Primary Muscle Diseases in Thammasat University Hospital: Muscle Biopsy Study of 12 Cases

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We reviewed retrospectively 12 muscle biopsies of patients who were clinically diagnosed with a primary muscle diseases from the clinical data base of Thammasat University Hospital from January 2005 to January 2007. Most patients were male and had median age of 30.5 years (range 14 to 56). The most common clinical presentation was proximal muscle weakness. Nine of eleven patients had elevated CK concentrations ranging from 338 to 1,023 IU/L. Clinicopathological correlation revealed specific diagnoses in nine patients. Suspected cases of mitochondrial neurogastrointestinal encephalopathy (MNGIE), myofibrillar myopathy (MFM) and distal myopathy with rimmed vacuoles (DMRV) were confirmed by molecular genetic studies examing thymidine phosphorylase, GNE, ZASP, myotilin, desmin, aβ-crystalline and filamin C genes. Specific histopathological findings on muscle biopsy help to select cases for advance molecular testing.

Keywords: *Muscle biopsy, Myopathy, Muscular dystrophy, Molecular genetic analysis*

J Med Assoc Thai 2010; 93 (Suppl. 7) : S236-S240
Full text. e-Journal: *http://www.mat.or.th/journal*

Primary muscle diseases are an uncommon group of neuromuscular disorders in clinical practice and are often difficult to diagnose, resulting in under diagnosis and under reporting. There are no curative treatments for most of primary muscle diseases; therefore, accurate diagnosis is essential to differentiate primary muscle diseases from secondary muscle diseases which may be amenable to treatment.

Whilst the clinical manifestations of PMDs and their routine laboratory investigations are helpful in the initial clinical assessment, muscle biopsy and non-invasive molecular genetic analysis are the definitive tools for accurate diagnosis[1-4]. Here, we report the role of muscle biopsy in order to select cases for advanced genetic studies in patients with primary muscle diseases diagnosed at Thammasat Universtiy Hospital, Bangkok, Thailand.

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Material and Method

We reviewed retrospectively the clinicopathological data of 12 patients who were clinically diagnosed with primary muscle diseases at Thammasat University Hospital from January 2005 to January 2007. Neurogenic muscular diseases and secondary muscular disorders were excluded. All muscle biopsies were studied for routine histopathology and enzyme activities using H & E and special stains, respectively, in collaboration with the Department of Pathology, Faculty of Medicine Siriraj Hospital, Bangkok. Blood samples for genetic analysis of patients with suspected mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), myofibrillar myopathy (MFM) and distal myopathy with rimmed vacuole (DMRV) were examined at the Department of Neurology, Columbia University College of Physicians and Surgeons, New York; the Department of Neurology and Neuromuscular Research Laboratory, Mayo Clinic, Minnesota; and the Department of Pathology and Neurogenetics Network, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok,

Thailand, respectively.

Results

Eight of 12 patients were male. The median age of all patients was 30.5 years (range 14 to 56 years). The most common clinical presentation was proximal muscle weakness (n = 8) resulting in difficulty standing up from sitting position and climbing up stairs. Initial clinical diagnoses included facioscapulohumeral muscular dystrophy (n = 2), myotonic dystrophy type 1 (n = 1), Duchenne muscular dystrophy (n = 1), distal myopathy with rimmed vacuoles (DMRV, n = 1), polymyositis (n = 1), mitochondrial neurogastro-intestinal encephalopathy (MNGIE, n = 1), unspecified myopathies (n = 3) and muscular diseases associated with connective tissue diseases (n = 2). CK data were available in eleven patients; of these nine had elevated CK level (Table 1).

Myopathic change was the most common pathological findings (n = 5). Severe end stage muscle disease and negative membrane staining for dystrophin 1 and dystrophin 2 were demonstrated in the suspected case of Duchenne muscular dystrophy. The clinically suspected case of DMRV displayed typical rimmed vacuoles on H & E staining and modified Gomori's trichrome staining (Fig. 1A, B). In the suspected case of MNGIE, the muscle biopsy revealed a few COX-negative fibers (Fig. 2) and rare ragged blue fibers. One of the patients with a clinical diagnosis of a non specific myopathy exhibited typical intracytoplasmic accumulation of MFM characterized by well-demarcated sarcoplasmic hyaline plaques which expressed a blue to purple in color with the modified Gomori trichrome staining (Fig. 3). Muscular dystrophy was found in one of two cases of clinically suspected FSHD. Two cases revealed no specific change on muscle biopsies.

Blood samples from the patients with MNGIE, MFM and DMRV were further studied by molecular analysis. The MNGIE case contained homozygous

Table 1. Patient data and clinical information

Case	Sex	Age	Clinical manifestation	CK level	Initial clinical diagnosis
1	male	14	Proximal muscle weakness and calf pseudohypertrophy	Not performed	Duchenne muscular dystrophy
2	male	18	Weakness of anterior and posterior compartment of distal leg	643	Myopathy cause?
3	male	20	Extra-ocular muscle weakness, ptosis, GI dysmotility, leukoencephalopathy	192	MNGIE
4	female	25	Proximal muscle weakness	585	FSHD
5	male	26	Wasting of limb muscle, suprascapular muscle atrophy, hand grip weakness, dystrophic face, pectus excavatum, fixed split S2, left ventricular hypertrophy	755	Myotonic dystrophy type 1
6	female	29	Proximal muscle weakness	1023	Myopathy cause?
7	female	32	Proximal muscle weakness	370	Unidentified connective tissue disease
8	male	37	Distal muscle weakness, weakness of foot dorsiflexion and foot drop	682	DMRV
9	female	38	Proximal muscle weakness	992	Overlapping syndrome (SLE & scleroderma)
10	male	48	Proximal muscle weakness	57	Myopathy cause?
11	male	55	Proximal muscle weakness, overlap syndrome SLE & scleroderma	953	Polymyositis
12	male	56	Proximal muscle weakness, wing scapular, atrophy of deltoid, left ventricular hypertrophy	338	FSHD

CK = Creatinine Kinase
Normal CK level = 21-232 IU/L

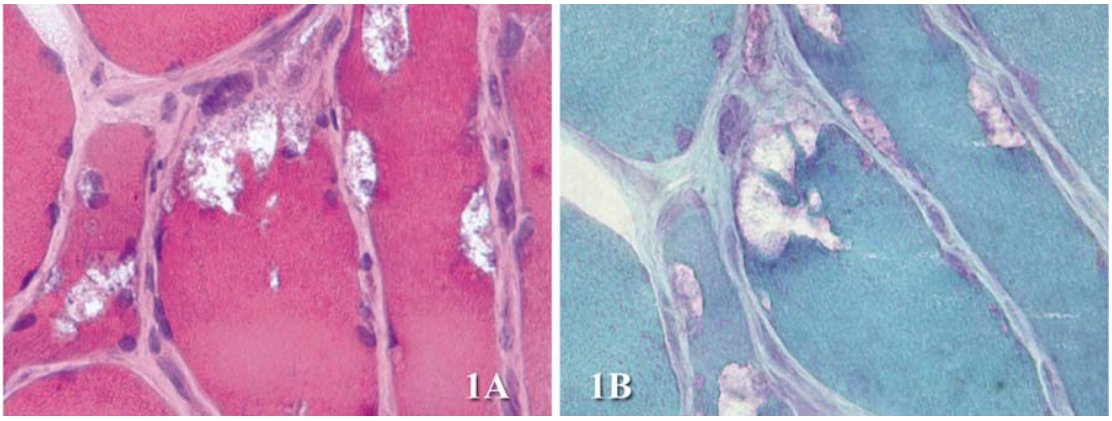

Fig. 1 (A) Muscle biopsy showed rimmed vacuoles on H&E (600x) and (B) modified Gomori Trichrome (600x) staining in DMRV patient.

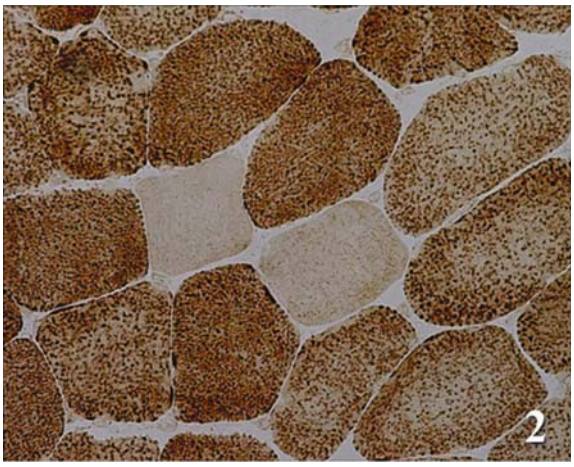

Fig. 2 A few scattered COX-negative fibers were found in muscle biopsy of MNGIE patient (COX, 400x)

c100insC on thymidine phosphorylase (TP) gene. The candidate genes of MFM including ZASP, myotilin, desmin, *a*β-crystalline and filamin C were analyzed but there were no demonstrable mutations. Compound heterozygous c869T > A (pL290X) and c2086G > A (pV696M) mutations were detected in the GNE gene of the DMRV patient (Table 2).

Discussion

In this small retrospective study, we set out to examine only patients with suspected primary muscle disease and excluded cases of neurogenic and secondary muscular disorders, in order to evaluate the true value of muscle biopsy[4]. Consistent with the findings of others[1-4], this study demonstrates the benefit of muscle biopsy in order to help in diagnosis of muscular disorders. We found that nine out of twelve patients provide specific diagnoses using clinical phenotypes, muscle biopsies and genetic analysis. One patient with the rare diagnosis of MFM has a significant change between pre- and post-biopsy diagnosis[5,6]. As a result, this allowed us to manage patients better by giving specific treatment and advising patients of the prognosis.

However, some studies have not found muscle biopsy helpful, especially in children[7]. In studies of children, congenital myopathies or muscular dystrophies are the most common final diagnoses, therefore, certain diagnoses do not lead to specific treatment or a change in the prognosis. Because muscle biopsy is significant event for parents, carries operative risks and contributes to the increasing cost of medical care, that study encourages the informed and selective use muscle biopsy in pediatric patients[7].

Based on knowledge that inflammatory myopathy is classically focalized process, increased diagnostic yield associated multiple simultaneous muscle biopsies has been reported[8]. In that study, potentially treatable inflammatory myopathies or vasculitis were diagnosable in only 1 out of 2 simultaneous biopsies. However, we did not (use this technique or) repeat muscle biopsy in our two patients with no specific change as we considered that multiple biopsies were not useful in suspected cases of non-inflammatory myopathies such as FSHD and myotonic dystrophy.

Two of three cases which are performed molecular genetic studies had mutations of specific genes. In our MNGIE case, we found a novel mutation on the *ECGF1* gene and is the first time this has been

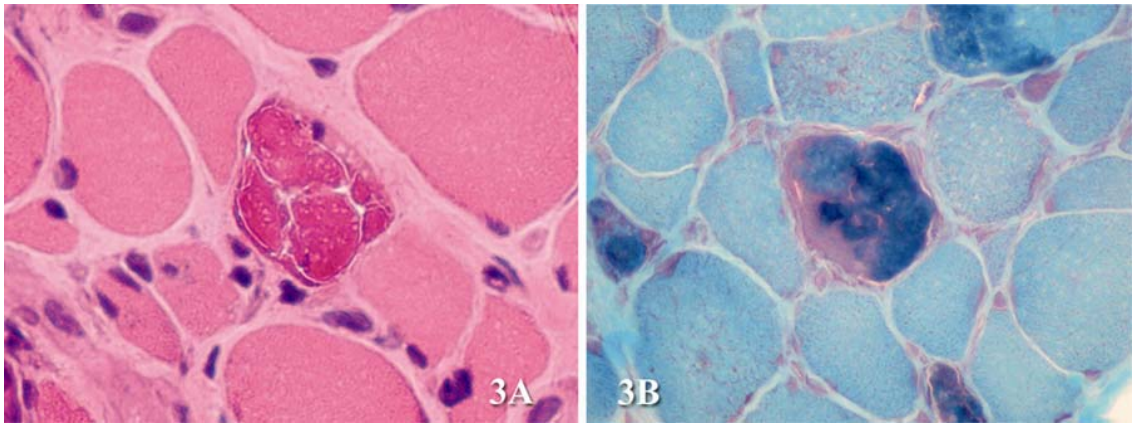

Fig. 3 (A) Sarcoplasmic amorphous hyaline plaques appeared pink on H&E (400x) and (B) blue to purple on modified Gomori Trichrome staining (400x) in MFM patient

Table 2. Muscle biopsy findings and genetic study

Case	Site of biopsy	Muscle biopsy	Genetic study	Clinicopathological diagnosis
1	Calf and thigh	Endstage muscle disease, negative membrane staining Dys1 and Dys2	Not performed	Duchenne muscular dystrophy
2	Anterior tibia	Myopathic change	Not performed	Myopathy cause?
3	Biceps	A few COX-negative fibers, rare ragged blue fibers	Homozygous c.100insC on TP gene	MNGIE
4	Quadriceps	No specific change	Not performed	FSHD
5	Quadriceps	No specific change	Not performed	Myotonic dystrophy type 1
6	Biceps	Myofibrillar myopathy (MFM)	No identified k nown mutations (ZASP, myotilin, desmin, *ab*-crytalline and filamin genes	MFM
7	Quadriceps, right	Myopathic change	Not performed	Unidentified connective tissue disease
8	Anterior tibia	Rimmed vacuoles	Heterozygous c.869T>A (p.L290X) and c.2086G>A (p.V696M) on GNE gene	DMRV
9	Thigh, left	Myopathic change	Not performed	Overlapping syndrome (SLE + scleroderma)
10	Deltoid	Myopathic change, presence of type 2 fiber atrophy	Not performed	Myopathy cause?
11	Biceps	Myopathic change	Not performed	Polymyositis
12	Deltoid	Muscular dystrophy	Not performed	FSHD

described in a Thai patient[9]. The DMRV patient had two mutations of the GNE genes. One is a common mutation found in Thailand (c2086G>A)[10] whilst the other has not been reported previously in Thai patients (c869T > A). These data support our approach of obtaining the muscle biopsy result in order to select

the appropriate patients for advance and expensive molecular studies.

Acknowledgements

The authors are very grateful to Prof. Duygu Selcen, Prof. Ichizo Nishino, Prof. Ikuya Nonaka and Dr. Chanin Limwongse for their expert consultant. The authors also wish to thank Mrs. Naree Choosrichom, Mrs. Pimchanok Sehamo and Mr. Theeraphong Phoiam for technical assistance.

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โรคกล้ามเนื้อชนิดปฐมภูมิในโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ: การศึกษา muscle biopsy ในผู้ป่วย 12 ราย

จุฑาทิพย์ คินทรักษ์, ตุ้มทิพย์ แสงรุจิ, ธีริน ลิ่วลักษณ์, ก้องเกียรติ กูณฑ์กันทรากร, สมบัติ มุ่งทวีพงษา

ผู้วิจัยทำการศึกษาข้อมูลย้อนหลังในผู้ป่วยโรคกล้ามเนื้อชนิดปฐมภูมิ 12 ราย ในโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติจากเวชระเบียนตั้งแต่เดือนมกราคม พ.ศ.2548 ถึง มกราคม พ.ศ. 2550 พบมีผู้ป่วยส่วนใหญ่เป็นเพศชาย อายุเฉลี่ย 30.5 ปี อาการทางคลินิกที่พบส่วนใหญ่คือภาวะกล้ามเนื้อส่วนต้นอ่อนแรง ผู้ป่วยเก้าในสิบเอ็ดรายที่ได้รับการตรวจระดับ CK พบมีระดับ CK สูงขึ้นโดยมีค่าอยู่ระหว่าง 338 ถึง 1,023 IU/L ผู้ป่วยจำนวน 9 ราย ได้รับการวินิจฉัยโรคกล้ามเนื้อชนิดปฐมภูมิด้วยข้อมูลทางคลินิกร่วมกับการส่งตรวจ muscle biopsy ตัวอย่างเลือดของผู้ป่วยที่ได้รับการวินิจฉัยเบื้องต้นว่าเป็น mitochondrial neurogastrointestinal encephalopathy (MNGIE), myofibrillar myopathy (MFM) และ distal myopathy with rimmed vacuoles (DMRV) นำไปศึกษา molecular genetic testing เพื่อหาการกลายพันธุ์ของยีน thymidine phosphorylase, GNE, ZASP, myotilin, desmin, aβ-crystalline และ filamin C genes