Comparison of Troponin I and Cardiac Enzymes as Diagnostic Markers for Acute Myocardial Infarction (AMI) in Cardiac Patients from a Tertiary Care Hospital of Lahore-Pakistan

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Abstract

Background Acute Myocardial Infarction (AMI) is a serious medical condition in which sudden damage occurs to heart muscles due to blockage of blood supply in coronary arteries. The clinical investigation of AMI is based on the evaluation of specific heart markers i.e. Troponin I and cardiac enzymes with variable efficacy and specificity.

Materials and Methods: A cross-sectional study was conducted on 130 subjects, including 100 patients suffering with Acute Myocardial Infarction (AMI) and 30 healthy subjects. The whole research work samples were collected during Februray 2022 to March 2022 and completed in Accident and Emergency Diagnostic Laboratory, Mayo Hospital Lahore. The Troponin I serum levels were determined by using Chemiluminescence Access2 reagents kit (Beckman Coulter, USA) on chemistry analyzer. Whereas, cardiac enzymes i.e. serum CPK, CK2 (CK-MB) and LDH were determined by using chemical reagents manufactured by (Beckman Coulter, USA) spectrophotometrically on fully automated chemistry analyzer AU-480.

Results: The study was conducted on 77% of positive group patients (*n*=100) diagnosed with AMI, moreover, 23% healthy individuals (*n*=30) were taken as control group. In control group the mean value of serum Troponin I and cardiac enzymes including CK-MB, CPK and LDH were 0.0160±0.001 ng/mL, 13.70±1.030 U/L, 105.87±4.454 U/L and 141.70±2.396 U/L respectively, whereas for positive group these values were 2.3957±0.783 ng/mL, 48.56±4.997 U/L, 327.42±53.68 U/L and 575.09±38.008 U/L respectively. In control group serum Troponin-I showed negative correlation with CK-MB, CPK and LDH by

applying Pearson Correlation i.e. ($p>0.05$) respectively. Whereas, in positive subjects serum Troponin I showed positive correlation with CPK and CK-MB ($p<0.05$) while negative correlation with LDH.
Conclusion: The present study demonstrated that serum cardiac Troponin I and cardiac enzymes i.e. serum CK-MB and CPK prove to be superior cardiac biomarker as compared serum LDH for the evaluation of Acute Myocardial Infarction in the effected population.
Key words: Acute Myocardial Infarction (AMI), Serum Troponin I, CK-MB, CPK, LDH.

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Introduction

Acute Myocardial Infarction (AMI) is a serious cardiac trouble where a sudden damage occurs to heart muscles due to blockage of blood supply in coronary arteries. As a consequence, efficiency of heart get disturbed depending upon the severity of the attack. The investigation of AMI is based on the evaluation of the proteins released from heart i.e. Troponin I and cardiac enzymes with variable efficacy and specificity [1]. Acute myocardial infarction (AMI) is a conditional term that can be caused due to ischemic and coronary cardiac disease and it becomes significant when an atherosclerotic plate is ruptured and a clot is formed. A growing thrombus can completely or partially obstructs the coronary artery that completely reduces the blood flow to the heart muscles [2, 3]. The world health organization (WHO) defined acute heart attack condition with the following features, (1) Acute ischemic sign and symptoms (chest discomfort) (2) appearance of Q waves in ECG (3) increase level of diagnostic enzymes (CPK, CK-MB and LDH) in the bloodstream. Acute heart attack is irreversible devastation of the heart muscle cells that also investigated prolonged absence of oxygen delivery to the cardiac tissues [4]. The serum Troponin I has been proven to be the most reliable diagnostic marker for Acute Myocardial Infarction (AMI). It is being increased in the cardiac tissues and it indicates the higher incidence of heart attack. Mostly patients with heart attack have high level of serum Troponin I within first 6 hours of AMI. Cardiac Troponin level is very specific and sensitive to cardiac ischemia, and an ideal choice of blood analysis for patients that are assumed to have Acute Myocardial Infarction. The levels of Troponin increase within 2–4 hours after the initiation of AMI [5]. Only in United States, annually about 1.5 million people are diagnosed with Myocardial Infarction [6]. The isoenzyme of CK i.e. CK2 (CK-MB) is considered less sensitive and specific than troponin, but during the unavailability of a cTn assay, CK2 is considered as a best substitute. A measurement of CK2 above the 99th percentile of the upper reference limit (URL) should be designated as the decision level for the diagnosis of AMI [7, 8]. The serum CK2 test is of diagnostic value in both cardiac and non-cardiac conditions. Its levels start to rise in 4 hours following MI that further peaks at 24 hours and remains elevated for 48 to 72 hours post infarction [9]. Apart from its elevated levels in non-cardiac disorders like skeletal muscle injury, hypothyroidism and CRF, still

it is a valuable enzymatic marker for detection of acute AMI due to its highly specific and sensitive outcomes [10]. Moreover, serum CK2 shows a abrupt decline pattern after MI and can be helpful in detection of early Myocardial reinfarction. LDH, another cardiac marker, is composed of four tetramer that encompass H and M subunit, that combine to form 5 isozymes (LDH-1 to LDH-5) with tissue specific distributions [11, 12]. LDH-1 is the predominant type found in heart tissues, which has four heart subunits (4H). The LDH-2 is the primary isozyme of the reticulo-endothelial system and RBCs with three heart and one muscle subunit (3H1M) [13]. The primary isozyme of the lungs, LDH-3 is made up of two heart and two muscle subunits (2H2M). LDH-4 is the major isozyme found in the kidneys with one heart and three muscle subunits (1H3M). The LDH-5 isozyme is composed of four muscle subunits (4M) and is expressed in the liver and skeletal muscle [14].

Materials and Methods

It is a cross-sectional study that was conducted on total 130 subjects including 100 subjects with Acute Myocardial Infarction (AMI) and 30 healthy control subjects, from Accident and Emergency Department, Mayo Hospital Lahore during the two months period (February to March 2022) with a prior approval from the Ethical Committee of School of Biochemistry, Minhaj University, Lahore. The patients with AMI were further divided into 13 different age groups i.e. 15-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80 years. The 3 mL blood samples were collected both from test and control group patients using sterile (5cc BD, USA) syringes in gel vials and incubated for 30 minutes at 37^{o}C in an incubator. These gel vials with clotted blood were centrifuged at 4000 rpm for 10 minutes to obtain serum which was collected in separate plastic tubes and subsequently used for determination of serum cTn I, CK-MB, CPK and LDH levels. The serum level of Troponin I were determine by Chemiluminescence reagent kit on fully automated special chemistry analyzer Access2 (Beckman Coulter, USA) while cardiac enzymes i.e. serum creatine phosphokinase, CK2 and LDH were determined spectrophotometrically on fully automated chemistry analyzer AU-480 (Beckman Coulter, USA).

Statistical Analysis

The data obtained from experimental work was analyzed statistically using automated software IBM SPSS (Version 25.0) for Pearson's correlation respectively, and p value were calculated to check significance level.

Results

This study was done to evaluate the levels of serum Troponin I and other cardiac biomarkers sensitivity i.e. serum CPK, CK2 and LDH. In order to compare the healthy control group and positive group with Acute Myocardial Infarction (AMI), observations were evaluated using descriptive statistics analysis. In healthy group 30 healthy subjects and 100 patients with Acute

MI were analyzed and their mean and standard error along with the minimum and maximum readings of cardiac enzymes and Troponin-I were noted.

The observed frequency of AMI in studied individuals is shown in table 1. Surprisingly, our data showed the highest frequency of AMI cases (0.09) among female subjects of age group 41-45, followed by a second highest frequency (0.08) in females of 46-50 years. The third highest frequency (0.07) was observed in 51-55 years old females. Whereas, in male subjects, the highest frequency of AMI (0.07) of AMI was observed which was comparable with female group (41-45 years) followed by second highest frequency of AMI (0.06) in young age males (36-40 years), while the same pattern of AMI frequency (0.06) was observed in males of age groups (46-50 years), (51-55 years), (56-60 years) and (66-70 years), respectively. Our data showed the lowest frequency of AMI (0.01) among (76-80 years) and (71-75 years) age groups of both females and males, respectively.

Table 1: Gender wise frequency distribution of Acute Myocardial Infarction (AMI) among patients

Sr. No	Age Group (Years)	Male frequency (n=100)	Female frequency (n=100)	Total (M + F)
1	15-20	(0.02)	(0.00)	(0.02)
2	21-25	(0.02)	(0.00)	(0.02)
3	26-30	(0.03)	(0.02)	(0.05)
4	31-35	(0.04)	(0.02)	(0.06)
5	36-40	(0.06)	(0.04)	(0.01)
6	41-45	(0.07)	(0.09)	(0.16)
7	46-50	(0.06)	(0.08)	(0.14)
8	51-55	(0.06)	(0.07)	(0.13)
9	56-60	(0.06)	(0.02)	(0.08)
10	61-65	(0.05)	(0.03)	(0.08)
11	66-70	(0.06)	(0.03)	(0.09)
12	71-75	(0.01)	(0.03)	(0.04)
13	76-80	(0.02)	(0.01)	(0.03)
Total	13	(0.56)	(0.44)	(1.0)

The descriptive statistics for healthy subjects and positive subjects with AMI has been shown in Table 2 and 3, respectively. In control group the mean values of serum Troponin-I and cardiac enzyme i.e. CK-MB, CPK and LDH were 0.0160±0.001 ng/ml, 13.70±1.030 U/L, 105.87±4.454 U/L and 141.70±2.396 U/L, respectively. Whereas, the mean values of serum Troponin-I and aforementioned cardiac enzyme for positive subjects with AMI were

2.3957±0.783 ng/ml, 48.56±4.997 U/L, 327.42±53.68 U/L and 575.09±38.008 U/L, respectively and Ratio scale was used for these variables.

Table 2: Descriptive Statistics for Healthy Control Subjects

Variables	N	Minimum	Maximum	Mean ± SE
Troponin I ng/mL	30	0.0010	0.0370	0.0160 ± 0.001
CK-MB U/L	30	7	35	13.70 ± 1.030
CPK U/L	30	35	162	105.87 ± 4.454
LDH U/L	30	120	171	141.70 ± 2.396

***SE = standard error**

Table 3: Descriptive Statistics for Positive Subjects with Acute Myocardial Infarction (AMI)

Variables	N	Minimum	Maximum	Mean±SE
Troponin-I ng/mL	100	0.006	64.664	2.3957 ± 0.783
CK-MB U/L	100	10	310	48.56 ± 4.997
CPK U/L	100	14	3700	327. 42 ± 53.68
LDH U/L	100	114	2321	575.09 ± 38.008

Negative correlations of serum Troponin-I with serum CK-MB, CPK and LDH for healthy controls group were represented in table 4. Moreover, Troponin-I levels also depicts statistically insignificant relationship with serum CK-MB, CPK and LDH in controls group. It has been demonstrated that p-value was greater than the specified level of significance i.e. (p>0.05).

Table 4: Correlation of Troponin-I with CK-MB, CPK and LDH in Healthy Subjects

Parameters	CK-MB	CPK	LDH
Pearson Correlation Sig. (2-tailed) Troponin-I	r = -0.089 p = 0.639	r = -0.023 p = 0.906	r = -0.274 p = 0.143

*Troponin-I = It is acute phase protein which becomes increased in serum of patients with Acute Myocardial Infarction (AMI) and is being used as diagnostic marker for AMI

**Positive Correlation= It is a type of Pearson correlation which indicates that if value of one parameter under study becomes increase then value of other compared parameter also increases

***Negative Correlation= It is a type of Pearson correlation which indicates that if value of one parameter under study become increase then value of other compared parameter become decreased

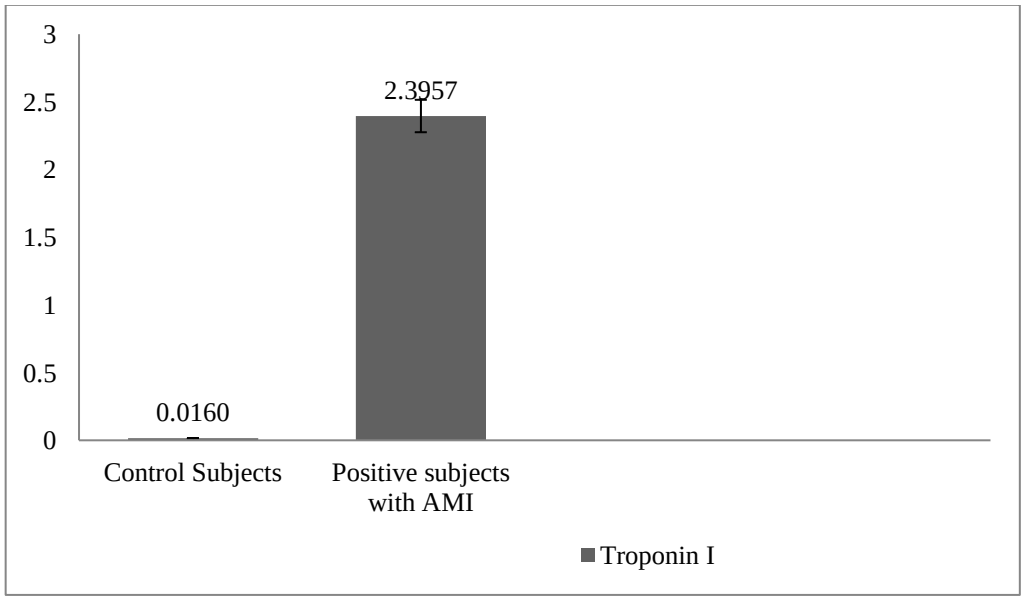
Positive correlation of serum Troponin I concentration with serum CK-MB and CPK were shown in table 5 and was statistically significant ($p<0.05$). The serum Troponin I showed negative correlation to serum LDH and this correlation was statistically non-significant i.e. ($p>0.05$). Serum Troponin I showed highly sensitive results to diagnose AMI as compared to other cardiac markers i.e. serum CK-MB, CPK and LDH, as serum Troponin-I showed a negative correlation with other cardiac markers in case of healthy control subjects and this correlation becomes positive for diseased subjects with AMI except LDH.

Table 5: Correlation of Troponin-I with CK-MB, CPK and LDH for Positive Subjects with Acute Myocardial Infarction (AMI)

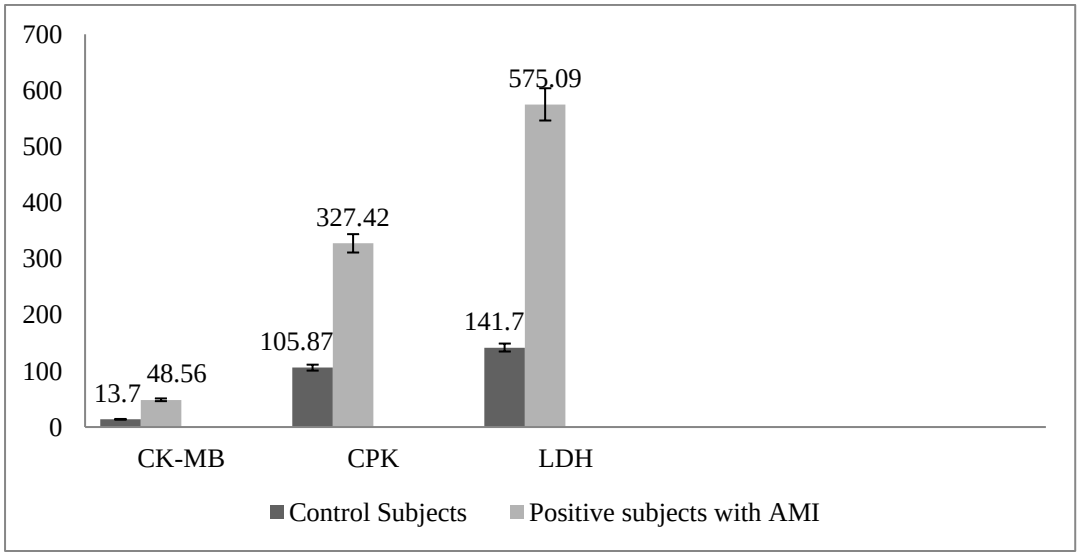
Parameters	CK-MB	CPK	LDH
Pearson Correlation Sig. (2-tailed) Troponin-I	r = 0.429 p = 0.000	r = 0.241 p = 0.016	r = -0.017 p = 0.863

*r = Pearson Correlation Coefficient **p = p-value

Comparison of Troponin I for control and AMI patients is shown in Figure 1, there is a statistically significant difference in serum Troponin I level ($p<0.05$) between control group and positive subjects with AMI. Whereas, the comparison of serum level of other three cardiac enzymes i.e. CK-MB, CPK and LDH, for control group and AMI patients, is shown in Figure 2. There is statistically significant difference ($p<0.05$) between control group and positive subjects with AMI.



Graph 1: Comparison of Troponin I in Control group and Positive Subjects with AMI



Graph 2: Comparison of Cardiac enzymes level i.e. CK-MB, CPK and LDH in Control group and Positive Subjects with AMI.

Discussion

AMI is a medical emergency condition which results in serious health consequences in the form of heart failure and ultimately the demise of patients. The clinical evaluation of cardiac markers including serum Troponin-I, CK-MB, CPK and LDH is vital in diagnosing the severity of AMI. The current study was conducted in patients diagnosed with AMI, and aim was to compare cardiac marker serum Troponin I with cardiac enzymes i.e. serum CK-MB, CPK and LDH to establish their correlation. The present study results indicated that serum Troponin-I showed significant correlation with serum CK-MB and CPK whereas it depicted statistically non-significant correlation with LDH. Serum CK-MB also showed positive correlation with CPK and LDH, as there is statistically significant difference among them. Moreover, serum CPK was also correlated positively with serum LDH due to statistically significant difference between them. Finally, the results of our study demonstrated that serum cardiac Troponin-I, serum CK-MB and CPK have been proven to be valuable diagnostic markers for AMI in emergency conditions while serum LDH proved to be less specific marker of AMI as compared to other three aforementioned markers.

Regarding correlation of Troponin I and CK-MB, our findings are similar to that of [15], who also established that cTnI and CK-MB prove to be worthful markers for the diagnosis of AMI as there is a positive correlation of cTnI with CK-MB.

Regarding determination of correlation between Troponin I and LDH, our findings are similar to [16], who made a comparative analysis of cardiac Troponin I and LDH isoenzymes for the delayed diagnosis of myocardial injury. They also concluded that LDH has negative correlation with cTnI, as there is statistically non-significant difference between them i.e. ($p>0.05$). Whereas, cardiac Troponin I showed greater sensitivity than isoenzyme of LDH for delayed diagnosis of

Acute Myocardial injury. Moreover, the sensitivity of LDH for diagnosis of myocardial infarction is less due to its poor prognostic importance.

Furthermore, regarding determination of correlation between Troponin I and CPK, our findings are similar to [17], who made a comparative analysis of cardiac Troponin I and CPK as diagnostic markers for cardiac injury. They also concluded that cardiac Troponin I (cTnI) has positive correlation with CPK and showed statistically significant difference with $p<0.05$.

Conclusion

On the basis of current study findings, it has been concluded that serum cardiac Troponin I and cardiac enzymes i.e. serum CK-MB and CPK prove to be superior cardiac biomarkers as compared serum LDH for the evaluation of Acute Myocardial Infarction in the effected population in emergency conditions for speedy treatment of such patients to save precious lives.

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