The necropsy obtaining from a man dying of hepatocellular carcinoma was performed in 1989 when snap frozen technique was commenced.

12. Non-specific changes. Non-specific changes accounted for the largest group in this study (25.66 %). No sexual preference is reported. According to the request form, clinical diagnoses of this group were diverse and usually nonspecific, including weakness, hypotonia, delayed development, etc. Received clinical information was of little help in drawing any diagnostic conclusion. To make diagnosis of neuromuscular dieases, we earnestly required some fundamental data encompassing the onset of symptoms in chronology, familial history, drug exposure and a physical examination. EMG, serum creatinine kinase (CK) level, and both serum and CSF lactate level are also important.

To minimize these problems, a muscle biopsy request form was recently designed in May, 2003. The main purpose was to obtain significant clinical data. Morphology per se was not adequate for pathological diagnosis of neuromuscular diseases.

13. Unclassified muscular dystrophies. Twenty-one patients (seven males and fourteen females) had dystrophic muscle, i.e. marked variation in fiber sizes, muscle necrosis, and regeneration and muscle fiber loss. According to the onset, muscular dystrophy can be classified into congenital form

in cases of symptoms occurring since birth. Other dystrophies are previously named by the distribution of affected muscles. Based on clinical information, only seven out of twenty one patients were subgrouped into limb girdle and congenital muscular dystrophy. Without clinical characteristics, family history, immunohistochemistry, and molecular study, precise diagnosis of muscular dystrophy is almost impossible. Since the immunohistochemistry was started in 2002, if antibodies are available, biopsy samples obtained later should not have equivocal interpretation. However, this benefit is still problematic for the archived samples that transferred to formalin prior to the year 2001.

13.1 *Limb girdle muscular dystrophy (LGMD).* This entity encompasses a heterogeneous group of muscular dystrophies. Various clinical criteria have been established to characterize LGMD[6]. Only five patients were clinicopathologically compatible with LGMD. All but one were female and were shown to have autosomal recessive inheritance. At first, one male patient was queried Becker muscular dystrophy (BMD) or LGMD, but subsequently dystrophin immunocytochemistry showed no defect of membrane staining.

13.2 *Congenital muscular dystrophy.* Two patients were in this group. Both were girls who had hypotonia and arthrogryposis since birth. Spinal muscular atrophy (SMA) was a differential diagnosis. Muscle biopsies demonstrated dystrophic features and end stage muscle disease. Generally, biopsy of SMA is rather clear-cut. Now, with available merosin, emerin and sarcoglycan antibodies, new cases of congenital dystrophy can be classified specifically.

14. Unclassified myopathy. These biopsies were taken from patients who had neuromuscular diseases and showed abnormal findings which were neither dystrophy nor neurogenic muscle diseases. But definite diagnoses could not be made. One exceptional case was a fifty-nine year old man who had sleep apnea and progressive muscle weakness within two years. The muscle biopsy revealed muscle necrosis, phagocytosis and extraordinary myofibrillary changes. There were numerous tubular aggregates and rimmed vacuoles. Tubular aggregates are observed in certain conditions, such as periodic paralysis, alcoholic myopathy, and familial occurrence[3-5]. Rimmed vacuoles were a hallmark of inclusion body myositis, oculopharyngeal muscular dystrophy and distal myopathy[5]. None of the aforementioned clinically applied to this patient. This myopathy remained unclassified.

15. Unsatisfactory specimens. Unsatisfactory specimens referred to specimens containing very little muscle tissue or specimens with artefacts occurring before transfer to a laboratory. For example, cauterized or soaked muscle, delayed freezing tissue, or specimen with artefacts during tissue processing. These are major problems of muscle biopsy interpretation. To get optimum quality of the samples, the following should be taken into consideration:

15.1 *Biopsy site.* If the disease is generalized, the biceps are preferable because most fibers are in parallel fashion. The deltoid is another good example for type I affected diseases. Previous injection or EMG sites should be avoided.

15.2 *Sample size.* A cross section of muscle is much more informative than a long section. A 0.5x0.5 cm cut surface and 1 cm or more in length of muscle is adequate.

15.3 *Transportation.* The specimen should be kept in moist gauze, tightly pack containers, and sent with ice cubes (not dry ice) to the laboratory as soon as possible.

15.4 *Freezing technique.* Rapid fixation is the fundamental and the most important factor. Improper freezing will result in unavoidable cutting and staining artefacts.

Although muscle biopsy is a rather simple procedure, it requires a keen physician to select the muscle group, perform a gentle biopsy, and still obtain adequate tissue. Needle biopsy is another standard technique performed in many institutes other than Siriraj Hospital. It may be suitable for very young patients. To solve the problems of unsatisfactory specimens we request at least one hour advance notification from the clinician. A guideline for muscle biopsy procedure will be given in return. Laboratory technicians will go to the operating room and carry specimens back to the laboratory. We have had no unsatisfactory samples since 1999.

16. End stage muscle diseases. End stage muscle informs nothing but inappropriate selected muscle for biopsy. Most muscle diseases are generalize involvement but differ in severity. For mild neuromuscular diseases, only severely affected muscle may reveal pathology, while in severe chronic longstanding muscle disease, moderately affected groups should be selected. For mitochondrial disease with ophthalmoplegia, deltoids are preferable to ocular muscles. Since most neurologists do not perform biopsy themselves, the biopsy site should be clearly specified to the surgeons.

17. Miscellaneous.

There were two disorders to be mentioned.

17.1 *Rhabdomyolysis.* This is a clinical and biochemical syndrome resulting from skeletal muscle injury that alters the integrity of the muscle cell membrane sufficiently to allow the release of muscle cell contents into the plasma[6]. Rhabdomyolysis can be classified in several ways, i.e. traumatic versus non-traumatic, exogenous versus endogenous, or hereditary versus non-hereditary. Patients should be catagorized by whether or not they have pre-existing muscle diseases. Our data contained 4 cases of clinical and histopathological characteristics of rhabdomyolysis without underlying myopathy. One patient was described in the text earlier as Statin-induced myopathy. One patient had bacterial myositis with extensive myonecrosis. One patient had history of myalgia after exercise then developed fever and renal shutdown. McArdle's disease was suspected, but was excluded by the absence of excess glycogen. The last patient had chronic renal failure, respiratory failure and acute rhabdomyolysis in an intensive care unit. Although we suspected critical illness myopathy in this patient, myosinolysis (the absence of myosin-ATPase reactivity in non-necrotic fibers) was not seen in the biopsy.

17.2 *Reye's syndrome.* One patient had fluliked symptoms and acute encephalopathy. The autopsy demonstrated microvesicular fatty changes in many organs, including striated muscle and cardiac muscle. Lipid droplets were clearly seen on oil red O and EM.

Future trend

Knowledge of muscle diseases is rapidly growing. At present, there are five neuromuscular laboratories in Thailand. Four of them, Siriraj Hospital, Ramathibodi Hospital, Prasart Hospital, and Chulalongkorn Hospital, are in Bangkok, and the other is at Maharaj Hospital in Chiang Mai. These centers provide standard methods of myopathology. Interconnection among laboratories should be made so that they can share their experience and laboratory resources. Clinicians and pathologists may refer fresh samples or slides to these laboratories. Even in remote areas, a mobile muscle biopsy unit is still possible.

We hope that, in the near future, we can elaborate study method to molecular pathology and can establish neuromuscular research center. We can obtain better understanding of neuromuscular diseases in Thais. We can provide more accurate diagnosis and appropriate treatment to our patients who suffer from neuromuscular diseases.

CONCLUSION

The myopathology laboratory has been gradually developed at Siriraj Hospital and is in progress. Although a few cases compare with other tissue samples in the department files, our data from a fourteen-year analysis clearly reveals the following:

- Muscle diseases are clinicopathologic entities. Collaboration of clinicians and pathologists is necessary.
- We have a wide variety of neuromucular diseases caused by both intrinsic and extrinsic factors.
- All age groups are affected, from intrauterine life to the elderly, with different preferences in each age group.
- At least twenty five percent of the cases are undiagnosed, and this percentage has to be reduced.

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