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**RESEARCH ARTICLE**

# Comparative analysis of Correlation among Creatine kinase (CK), Aldolase and Myoglobin (Mb) concentrations in patients suffering from Myopathies

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## *Abstract*

*Aim:* The study presented in this communication details the determination of possible correlation among various muscular enzymes such as creatine kinase (CK) and aldolase with myoglobin levels, in addition to the hypothesis that this combination of muscular components may precede individual assessment regiments in patients suffering from myopathies. *Methods*: The research study was performed during the period effective January 2006 to December 2008. Patients who met the laid down and referred criteria were eligible for the study. Standard protocols were followed for blood samples collection and estimation of aldolase, creatine kinase (CK) and myoglobin (Mb) levels. Regression correlations were determined and results were compared as per $R^2$ and significance level $P < 0.05$. *Results:* Seventy five patients including males = 46 (61.33%) and females = 29 (38.66%) were selected that were fulfilling the inclusion criteria. Notably CK and myoglobin levels, and aldolase and myoglobin levels, when plotted through regression, were leading with exceptional correlation. CK-Myo in total patients showed $R^2$ of 0.7116, whereas for males, $R^2$ 0.5157 and females $R^2$ 0.8686, thus depicting 71%, 51% and 86% existing correlation with elevating levels of both components. This also shows the positive correlation between these parameters and marked significance ($p<0.01$), however in group of females, strongest significant level of $p< 0.001$. In groups where aldolase was combined and correlated with myoglobin, $R^2$ also depicts considerable correlation activity in total patients as $R^2$ 0.7197, males $R^2$ 0.6939 and females $R^2$ 0.6893. *Conclusion*: It was concluded that the combination of muscle enzymes and proteins, such as CK and aldolase with myoglobin, especially in patients with myopathies, enhanced the diagnostic utility and significance of this combo of laboratory tests.



## Introduction

It is documented that muscle enzymes such as creatine kinase (CK), transaminase enzymes especially aspartate transaminase or aldolase and muscle component such as myoglobin and its determination can predict the presence and extent of myopathies (Borleffs *et al.*, 1987; Rider and Miller, 1995, Toledano *et al*, 2012). It was referred that concentration of circulating CK, one of the significant laboratory parameter has been suggested to facilitate the diagnosis and follow-up of the patients with DM or PM or related myopathies. The notion behind this selectiveness is the high dimension

of sensitivity, relative muscle specificity, and good correlation of CK with disease activity and muscle strength (Rider and Miller, 1995; Straus *et al.,* 1989; Targoff, 1988; Tymms *et al.,* 1990). Similarly increased plasma aldolase level was reported to identify patients with risk to develop subsequent myopathy (Toledano *et al.,* 2012). In addition, increase in aldolase concentration was found in myotonic muscle disease, such as progressive muscular dystrophy (Brancaccio *et al.,* 2010). However beside its intrinsic worth, it was also argued that independent assessment relating only to serum CK levels might never be a clinical or diagnostic alternate that can replace systematic review of the patient, which includes muscle strength and functional assessments, as done for the measurements of myositis disease activity (Rider and Miller, 1995). Therefore, aldolase is suggested to be assessed together with CK to evaluate the detailed status of muscle activity and dystrophy. Consequently, relating to aforementioned argument and to aid in the diagnosis and treatment in myopathies, researchers, scientists and clinicians assessed other parameters as well, suggesting combination of more than two enzymes or muscle components such as CK with myoglobin or CK and aldolase-combined, so that significant biochemical epitome can be obtained with simpler interventions.

Determination of serum myoglobin levels in patients of PM, DM and generic myopathies, in addition to serum enzyme such as CK and aldolase levels facilitating the in-depth assessment of clinical features, were the few significant preliminary studies reported earlier (Borleffs *et al.,* 1987; Brancaccio *et al.,* 2010; Giampietro *et al.,* 1987; Nishikai and Reichlin, 1977; Rider and Miller, 1995; Toledano *et al.,* 2012). Therefore the present study was undertaken to determine possible correlation between CK and aldolase in combination with myoglobin levels to help in distinct and vigor evaluation of onset and magnitude of myoptahies. It was also hypothesized that increased correlative activity of CK versus myoglobin and aldolase versus myoglobin concentrations may precede the changes detected and then possibly utilize in final diagnosis, as compared to CK and aldolase levels independently in patients suffering from myopathies.

## MATERIALS AND METHODS

### Research protocols:

The presented study was performed during the period effective from January 2006 to December 2008. The study was a collaborative venture between departments of biochemistry lab services and Physiotherapy services. Basic inclusion criteria were patients aged > 20 yrs to < 75 yrs and having the diagnosis of myopathies as per description of Hoogendijk *et al.,* (2004) and Toledano *et al.,* (2012). Exclusion criteria were mainly the patients with aged group < 18 years and > 75 years and with any known immunosuppressive condition including other clinical conditions such as infections, hypo-thyroid disorders, hepatic infections, hematological disorders, cardiac episodes, or any current extremities accident (Toledano *et al*. 2012).

### Collection of clinical and related data:

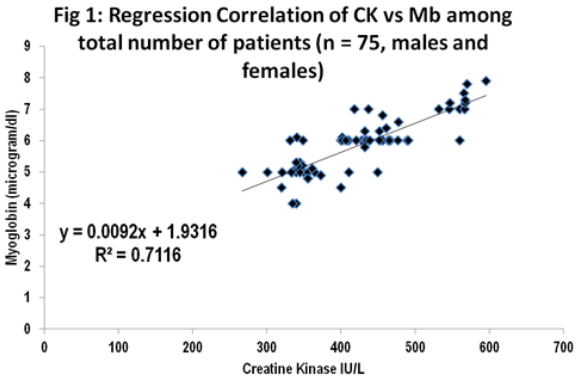
Data of a total of 75 patients (males = 46, females = 29) were obtained and complied as per described criteria mentioned above. Data were also collected from LIS and HIMS archives. Age, gender, description of myopathies, disease duration and current or any previous history of determination of CK, aldolase (or any muscle enzymes such as LDH, AST) and myoglobin levels were collected and analyzed.

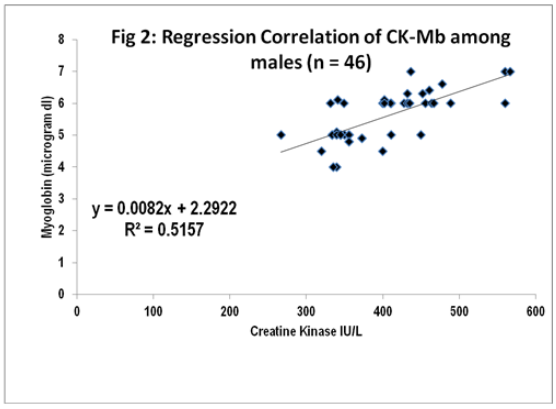
### Blood sampling and analysis:

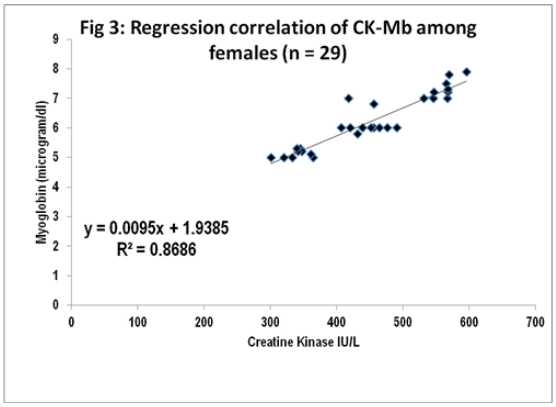
Differential biochemical and serological tests were performed on blood samples collected from the groups to facilitate extensive investigative and clinical assessments. The tests were antinuclear antibodies (ANA) and RA factor, aldolase, creatine kinase, C reactive protein and myoglobin that were performed using internationally recognized and prescribed methodology and as per protocols noted earlier (Borleffs *et al.,* 1987; Giampietro *et al.,* 1987; Toledano *et al.,* 2012). Blood sampling was done from patients through venipuncture (Li-heparinate 5 mL tubes) suggestively after an overnight fasting and prior resting period of three days. Analysis of aldolase, CK enzyme levels and myoglobin were done by standard methods at the time of 1$^{st}$ visit and when required at the time of diagnosis of myopathy. Aldolase was determined by standard commercial kit from Roche and Randox on Spectronic 1010 (Borenger Menhiem, Germany) and Randox-Monza (semi-automatic analyzer-Randox-UK). CK and CRP were measured on Hitachi 912 (Roche Diagnostics-Basel) using commercial kits and both internal and external quality controls. Myoglobin was measured on Hitachi 912 (Roche Diagnostics-Basel) by Tina-Quant Myoglobin Gen.2 (Roche)-immunoturbidimetric- latex–bound anti-myoglobin antibodies method. The ranges for myglobin (Mb) were Men: 2.3-7.2 µg/dL Women: 1.9-5.1 µg/dL. Normal reference ranges of all analytes are plasma aldolase, 0-16 years: <14.5 U/L and > 17 years: <7.7 U/L; CK, males > 18 years: 52-336 U/L, females > 18 years: 38-176 U/L and CRP, < 8.0 mg/L, respectively.

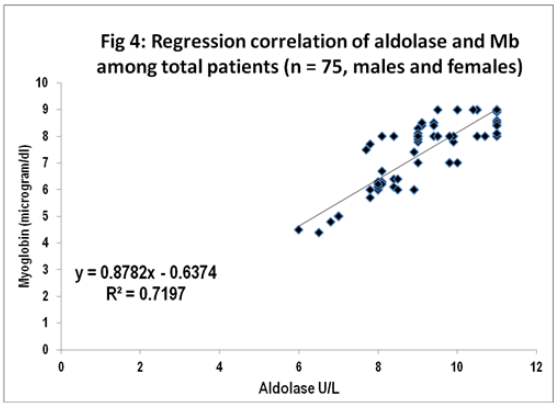
## RESULTS

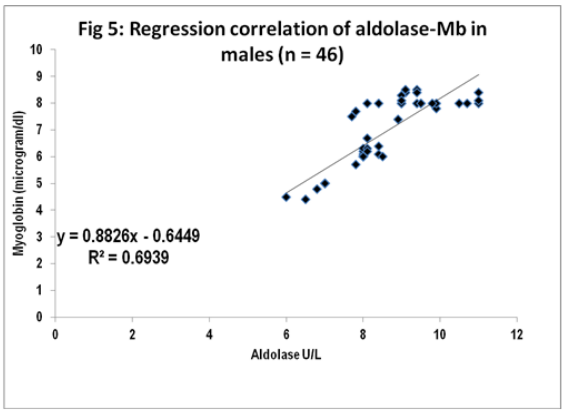
The results are summarized in Figures 1-9. Seventy five patients both males = 46 (61.33%) and females = 29 (38.66%) were selected and thus part of the presented research cohort. Levels of CK, CRP, aldolase and myoblobin were measured and both combination i.e. CK versus myoglobin and aldolase versus myoglobin showed significant $R^2$ correlation. Whilst combining enzymes and muscle components resulted in favorable correlation upto $R^2$ 0.86 (range 0.68 to 0.86), the grouping of CK versus aldolase showed certain low levels of correlation between $R^2$ 0.13 to $R^2$ 0.42. The mean CK levels for males was 399.54 ± 61.45 IU/L, for females was 449.3 ± 80.15 IU/L and for total patients, 418.78 ± 81.60 IU/L, where as myoglobin concentrations were 6.93 ± 1.50 □ g/dl, 7.75 ± 2.15 □ g/dl and 7.24 ± 1.35 □ g/dl for males and females and total patients. Similarly aldolase levels were 8.58 ± 2.20 U/L, 9.60 ± 2.43 U/L and 8.97 ± 1.98 U/L, respectively for males, females and total patients. Notably CK and myoglobin levels, and aldolase and myoglobin levels, when plotted through regression, were leading with exceptional correlation. CK-Myo in total patients showed $R^2$ of 0.7116 (Fig 1), whereas for males, $R^2$ 0.5157 (Fig 2) and females $R^2$ 0.8686 (Fig 3), thus depicting 71%, 51% and 86% existing correlation with elevating levels of both components. This also shows the positive correlation between these parameters and marked significance ($p<0.01$), however in group of females, strongest significant level of $p< 0.001$. In groups where aldolase was combined and correlated with myoglobin, $R^2$ also depicts considerable correlation activity in total patients as $R^2$ 0.7197 (Fig 4), males $R^2$ 0.6939 (Fig 5) and females $R^2$ 0.6893 (Fig 6). Somewhat lagging in correlative activity was the combination of CK with aldolase, especially when assessed in females ($R^2$ 0.1356-Fig 9), however moderate activity was observed in total ($R^2$ 0.4282-Fig 7) and male ($R^2$ 0.6808-Fig 8) groups. The results profoundly suggest that by means of the combining CK and aldolase with myoglobin assay, additional laboratory investigation might not be needed, since CK-aldolase-myoglobin levels showed around 51% to 86% positive correlation with occupying muscle disorders.

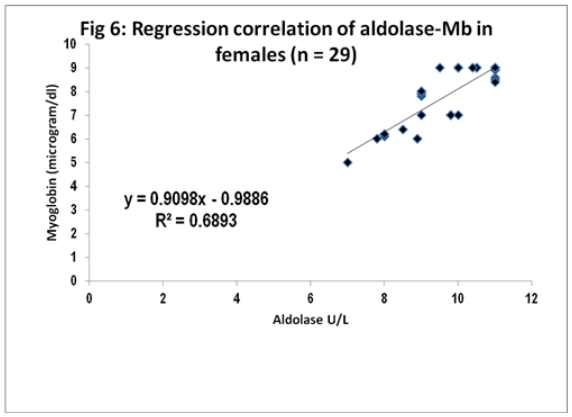


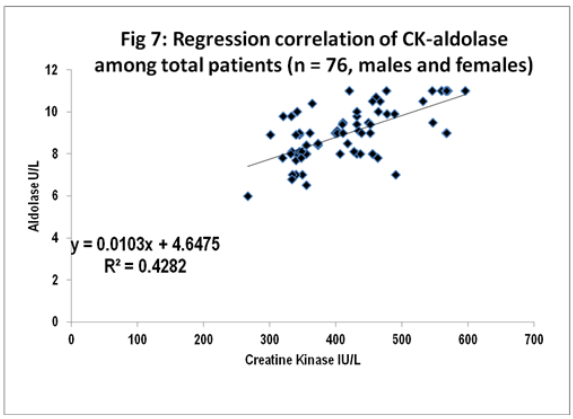



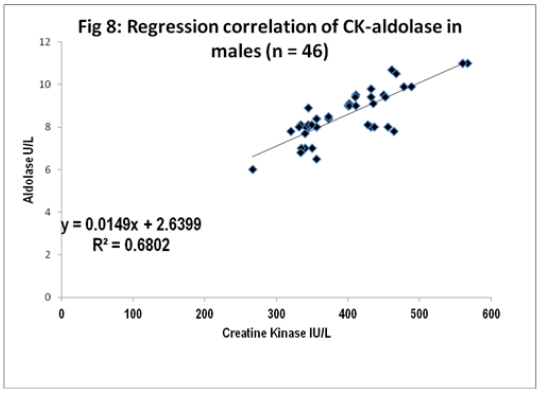



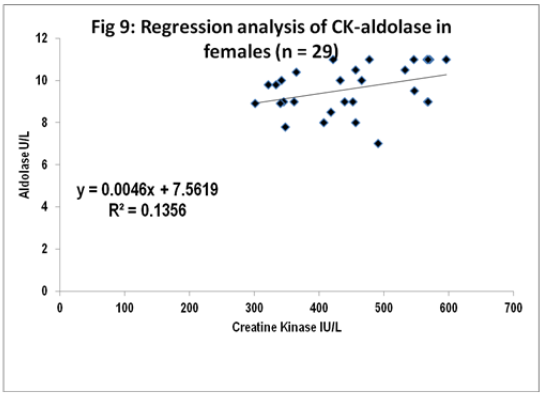



## DISCUSSION:

Generally, myopathies are grouped into several disease, conditions and syndrome forms such as familial myopathies, progressive degeneration of tissue and muscles, mitochondrial myopathies, muscle disease due to polysaccharide storage, dermatomyositis (DM), myositis ossificans, polymyositis (PM), inclusive of body myositis, and related myopathies and tetany (Alisa *et al.*, 2013; Bohan and Peter, 1975 a,b; Ungprasert *et al.*, 2013). Scientifically myopathies are known disorders of neuromuscular origin characterized by the foremost manifestation of muscle feebleness related to dysfunctional muscle fiber. Moreover, it could be innate (such as the muscular dystrophies) or acquired (such as common muscle cramps).

In this regard, when diagnostic and clinical analysis of myopathies were performed, it was noted that the levels of several enzymes such as aldolase, lactate dehydrogenase (LD) and its isoforms 5 and 1, and transaminases (both serum glutamic oxalacetic transaminase [SGOT]/aspartate aminotransferase [AST], serum glutamic pyruvic transaminase [SGPT]/alanine aminotransferase [ALT]) and carbonic anhydrase III were found to be increased in

patients (Borleffs *et al.,* 1987; Giampietro *et al.,* 1987; Nishikai and Reichlin, 1977; Rider and Miller, 1995; Toledano *et al.,* 2102). However, comparatively the extent of increase in all of such enzyme activities occurs less frequently and to a smaller extent than CK levels (Bohan *et al.,* 1977; Hochberg *et al.,* 1986; Rider and Miller, 1995).

Several past studies suggested that estimating CK and myoglobin in combination in patients with myopathies would be more beneficial than individual assessment as both correlated significantly with each other than any other enzymatic or muscle components (Alam *et al.,* 2013; Borleffs *et al.,* 1987; Diószeghy and Mechler, 1988; Giampietro *et al.,* 1984). Similarly it was notified earlier that determining serum Mb and CK concentrations would depict analogous clinical usefulness as symbol of muscle damage in foremost and then consequential skeletal muscle disorders (Diószeghy and Mechler, 1988). Nonetheless, it was argued that, the serum Mb is more of a detector of carrier patients than an assessor, thus can't be targeted as more precise than CK measurement (Diószeghy and Mechler, 1988). Additionally, it was documented that muscle damage due to excessive exercise may also induce an increase in myoglobin levels, thus depicting its importance as a direct parameter for assessing acute muscle damage (Borleffs *et al.,* 1987) and not for chronic cases such as PM and DM. Even so, it was envisaged that combining both CK and myoglobin estimation in patients with dermatomyositis or polymyositis shall provides better overall impression of muscle damage (Borleffs *et al.,* 1987; Diószeghy and Mechler, 1988). Regarding aldolase, a recent study argued that it can accurately indentify myopathy (Myo) occurring in systemic sclerosis (SCC) and in those patients who may have high risk of developing subsequent Myo-SCC (Toledano *et al.,* 2012). Similarly it was reported that aldolase level may increase in myotonic muscle diseases such as PM or progressive muscular dystrophy (Brancaccio *et al.,* 2010).

Henceforth, the present describes the results that were obtained after assessing a total of 75 patients [males = 46 (61.33%) and females = 29 (38.66%)]. Determination of CK, CRP, aldolase and myoblodin levels were measured and to evaluate the correlative efficacy of combination of more than one enzyme/components, three different sets of combination were used, i.e. CK versus myoglobin, aldolase versus myoglobin and CK versus aldolase. Out of all three combinations, CK versus Mb showed highest significant $R^2$ correlation. Whilst combining enzymes and muscle components resulted in favorable correlation upto $R^2$ 0.86 in case of CK-Mb, the grouping of CK versus aldolase showed certain low levels of correlation between $R^2$ 0.13 to $R^2$ 0.42. Particularly, CK and myoglobin levels, and aldolase and myoglobin levels, when plotted through regression, were leading with exceptional correlation. CK-Myo in total patients showed $R^2$ of 0.7116, whereas $R^2$ 0.5157 for males and $R^2$ 0.8686 for females, thus depicting 71%, 51% and 86% existing correlation with elevating levels of both components. The significance levels were $p<0.01$ which also shows the positive correlation between these parameters. Conversely, in group of females, strongest significant level of $p< 0.001$ was noted with $R^2$ of 0.86 in the combo of CK-Mb. In groups where aldolase was combined and correlated with myoglobin, $R^2$ also depicts considerable correlation activity in total patients as $R^2$ 0.7197, males $R^2$ 0.6939 and females $R^2$ 0.6893. Somewhat lagging in correlative activity was the combination of CK with aldolase, especially when assessed in females ($R^2$ 0.1356), however moderate activity was observed in total ($R^2$ 0.4282) and males ($R^2$ 0.6808) groups. The results profoundly suggest that by means of the combining CK and aldolase with myoglobin assay, additional laboratory investigation might not be needed, since CK-aldolase-myoglobin levels showed around 51% to 86% positive correlation with occupying muscle disorders. Our findings were supported by two previous studies mentioned earlier (Borleffs *et al.,* 1987; Diószeghy and Mechler, 1988) and yet another by Giampietro *et al* (1984) and a recent by Alam *et al.,* (2013) that reported significance of combining CK estimation with that of myoglobin concentration. Giampietro *et al* (1984) categorically stated that both, the muscle component "myoglobin" and the enzyme CK, have shown their worth as preeminent indicators of the myopathy related to hypo-thyroid disorders. Additionally, they are responsive in timely recognition of muscle involvement and are closely correlated to the metabolic conditions of patients (Alam *et al.,* 2013; Giampietro *et al.,* 1984). In addition, discovering the importance of aldolase in Myo-SCC assessments, it was suggested that elevated levels of aldolase may help clinicians to recommend biopsy and monitor progression (Ranque *et al.,* 2009; Toledano *et al.,* 2012).

It was initially deliberated that the levels of CK and other muscle-derived enzymes in serum such as LDH, aldolase, are generally useful in following myositis activity and responses to therapy, especially in PM/DM. However the magnitude of the increase in its level does not always correlate with disease activity (Rider and Miller, 1995; Straus *et al.,* 1989). Past and present studies thus suggested inclusion or assessing of other enzymatic components for the

same purpose, therefore supporting the objectives and results our study as well. For example in a cohort study reported last year, it was assessed and concluded that incremental spikes in blood aldolase at initial stage of disease progression was suggestively the best method to assist in diagnosis of systemic sclerosis patients. However, other components such as transaminases and inflammatory proteins, when assessed were not suitable to predict subsequent Myo-SSc occurrence (Toledano *et al*, 2012). Depicting this defiant elevation in serum CK levels in patients with myositis, it was suggestively argued that, it was due to increases in the MM-isoenzyme fraction which is released from skeletal muscle (Rider and Miller, 1995). Debatably continuing elevation of CK-MB levels is commonly seen in patients with skeletal muscle damage (Keshgegian and Feinberg, 1984; Rider and Miller, 1995; Tsung *et al.,* 1982). Similarly, the levels of macro-CK type 1, a complex of a CK isoenzyme and immunoglobulin, were found elevated in patients with myositis as well (Lee *et al*., 1994; Rider and Miller, 1995). In a very significant study reported earlier by Diószeghy and Mechler (1988), both CK and Mb levels were analyzed in patients with different neuronal and muscle diseases, carriers of X-linked Duchenne-type muscular dystrophy and also in normal volunteers, resulting in discovering of high levels in Duchenne dystrophy. In patients distressed by limb-girdle dystrophy, the incremental elevation in CK activity and Mb levels were also pronounced (Diószeghy and Mechler, 1988), thus supporting the outcome of our cohort as well.

**CONCLUSION**:

After assessing our resultant outcome and the riposte to our hypothesis, it was thus concluded that by combining both CK and myoglobin, in addition to aldolase and myoglobin combination in patients with myopathies especially dermatomyositis or polymyositis, the diagnostic utility and significance of this combo test has extensively enhanced thus providing a better overall impression of muscle damage and related conditions.

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