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A hospital based study on laboratory investigations on heriditary muscle disorders

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***Abstract*---**Background: Myopathies are a group of neuro muscular diseases that cause muscle weakness, cramps, and spasms due to a primary defect of the muscle fiber. We undertook this study of hereditary muscle disorders to identify the clinical patterns and laboratory findings in these conditions and study the correlation between them, which will help in recognizing them early for adequate management with rehabilitation measures and for prognostication. Aim: To assess the correlation between the clinical and investigational profile in Hereditary muscle disorders. Methods: A cross sectional study was conducted among 44 patients with clinical features suggestive of hereditary muscle disorders who sort health care at Institute of neurology, Madras Medical college, Chennai using various investigation techniques like Serum CK measurement, Nerve conduction Study, Electromyography, Muscle biopsy. Results: CK elevation was found in all patients in our study, but the degree of elevation differs among various hereditary muscle disorders. It was maximal (>25 times normal) in patients with DMD (50%), BMD (40%) and in LGMD (4%). High degree of correlation between clinical diagnosis of hereditary muscle disorders and myopathic EMG was found. Myopathic pattern was observed in all patients of Muscular dystrophy, congenital myopathy and distal myopathy. Conclusion: Hence, a structured clinical approach focusing on pattern of muscle involvement and on specific clinical signs along with investigations

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like serum CPK, EMG and Muscle biopsy, it is possible to make an accurate early diagnosis in hereditary muscle disorders, prognosticate and manage them appropriately to improve the quality of life in these patients.

***Keywords*---**Myopathy, Serum CPK elevation, Electromyography, Clinical Diagnosis.

Introduction

Myopathies are a group of neuro muscular diseases that cause muscle weakness, cramps, and spasms due to a primary defect of the muscle fiber. Myopathy means Greek: Myo- muscle; Patheia- suffering, that implies primary defect is in the muscle. Myopathies can be classified as Neuro mucular or musculo skeletal in nature. They may be a result of hereditary transmission or from several systemic diseases or maybe acquired. Various pathological processes, some genetically determined and others acquired, may affect the function of the skeletal muscles and Neuromuscular may manifest in different ways. Some, such as, the congenital myopathies, produce weakness and hypotonia at birth whereas others do not cause functional abnormalities until childhood, adolescence, or adult life (.F L Mastagliaet. Al,) 1 With the Clinical application of modem molecular biological techniques, major advances have taken place in, the identification of the genetic mutations responsible for many of the hereditary muscle diseases. This review focuses on the modern approach to the clinical and laboratory investigation of patients with muscle diseases with particular emphasis on the application of molecular techniques in diagnosis. We undertook this study of hereditary muscle disorders to identify the clinical patterns and laboratory findings in these conditions and study the correlation between them, which will help in recognizing them early for adequate management with rehabilitation measures and for prognostication.

Aims and Objectives

The aim of the study is to assess the correlation between the clinical and investigational profile in Hereditary Muscle Disorders.

Materials and Methods

Study Centre: Institute of Neurology, Madras Medical College, Chennai.
Study design: Cross sectional study.
Study period: July 2018 to February 2019(8 months).
Study Sample: 44 patients (Males-32, Females-12).

Clinical Evaluation: Clinical evaluation of all patients were done with,

- Detailed history taking
- Clinical examination
    i. General Examination with details about the presence of contractures, skeletal deformities, etc.

ii. CNS Examination which included higher mental functions, cranial nerves, spinomotor system, reflexes, in coordination, sensory system, gait, cerebellar and extra pyramidal system examinations.
iii. Muscle testing which included examination of muscle bulk, tone, power, reflexes, muscle tenderness, identifying specific pattern of muscle involvement and other characteristic signs (eg. polyhill sign in FSHD).

Investigations:

- ❖ All the patients were subjected to routine blood investigations like complete blood count, blood sugar, renal function test, liver function test, routine urine analysis, thyroid function test, ECG/ECHO study, ophthalmologic evaluation.
- ❖ Other specific investigations included,

1) Serum CPK measurement:

Test Procedure

- Blood is drawn from a vein, usually from the arm. The venipuncture area is cleaned with antiseptics. A tourniquet is placed around the upper arm to make the vein prominent. The needle is inserted into the vein, and the blood is allowed to collect in a blood collection tube.
- The blood samples were centrifuged at 3000 RPM for ten minutes immediately after collection, and the serum was removed for analysis of CPK.
- Total enzymatic activity was determined by spectrophotometry and kinetic method. The results are expressed in IU/L.

2) Nerve conduction study(NCS):

Nerve conduction studies were done to evaluate the functioning of motor and sensory nerves.

- The muscle electrical signal was recorded and the time from electrical stimulus to muscle contraction (latency), NCV, amplitude determined for both motor and sensory nerves.

3) Electromyography(EMG):

The EMG evaluation to determine the electrical function of individual muscle motor unit potentials at rest and during muscle contraction was done for all patients.

Technique:

- It is performed by inserting a recording needle electrode into the belly of a muscle. The needle tip is the recording electrode and the needle shaft is the reference electrode in a concentric needle.
- Electrical activity from muscle fibers is recorded and amplified to appear on an oscilloscope as a tracing of voltages versus time with accompanying sound.
- Spontaneous activity, Motor unit action potentials (MUAPS), Interference pattern were observed and interpreted as normal, myopathic or neurogenic patterns.

Interpretation:

- Normal-no spontaneous activity, MUAPs with 3-4 phases, amplitude of 0.5 to 2 mV, duration of 5-15ms and a normal interference pattern.

- Neurogenic: spontaneous activity-present (positive sharp waves, fibrillation potentials, fasciculations, MUAPs-large amplitude, polyphasic, longerduration, interference pattern-incomplete.
- **Myopathic**- no spontaneous activity (except in myotonic dystrophy, where myotonic discharges are seen), MUAPs-normal to low amplitude, polyphasic, shorter duration, interference pattern-complete with early recruitment.

4) Muscle Biopsy:

Technique:

- Open biopsy procedure was used to obtain muscle specimen in all patients. Under local anesthesia, a linear piece of muscle tissue of 1.5×0.5cms size obtained.
- Moderately affected muscles were selected for biopsy. In most of the occasion, Vastus lateral is was sampled and in some patients tibialis anterior was biopsied.
- Precautions like avoiding severely affected muscles, muscles tested by EMG etc. were followed.
- Muscle sample was preserved in saline moistened gauze for transportation to the lab.
- One portion the specimen was flash freezed in isopentane and the cryosectioned specimen used for routine staining (HE/MGT), Enzyme staining (SDH/ATPase).
- 10% formalin fixed paraffin sections were used for routine staining (HE/MAT/PTAH).

Inclusion Criteria:

- Patients with clinical features suggestive of Hereditary muscle disorders.

Exclusion criteria:

The following patients are excluded from the study,

- Patients with clinical features, electro diagnostic tests (NCV/EMG) suggestive of neuropathies or neuromuscular junction disorders.
- Patients with features suggestive of drug-induced myopathies.
- Patients with features suggestive of toxic myopathies.
- Patients with features suggestive of Endocrine myopathies.
- Patients with features suggestive of myositis (infective/inflammatory).

Data Analysis:

All the data were tabulated in Microsoft XL sheet, followed by analysis using SPSS software (version 20.0).

Results

Table 1: Distribution of subjects with diagnosis of Hereditary Muscle Disorders and Serum Creatine Kinase value

DIAGNOSIS OF MUSCLE DISORDERS	VALUE OF SERUM CREATINE KINASE							Total	
	<250	251 – 1000	1001 – 2000	2001 – 3000	3001 - 4000	5001 - 6000	8001 - 9000		
DMD (n = 2)	0	0	1	0	0	1	0	2	p<0.031
BMD (n = 5)	0	0	1	1	1	2	0	5	
FSHD (n = 5)	2	3	0	0	0	0	0	5	
LGMD (n = 25)	0	12	9	0	3	0	1	25	
MYOTONIC DYSTROPHY (n = 3)	0	1	2	0	0	0	0	3	
DISTAL MYOPATHY (n = 2)	0	2	0	0	0	0	0	2	
CONGENITAL MYOPATHY (n = 2)	0	2	0	0	0	0	0	2	
Total	2	20	13	1	4	3	1	44	

Among 25 patients with LGMD 12(48%) had serum creatinine kinase (CK) level between 251-1000, 9(36%) between1001-2000 and 3(12%) have between 3001 – 4000. Out of 5 with BMD 2(40%) had CPK between 5001 – 6000, 1(20%) had between 1001-2000 and 1(20%) between 2001-3000 and 1(20%) had between 3001-4000. Among 5 with FSHD 2(40%) have CK level < 250 and 3(60%) have between 251 to 1000. Out of 3 with Myotonic dystrophy, 2(66.67%) have CK level between1001- 2000 and 1(33.33%) had between 251-1000. All 2(100%) with distal myopathy have CK level between 251-1000. All 2 (100%) with Congenital myopathy had CK level between 251-1000. Out of 2 with DMD, 1(50%) had CK level between 1001-2000 and 1(50%) had between 5001-6000.The differences in the CPK level among patients with muscle disorders was found to be statistically significant (p< 0.031).

Table 2: Distribution of subjects with diagnosis of Hereditary Muscle Disorders and ecg and echo

DIAGNOSIS OF MUSCLE DISORDERS	ECG / ECHO		Total	
	Normal	Abnormal		
DMD (n = 2)	0	2	2	p<0.006
BMD (n = 5)	4	1	5	
FSHD (n = 5)	4	1	5	
LGMD (n = 25)	24	1	25	
MYOTONIC	3	0	3	

DYSTROPHY (n = 3)				
DISTAL MYOPATHY (n = 2)	2	0	2	
CONGENITAL MYOPATHY (n = 2)	1	1	2	
Total	38	6	44	

Out of 25 patients with LGMD, 24(96%) had normal cardiac status, while 1(4%) had abnormal cardiac status. Among 5 with FSHD, 4(80%) had normal cardiac status and 1(20%) had abnormality. Among 5 with BMD, 4(80%) had normal cardiac status and (20%) had abnormality. All 3(100%) with Myotonic dystrophy had normal cardiac status. All 2(100%) with DMD had normal cardiac status. All 2(100%) with Distal myopathy had normal status. Among 2 with Congenital myopathy, 1(50%) had normal and 1(50%) had abnormal cardiac status. The difference was found to be statistically significant (p<0.006).

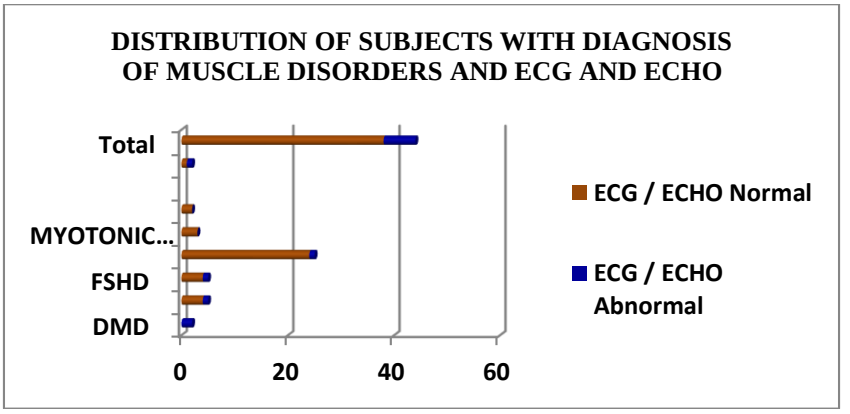

Figure 1: Distribution of subjects with diagnosis of muscle disorders and ecg and echo

Table 3: Distribution of subjects with diagnosis of muscle disorders and emg

DIAGNOSIS OF MUSCLE DISORDERS	EMG		Total	
	Myopathic	Myotonic		
DMD (n = 2)	2	0	2	
BMD (n = 5)	5	0	5	
FSHD (n = 5)	5	0	5	
LGMD (n = 25)	25	0	25	p<0.001
MYOTOINIC DYSTROPHY (n = 3)	0	3	3	
DISTAL MYOPATHY (n = 2)	2	0	2	
CONGENITAL MYOPATHY (n = 2)	2	0	2	
Total	41	3	44	

All 25(100%) with LGMD had myopathic EMG pattern. All 5(100%) with FSHD had myopathic pattern. All 5(100%) with BMD had myopathic pattern. All 3 (100%) with Myotonic dystrophy had myotonic picture. All 2 (100%) with DMD had myopathic picture in EMG. All 2(100%) with Distal myopathy had myopathic picture. All 2(100%) with Congenital myopathy had myopathic picture. The differences was found to be statistically significant (p<0.001).

Table 4: Distribution of subjects with diagnosis of muscle disorders and muscle biopsy

DIAGNOSIS OF MUSCLE DISORDERS	MUSCLE BIOPSY		Total	
	MD	No dystrophy		
DMD (n = 2)	2	0	2	
BMD (n = 5)	5	0	5	
FSHD (n = 5)	5	0	5	
LGMD (n = 25)	25	0	25	p<0.009
MYOTONIC DYSTROPHY (n = 3)	2	1	3	
DISTAL MYOPATHY (n = 2)	2	0	2	
CONGENITAL MYOPATHY (n = 2)	1	1	2	
Total	42	2	44	

MD-Muscular dystrophy

All 25(100%) patients with LGMD showed muscle dystrophy in muscle biopsy. All 5(100%) with FSHD showed muscle dystrophy. All 5(100%) with BMD showed muscle dystrophy. Out of 3 with Myotonic dystrophy, 2(66.67%) showed muscle dystrophy and 1(33.33%) showed no muscle dystrophy. Out of 2 with congenital myopathy, 1(50%) showed muscle dystrophy and the other (50%) had specific feature (Nemaline rods) but without dystrophy. All 2(100%) with DMD showed muscle dystrophic pattern. All the 2 (100%) with distal myopathy showed muscle dystrophy and the differences was statistically significant (p<0.009).

Table 5: Correlation between the clinical diagnosis of Hereditary Muscle Disorders and elevated Serum Creatine Kinase value

		ELEVATED SERUM CREATINE KINASE VALUE	CLINICAL DIAGNOSIS
ELEVATED SERUM CREATINE KINASEVALUE	Pearson Correlation	1	.413**
	Sig. (2-tailed)	-	.005
	N	44	44
CLINICAL DIAGNOSIS	Pearson Correlation	.413**	1

	Sig. (2-tailed)	.005	-
	N	44	44
**. Correlation is significant at the 0.01 level (2-tailed).			

A high degree of correlation was found between the clinical diagnosis of hereditary muscle disorders and elevated serum creatine kinase which was statistically significant with a p value of <0.005.

Table 6: Correlation between the clinical diagnosis of Hereditary Muscle Disorders and myopathic emg

			MYOPATHIC EMG	CLINICAL DIAGNOSIS
Spearman's rho	MYOPATHIC EMG	Correlation Coefficient	1.000	.487**
		Sig. (2-tailed)	-	.001
		N	44	44
	CLINICAL DIAGNOSIS	Correlation Coefficient	.487**	1.000
		Sig. (2-tailed)	.001	-
		N	44	44
**. Correlation is significant at the 0.01 level (2-tailed).				

A high degree of correlation was found between the clinical diagnosis of hereditary muscle disorders and myopathic EMG which was statistically significant with a p value of <0.001.

Table 7: Correlation between the clinical diagnosis of hereditary muscle disorders and myopathic pattern of muscle biopsy

			MYOPATHIC PATTERN OF MUSCLE BIOPSY	CLINICAL DIAGNOSIS
Spearman's rho	MYOPATHIC PATTERN OF MUSCLE BIOPSY	Correlation Coefficient	1.000	.357*
		Sig. (2-tailed)	-	.017
		N	44	44
	CLINICAL DIAGNOSIS	Correlation Coefficient	.357*	1.000
		Sig. (2-tailed)	.017	-
		N	44	44
*. Correlation is significant at the 0.05 level (2-tailed).				

A high degree of correlation was found between the clinical diagnosis of hereditary muscle disorders and myopathic pattern in muscle biopsy which was statistically significant with a p value of <0.005.

Discussion

CK elevation was found in all patients in our study, but the degree of elevation differs among various hereditary muscle disorders (Wong E T et al).[2] It was maximal (>25 times normal)in patients with DMD(50%),BMD(40%) and in LGMD(4%).All these patients had similar phenotype with severe degree of weakness. Mild to moderate elevations (2-25 times normal) was found in most of our patients with LGMD, congenital myopathy and distal myopathy. Minimal elevation was found in FSHD patients as described in literature.[3,5]

In a previous study by (Mansur et al)[3] ,72% of BMD patients had cardiac involvement which was not found in our study where only 20% had abnormal ECG/ECHO findings. Whereas all 2(100%) DMD patients, 1(20%) FSHD, 1(4%) of LGMD and1 (50%) of congenital myopathy had cardiac involvement. High degree of correlation between clinical diagnosis of hereditary muscle disorders and myopathic EMG was found. Myopathic pattern was observed in all patients of Muscular dystrophy, congenital myopathy and distal myopathy. All 3(100%) myotonic dystrophy patients showed spontaneous activity (myotonic discharges) in EMG study. This is consistent with other previous studies (Garima Shukla et al, Black JT et al., Peter K et al., and Buchthal et al.).[4,5,6,7]

Muscle biopsy study in our patients showed that, all patients with muscular dystrophy(100%),1(50%) patient with congenital myopathy and in 2(66.7%) patients with myotonic dystrophy had myopathic pattern. 1(50%) patient with congenital myopathy had specific changes of internal structure in muscle fibres(Nemaline rods)8. Clinical diagnosis of hereditary muscle disorders and concordant myopathic pattern in muscle biopsy study was found in more than 90% of our patients as comparable to many previous studies (Buchthal et al., Peter K et al..Schwartz et al,).[7,6,9]

Conclusion

- Serum CK elevation is maximum (>25 times normal) in DMD, BMD, LGMD (AR inheritance) and minimum(1-2 times normal) in FSHD and the CK elevation correlates well with the clinical phenotype among various hereditary muscle disorders in our population. A strong correlation between elevated serum CK and clinical diagnosis of hereditary muscle disorders was noted in our study.
- High degree of concordance of clinical diagnosis of hereditary muscle disorders with EMG was observed in our study.
- High degree of concordance of clinical diagnosis of hereditary muscle disorders with muscle biopsy was observed in our study.

Hence, a structured clinical approach focusing on pattern of muscle involvement and on specific clinical signs along with investigations like serum CPK, EMG and Muscle biopsy, it is possible to make an accurate early diagnosis in hereditary

muscle disorders, prognosticate and manage them appropriately to improve the quality of life in these patients.

Abbreviations:
CK: Creatine Kinase
EMG: Electromyograpy
MD: Muscular Dystrophy

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