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Chue-Fun Chen

I-Nan Lien

Fung-Jou Lu

SERUM CREATINE KINASE ACTIVITY AND ITS ISOENZYMES IN DUCHENNE MUSCULAR DYSTROPHY

CHUE-FUN CHEN, I-NAN LIEN and FUNG-JOU LU*

Serum creatine phosphokinase (CK) and its three isoenzymes (CK–MM, CK–MB and CK–BB types) were studied on 23 patients with well-defined Duchenne muscular dystrophy and 24 normal subjects. The cardiac status of the patients was evaluated with ECG examination. The functional capacity of each patient was evaluated and divided into 9 calsses.

The CK activities (IU/L) of the patients were significantly higher than those of the normal subjects ($p<0.05$). CK isoenzyme analysis with electrophoresis on cellulose acetate revealed that the sera of the 24 normal subjects contained only CK–MM isoenzyme. In addition to CK–MM isoenzyme, CK–MB isoenzyme was present in 20 cases among the 23 patients (87%). No CK–BB isoenzyme was present in the sera of either normal subjects or the patients.

The results showed that CK activity (IU/L) and MB isoenzyme activity (IU/L) had an inverse relationship with the patient's age and functional capacity ($p<0.05$), while MB% (MB/total CK x %) had no relationship with the clinical status of the patient. Therefore, it is recommended to use the MB activity in IU/L rather than MB% in the clinical evaluation of the patients. Besides, all of the patients in the present study were free from any sign of cardiac damage on ECG examination, and therefore the MB isoenzyme shown in the serum of Duchenne muscular dystrophy does not originate from dystrophic cardiac muscle.

Key words: ***creatine kinase, creatine kinase isoenzyme, Duchenne muscular dystrophy.***

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Increased activity of serum creatine kinase (CK: EC 2.7.3.2.) has been reported in patients with Duchenne muscular dystrophy (DMD) and used extensively in monitoring their progression.[1-4] The enzyme is a dimeric molecule consisting of two subunits: M (muscle type) subunit and B (brain type) subunit. The dimeric enzyme molecule has three isoenzymes in human tissue: CK–BB (CK–1; brain type), CK–MB(CK–2; myocardial type) and CK–MM (CK–3; muscle type) isoenzymes.[5-6] CK activity is abundant in heart and skeletal muscles, but the proportion of isoenzyme in these two tissues is quite different. Several investigations[6-9] have revealed that the heart muscle contains 25%-52% MB and 48%-75% MM isoenzymes. Skeletal muscle contains 0%-28% MB and 72%-100% MM isoenzymes. In the sera of normal healthy subjects, only CK–MM isoenzyme is present.[7] The CK–MB isoenzyme is found in the sera of patients with acute myocardial infarction[10-12] or various neuromuscular diseases,[7-8,13-15] especially DMD.[2,4]

It is still very controversial whether the MB isoenzyme in the serum of DMD patients reflects the dystrophic process

Department of Physical Medicine and Rehabilitation, National Taiwan University Hospital, and Department of Biochemistry*, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.
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in the myocardium,[7] although cardiomyopathy is well known to be present in DMD.[16] The purpose of this study is to identify the tissue origin of MB isoenzyme and the patterns of elevated CK and its isoenzyme activity in the sera of patients with DMD.

MATERIALS AND METHODS

Sera were obtained from 23 male patients, with ages ranging from 4 to 33 years, who had a well-documented history of Duchenne muscular dystrophy confirmed by clinical examination, electromyography and muscle biopsy. Control sera were obtained from 24 healthy volunteers without any evidence of neuromuscular disorder or heart problems. Among the volunteers there were 13 males and 11 females, with ages ranging from 21 to 60 years. The blood samples were taken from the subjects via venopuncture. The serum was then separated from the whole blood after clotting and divided into two portions. One was analysed immediately for the total activity of CK, and the other was analysed immediately or stored at -20°C and analysed within one week for the CK isoenzymes. The CK total activity was measured with Gilford diagnostics kits at 25°C. The measurement of CK isoenzymes was performed by electrophoresis. It was performed on cellulose acetate with the procedure developed by Helena Laboratories. About 10 μl of serum was applied on the plate and was electrophoresed for 10 minutes at 350 volts. Simultaneously, a control reagent, containing all MM, MB and BB isoenzymes was also applied for identification of the location of each isoenzyme during electrophoresis. The electrophoretic pattern was scanned on a Flu-Vis-Quick-Scan (Helna Laboratories). The result of CK activity was expressed in international units per liter (IU/L). The MB isoenzyme was expressed in MB% (MB/total CK x %) or IU/L (CK activity x MB%). The patient's functional capacity was divided into 9 classes according to the method of Vignos[17] (Table I). There were 12 cases in classes 1 to 4 and 11 cases in class 9. No case was in classes 5 to 8. Accordingly the patients were divided further into two groups: ambulatory group (Classes 1 to 5) and nonambulatory group (Class 9). Besides, the patient's cardiac status was evaluated by cardiac auscultation and ECG recorded with standard 12 leads.

RESULTS

The average value of serum CK total activity of normal healthy subjects was 36.3±8.7 IU/L (mean±ISD) and that of DMD patient was 2296.9±1982.2 IU/L.

Table 1. Classification of Functional Ability in Patients with Duchenne Muscular Dystrophy by Vignos *et al.*[17]

Class	Physical activity capacity
1	Walks and climbs stairs without assistance
2	Walks and climbs stairs with aid of railing
3	Walks and climbs stairs slowly with aid of railing (25 seconds for eight standard steps)
4	Walks unassisted and rises from chair but can not climb stairs
5	Walks unassisted but can not rise from chair or climb stairs
6	Walks only with assistance or walks independently with long leg braces
7	Walks in long leg braces but requires assistance for balance
8	Stands in long leg braces but unable to walk even with assistance
9	In wheelchair or bed

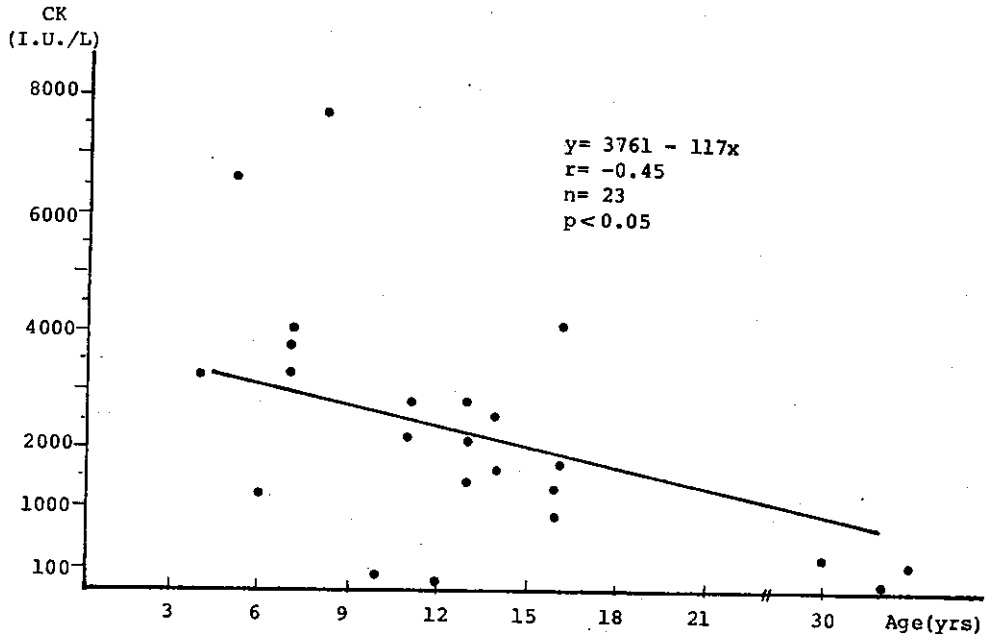

Fig. 1. Correlation between serum CK activity and the age of patients with Duchenne muscular dystrophy

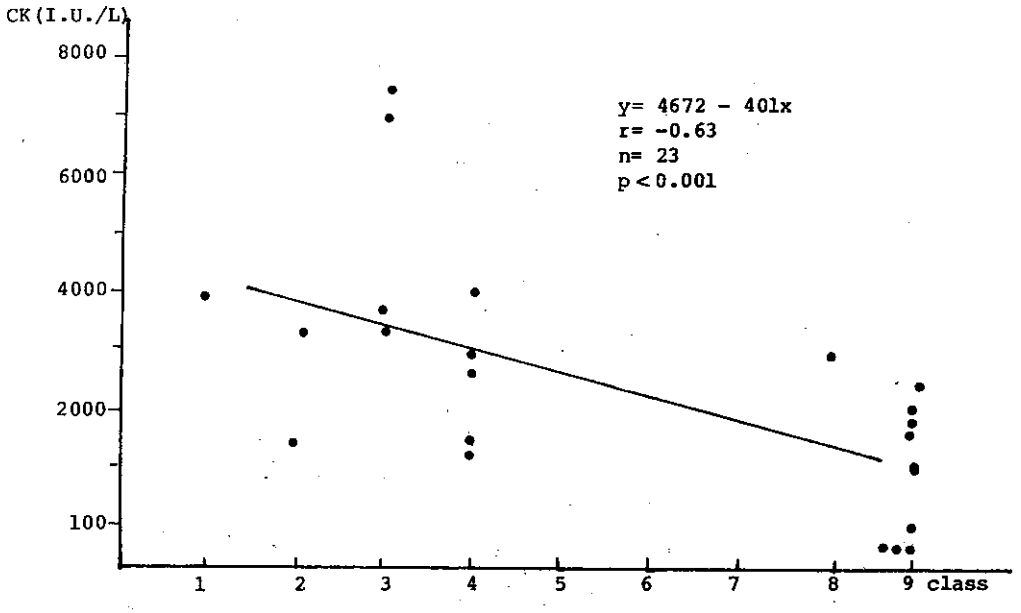

Fig. 2. Correlation between serum CK activity and functional class of patients with Duchenne muscular dystrophy.

The highest CK activity of the patients was 150 times the normal. Among the 23 patients, CK activity was elevated in 20 cases (87%), being normal in 3 cases. These 3 cases all belonged to class 9 functional capacity and their ages were 10, 12 and 32 years respectively. There was a negative correlation ($p<0.05$) between the serum CK activity and the age of patients (Fig. 1). The functional capacity of patients deteriorated significantly with increasing age ($p<0.05$). Statistically it was also shown that CK activity was significantly higher in the initial functional class of the disease and decreased gradually when the disability progressed (Fig. 2).

The electrophoretic scans of the control reagent demonstrating locations of the 3 isoenzymes are shown in Fig. 3A: MM isozyme was the slowest moving band and MB isoenzyme migrated intermediate to MM and BB isoenzymes. In all the sera of normal subjects, only MM isoenzyme was detected (Fig. 3B). Occasionally an albumin band appeared between MB and BB isoenzymes, but it was excluded from the calculation of isoenzyme distribution. In the sera of DMD patients, MB isoenzyme (Fig. 3C) was evident in 20 cases (87%). Among these 20 cases there were 2 cases whose CK activities remained in the normal range. The CK activities of the other 3 patients without MB activity in their sera were 39.4, 95 and 132.7 IU/L, respectively.

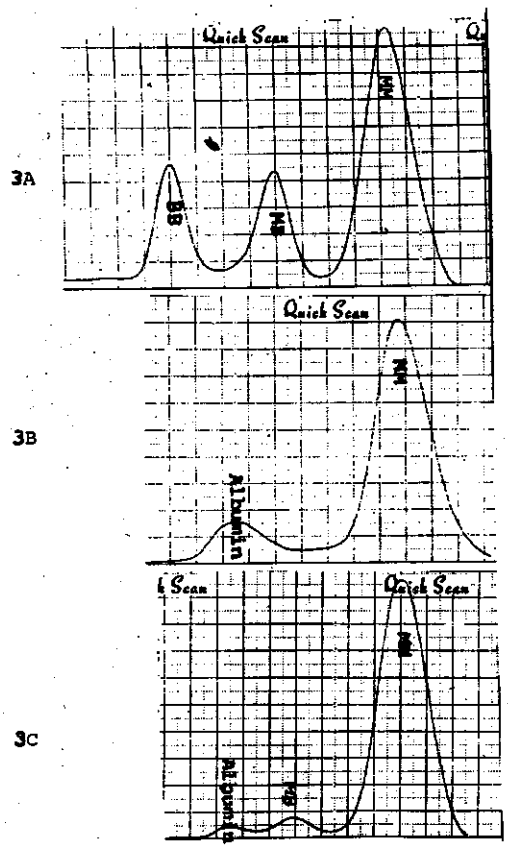

Fig. 3. The electrophoretic scan of serum CK isoenzyme pattern of control reagent (3A), normal subject (3B) and patient with Duchenne muscular dystrophy (3C).

The above findings revealed that MB isoenzyme could only be demonstrated in the sera of DMD patients, but the ap-

Table 2. Serum CK Activity, MB(%) and MB Activity (I.U./L) in Normal Subjects and Patients with Duchenne Muscular Dystrophy with Different Functional Capacity

Serum level	Normal subject (N=24)	Duchenne muscular dystrophy		
		Ambulatory (N=12)	Non-Ambulatory (N=11)	P value
CK activity (I.U./L)	36.3±8.7	3435.7±1977.8	1050.7±1041.4	< 0.05
MB (%)	0	10.3±4.6	6.5±7.8	> 0.1
MB activity (I.U./L)	0	358.9±252.9	119.6±156	< 0.02

pearance of MB isoenzyme in the serum did not correspond to the increase of CK activity. No BB isoenzyme was detected in normal subjects or the patients. The proprotion of MB isoenzyme in DMD patients ranged from 2.4% to 27.0% (mean=9.7%) of the total CK activity. There was no relationship between the proportion of MB isoenzyme (MB%) and the patient's age, or MB% and the patient's functional class ($P>0.1$). However, the activity of MB isoenzyme (CK activity x MB%) had an inverse relationship ($P<0.01$) with the patient's age and functional capacity (Fig. 4). As shown in Table 2, sera of DMD patients had abnormally high CK activity and appearance of MB isoenzyme. This phenomenon was more prominent in the ambulatory group of patients than in the non-ambulatory group.

In detailed cardiac evaluation, all the DMD patients were found to be free from cardiac symptoms, and the ECG examinations revealed no evidence of myocardial infarction.

DISCUSSION

Duchenne muscular dystrophy is a sex-linked recessive hereditary disease, affecting primarily the skeletal muscular system. The age at onset is usually around 3 years. The common symptoms are clumsiness in walking, difficulty in climbing stairs and running. The course is slowly progressive and eventually the patient becomes wheelchair bound or bed-ridden before the age of 20 years.[18] The sequence of deterioration is shown in Table 1 (from Class 1 down to 9). The breakdown of skeletal muscle contributes to the elevated CK activity in the serum of the DMD patient. In our study, the sera were taken from subjects at outpatient clinics. Since a previous study by Pearce[19] had shown that the CK activity of normal or dystrophic individuals does

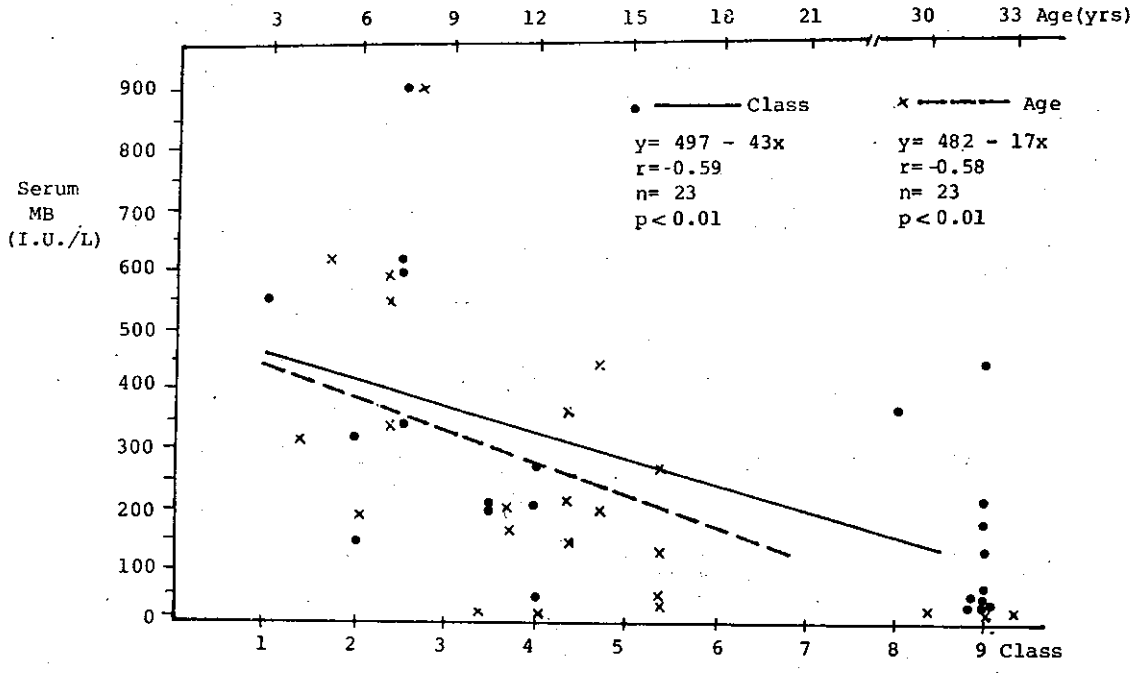

Fig. 4. Correlation between serum MB isoenzyme activity and the age and functional class of patients with muscular dystrophy.

not vary significantly with physical activity or the taking of food, the above factors will not influence the estimation of the CK activity of an out-patient. The results of CK activity analysis showed that the CK activity was elevated in 20 patients (87%) and it showed an inverse relationship with the age and functional capacity of the patients as shown in Fig. 1 and Fig. 2. These findings are similar to many previous studies.[1-4,19] It agrees with the explanation that the drop of CK activity with the patient's age and functional capacity are due to diminution of functional skeletal muscle mass[20] and restriction of activity.[21] In the late stage of the clinical course (Class 9), the patient's activity is markedly restricted and the muscle mass is markedly atrophic (Fig. 5). Only small amounts of CK can be released from the muscle, and therefore, the CK activity in the circulation is no more elevated. Practically, we can measure the CK activity in the serum as a reference for the patient's condition.

In this study, analysis of serum CK isoenzyme showed that MB isoenzyme was detected in 20 out of 23 patients. The detection rate of MB isoenzyme in DMD patients was 87%. The average MB% was 9% (2-27%). Although the MB% did not have any relationship with the patient's age and functional capacity in this study, we did demonstrate an inverse relationship of MB activity (IU/L) with the patient's condition as shown in Fig. 4 and Table 2. The reasons for the drop in MB activity with patient's age and functional capacity are similar to those of the drop in CK activity. The appearance of MB isoenzyme is not simply due to increased CK activity, because among 20 MB positive cases there were two cases with normal CK activity. On the other hand among the 3 MB negative patients, two cases had elevated CK activity. Carcia[13] in 1974 did special research to obtain sera with increased total CK activity, presumbly of muscular origin, and did not show any MB isoenzyme activity. Therefore, there must be some reason responsible for the discrepancy between CK activity and the appearance of MB isoenzyme in the sera of DMD patients.

Because of abundant MB isoenzyme content in heart muscle, transient elevation of serum MB isoenzyme in acute

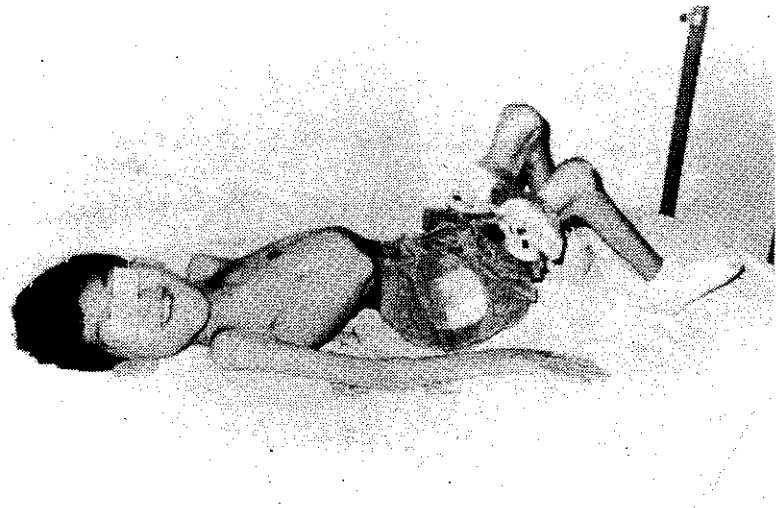

Fig. 5. Terminal stage of patient with Duchenne muscular dystrophy. Remarkable muscle atrophy and activity restriction were noted.

myocardial infarction is well known.[8-12] Goto[14] considers the appearance of MB isoenzyme in the serum of DMD patients reflects damaged heart muscle. In previous studies[16,20] cardiomyopathy associated with DMD has been recognized and the ECG was thought to be the most sensitive parameter of cardiac disease in DMD.[23] But in our study, ECG examination failed to detect any sign of cardiac damage in all the DMD patients. Therefore, the cardiac involvement is probably not a proper explanation for the appearance of serum MB isoenzyme in DMD patients. The most probable reason for serum MB isoenzyme in DMD patients is the breakdown and regeneration of skeletal muscle.[3-4,7,15,22]

It was observed by Uedak[24] that the white fiber (type II fiber) of skeletal muscle was mainly involved in the early stages (until 4 years old), followed by red muscle fiber (type I fiber) and heart muscle in the advanced stages (10 years to adult age). Rosalki[25] reported that the type II muscle fiber contains only MM isoenzyme and type I muscle fiber contains both MM and MB isoenzymes. Therefore in the later stages of DMD, relatively more MB isoenzyme will be released from the type I fiber into the circulation; in the early stage, however, there will be elevated CK activity containing mainly MM isoenzyme. The above phenomenon may partly explain the discrepancy between serum CK actiivity and the appearance of MB isoenzyme.

Another explanation is the presence of immature regenerative muscle fiber in DMD patients.[15] During embryonic development, skeletal muscle first synthesizes the B subunit of CK and then switches to the M subunit, so that BB, MB or MM is present at various stages of the developing skeletal muscle.[26] Muscle fiber regeneration is noted in the dystrophic muscle.[27] Adornato and Engel[15] observed their dystrophic patients and reported that the amount of regenerative fiber generally correlated with the amount of MB in the serum. Their finding favors the regenerative skeletal muscle fiber being the major source which contributes to the appearance of MB isoenzyme in the serum of DMD patients. In conclusion, our study revealed that the presence of MB isoenzyme in ther serum of DMD patients did not originate from cardiac muscle, but was possibly released from the immature regenerating skeletal muscle and the breakdown of type I skeletal muscle fiber into the circulation.

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