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ASSESSMENT OF SERUM ENZYME LEVELS IN VARIOUS MUSCULAR DYSTROPHY CONDITIONS

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ABSTRACT

Muscular dystrophy (MD) is known to be heterogeneous group of myopathies, sharing somewhat similar clinical symptoms and manifest comparable myopathic changes. However, regardless of clearly manifested signs, well known documented symptoms and several invasive and non-invasive (CT, MRI scans) diagnostic techniques, comprehensible diagnoses of MDs remains inconclusive. There are several enzymes that are known to provide information regarding muscle damage such as Creatine Kinase (CK), Aldolase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and even metabolic parameter such as lactate. Therefore present study was undertaken to provide base-line data regarding serum enzymes variations in few types of MDs in order to suggest their diagnostic efficacy and probability of routine usage as serum markers of MDs. A total of 31 patients (Males = 19, females 12) diagnosed with Duchenne muscular dystrophy, Becker's muscular dystrophy, Facioscapulohumeral dystrophy, limb girdle muscular dystrophy, Emery-Dreifuss muscular dystrophy were selected during the study period of January 2014 to January 2016. Blood samples were analyzed in blood samples collected in clot-activated tubes, centrifuged at 5000 rpm, serum separated and stored at -20^{o}C until processed. Biochemical enzymes CK, AST, ALT, LDH and ALP were determined in serum by prescribed methods. All enzymes manifested elevated levels in Duchenne muscular dystrophy and Becker's muscular dystrophy, whereas only CK and LDH manifested elevating trend as compared to ALT, AST and ALP in Facioscapulohumeral dystrophy and limb girdle muscular dystrophy. Nonetheless, capricious pattern of variations in serum enzyme levels suggested apparent alterations due to muscular disease-dependent activity. In conclusion, alteration pattern of serum enzymes, assessed in various types of MDs, may provide supportive inference for the differential diagnosis of designated MD.

Key-words: Muscular dystrophy, Serum enzymes, Creatine Kinase, Aldolase, aspartate aminotransferase, alanine aminotransferase.

Abbreviations: MD=Muscular dystrophy; CT = Computerized Tomography; MRI = Magnetic Resonance Imaging; CK= Creatine kinase; AST= Aspirate aminotransferase; ALT= Alanine aminotransferase; ALP= alkaline phosphatase; CPK= creatine phosphor-kinase; LDH= lactate dehydrogenase.

INTRODUCTION

It is a stated that there are several types of documented muscular dystrophy (MD) conditions and diseases that cause muscle weakness, myopathies on several areas of body and even extremities. It is well researched and documented that MD is a heterogeneous group of myopathies, some congenital and some acquired (Begum *et al.*, 2000; Zhu *et al.*, 2015). Interestingly, all types of MDs share somewhat similar clinical symptoms and manifest comparable myopathic changes in histopathological findings (Mercuri and Muntoni, 2013; Zhu *et al.*, 2015). Nonetheless, regardless of clearly manifested signs, well known and documented symptoms and several invasive (histopathology) and non-invasive (CT, MRI scans) diagnostic techniques, comprehensible diagnoses of MDs remains inconclusive in many settings, specially population from under-developed and rural areas (Begum *et al.*, 2000; Bradley *et al.*, 2002; Kamath *et al.*, 2000; Kohli *et al.*, 2005; Zhu *et al.*, 2015). Reason behind is the inaccessibility to such advanced technologies, un-trained staff for such procedures, expenses and most of the time reluctance from the patients side to go under invasive procedure (Zhu *et al.*, 2015). Moreover, since MDs assessment requires biopsy and histological examinations, it is sometimes difficult to get good biopsy samples without damaging to-be examined tissues (Kanda *et al.*, 2014). Another reason is the unavailability of such services even in developed city hospitals due to financial constraints or unavailability of trained medical staff (Armstrong *et al.*, 1984; Beaton *et al.*, 2002a,b; Kanda *et al.*, 2014). Therefore, as a result, clinicians remain in dismay as how to clearly diagnose and define the prevailing MDs.

There are several enzymes that are known to provide information regarding muscle damage such as creatine Kinase (CK) or creatine phosphor-kinase (CPK), Aldolase and even metabolic parameter such as lactate (Armstrong *et al.*, 1984; Beaton *et al.*, 2002a,b; Begum *et al.*, 2000; Bradley *et al.*, 2002; Kamath *et al.*, 2000; Kanda *et al.*,

2014; Kohli *et al.*, 2005; Korones *et al.*, 2001; Morse *et al.*, 1993; Tay *et al.*, 2000; Urganci *et al.*, 2006; Zhu *et al.*, 2015). Furthermore, some other serum enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) are also identified as markers of muscle damage to some extent and prime marker of muscle damages during extensive exercise (Brancaccio *et al.*, 2008; Kanda *et al.* 2014; Paulsen *et al.*, 2012). Therefore present study was undertaken to provide base-line data regarding serum enzymes variations in few types of MDs in order to suggest their diagnostic efficacy and probability of routine usage as serum markers of MDs.

MATERIALS AND METHODS

Patient's selections:

A total of 31 patients (Males = 19, females 12) diagnosed with Duchenne muscular dystrophy (n = 7), Becker's muscular dystrophy (n = 6), Facioscapulohumeral dystrophy (n = 5), limb girdle muscular dystrophy (n = 7), Emery-Dreifuss muscular dystrophy (n = 6) were selected during the study period of January 2014 to January 2016 from Gov Lyari general hospital, Karachi Dock Labor Board and LNH. Aged matched Normal healthy controls (n = 10, F = 05, M = 05) were also included. Diagnoses were made by physical examination, CT, MRI, X-rays, lab reports, history and related clinical findings. Most of the patients belong to rural areas of Baluchistan and FATA. Their clinical and demographic data were also retrieved by reviewing files and where needed additional information were collected and archived.

Analytical determination of enzymes:

Enzymes were analyzed in blood samples collected in clot-activated tubes, centrifuged at 5000 rpm, serum separated and stored at -20^{o}C until processed. Biochemical enzymes such as creatine Kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were determined in serum by methods described earlier (Huang *et al.*, 2006; Tietz *et al.*, 1983; Chemnitz *et al.*, 1979; IFCC, 2002) on fully automated chemistry analyzers Cobas c501 (Roche Diagnostics, Basil). Data is reported as mean ± SD. Each sample was run in triplicate and results were expressed as mean ± SD. Data of serum enzyme from normal-healthy individuals were considered as comparable results and compared with those obtained from MDs group. However known normal reference ranges of these estimated serum enzyme are; CK = Males (7-12 years and adults) < 247 IU/l, Females < 154 IU/L; AST = Males < 35 IU/L, females < 50 IU/L; ALT = Males < 41 IU/L, Female < 31 IU/L; Alkaline Phosphatase = Males and females (7-12 years) < 300 IU/L, Male adults < 129 IU/L, Female adults < 104 IU/L; LDH = Males and females adult 135-225 IU/L; Children (2-15 years) 120-300 IU/L.

Statistical analysis:

Serum enzyme parametric data of all MDs group were compared and analyzed by SPSS ver 13.0. Results were considered significant when $P < 0.05$.

RESULTS

Results are summarized in table 1. All patients goruped in each MDs category manifested abnormal enzyme levels exhibiting comparative significance variability from < 0.05 to P < 0.001. Analysis of serum enzymes in Duchenne muscular dystrophy (n = 7) age range 15-20 (all males) group shows considerably high results as 116 ± 23.0; 105 ± 18.05, 116 ± 21.40, 484 ± 55.50, 505 ± 50.15 for ALT (IU/L), AST (IU/L), ALP (IU/L), LDH (IU/L), CK (IU/L) respectively. Similarly sample of patients in Becker's muscular dystrophy group (n = 6) all male, with age range 28-43 years exhibited elevate levels of enzyme viz 262 ± 45.00 IU/L, 196 ± 25.45 IU/L, 121 ± 20.65 IU/L, 1027 ± 101.20 IU/L, 1070 ± 100.55 IU/L for ALT, AST, ALP, LDH, CK respectively. Interestingly variations were considerably significant in serum levels of CK, LDH, AST, ALT and partially in ALP, in same order in Duchenne muscular dystrophy (P< 0.001), Becker's muscular dystrophy (P< 0.001) as compared to Facioscapulohumeral dystrophy (P< 0.05), limb girdle muscular dystrophy (P <0.05), Emery-Dreifuss muscular dystrophy (P< 0.05) as compared to normal healthy controls. Results were comparable amongst enzyme levels of various MDs within the groups. However, all enzymes manifested elevated levels in Duchenne muscular dystrophy and Becker's muscular dystrophy, whereas only CK and LDH manifested elevating trend as compared to ALT, AST and ALP in Facioscapulohumeral dystrophy and limb girdle muscular dystrophy, where in both later MD groups, it manifested lower than data as compared to control groups. Capricious pattern of variations in serum enzyme levels advocate the

suggestion that apparent alterations were muscular disease-dependent activity of physiological and metabolic nature, which makes this assessment unique and independent as per diagnosing MDs.

Table 1. Serum enzyme variations in various muscular dystrophic conditions.

Category (& patients number)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)	CK (IU/L)
Duchenne muscular dystrophy (n = 7) age range 15-20 (all males)	116 ± 23.0	105 ± 18.05	116 ± 21.40	484 ± 55.50	505 ± 50.15
Becker's muscular dystrophy n = 6) (all men) age range 28-43	262 ± 45.00	196 ± 25.45	121 ± 20.65	1027 ± 101.20	1070 ± 100.55
Facioscapulohumeral dystrophy (n = 5) age range 32-41	20 ± 2.55	27 ± 3.50	126 ± 21.35	254 ± 49.65	265 ± 45.50
limb girdle muscular dystrophy (n = 7) age range 32-45	41 ± 8.50	37 ± 4.65	66 ± 5.60	244 ± 45.70	255 ± 42.35
Emery-Dreifuss muscular dystrophy (n = 6) age range 28-48	66 ± 9.30	62 ± 9.15	60 ± 6.65	357 ± 41.90	272 ± 47.40
Normal controls (n = 10) Age range 22-50	40 ± 3.50	37 ± 3.40	110 ± 15.15	190 ± 20.40	198 ± 25.60
Level of significance P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.001	P < 0.001

Results are expressed as mean ± SD; CK= Creatine kinase; AST= Aldolase aspirate aminotransferase; ALT= Alanine aminotransferase; ALP= alkaline phosphatase; LDH= lactate dehydrogenase.

DISCUSSION

There are several types of muscular dystrophy and dystrophic conditions that result in difficulty in walking, standing, breathing problems, even complexity in speaking and normal day activities (Kanda *et al.* 2014, Zhu *et al.*, 2015). In current study patients suffering from five types of MDs viz., Duchenne muscular dystrophy, Becker's muscular dystrophy, Facioscapulohumeral dystrophy, limb girdle muscular dystrophy, Emery-Dreifuss muscular dystrophy, were selected for assessment of their serum enzymes levels such as CK, AST, ALT, LDH and alkaline phosphatase (ALP). Alterations were considerably significant in serum levels of CK, LDH, AST, ALT and partially in ALP, in same order in Duchenne muscular dystrophy ($P < 0.001$), Becker's muscular dystrophy ($P < 0.001$) as compared to Facioscapulohumeral dystrophy ($P < 0.05$), limb girdle muscular dystrophy ($P < 0.05$), Emery-Dreifuss muscular dystrophy ($P < 0.05$) and normal healthy controls. No significance (or if exist than it was minor significance level) was noted amongst enzyme levels of various MDs. Notably, all assessed enzymes ALT, AST, ALP CK, LDH exhibited elevated levels in Duchenne muscular dystrophy and Becker's muscular dystrophy, whereas only CK and LDH manifested elevating trend as compared to ALT, AST and ALP which exhibited lower than normal levels in Facioscapulohumeral dystrophy and limb girdle muscular dystrophy. This variable pattern of alteration in serum enzyme levels supports the suggestion that resultant alterations were MD-dependent muscular and metabolic activity, which makes this assessment unique and independent as per diagnosing MDs.

It is well documented that Duchenne muscular dystrophy inherited disorder mostly affecting boys; however girls can occasionally be affected, with milder anomalies. Duchenne MD patients start to develop noticeable symptoms between the age of one and three years. Additionally, some teens with Duchenne MD might develop dilated cardiomyopathy at later stages. Contrarily facioscapulohumeral MD affects both gender, however males tend to be affected earlier and more severely than females. Most dangerous part of facioscapulohumeral MD is non-

manifestation of any symptoms till adulthood. Becker MD like Duchenne mostly affects male at an early age and cause muscle damage to similar areas of the body, except that symptoms are less severe. Nonetheless, Limb-girdle MD is not a single MD but collage of related conditions that cause weakness in the big muscle groups at the base of the arms and legs (around the shoulders and hips) and its symptoms usually begin in late childhood or early adulthood, whereas patients with Emery-Dreifuss MD often begin to develop symptoms during childhood or adolescence, where the muscles and tendons become shortened and tightened, limiting the range of movement at nearby joints. However, due to the risk of serious heart and respiratory problems in Emery-Dreifuss MD, patient might have a shortened life expectancy than other MDs.

Some recent past studies regarding serum enzyme assessments in various form of MDs suggested its usage more often to compliment or support clinical diagnoses and replacing (or helping) mostly expensive (CT, MRI) or invasive (Biopsies) diagnostic measures (Begum *et al.*, 2000; Kamath *et al.*, 2000; Kohli *et al.*, 2005; Korones *et al.*, 2001; Zhu *et al.*, 2015). Our results are also in agreement with previous studies where ALT, AST, LDH and ALP were analyzed to assess its efficacy or any alterations in various MDs (Zhu *et al.*, 2015) and patients with Becker's muscular dystrophy and Duchenne MD manifested high levels of ALT, AST and LDH (Kanda *at el*., 2014; Zhu *et al.*, 2015) as compared to healthy controls. Arguably, some studies suggested that since patients with MDs often mistakenly considered as cryptogenic liver diseases, levels of AST, ALT were of lesser importance whereas in combination with ALP and LDH would increase its credibility and clinical significance (Begum *et al.*, 2000; Kamath *et al.*, 2000; Kohli *et al.*, 2005; Korones *et al.*, 2001; Pratt and Kaplan, 2000; Urganci *et al.*, 2006; Vajro *et al.*, 2010; Zhu *et al.*, 2015).

Related research studies done earlier showed that subtypes of MDs exhibited variable degree of alterations such as upto 22.6 times increase in case of ALT amongst Duchenne MD patients (McMillan *et al.*, 2011; Zhu *et al.*, 2015), whereas another study suggested that MDs can be graded as per their potency according to assessed levels of AST or ALT (e.g DMD/BMD > LGMD > FSHD) (Zhang *et al.*, 2012; Zhu *et al.*, 2015). Furthermore LDH was also stated to notable amongst serum enzyme that can manifest higher levels upto 3.4-folds in cases of DMDs (Yasmineh *et al.*, 1978; Zhu *et al.*, 2015). As far as ALP is concern, it is not often considered as a MD marker however, its elevation (although not very substantial) in various types of MDs, along with other serum enzymes is or merit and needed further exploration (Tawil *et al.*, 1998; Zhu *et al.*, 2015). Nonetheless, CK does provide differential diagnosis to some extent in all cases of MDs, as suggested, however each type of MD provide a different set of serum enzymes alterations (Tawil *et al.*, 1998; Zhu *et al.*, 2015).

CONCLUSION

In conclusion, the distribution or alteration pattern of serum enzymes, assessed in various types of MDs may provide supportive inference for the differential diagnosis of designated MD. Furthermore, patients with various MDs although shared the similar altered serum enzyme profiles, characteristic evaluation amongst these diseases would be made easier based on both enzymes results and clinical features.

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