Scientific Research and Essays Vol. 7(32), pp. 2882-2890, 16 August, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE12.125
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

Certain morphological features in limb-girdle muscular dystrophies are not shared by polymyositis: Useful diagnostic clues

Omar A. Mahmood, Wen Hu Wang, Li Mei Tan, Yan Li Zhang and Xin Mei Jiang*

Department of Neurology, 1st Hospital of Jilin University, 130021, Changchun, China.

Accepted 11 June, 2012

Some hereditary myopathies can mimic acquired polymyositis (PM) in particular when they first present in adulthood with a limb-girdle distribution of weakness. The diagnosis is made yet more complex when inflammatory infiltrates attend on muscle biopsy. This is common in certain dystrophies in particular limb-girdle muscular dystrophies (LGMD). For better distinction between the two diseases, histochemical morphometric analysis for twenty biopsies of proteomically confirmed LGMD and eight PM biopsies was performed. Each parameter of myopathy was evaluated in five or ten randomly, selected fields, using Image-Pro plus bioanalytical software. Comparison between groups was tested using Student's *t*-test analysis. The muscle of LGMD patients betrayed more splitting fibers and larger connective tissue surface area in comparison to PM patients (p = 0.02, 0.01, respectively). On the opposite, the number of regenerating fibers was higher in myositis biopsies (p = 0.04). Other parameters were akin to those of PM patients. We also described different patterns MHC-I up regulation on LGMD biopsies. We reported some tips of difference between LGMD biopsies and PM.

Key words: Limb-girdle muscular dystrophies (LGMD), polymyositis (PM), histocompatibility complex class I (MHC-I), morphometric.

INTRODUCTION

The limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of disorders described by weakness and wasting of the pelvic and shoulder girdle muscles (Wildförster and Dekomien, 2009; Ojima et al., 2010). The clinical course of LGMD ranges from severe forms and rapid progression within the first decade of life, to milder forms with later onset and slower progression (Zatz et al., 2000). There are now 21 identified forms of autosomal dominant and autosomal recessive LGMD (Gordon et al., 2009) and it is often difficult to discriminate between the various LGMD subtypes (Laval and Bushby, 2004). Therefore, diagnosis of LGMD increasingly relies on a combination of proteomic analyses, followed by DNA sequencing to identify the primary mutation, which is essential for the provision of precise genetic and prognostic counseling. Even with these comprehensive studies, the genetic etiology of many cases of LGMD is not yet recognized (Bushby and Beckmann, 2003).

Non-specific inflammatory infiltrates are observed in many LGMD subtypes, e.g. dysferlinopathy (LGMD2B) (Vinit et al., 2010), LGMD2L with *fukutin* gene mutations (Godfrey et al., 2006), LGMD2I with *FKRP* gene mutation (Darin et al., 2007), and LGMD2N with *POMT2* gene mutation (Biancheri et al., 2007). In these cases, infiltrates consist of macrophages, T cells, and B-cells. In some cases, eosinophilia was reported in patients with *CAPN3* mutations (LGMD2A) (Krahn et al., 2006) and sarcoglycanopathy (Baumeistera et al., 2009). Moreover, LMNA mutations (autosomal dominant and rare recessive forms of Emery–Dreifuss as well as LGMD1B) were reported in patients showing extensive inflammatory infiltrates in the muscle biopsy, which could lead to diagnostic mistake (Lubieniecki et al., 2007).

*Corresponding author. E-mail: jiangxinmei55@yahoo.com.cn Tel: 0086-13843146820, +86-431-82787755. Fax: 0431-88782378

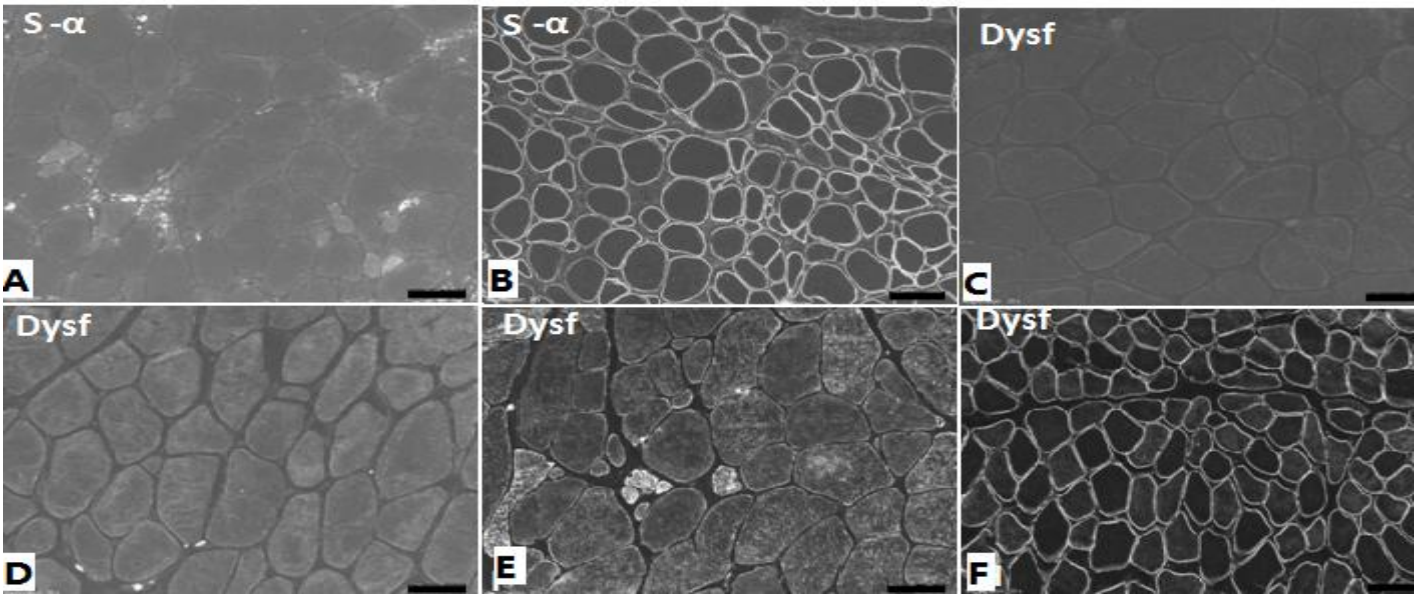

Figure 1. Immunohistochemical labeling of muscle biopsies of LGMD and CN patients. (A) sarcoglycan-α lack; (B) normal control sarcoglycan-α; (C) Dysferlin lack; (D) Dysferlin severe deficiency; (E) Abnormal localization of dysferlin, and (F) Normal control dysferlin. Bar: 50 μm.

The clinical picture of LGMD and polymyositis (PM) is often confusing the clinicians. Muscle biopsy from both patients, as aforementioned, may demonstrate inflammatory infiltrates. Dramatic progress in proteomics and molecular biology in the last decade had made pathologist somewhat entirely dependent on immune-histochemical, immunoblot, and genetic screening results in differentiating the two diseases. However, in some undeveloped areas in the world where such advanced approaches are out of stock, muscle biopsy and routine histochemical stain are often used to help diagnose muscle diseases. Present literatures focus little on morphological findings of muscular diseases (Figarella-Brangera et al., 2002; Comerlato et al., 2005; Cenacchi et al., 2005; Gayathri et al., 2011), hence, a comprehensive study including various parameters of myopathy and dystrophy, comparing between the most confusing myopathic diseases was necessary. We did morphological analysis of muscle biopsies taken from 20 LGMD patients with inflammatory components. We assessed the mean surface area, mean diameter, muscle fibers' mean density, number of cellular infiltrates, membrane and internal nuclei per single muscle fiber. Mean connective tissue surface area, number of necrotic, regenerating, opaque, and splitting muscle fibers was calculated per field. We also determined fiber type predominance, abnormal NADH-TR fibers, and some other abnormal recognized histological features. We compared these findings with those observed in PM with the aim of making some pathological distinctions between the two diseases.

MATERIALS AND METHODS

Patients

Twenty LGMD patients with inflammatory components were ascertained through the Department of Neurology, First Hospital of Jilin University, Changchun, China. Eleven were diagnosed as dysferlinopathy where dysferlin is absent or severely reduced. Four cases were diagnosed as sarcoglycanopathy where sarcoglycan-α is deficient. The remaining five cases remained unclassified LGMD where calpain-3, caveolin-3, and sarcoglycan-α all was normal on immunoblot, but showed reduction and abnormal localization of dysferlin staining (Figure 1). We further examined muscle biopsies from eight patients with PM. Dalakas and Hohlfeld (2003) definite criteria for PM were used for diagnosis of PM patients. Electrophysiological studies (nerve conduction study and electromyography) reported myopathic changes in both LGMD and PM patients. Clinical, biochemical and electrophysiological features for both LGMD and PM patients are provided on Supplementary Tables 1 and 2.

Muscle biopsies

At the time of diagnosis, an open biopsy from the biceps brachii muscle was obtained under local anaesthesia after written informed consent. Samples were frozen, and stored in liquid nitrogen pending assay.

Histochemical staining

Serial frozen sections were stained with hematoxylin and eosin (H&E), modified Gomori trichrome (MGT), periodic acidic-Schiff, oil red O, nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), cytochrome c oxidase (COX), and acid phosphatase.

None but one patient had received immunosuppressive drugs

Table 1. Comparison of parameters of myopathy in 20 LGMD patients X8 PM cases.

S/N	Parameter	LGMD (mean ± SD)	PM(mean ± SD)	p-value
1	Surface area/muscle fiber (µm²) (X20)	4375.51 ± 2190.10	3146.83 ± 2051.56	0.19
2	Diameter/muscle fiber (µm) (X20)	66.97 ± 18.13	56.38 ± 19.99	0.19
3	Density/muscle fiber(1.98 for black and 255 for white) (X20)	114.66 ± 16.28	105.05 ± 37.54	0.53
4	Inflammatory cells/muscle fiber (X40)	1.03 ± 0.59	1.24 ± 0.81	0.46
5	Membrane nuclei/muscle fiber (X40)	2.79 ± 0.73	2.44 ± 1.03	0.33
6	Internal nuclei/muscle fiber (X40)	0.21 ± 0.16	0.13 ± 0.10	0.18
7	Degenerating fibers /field (X20)	0.75 ± 0.99	2.76 ± 2.68	0.08
8	***Regenerating fibers/field (X20)***	***1.64 ± 1.95***	***3.81 ± 3.13***	***0.04***
9	Opaque fibers/field (X20)	0.1 ± 0.35	0.28 ± 0.44	0.28
10	***Splitting fibers/field (X20)***	***0.85 ± 0.99***	***0.18 ± 0.18***	***0.02***
11	***Connective tissue surface area/field (µm²) (X20)***	***70675.83 ± 32385.95***	***36705.45 ± 16493.34***	***0.01***
12	Type I fiber predominance (%) (X20)	50.1 ± 15.59	41.27 ± 10.65	0.22

Parameters in Bold Italics indicate significant differences.

prior to biopsy.

Immunohistochemistry

Further serial sections were placed on gelatinised slides and were processed for immunohistochemical analyses, using a panel of different antibodies to the following proteins: Dystrophin (Rod C-&N-terminus) (NCL-DYS1,2,3), dysferlin (NCL-Hamlet), from Novocastra Laboratories (Newcastle, UK), α-sarcoglycan (Vector Laboratories, Inc. Burlingame, CA, USA), and mouse monoclonal antibodies were used to identify MHC class I (ABC) (Biolegend, San Deigo, CA). A secondary peroxidase-conjugated goat anti-mouse immunoglobin G (IgG) antibody (KPL, Gaithersburg, USA) was added for 30 min at room temperature.

Western blotting

Muscle biopsy sections were dissolved in Laemmli loading buffer (0.05 mol/L dithiothreitol, 0.1 mol/L ethylenediaminetetraacetic acids (EDTA), 0.125 mol/L Tris, and 4% sodium dodecyl sulfate (SDS), 0.005% bromophenol blue) and were centrifuged. Proteins were resolved by SDS-PAGE and electroblotted to hybond polyvinylidene difluoride (PVDF) membrane (Amersham bioscience, USA). Blots were blocked with bovine serum albumin (fraction V) and then incubated with antibodies against caveolin-3 purchased from BD transduction Laboratory (1:1000), calpain-3 (Calp3d/2C4), diluted 1:400, sarcoglycan-α(1/250), and heavy chain myosin (1/250) were used to normalize the amount of protein loaded in each lane. The latter were purchased from Santa Cruz Biotechnology Inc (California, USA). Immunoreactive bands were visualized using Cy3-conjucated Affinipure goat anti-mouse IgG (1/1000) and Typhoon fluorescent scanner (Amersham bioscience, USA).

Histopathological and immunohistochemical morphological analysis

Each parameter of myopathy in this study was evaluated in five or ten unoverlapped, randomly, selected fields (at X200 or X400 magnification, respectively). During the process of counting the total number of muscle fibers in field, those cut on the left and lower borders were ignored. Image–Pro plus bioanalytical software was used for histochemical morphometric analysis (Figure 2).

Statistical analysis

The SPSS statistical software was used for statistical analysis. Differences between groups were tested by Student's *t*-tests. Two-sided values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The number of splitting fibers was higher in LGMD patients (Table 1 and Figure 3). Connective tissue demonstrated considerable proliferation in LGMD with p value of 0.01 from PM. Abnormal NADH-TR stained fibers include: moth-eaten, lobulated and intermyofibrillar network disorder. The fibers were only detected in LGMD patients with prevalence ranging from 0 to 29.7% (8.71 ± 11.64%) of muscle fiber cross sections, and were found in type I fibers. These features are missing in PM (Figure 4). The regenerating, necrotic, and opaque fibers were fewer in LGMD than PM patients, though, only regenerating fibers showed significant difference ($p = 0.04$).

There was larger mean surface area and longer mean diameter of muscle fibers in the muscle of LGMD patients than in PM. However, the differences were irrelevant ($p > 0.05$). We also measured muscle fibers' mean density using COX stain to check whether dystrophic process causes change in mitochondrial enzyme activity. Despite that, there was no large difference. In terms of cellular infiltrates, membrane and internal nuclei per muscle fiber, less inflammatory cells, and more nuclei were observed in LGMD patients. Yet, no statistical importance was reported ($p = 0.46$, 0.33, and 0.18, respectively). Type I muscle fibers were selectively atrophic and predominant on LGMD biopsies. Quantitation study betrayed Rimmed vacuoles were noticed in only one LGMD biopsy, mainly

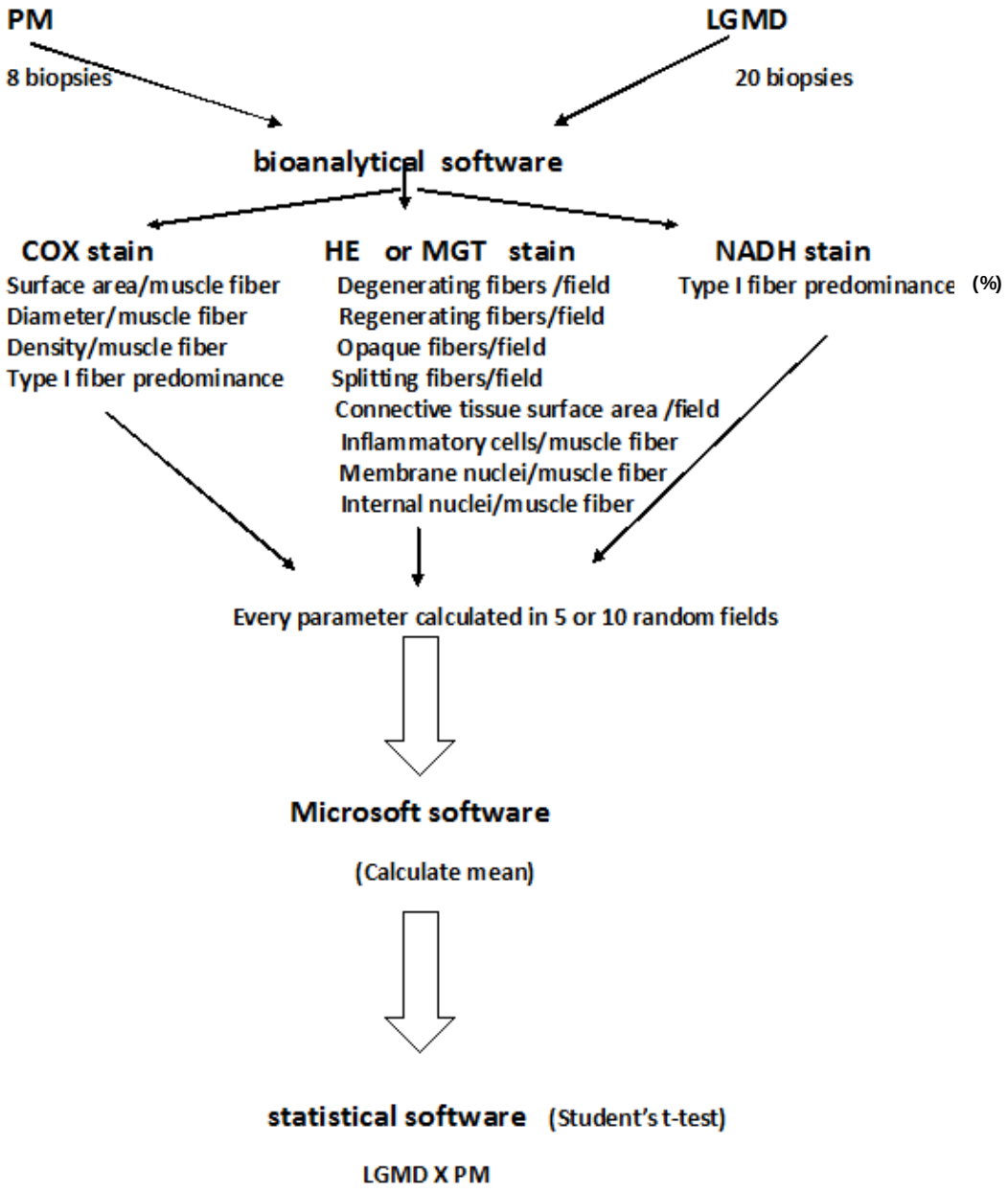

Figure 2. Flow chart shows the general design of the strategy.

in the membrane nuclei, accounting for 10.1% of muscle fiber cross sections. Pyknotic nuclear clumps and angular fibers were detected in eight biopsies; nevertheless, none displayed neurogenic muscle damage electro-physiologically. PM biopsies did not reveal such features. We also reported mononuclear inflammatory infiltrates of non-necrotic fibers on one LGMD biopsy (Figure 5), a finding thought to be specific for idiopathic PM (Hilton-Jones, 2011). Regardless of the underlying pathophysiology, these aspects were the most crucial tips differentiating the two diseases.

MHC class I was presented in LGMD biopsies either as negative (ten biopsies) where only blood vessels, necrotic cells and nuclei were stained; or as up-regulated. We grouped up-regulated MHC-I biopsies into three categories based on structure involved: group I (4/20 biopsies), in addition to blood vessels, necrotic cells and nuclei, plasma membranes of muscle fibers were stained; group II (3/20 biopsies), besides the aforementioned structures, sarcoplasm of some intact muscle fibers was stained; group III (3/20 biopsies), sarcoplasm of all intact muscle fibers was stained (Figure 5).

Clinically, the mean age of LGMD patients was 35.45 ± 13.48 years lower than PM patients (40.88 ± 17.05 years), but was not considerably different ($p = 0.38$), the upper age limit was 71 years, unusual for dystrophic

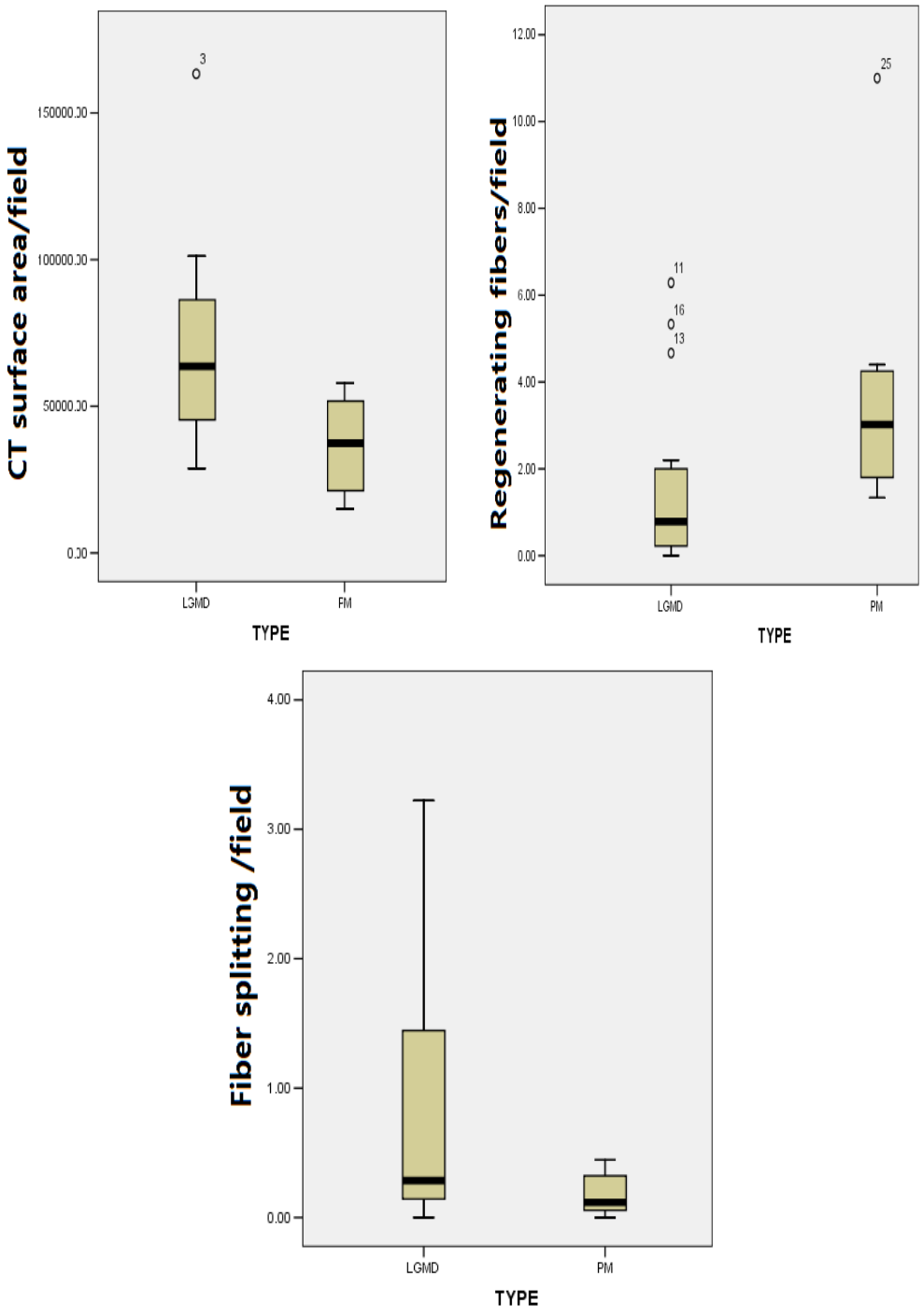

Figure 3. Box plots showing significant differences between LGMD and PM patients in certain parameters of myopathy (95% CI).

diseases. All, but three cases were sporadic; familial cases showed autosomal recessive mode of inheritance, the horizontal distribution or history of consanguinity. Creatine kinase level was greater in myositis patients (27.63 ± 23.74 versus 17.65 ± 20.13 folds) with 0.27 significance. Five of our LGMD patients betrayed electro-cardiac abnormalities, all but one were subclinical.

DISCUSSION

In the face of all this modern progress in diagnostic

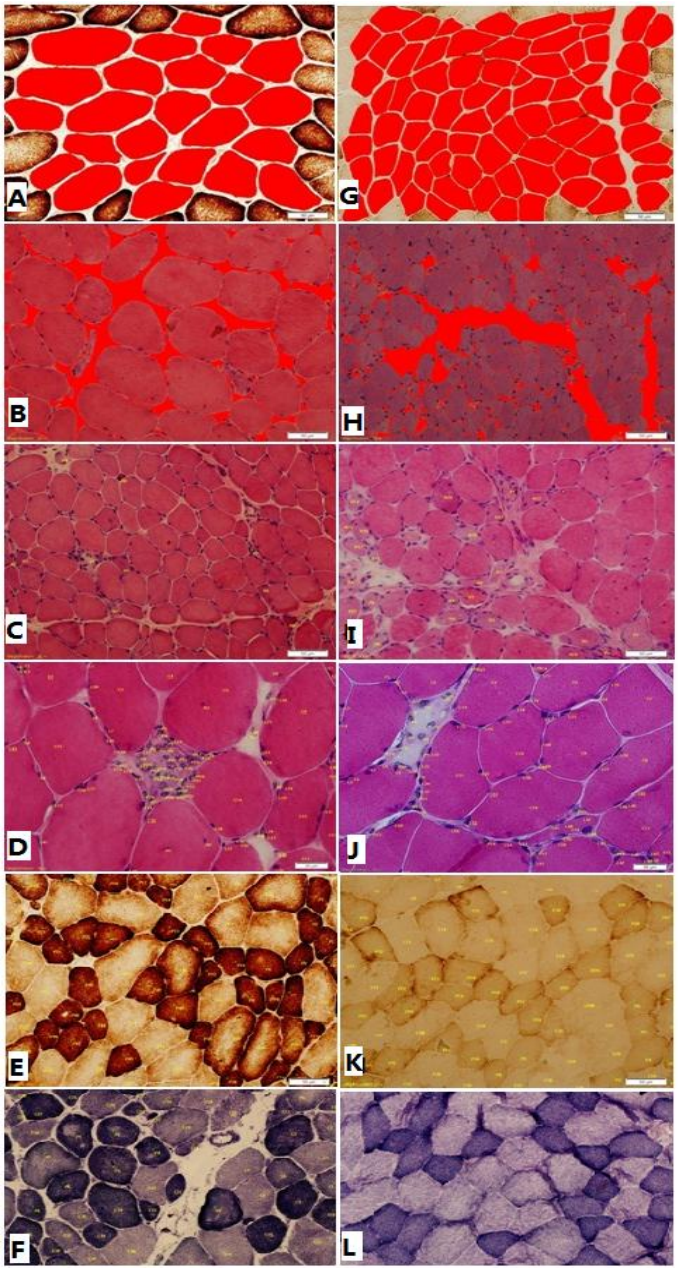

Figure 4. A to F: LGMD biopsies and G to L: PM biopsies; (A and G) surface area, diameter, and density measurements (COX stain used); (B and H) connective tissue measurements (HE stain used); (C and I) degenerating [P], regenerating [R], opaque [L], and splitting fibers [C] (HE stain used); (D and J) inflammatory cells [P], membrane nuclei [L], internal nuclei [R], and muscle fiber [C] (HE stain used); (E and K) fiber-I Predominance, atrophy: fiber-I [P] and fiber-II [C] (COX stain used); (F and L) abnormal NADH fibers [P] and normal fibers [C] (NADH-TR stain used). Bar in all are 50 μm, except for D,J that are 20 μm.

techniques, all muscle specialists have faced troubles with diagnosis leading to over-treat of patients with dystrophy, thinking that it is an inflammatory myopathy or, conversely, to therapeutic abstention in a patient with treatable acquired myopathy believing that it is a dystrophy (Vinit et al., 2010; Benveniste et al., 2011; Dalakas, 2011). Muscle enzymes and electrophysiological study are of little diagnostic

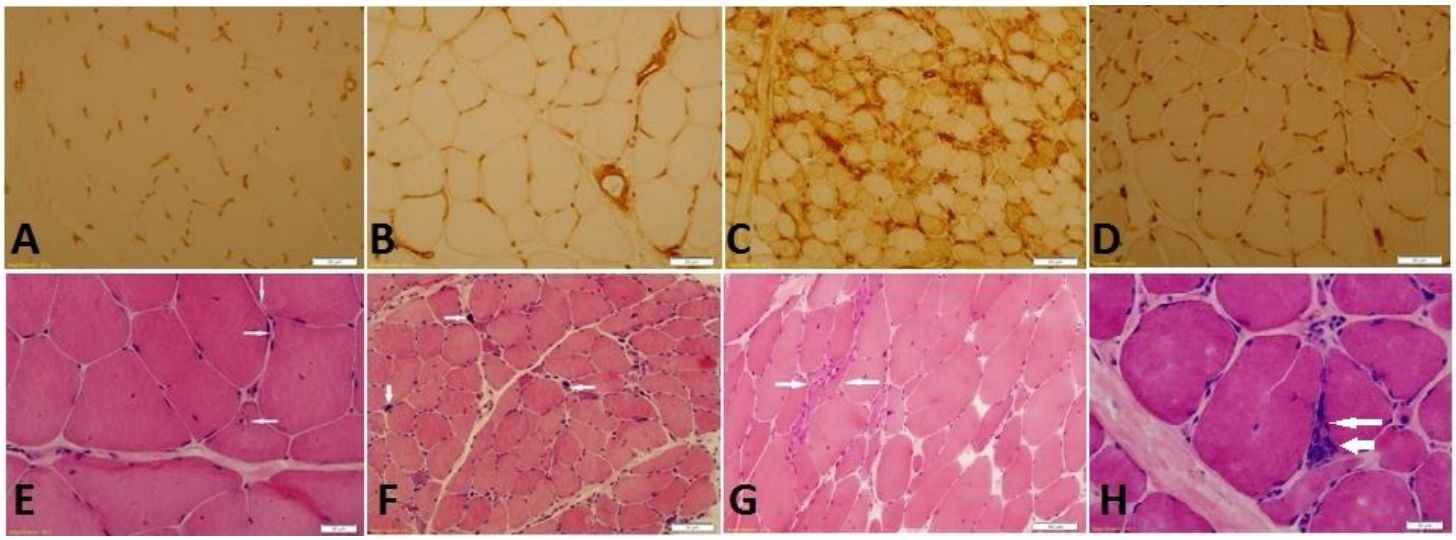

Figure 5. (A to D) MHC-I expression: variable patterns of expression (Text) and (E to H) Abnormal features: HE stain used (E) rimmed vacuoles. (F) Pyknotic nuclear clumps, (G) Small angular fibers, (H) Mononuclear inflammatory infiltrates of non-necrotic fibers. All indicated by white arrows. Bar in all, 50 μm, except E and H, 20 μm.

significance in differentiating between the two diseases.

Recently, Zhang et al. (2011) established the value of muscle enzymes in differentiating various neuromuscular diseases. Though, the study reported no significant difference between the two diseases in terms of creatine kinase (CK) level.

At early times after acute damage, inflammatory macrophages are predominant, and play a non-outdated role in the clearance of cellular debris. At later time points, when fiber regeneration occurs, macrophages acquire a switch off phenotype, which has been linked to tissue remodeling (Brunellia et al., 2008). A series of fiber damage and tissue healing with slow progression of symptoms and cumulative loss of function results in more hypertrophied muscle fibers. Calf and deltoid hypertrophy (Rocha et al., 2010; Rosales et al., 2010) are the main phenotypic feature. The latter acts as compensatory mechanism for the disused dystrophic tissue. Hypertrophied muscle fiber results in more splitting fibers. This finding is considered as valuable diagnostic hint that helps to separate the two diseases and act as clue to pathogenesis. On LGMD biopsies, we suggest that degenerating and regenerating fibers may decrease in number when macrophages play deactivation role. While in inflammatory myopathy, infiltrating leukocytes stay alive and are continuously activated and may explain higher CK level reported in PM.

A quantitation study shows that the overall dysferlinopathic (LGMD2B) muscles had less marked inflammatory response than PM muscles, with numbers of inflammatory cells, all significantly lower (Confalonieri et al., 2003). The inflammatory cells in our LGMD biopsies were insignificantly lower than PM. Different quantification technique may be responsible for this dissimilarity, the inflammatory cells per muscle fiber was measured, while the former study focuses on per field. Furthermore, their study is on one LGMD subtype which was not the case in our study (many LGMD subtypes).

Connective tissue growth factor is over expressed in dystrophic muscles, especially in muscle fibers and activated fibroblasts (Sun et al., 2008). Although, an alternative method for measuring surface area was implemented in our study, the results coincided with the former study considering dystrophic myopathy's high connective tissue mean surface area. A previous study reports type I fiber predominance and atrophy (Figarella-Brangera et al., 2002). Still, no quantitative study has been conducted. Our quantitation study revealed that type I fibers on LGMD biopsies were not so different from PM patients. Moth-eaten, lobulated fibers and intermyofibrillar network disorder were detected only in LGMD, a finding that allows us to differentiate LGMD from PM.

The pyknotic nuclear clumps and angular fibers were hypothesized to be specific to neurogenic muscle disease. These findings were observed in some cases of LGMD, consequently, these findings would let diagnosticians and pathologists make diagnosis without hesitation. The same applies for mononuclear inflammatory infiltrates of non-necrotic fibers that are often detected in idiopathic inflammatory myopathy (Pumarola et al., 2004; Creus et al., 2009; Hilton-Jones, 2011).

Further work is required to identify other components of muscle inflammatory mechanism. This may aid to explain the disparity observed in the inflammatory cell localization in different LGMD biopsies and patterns of MHC class I up-regulation. These findings will open new insight into

understanding pathophysiology, inflammatory mechanisms, and thwart disease progression owing to specific pathway.

Conclusively, the results attained in this present paper have a considerable diagnostic application, wide area of connective tissue combined with high number of splitting fibers and less regenerating fibers should alarm pathologist about dystrophic process. This quantitation study presents the first, involving many parameters of dystrophy comparing them with those of inflammatory myopathy.

ACKNOWLEDGEMENT

The authors are grateful to all the patients who participated in this study. We thank Mr. Yu Z. Ma for the technical support.

REFERENCES

Baumeister SK, Todorovic S, Milić-Rasić V, Dekomien G, Lochmüller H, Walter MC (2009). Eosinophilic myositis as presenting symptom in γ-sarcoglycanopathy. Neuromuscul. Disord. 19: 167-171.

Benveniste O, Romero NB (2011). Myositisor dystrophy? Traps and pitfalls. Presse Med. 40:249-255.

Biancheri R, Falace A, Tessa A, Pedemonte M, Scapolan S, Cassandrini D, Aiello C, Rossi A, Broda P, Zara F, Santorelli FM, Minetti C, Bruno C (2007). POMT2 gene mutation in limb-girdle muscular dystrophy with inflammatory changes. Biochem. Biophys. Res. Commun., 363(4):1033-1037.

Brunellia S, Rovere-Querinic P (2008). The immune system and the repair of skeletal muscle. Pharmacol. Res. 58: 117-121.

Bushby KM, Beckmann JS (2003). The 105th ENMC sponsored workshop: pathogenesis in the non-sarcoglycan limb–girdle muscular dystrophies, Naarden, April 12–14, 2002. Neuromuscul. Disord. 13: 80–90.

Cenacchi G, Fanin M, De Giorgi LB, Angelini C (2005). Ultrastructural changes in dysferlinopathy support defectivemembrane repair mechanism. J. Clin. Pathol. 58: 190–195.

Comerlato EA, Scola RH, Werneck LC (2005). Limb-girdle muscular dystrophies: An immunohistochemical diagnostic approach. Arq. Neuropsiquiatr. 63(2A): 235-245.

Confalonieri P, Oliva L, Andreetta F, Lorenzoni R, Dassi P, Mariani E, Morandi L, Mora M, Cornelio F, Mantegazza R (2003). Muscle inflammation and MHC class I up-regulation in muscular dystrophy with lack of dysferlin: an immunopathological study. J. Neuroimmunol. 142: 130-136.

Creus KK, De Paepe B, Werbrouck BF, Vervaet V, Weis J, De Bleecker JL (2009). Distribution of the NF-κB Complex in theInflammatory Exudates Characterizing the Idiopathic Inflammatory Myopathies. Ann. N Y Acad. Sci. 1173: 370-377.

Dalakas MC, Hohlfeld R (2003). Diagnostic criteria for polymyositis and dermatomyositis. Lancet, 362: 1763.

Dalakas MC (2011). Pathophysiology of inflammatory and autoimmune myopathies. Presse Med. 40: e237–e247.

Darin N, Kroksmark AK, Ahlander AC, Moslemi AR, Oldfors A, Tulinius M (2007). Inflammation and response to steroid treatment in limb-girdle muscular dystrophy 2I. Eur. J. Paediatr. Neurol. 11(6): 353-357.

Figarella-Brangera D, El-Dassoukia M, Saenz A, Cobo AM, Malzac P, Tong S, Cassotte E, Azulay JP, Pouget J, Pellissier JF (2002). Myopathy with lobulated muscle fibers: evidence for heterogeneous etiology and clinical presentation. Neuromuscul. Disord. 12: 4-12.

Gayathri N, Alefia R, Nalini A, Yasha TC, Anita M, Santosh V, Shankar SK (2011). Dysferlinopathy: spectrum of pathological changes in skeletal muscle tissue. Indian J. Pathol. Microbiol. 54(2): 350-354.

Godfrey C, Escolar D, Brockington M, Clement EM, Mein R, Jimenez-Mallebrera C, Torelli S, Feng L, Brown SC, Sewry CA, Rutherford M, Shapira Y, Abbs S, Muntoni F (2006). Fukutin gene mutations in steroid-responsive limb girdle muscular dystrophy. Ann. Neurol. 60(5): 603-610.

Gordon E, Pegoraro E, Hoffman EP. Gene Reviews [Internet]. [Updated 2009 Jul 23; cited 2000 Jun 08]. Limb-Girdle Muscular Dystrophy Overview. In: Pagon RA, Bird TD, Dolan CR, Stephens K, editors. Seattle (WA): University of Washington, Seattle; 1993- 2011. Available from: http://www.ncbi.nlm.nih.gov/pubmed. PMID: [20301582]

Hilton-Jones D (2011). Observations on the classification of the inflammatory myopathies. Presse Med. 40:199

Krahn M, Lopez de Munain A, Streichenberger N, Bernard R, Pécheux C, Testard H, Pena-Segura JL, Yoldi E, Cabello A, Romero NB, Poza JJ, Bouillot-Eimer S, Ferrer X, Goicoechea M, Garcia-Bragado F, Leturcq F, Urtizberea JA, Lévy N (2006). CAPN3 mutations in patients with idiopathic eosinophilic myositis. Ann Neurol., 59(6): 905-911.

Laval SH, Bushby KM (2004). Limb–girdle muscular dystrophies – from genetics to molecular pathology. Neuropathol. Appl. Neurobiol. 30:91–105.

Lubieniecki F, Monges S, Dubrovsky A, Stewart-Harris A, Ruggieri V, Barroso F, Espada G, Ferrea M, Robertella M, Richard P, Bonne G, Romero N, Guicheney P, Sacolitti M, Taratuto A (2007). G.P.5.01 Inflammatory or 'congenital myopathy' type findings in muscle biopsies of patients with LMNA mutations. Neuromuscul. Disord. pp. 17-797 .

Ojima K, Kawabata Y, Nakao H, Nakao K, Doi N, Kitamura F, Ono Y, Hata S, Suzuki H, Kawahara H, Bogomolovas J, Witt C, Ottenheijm C, Labeit S, Granzier H, Toyama-Sorimachi N, Sorimachi M, Suzuki K, Maeda T, Abe K, Aiba A, Sorimachi H (2010). Dynamic distribution of muscle-specific calpain in mice has a key role in physical-stress adaptation and is impaired in muscular dystrophy. J. Clin. Invest. 120(8): 2672–2683.

Pumarola M, Moore PF, Shelton GD (2004). Canine inflammatory myopathy: analysis of cellular infiltrates. Muscle Nerve 29(6):782-789.

Rocha CT, Hoffman EP (2010). Limb–Girdle and Congenital Muscular Dystrophies: Current Diagnostics, Management, and Emerging Technologies. Curr. Neurol. Neurosci, Rep. 10(4):267-276.

Rosales XQ, Gastier Foster JM, Lewis S,mVinod M, Thrush DL, Astbury C, Pyatt R, Reshmi S, Sahenk Z, Mendell JR (2010). Novel Diagnostic Features of Dysferlinopathies. Muscle Nerve 42:14–21.

Sun G, Haginoya K, Wu Y Chiba Y, Nakanishi T, Onuma A, Sato Y, Takigawa M, Iinuma K, Tsuchiya S (2008). Connective tissue growth factor is over expressed in muscles of human muscular dystrophy. J Neurol. Sci. 267: 48-56.

Vinit J, Samson M Jr, Gaultier JB, Laquerriere A, Ollagnon E, Petiot P, Marie I, Levesque H, Rousset H (2010). Dysferlin deficiency treated like refractory polymyositis. Clin. Rheumatol. 29(1):103-106.

Wildförster V, Dekomien G (2009). Detecting copy number variations in autosomal recessive limb-girdle muscular dystrophies using a multiplex ligation-dependent probe amplification (MLPA) assay. Mol. Cell Probes 23(1): 55-59.

Zatz M, Vainzof M, Passos-Bueno MR (2000). Limb–girdle muscular dystrophy: one gene with different phenotypes, one phenotype with different genes. Curr. Opin. Neurol. 13:511–517.

Zhang Y, Huang JJ, Wang ZQ, Wang N, Wu ZY (2011). Value of muscle enzyme measurement in evaluating different neuromuscular diseases. Clin, Chim, Acta. (3-4):520-524.

Supplementary Table 1. Clinical and pathological features of 20 patients with LGMD.

S/N	Gender/age	Onset mode	Family Hx	CK (folds)	ECG	Histochemical (Abnormal features)
1	M/58	PLE	**Brother**	3	Not done	-
2	M/51	Weakness	Negative	14	Not done	-
3	M/39	PLE	Negative	4	VT	-
4	F/26	PLE	Negative	N	AE	-
5	M/18	PLE	Negative	5	N	-
6	M/23	PLE	Negative	12	Not done	-
7	F/42	PLE	Negative	13	Not done	Lobulated
8	M/22	PLE	Negative	16	ST - T↓,LVH	-
9	M/32	PUE	Negative	N	N	Lobulated
10	M/28	PUE	Negative	2	V_{1-3}: ST↑	Lobulated
11	M/18	PLE	Negative	28	N	-
12	M/30	PLE	Negative	17	Not done	Lobulated
13	F/35	PLE	Negative	2	N	Lobulated
14	F/37	PLE+PUE	Negative	53	Not done	-
15	M/41	PLE+PUE	**Sister**	4	N	-
16	M/**71**	PLE+PUE	Negative	80	Not done	-
17	M/37	hyperCKemia	Negative	23	MAPC	-
18	M/22	PLE	**Consanguinity**	41	N	-
19	M/39	PLE	Negative	14	N	RV
20	M/40	PLE	Negative	20	N	-

PLE, Proximal lower extremities; PUE, proximal upper extremities; N, normal; VT, ventricular tachycardia; AE, atrial ectopic; MAPC, multiple atrial premature contractions; RV, rimmed vacuoles.

Supplementary Table 2. Clinical features of 8 patients with PM.

S/N	Gender/age	Onset mode	Family Hx	CK (folds)	ECG
1	F/17	PUE	Negative	16	N
2	M/50	PUE+PLE	Negative	57	Not done
3	F/17	Limitation of movement	Negative	8	Not done
4	M/44	PUE	Negative	33	N
5	F/59	PUE+PLE	Negative	39	LBBB, ST-Tchanges
6	M/12	Fatigue, pain	Negative	62	N
7	F/51	Weakness	Negative	5	T wave changes
8	M/51	PUE+PLE	Negative	N	Not done

PLE: proximal lower extremities; PUE: proximal upper extremities; N: normal; LBBB: left bundle branch block.