The Spectrum of Muscle Disorders Diagnosed at King Hussein Medical Center: An 8 Year Experience

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ABSTRACT

Objective: To report on the prevalence of various muscle disorders encountered at King Hussein Medical Center in terms of diagnosis, age and gender distribution.

Method: This is a retrospective study of 636 cases of suspected muscle diseases that were biopsied over a period of eight years at King Hussein Medical Center between January 2006 and December 2013. The biopsies were examined by several methods including Haematoxylin and Eosin stained frozen tissue sections, muscle enzyme histochemistry, immunohistochemistry, and electron microscopic examination. The different disease diagnoses that were encountered were classified and analyzed.

Results: A positive biopsy with significant changes was encountered in 437(68.7%) of the patients. There were 168 (26.4%) normal biopsies and 31 (4.8%) inadequate specimens. Of specimens with positive findings there were 169 (38.7%) cases of dystrophy, 72 (16.4%) cases of inflammatory myositis, 70 (16%) cases of neurogenic atrophy, 36 (8.2%) cases of congenital myopathy, 19 (4.3%) cases of mitochondrial myopathy, and 71 (16.2%) cases that were grouped together as having various other myopathic changes. A total of 272 positive biopsies belonged to male patients, and 165 belonged to female patients. The age range of patients varied from 1 month to 75 year old.

Conclusion: Muscle biopsies are frequently encountered at King Hussein Medical Center practice. Accurate histopathologic diagnosis and classification of myopathies requires several advanced techniques which can only be carried out at a fully equipped laboratory center. In this study the largest groups of patients were diagnosed to with dystrophy, followed by inflammatory myositis and neurogenic muscular atrophy.

Key words: Dystrophy, Myopathy, Myositis, Neurogenic atrophy.

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INTRODUCTION

Diseases of skeletal muscle are common diseases which are encountered in all regions of the world, and are associated with significant morbidity and mortality. Accurate interpretation of a muscle biopsy requires prior knowledge of patient's clinical history, physical examination, and salient tests such as EMG, nerve conduction studies, and serum creatinine phosphokinase (CPK).[1]

Genetic testing for certain inherited muscular and neurogenic muscular diseases is also carried out under certain circumstances for confirmation and sometimes for prenatal diagnosis.

Much of the literature regarding muscle

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diseases is available from series conducted in the United States, Europe, and the Far East, and most epidemiologic studies carried are out on dystrophic muscle diseases.[2,3] There are few old and recent published studies on these diseases from the region of the Middle East, and across the Arabic World, mostly describing their pattern of presentation.[4-7] Prior to 2006, like other laboratory centers in Jordan, the Histopathology Laboratory Department at King Hussein Medical Center (KHMC) handled tissues from muscle biopsies in similar fashion to ordinary tissues received from all other sites in formalin fixation and interpreted only the Haematoxylin and Eosin stained sections. The majority of the muscle disease diagnosis was established with limited other techniques and ancillary studies. The first pathology muscle laboratory in Jordan was established in 2006 at Princes Iman Center at KHMC in order to handle muscle biopsies in alignment with the gold standards as described in literature, through the use of electron microscopy from 2006, followed by muscle enzyme histochemical stains in 2007, and lastly the introduction of immunohistochemistry in 2008 in order to sub classify the various muscle diseases according to the best working classification. [8, 9]

The aim of this study is to describe the various muscle diseases encountered at KHMC in the hope that this would be an introduction to further studies in Jordan and the region.

Methods

This is a retrospective study of all 636 muscle biopsies that were diagnosed at Princes Iman Center from specimens that were mostly biopsies at KHMC and from freshly received specimens that were referred to KHMC from other centers and private hospitals in Jordan. The biopsies belonged to patients complaining of various neuromuscular symptoms such as hypotonia, muscle weakness, pain, abnormal gait, elevated CPK, and others. The biopsies belonged mostly to Jordanians and non Jordanian patients from neighboring seeking medical advice in Jordan including the Gulf region, and North Africa. All specimens were received fresh and were handled in a similar fashion. Part of each specimen was dissected out for electron microscopic examination and fixed in glutaradehyde for later use if indicated. The remainder of the specimen was cut into horizontal and vertical sections and then snaps frozen in liquid nitrogen followed by isopentane fixation prior to serial cryostat sectionining. The sections were cut between 6 and 7 microns, stained by Haematoxylin and Eosin stain, and then examined primarily before further studies were carried out using muscle enzyme histochemical stains, immunohistochemistry, or electron microscopy, in combination, or individually depending on each particular case. Enzyme histochemical stains included ATPase 9.4 and 4.6 to differentiate fiber types (1, 2A, and 2B), NADH-TR, SDH, Modified Gomori trichrome stain, PAS, Oil red O, and muscle phosphorylase. Depending on availability at time of the biopsy, the following panels of immunohistochemical markers were performed: Dystrophin (1, 2 and 3), Dysferlin, Spectrin, Calpaine, Merosin, Emerin, Caveolin, and the Sarcoglycan group of proteins (alpha, beta, gamma, and delta), which were carried out on cryostat cut sections using antibody clones from various manufacturers namely Novocastra, Biogenex, Leica, and Dako.

Results

Of the 636 cases received, a total of 400 biopsies belonged to male patients, and 236 belonged to female patients with a male to female ratio of (1.7:1). The age range of patients varied from 1 month to 75 year old. A positive biopsy with significant changes was encountered in 437(68.7%) of the patients whom were biopsied (Table I & Fig. 1). There were 168 (26.4%) normal biopsies. There were 31 (4.8%) biopsies which were considered inadequate, as the specimens were composed of fat and fibrous tissue only.

A total of 169 (26.6%) of total cases and almost 40% of positive biopsies were categorized as having a muscular dystrophy and were grouped according to (Table II). Of these, there were 132 males and 37 females, with a mean age of 12, and age range between 2 months and 67 years. There were 8 patients (all males) with end stage muscular dystrophy and

Table I: Distribution of 437 positive cases of muscle biopsies by category

Category	Number of cases	% of total	Male/Female	Mean age/Years
Dystrophy	169	38.7	132 / 37	12
Inflammatory myopathy	72	16.4	27 / 45	18
Neurogenic atrophy	70	16	42 / 28	18
Congenital myopathy	36	8.2	20 / 16	3.7
Mitochondrial myopathy	19	4.3	8 / 11	15
Other Myopathic pathology	71	16.2	40 / 31	18
Total	437	100	269 / 168	16

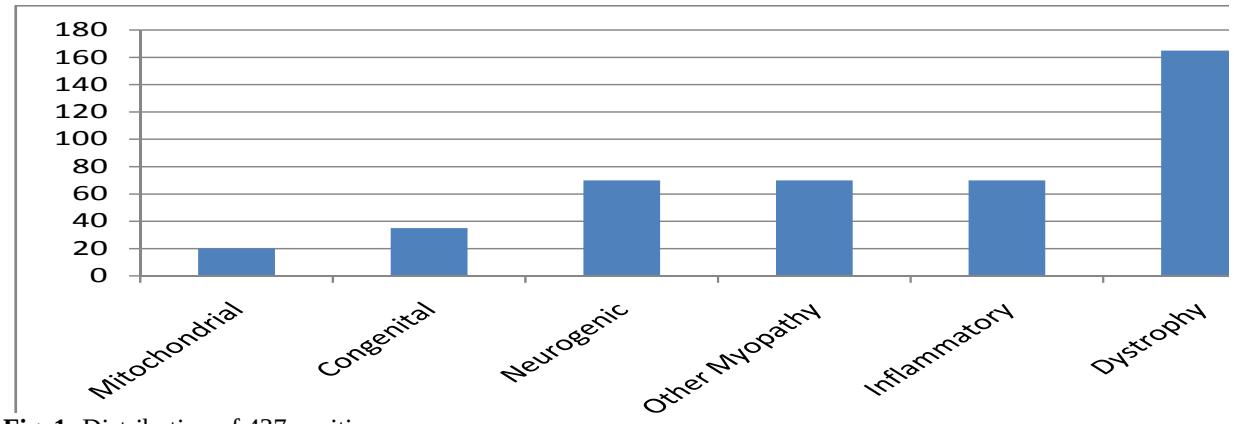

Fig. 1: Distribution of 437 positive cases

further sub typing was not possible as the specimens showed severe fat and fibrous tissue replacement with minimal residual muscle fibers showing dystrophic features.

Twenty three patients were classified as having a dystrophy not otherwise specified as these patients' biopsies were carried out prior to the availability of the immune markers with the exception of dystrophin, in the years 2006 to 2008. Of the latter group the mean age of patients was 16 years (13 males, 10 females) with an age range between 6 and 34 years.

A total of 25 patients were classified as having a congenital muscular dystrophy (15 male, 10 females). Of the 25 patients, 4 patients were diagnosed before markers were available at the laboratory. Twenty one patients were tested for Merosin and other markers listed in the methods. 6 patients were diagnosed as having a Merosin negative congenital muscular dystrophy (CMD) and 15 patients as having a Merosin positive CMD. The mean age of this group of patients was 2.4 years with an age range between 2 months and 6 years.

Dystrophin deficient patients (all males) accounted for 63 (37%) of the 170 patients under the category of muscular dystrophies. There were 38 patients suffering from Duchenne and 25 patients with Becker muscular dystrophy as evident by either total lack or partial/weak staining for one of the dystrophin immunohistochemical markers respectively. The mean age of patients with the former was 6 years and with the latter 8.4 years.

Limb girdle muscular dystrophy (LGMD) affected a large portion of the cases examined (48 patients; 31 males, 17 females) with a mean age of 19 years and age distribution between 3 years and 67 years. Of those patients; 26 were tested positive for all markers available, 7 patients tested negative for all sarcoglycan group of proteins, as deficiency of one of these proteins can lead to deficiency of all other proteins, 5 patients had a dysferlinopathy (LGMD Type 2B), 7 patients with a gamma sarcoglycanopathy (LGMD Type 2C), and 3 patients had an alpha sarcoglycanopathy (LGMD Type 2D). Two male patients in our series had adult onset myotonic dystrophy with a mean age of 25 years.

The second largest category of muscle diseases belonged to the inflammatory myopathies (Table III). There were a total of 72 patients accounting for 16.4% of positive cases.

Table II: Distribution of 169 cases of dystrophies

Diagnosis	Number	M/F	Mean age/ Years
Duchenne muscle dystrophy	38	38 / 0	6
Becker muscle dystrophy	25	25 / 0	8.4
Congenital muscle dystrophy NOS	4	2 / 2	1.8
Congenital dystrophy Merozin +	15	11 / 4	1.8
Congenital dystrophy Merozin -	6	2 / 4	3
LGMD all markers +	26	19 / 7	24
LGMD – for all sarcoglycans	7	2 / 5	7
LGMD 2B- Dysferlin	5	3 / 2	25
LGMD 2C- Gamma sarcoglycan	7	4 / 3	8
LGMD 2D- Alpha sarcoglycan	3	3 / 0	9
Myotonic dystrophy	2	2 / 0	25
Dystrophy NOS	23	13 / 10	16
End stage	8	8 / 0	21

Table III: Distribution of 72 cases of myositis

Diagnosis	Number	M/F	Mean age Yrs
Polymyositis	31	9 / 22	32
Dermatomyositis	22	7 / 15	29
Inclusion body myositis	5	3 / 2	50
Mild myositis	14	4 / 10	17

Table IV: Distribution of cases of 70 cases of neurogenic muscular atrophy

Diagnosis	Number	M/F	Mean age
SMA 1	17	8 / 9	6 months
SMA2	7	6 / 1	3.4 yrs
SMA3	9	7 / 2	5.6 yrs
Motor neuron dis.	8	5 / 3	40 yrs
Neurogenic atrophy	29	15 / 14	32 yrs

31 patients had polymyositis (9 males, 22 females), 22 patients had dermatomyositis (7 males, 15 females), 5 patients had an inclusion body myositis (3 males, 2 females), and 14 patients had either a non specific mild myositis, or various inflammatory changes associated with a known connective tissue or vasculitic diseases. The mean age for these inflammatory myopathies was 30 years, and the standard H&E stained cryostat sections, and electron microscopy was used to diagnose this group of patients.

Neurogenic muscular atrophy accounted for 70 (16%) of positive cases in this study (Table IV). There were 29 patients, mean age 32 (15 males, 14 females) whom were diagnosed to have some degree of neurogenic atrophy not belonging to the remainder of the diseases in this group and characterized by have few single or small groups of angulated atrophic fibers, in conjunction with the clinical or EMG findings and absent other myopathic changes. Eight patients (5 males, 3 females) with a mean age of 40 were diagnosed as a motor neuron disease (MND) based on the histopathologic changes of group atrophy with fiber type grouping, in conjunction with the clinical picture. Thirty three patients were grouped as having spinal muscular atrophy (SMA) in conjunction with disease onset clinically and progression of the disease. Of the patients with SMA; 17 children (8 males, 9 females) suffered from type 1 SMA (Acute infantile/ Werdnig-Hoffmann disease) with a mean age of 6 months at the time of biopsy, and an age range between from 1 month to a year. Seven (6 males, 1 female) patients were categorized as SMA type 2 (severe and intermediate chronic childhood) with a mean age of 3.4 years at time of biopsy and an age range between 1.5 to 6 years. Nine patients (7 male, 2 female) were labeled as SMA type 3 (mild SMA/ Kugelberg-Welander disease), with a mean age of 5.6 years at time of presentation and an age range between 2 and 10 years.

The study demonstrated a subset of patients suffering from a well characterized group of

Table V: Distribution of 36 cases of congenital myopathy

Diagnosis	Number	M/F	Mean age Yrs
Fiber type diproportion	24	12 / 12	4.5
Nemaline rod myopathy	6	4 / 2	2.3
Centronuclear myopathy	5	3 / 2	11
Multi core disease	1	1 / 0	1

disorders belonging to a congenital myopathy (Table V), diagnosed from a combination of all tests available including enzyme histochemistry and electron microscopy. Of the 36 (8.2%) patients (20 males, 16 females), 24 patients had a congenital fiber type disproportion, 5 patients were labeled as centronuclear myopathy, 6 as Nemaline rod myopathy, and 1 as multicore disease. The mean age of patients in this group was 3.7 years and an age range between 7 months and 13 years.

There were 19 patients (8 males, 11 females) diagnosed as having a mitochondrial myopathy, 11 of whom were confirmed, based on the presence of ragged red fibers on modified Gomori stain, with positive electron microscopic features of abnormal mitochondria containing parking lot or crystalline inclusion. Eight patients had ragged red fibers but the electron microscopic findings only revealed abnormal aggregation of mitochondria with no obvious inclusions and therefore this diagnosis was highly suggested. The mean of this group was 15 years with an age range between 2 years and 41 years.

The last category of diseases was grouped as patients having various other myopathic features. This group included 71 patients (40 males, 31 females) with a mean age of 18 years. The largest in this group comprised 24 patients (mean age 21 years) with elevated CPK, but the only histopathologic changes was focal myonecrosis. 11 patients (mean age 1.6 years) had hypotrophic muscle changes which were attributed to disuse. Seven patients had type 2 fiber atrophy secondary to steroid treatment. Three patients had a glycogen storage disease, 1 patient had a lipid myopathy, 1 patient had spectrin B deficiency, and 16 had various mixed type 1 or type 2 atrophy or predominance not typical of a congenital fiber type disproportion. Two patients each aged 1 year were also diagnosed to have with other congenital muscle diseases: A case of myotonia congenita and a case of inherited variant of inclusion body myopathy. Three had increased internalized nuclei of uncertain significance, and 4 patients had advanced atrophic changes and fat replacement with no obvious other changes to place them in a particular subset.

Discussion

The spectrum of muscle disorders in the Middle East is poorly documented with few well described old studies largely based on clinical findings with secondary review of histopathologic sections such as the Saudi study carried out on a series of 84 children between 1982-1992 where dystrophies accounted for 48% of muscular diseases.[4]

Similarly a study on 40 patients in Egypt in 1997 found dystrophies without further specification as the commonest muscular disease. [5] More recent studies from the region have addressed the molecular changes associated with well known neuromuscular diseases such as Duchenne muscular and SMA from countries such as Egypt, Tunisia, and Saudi Arabia.

Muscular diseases in Jordan have long been known but accurate classification of various diseases and in particular the congenital myopathies, mitochondrial myopathies and the subsets of various limb girdle muscular dystrophies have never been carried out in Jordan, due to lack of a well equipped muscle laboratory. A study from Jordan University from 1998, reported a collection of 75 children over a 7 year period with muscle diseases. [6] In their study 55 (73.3%) had muscular dystrophy, 28 of whom had congenital a congenital muscular dystrophy, 11 (20%) had Duchenne muscular dystrophy, 9 (16.4%) had Becker muscular dystrophy, 4 (7.3%) had myotonic dystrophy, 2 (3.6%) had limb-girdle dystrophy, and 1 (1.8%) patient had facioscapulohumeral dystrophy. The latter study was based on routine H& E diagnosis and clinical history only. Our study relating to dystrophy showed

similar results as to the percentage of Duchenne (22.5%) and Becker dystrophy (15%). However; the percentage of congenital dystrophy is significantly less representing 15% in our study as compared to 50% in their study. This could be related to age selection in their study or might be related to missed cases of a LGMD, since our study showed a significantly higher subset of patients with LGMD (29%) compared with their rate of 6%. The introduction of immune markers in characterization of these disorders and the exclusion of various congenital myopathies by electron microscopic examination is a plausible explanation to the previous findings. A more recent study from Copenhagen-Denmark, on 123 patients in collaboration with a private laboratory from Jordan, was published in 2005 on selected referral cases from Jordan. In their study, dystrophies were the commonest disorder followed by myositis. [7]

Our study showed a significant number of patients with idiopathic inflammatory myopathy 72 cases (16.4 % of positive results). Compared with other studies from a large center in Jordan [10] over a period of 12 years and a collection of 30 cases, our percentage of cases of polymyositis 58% and dermatomyositis 43% when taken in isolation are almost exactly reversed, which could be explained by differences between formalin fixed tissue interpretation which causes artfactual retraction of fibers to resemble the perifasicular atrophy seen truly on cryostat prepared sections as should be done. Furthermore in their series there were no cases of inclusion body myositis compared with 5 cases in our series representing 7% of inflammatory myopathies. To the best of our knowledge the latter group of patients has never been reported in the Middle East. There was few case reports from Turkey with an incidence of 0.2% of total muscle biopsies compared with 0.7% in our study.[11]

Accurate sub typing of congenital myopathies requires proper examination by enzyme histochemistry and electron microscopy. This series is the first from Jordan and includes 36 patients. A study from Israel [12] on 32 families identified also 38 patients: 13 children were diagnosed with congenital fiber type disproportion, 10 had myotubular myopathy (centronuclear myopathy), 7 had Nemaline myopathy, 5 had central core disease, 1 had actin myopathy, and 1 had multi-minicore disease.[13] Our results were comparable although we had a higher percentage of fiber type disproportion (24 cases) and no cases of central core disease. In our series patients with centronuclear myopathy had a wider age group with slightly later clinical presentation and milder form of the disease.

Mitochondrial myopathies have never been reported in Jordanians. This is the first series from our region with 19 cases identified.

Patients with neurogenic muscular atrophy (70 cases) represented a significant number of cases, while most adult patients had non-specific neurogenic changes, almost half of our patients had a subtype of spinal muscular atrophy which would need further genetic testing in order to characterize the gene deletions associated with these disorders in our population and compare them with other studies done in nearby countries such as Saudi Arabia, Egypt, and Oman.[13-16]

Finally the patients that were grouped as other myopathies (68 cases) were previously subcategorized in the result section. The majority of patients had focal myonecrosis (23 patients) and the exact etiology remained undetermined, but could be explained by misrepresentation of the biopsy from a less affected site, a fact to remember when a muscle biopsy is being planned for. Disuse atrophy related to CNS abnormalities especially in patients with cerebral palsy was seen. Steroid induced myopathy is well known and remains a cause of weakness in a subset of patients. The few cases of glycogen storage diseases and lipid myopathies is explained by other modalities and biochemical testing used to diagnose these patients in clinical practice.

Limitation of the study

This study was based on the histopathologic changes that are associated with the various diseases encountered. The use of genetic studies to detect various DNA mutations to confirm the diagnosis especially in certain dystrophies and spinal muscular atrophy was

not carried out in this study due to lack of facilities.

Conclusion

This is the largest conducted study on patients with various muscular disorders primarily in Jordanians and from this region of the world. It is only by proper handling and examination of muscle biopsies using enzyme histochemistry, electron microscopy and the enlarging panel of antibodies available in the market for tissue diagnosis of dystrophies, we are able to further characterize these disorders and in particular the inherited diseases. We hope that this study can be used as a core study to help offering families grounds for genetic counseling and prenatal diagnosis.

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