Study of Serum Isoenzymes of Creatine Phosphokinase and Lactate Dehydrogenase in Myocardial Infarction

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SUMMARY

Total serum enzyme levels of creatine phosphokinase and lactate dehydrogenase as well as their isoenzymes CKMB and HBDH $(LDH)_1$ were measured in 92 patients (74 males and 18 females) admitted consecutively to coronary care unit. The isoenzymes were determined on first day of admission while the total enzymes were measured on first three consecutive days of admission.

The patients were devided into two main groups, which were further classified in four subgroups according to age, E.C.G changes, location of infarct and the frequency of attack and its repetition . The combined use of CPK and LDH isoenzymes provided the best specificity for the diagnosis of acute myocardial infarction, and a good correlation with clinical and electrocardiographic findings.

INTRODUCTION

The serum enzymes, creatine phosphokinase, lactate dehydrogenase and aspartate aminotransferase are measured routinely for the diagnosis of acute myocardial infarction in local hospitals. It is well established that all the three enzymes are sensitive indicators of acute myocardial infarction. CPK level is raised however, in muscle diseases skeletal muscle injury (particularly I/M injections), brain damage, alcoholism, pulmonary embolism and after cardioversion.

LDH level is elevated in diseases of the liver, biliary and pancreatic tracts and skeletal muscle, (Varley et al, 1981). The diagnostic specificity can be improved by the determination of CPK and LDH isoenzymes (CKMB and HBDH), since these are found particularly in myocardium (Vanderveen and Willebrands, 1966).

The blood was collected with great care and in case of any suspicion of hemolysis, the sample was discarded, as HBDH (LDH_1) enzyme is also found in RBC. The present study was undertaken to evaluate the specificity and sensitivity of serum CPK and LDH isoenzymes and to correlate them with total enzyme levels, clinical and electrocardiographic findings.

PATIENTS AND METHODS

Ninety two patients suffering from acute myocardial infarction admitted to coronary care unit Mayo Hospital Lahore, were included in the study. The diagnosis of acute myocardial infarction was based on typical history, electrocardiogram and serum enzyme and isoenzyme changes.

Three consecutive specimens of blood were collected from the anterior cubital vein of each patient within 24 hours, 48 hours and 72 hours after admission respectively. The blood was allowed to clot. The clotted blood was poured into a centrifuge tube and centrifuged at 3000 r. p. m. The serum thus separated was analyzed for enzymatic levels of creatine phosphokinase,

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lactate dehydrogenase, CKMB & HDBH (LDH_1).

The combined use of CPK and LDH isoenzyme levels provided the best specificity for the diagnosis of acute myocardial infarction.

Serum isoenzymes, CKMB and HBDH (LDH_1) were determined only on the first day of admission while the total enzymes CPK and LDH were measured on first three consecutive days of admission. The enzyme CPK and LDH were estimated by Fiske, Subbarow (1925) and Spiegel and Symmington (1974) methods respectively. The isoenzyme CKMB was measured with the ultraviolet test sets of Merck. While HBDH was measured with the U. V test sets of Roche Diagnostica.

The upper normal limits of the enzymes and isoenzyme according to the above methods were CPK, 0-IQu/ (Sigma) LDH, 24-78u/l (General diagnostics), CKMB,0–10u/l and HBDH 55-140u/l respectively. The results were compared with age matched 38 healthy subjects. In each case a detailed history was taken and appropriate physical examination was performed. Blood pressure and serial electrocardiograms were recorded in every patient. The data was collected on a special proforma and was analysed by standard statistical methods.

RESULTS

The data was analysed by analysis of variance (F test) test. The results are shown in Table I-VIII.

DISCUSSION

The total serum CPK test has been advocated as an indicator of myocardial infarction (Smith, 1967), principally because this enzyme is not found in the liver and erythrocytes (Colombo et al., 1962), both of them are common sources of error in infarct diagnosis by means of serum enzym tests. The AST (SGOT) was not determined in this study due to its well documented non-

Table-I.

MEAN LEVELS OF SERUM CPK, LDH, CKMB AND HBDH MALE AND FEMALES) IN CONTROL GROUP.

CONTROLS	IST DAY				2ND DAY		3RD DAY	
	CPK u/ml	CKMB u/1	LDH u/1	HBDH u/1	CPK u/m1	LDH u/1	CPK u/ml	LDH u/1
C_1 n=10	4.8 (0-12)	4.29 (2.5-7.5)	61.7 (30-90)	80.0 (50-135)	3.9 (0-12)	57.4 (42-75)	4.1 (0-11)	58.4 (35-75)
C_2 n=10	3.9 (0-9)	4.21 (0-10)	74.4 (60-85)	91.6 (45-134)	3.6 (2-10)	65.4 (55-70)	4.6 (2-10)	69.1 (65-78)
D_1 n=10	3.0 (0-6)	2.1 (2-2.2)	82.0 (68-86)	101.0 (80-122)	4.0 (2-6)	63.5 (55-72)	1.5 (0-3)	68.5 (62-75)
D_2 n= 8	3.0 (0-6)	5.71 (1.5-10)	71.6 (50-92)	91.62 (55.5-135)	4.0 (2-6)	72.0 (60-85)	2.0 (0-6)	71.0 (60-82)

C_1 = males aged 41-50

C_2 = males aged 51-60

D_1 = females aged 41-50

D_2 = females aged 51-60

n = Number of subjects

The figure in Parenthesis show the Range of Values.

specificity (Castrini and Thomson, 1977), Although total serum LDH test is sensitive enough, it is less specific for infarct diagnosis. The isoenzyme CKMB and HBDH appear to constitute a quite specific indictors of heart damage, since the CKMB is found in skeletal muscle in very small amounts. (Somer and Konttinen, 1972). Other organs with high CPK activities are the brain and skeletal muscles. The former contains BB isoenzyme while the latter contains MM isoenzyme.

The LDH_1 isoenzyme (HBDH) is also considered to be the most specific infarct detector (Accvinen, 1972) but occurs in addition to myocardium in large amounts in RBC and Kidney. The error from erythrocytes can be eliminated by using unhemolysed sera, while the kidney disease can easily be differentiated from myocardial infarction. The mean enzyme and isoenzyme levels were 5-10 times higher in patients with acute myocardial infarction than in normal controls.

The mean serum CPK and LDH levels in male and female patients with unequivocal ECG changes in the Present study were higher than in patients with equivocal E. C. G. changes on first three consecutive days of admission. This difference in the serum CPK and LDH values between the two groups was statistically significant.

The serum CKMB and HBDH levels showed a significant difference ($P < .05$) ($P < .01$) between male patients with unequivocal and equivocal E. C. G. changes (II) while this difference was statistically non significant for CKMB between female patients with unequivocal and

Table-2.

MEAN LEVELS OF SERUM CPK,LDH, CKMB AND HBDH IN PATIENTS (MALE AND FEMALES) WITH CARDIAC INFARCTION ACCORDING TO ECG ASSESSMENT.

PATIENTS	1ST DAY				2ND DAY		3RD DAY	
	CPK u/ml	CKMB u/1	LDH u/1	HBDH u/1	CPK u/m1	LDH u/1	CPK u/m1	LDH u/1
A_3 n = 54	37.96 (0-184)	44.0 (2.8-240)	211.4 (50-420)	440.7 (0-1619)	26.79 (2-90)	221.9 (53-420)	11.62 (0-73)	209.6 (75-420)
A_4 n = 20	15.00 (0-57)	18.75 (0-70)	124.3 (65-282)	162.6 (64-512)	19.0 (0-84)	134.2 (65-235)	9.55 (0-50)	126.2 (77-212)
P Value	.05	.05	.05	.01	NS	NS	.01	.01
B_3 n = 8	27.12 (7-92)	35.17 (4-120)	317.6 (142-420)	448.3 (170-1106)	27.25 (12-40)	282.3 (105-420)	10.25 (4-21)	271.3 (112-420)
B_4 n = 10	9.2 (0-26)	13.95 (0-247)	177.0 (67-420)	194.7 (0-755)	4.6 (0-15)	172.5 (68-420)	5.9 (0-21)	129.2 (63-375)
P Value	NS	NS	NS	.05	NS	.01	.05	.05

A_3 = Male Petients with unequivocal ECG.
A_4 = Male Patients with Equivocal ECG.
B_3 = Female Patients with Unequivocal ECG.
B_4 = Female Petients with Equivocal ECG.
NS = Non Significant.

The Figures in Parenthesis show the Range of Values.
n = Number of patients.

equivocal E. C. G changes. However, difference in serum HBDH levels in female patients was statistically singnificant ($P < .05$) between patients with unequivocal and equivocal ECG changes. These findings may be consistent with the observation that the risk of death for CHD are less for women than for men throughout adult life and this difference in especially marked below 55 years of age.

Out of 92 patients (both sexes) studied, 62 patients showed unequivocal E. C. G changes while 30 patients showed equivocal E. C. G changes (Table-V). It has been shown by various workers that between 90-100 per cent of patients with acute myocardial infarction have elevated levels of CPK within 24-72 hours of the onset of chest + pain (Galen, 1975, Richard, 1984). This study showed that 79 percent patients with unequivocal E. C. G changes of infarction showed elevated CPK levels, while 67 per cent patients with equivocal E.C.G changes showed increased levels of CPK (Table-V). This shows that sensitivity of CPK was in close approximation with the previous studies. The major drawbacks of serum CPK determination are the relatively short time period during which CPK level is elevated after the onset of infarction and secondly the problem of false positive elevation due to skeletal muscle injury especially after intramuscular infections.

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Table-3.

MEAN LEVELS OF SERUM CPK, LDH, CKMB AND HBDH IN PATIENTS (MALE AND FEMALES) WITH CARDIAC INFARCTION ACCORDING TO TYPE OF INFARCT.

Patients	1st Day				2nd Day		3rd Day		Infarction
	CPK u/ml	CKMB u/l	LDH u/l	HBDH u/l	CPK u/ml	LDH u/l	CPK u/ml	LDH u/l	
A_5 n = 34	46.26 (0-184)	52.57 (4.5-126)	204.65 (36-400)	467.0 (0-995)	27.7 (2-108)	231.2 (80-420)	13.41 (0-73)	219.1 (75-420)	Anterior Wall
A_6 n = 20	19.85 (0-54)	37.9 (2.8-240)	197.8 (50-420)	425.0 (53-1619)	22.55 (0-90)	222.9 (62-420)	8.95 (0-21)	178.5 (8-400)	Inferior Wall
P Value	NS	NS	NS	NS	NS	.05	NS	NS	
B_5 n = 5	28.6 (0-84)	42.0 (4-120)	352.4 (142-420)	495.8 (175-1100)	28.8 (7-92)	290.6 (105-420)	12.4 (2-21)	239.6 (112-420)	Anterior Wall
B_6 n = 3	22.2 (4-84)	23.8 (20-26.4)	259.6 (145-317)	369.3 (170-425)	22.6 (4-40)	279.0 (130-240)	5.3 (0-9)	224.3 (133-420)	Inferior Wall
P Value	NS	NS	NS	NS	NS	NS	.01	NS	

A_5 = Male Patients with Anterior Wall Infarction.

A_6 = Male Patients with Inferior Wall Infarction.

B_5 = Female Patients with Anterior Wall Infarction.

B_6 = Female Patients with Inferior Wall Infarction NS = Non Significant.

The Figures in Parenthesis show the Range of Values.
n = Number of Patients.

(1960) cited by Braunwald (1984) elevated LDH level was elevated in 92-95 per cent of cases tients with the clinical disgnosis of acute myocardial infarction. Almost similar findings were reported by Richard (1984) and serum LDH level was elevated in 92-95 percent of cases with acute myocardial infarction. Present findings are in conformity with the above studies and 100 per cent of patients with unequivocal E.C.G. changes had elevated LDH levels. This study indicates that LDH is comparatively more sensitive than CPK. This could be due to the fact that CPK level remains elevated for upto 3 days after infarction while LDH level remains elevated for upto 11-14 days and some patients studied during this work were admitted in the hospital after 2 to 3 days of the onset of chest discomfort, and by then their serum CPK level had already come to the base line.

The mean serum CPK and LDH values in patients of both sexes with anterior infarction were higher but not significantly, than in patients with inferior infarct (Table III). These findings are in conformity with those reported by Ryan and his co-owrkers (1981). Similarly the corresponding isoenzyme, CKMB and HBDH values were not statistically different between the two groups of patients, though they were higher in patients with anterior than those in patients with inferior infarction (Table III). This is due to anatomical distribution of the anterior des-

Table-IV

MEAN LEVELS OF SERUM CPK, LDH, CKMB, AND HBDH IN PATIENTS (MALE AND FEMALES) WITH CARDIAC INFARCTION ACCORDING TO FREQUENCY OF HEART ATTACK.

Patients	1st Day				2nd Day		3rd Day		Number of Attacks.
	CPK u/ml	CKMB u/ml	LDH u/ml	HBDH u/ml	CPK u/ml	LDH u/ml	CPK u/ml	LDH u/ml	
A - 7 n = 52	32.0 (0-184)	36.66 (0-240)	215.1 (50-420)	350.0 (64-995)	28.0 (0-108)	218.6 (65-420)	11.61 (0-50)	183.1 (75-420)	Single
A - 8 n = 22	32.0 (0-156)	32.52 (2-126)	172.0 (50-440)	324.2 (53-944)	18.31 (0-68)	197.2 (62-420)	10.22 (0-73)	178.6 (78-420)	Mutiple
P Value	NS	NS	NS	NS	NS	NS	NS	NS	
B-7 n = 14	18.0 (0-84)	23.9 (0-120)	280.0 (67-420)	345.4 (56-1106)	17.0 (0-92)	254.0 (95-420)	9.0 (0-21)	222.0 (80-420)	Single
B-8	6.0 (2-16)	21.4 (16.5-24.7)	99.0 (68-145)	108.2 (68-165)	3.0 (2-7)	108.2 (68-165	3.5 (0-12)	89.0 (62-133)	Mutiple
P Value	NS	NS	.05	NS	NS	.05	.01	NS	

A - 7 = Male Patients with first attack of cardiac infarction.

A - 8 = Male patients with Multiple/attacks of cardiac infarction.

B - 7 = Female Patients with first attack of cardiac infarction.

B - 8 = Female Patients with Multiple attack of cardiac infarction.

n = Number of Patients

NS = Non Significant.

The figures in Parenthesis show the range of values.

cending branch of left coronary artery which when occluded acutely involves greater portion of the cardiac tissue and so the blood level of enzymes and isoenzymes is higher in anterior infarction than in inferior infarction (Holzner, 1983).

No statistically significant difference was seen in the serum isoenzyme values (CKMB and HBDH) of male and female patients after first attack as compared with the values after multiple attacks of MI in present study (Table IV). However it was found that both CKMB and HBDH levels were higher in patients after first attack than in those after multiple attacks (Table IV). Higher values were also found for serum CPK and LDH in male and female patients after first attack of MI than after multiple attacks (Table IV). These findings confirm the results observed by Ryan and his co-workers (1981). The higher level of isoenzyme (CKMB-HBDH) in patients after first attack than in those after multiple attacks could be due to the fact that cardiac tissue get so much damaged that the tissue level of these enzymes and isoenzymes decreases and so is the blood level after multiple attacks. Leung and Henderson, (1979) in their study report that the serum level of CKMB is 100 per cent sensitive and 96 per cent specific while serum level of LDH_1 has the same sensitivity and a little lower specificity (90%) for the diagnosis of cardiac infarction. The results of this study confirm the above results as in 52 patients of MI with unequivocal E. C. G change, 49 patients had both elevated CKMB and HBDH serum levels while only 7 patients out of 30 (23%) with equivocal ECG changes had increased serum isoenzyme values. When considered alone serum CKMB value was elevated in 52 patients (84%) while HBDH values were elevated in 58 patients (93%) out of 62 patients with unequivocal E. C. G changes (Table-VII, VIII). It means that CKMB had a sensitivity of 84 per cent while HBDH was 93% sensitive for the diagnosis of MI.

In 30 patients of MI with equivocal ECG changes 18 patients (60%) had elevated serum CKMB levels while 7 patients (23%) had elevated serum HBDH values (Table-VII,VIII). Value of serum HBDH in this study was found to be more specific for the diagnosis of MI, than the CKMB values within 24 hours after admission and this deviation from the results of previous studies could be due to the fact that sample for the isoenzyime analysis was obtained within 24 hours of admission and CKMB level may have come to the base line while LDH_1 isoenzyme on the contrary remains elevated for 12 days (Richard 1984).

In conclusion the present study shows that the serum isoenzymes CKMB and HBDH (LDH) levels are more sensitive and specific for the diagnosis of acute myocardial infarction than the total CPK and LDH enzyme levels. As the

Table - V

Distribution of patients (Both Sexes) with unequivocal and equivocal ECG changes of cariac infarction according to the results of serum levels of CPK and LDH.

Serum CPK Test Result	ECG	Number of Patients	Total	Percentage
Positive	Unequivocal	49		79
Negative	Unequivocal	13	62	21
Positive	Equivocal	20		67
Negative	Equivocal	10	30	33
Grand Total:		92	92	100

Table-VI

Serum LDH Test result	ECG	Number	Total	Percentage
Positive	Unequivocal	62		100
Negative	Unequivocal	0	62	0
Positive	Equivocal	29		98
Negative	Equivocal	1	30	2
Grand Total:			92	100

Note:

Normal range of serum CPK and LDH according to this method is 0-12/uml and 24/78 u/l respectively.

CPK Test: Positive 12 u/ml Negative 12 u/ml

LDH Test: Positive 78 u/l Negative 78 u/l

Table - VII

Distribution of Patients (Both Sexes) with unequivocal and equivocal ECG changes of cardiac infarction according to the results of serum levels of CKMB and HBDH.

Serum CKMB Test Result.	ECG	Number	Total	Percentage.
Positive	Unequivocal	52		84
Negative	Unequivocal	10	62	16
Positive	Equivocal	18		60
Negative	Equivocal	12	30	40
Grand Total:			92	100

Table - VIII

Serum HBDH Test Result	ECG	Number	Total	Percentage
Positive	Unequivocal	58		93
Negative	Unequivocal	4	62	7
Positive	Equivocal	7		23
Negative	Equivocal	23	30	77
Grand Total:			92	100

Note:

Normal range of CKMB and HBDH according to this method is 0-10 u/l and 55-140 u/l respectively.

CKMB Test:

Positive 10 u/l Negative 10 u/l

HBDH Test:

Positive 140 u/l Negative 140 u/l

electrophoretic separation of CKMB and LDH_1 isoenzymes is a time consuming procedure especially when many plasma samples have to be analysed, it is suggested that, these isoenzymes (CKMB and HBDH) should be estimated by rapid ultraviolet spectrophotometric methods rather than the former one, in a routine clinical laboratory. As the facilities of chromatography and Gel Electrophoresis (Starch, Agarose and Polyacrylamid) at present, are not available in our teaching Hospital Laboratories, this simple though relatively less sensitive method for the determination of isoenzymes will help a lot to improve the diagnosis. The present work showed that HBDH (LDH_1) is more sensitive and specific than CKMB, this is particularly useful in patients admitted in the hospital, 48-72 hours after the onset of symptoms indicating an acute myocardial infarction as is usually the case in majority of patients admitted in local hospitals. Keeping in mind the conditions in local hospitals, it is suggested that determination of both serum CKMB and HBDH should be carried out along with total enzyme level of serum CPK, LDH and SGOT for the proper diagnosis of acute myocardial infarction.

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