Original Article

Cardiac Enzymes in Acute Coronary Syndrome

Khuram Shazad[1], Rashid Mahmood[1], Abdullah Mashori[2], Syeda Asya Parveen[3] and Farooq Muanfat[4]

ABSTRACT

Objective: The present study was conducted on specific population of Mirpur Azad Kashmir to observe the behavior of the MI evaluating various cardiac biomarkers for the detection of ACS in relation to age, gender and risk factors.

Study Design: Case control study

Place and Duration of Study: This study was conducted at the Indoor Patient Department Kashmir Institute of Cardiology Mirpur, Azad Jammu and Kashmir from June 2013 to July 2014.

Materials and Methods: Total 240 patients were recruited in the study. 120 admitted patients of Acute Myocardial Infarction (AMI) in Kashmir Institute of Cardiology were registered as cases. 120 age and sex matched controls were included in the study. Informed written consent was obtained by the participants of study. Detailed medical/surgical history was obtained and recorded.

Results: The cardiac enzymes used as biomarkers of myocardial infarction (MI) have been assayed in MI affected and compatible non MI control subjects. The pertinent cardiac enzymes in related to MI included CK-NAC and LDH. The responses of the cardiac enzymes have been analyzed in relation to age.

Conclusion: It is concluded in this study that, in all the subjects of study, main cardiac enzymes CKNAC and LDH concentrations were increased in MI patients as compared non-MI subjects. This study also concludes this significant rise of CKNAC in younger group and that of LDH in oldest age group.

Key Words: CK-NAC, LDH, Cardiac Enzymes, Acute Coronary Syndrome

Citation of article: Shazad K, Mahmood R, Mashori A, Parveen SA, Muanfat F. Cardiac Enzymes in Acute Coronary Syndrome. Med Forum 2020;31(1):38-42.

INTRODUCTION

Coronary artery disease (CAD) is one of the major causes of mortality worldwide. In developing countries, CAD affected population is increasing more rapidly as compared to developed countries. CAD is anticipated to be the leading cause of death in developing countries in near future (Jafaryet al., 2005 & Ali et al., 2009)[1]. Ischemic heart disease (IHD) is the most common cause of mortality in developed world, followed by developing countries in which incidence is increasing rapidly (Munir, 2007)[2].

1. Department of Physiology, Shaheed Benazir Medical College, Mirpur Azad Kashmir.
2. Department of Biochemistry, Gambat Medical College, Gambat.
3. Department of Biochemistry, Karachi Medical and Dental College, Karachi.
4. Department of Physiology, Multan Medical and Dental College, Multan.

Correspondence: Dr. Khurram Shahzad Kh, Assistant Professor, Department of Physiology, Shaheed Benazir Medical College, Mirpur Azad Kashmir.
Contact No: 0342-5972414
Email: khuram60@hotmail.com

Received: August, 2019
Accepted: November, 2019
Printed: January, 2020

Until recently, diagnosis of AMI was based on the revised World Health Organization (WHO) criteria that require 2 of the following 3 features for diagnosis i.e. signs and symptoms of ischemia, ECG changes consistent with severity/degree of ischemia and elevated enzyme levels usually Creatine Kinase Myocardial Band (CK-MB) (Meier et al., 2002)[3].

Clinicians face a common and difficult challenge in identifying patients with acute chest pain who are at high risk for developing cardiovascular complications (Johnson et al., 1999)[4]. Around 2-10% cases of AMI remains undiagnosed and are missed. Conversely, a large number of patients admitted with the complaint of chest pain do not turn out to have Acute Coronary Syndrome (ACS). In addition to ACS, other life threatening diseases include pulmonary embolism, aortic dissection and tension Pneumothorax requiring rapid diagnosis and different treatment than ACS (Swap, 2005)[5].

The modified WHO criterion to differentiate patients of AMI presenting with chest pain from those presenting with other non-cardiac causes in the following way of chest pain/ discomfort for duration of at least 20 minutes; ECG changes consistent with AMI, defined as ST-segment deviation (ST segment depression or elevation $\geq$ 0.1mV on at least two adjacent leads) or new symmetric T wave inversion $\geq$ 0.1mV or both and changes in serial plasma or serum, protein markers

(LDH or CTnI) associated with AMI (Apple et al., 1999). [6] Immediately after an acute coronary occlusion, blood flow ceases in the coronary vessels beyond the occlusion except for small amounts of collateral flow from surrounding vessels. The area of muscle that has either zero flow or so little flow that it cannot sustain cardiac muscle function is said to be infracted, and the condition is known as MI (White, 2009).[7]

Cardiac enzymes are found in heart tissue, serving as catalysts for the heart's biochemical reactions. Key cardiac enzymes are troponin and creatine kinase. Troponin-T is a part of the troponin complex and binds to tropomyosin, interlocking them to form a troponin-tropomyosin complex. Cardiac troponin regulates the cardiac muscle contraction in response to changes in calcium concentration (Layland, 2005).[8]

Creatine kinase (CK), aspartate transferase (AST) and lactate dehydrogenase (LDH) are also biochemical markers for the diagnosis of acute myocardial infarction (Fonteset al., 1999).[9]

In Pakistan, IHD is the 2nd leading cause of death at all ages contributing to 11% of all deaths (Andrieuet al., 2000).[10] The incidence of AMI has been found to prevail in differing age although with greater magnitude in aging (Pfefferet al., 2000 &Morillaset al., 2007).[11] Similarly there is evident difference in the incidence of AMI in the different genders (Merzet al., 2006).[12]

MATERIALS AND METHODS

This Case control study was conducted at indoor patient department Kashmir Institute of Cardiology Mirpur, Azad Jammu and Kashmir in 12 months.

Sampling Technique: Non-probability purposive sampling technique was used.

population selection and sample size: Total 240 patients were recruited in the study. 120 admitted patients of Acute Myocardial Infarction (AMI) in Kashmir Institute of Cardiology were registered as cases. The span of study was from June 2013 to July 2014. 120 age and sex matched controls were included in the study. Informed written consent was obtained by the participants of study. Detailed medical/surgical history was obtained and recorded.

SAMPLE SELECTION

Inclusion criteria

- Patients with chest pain suggestive of myocardial ischemia within 12 hours after the onset of symptoms on the basis of ECG changes.
- Persons presenting with non-infracted states of ischemia, routine, general health check-up were taken as controls.

Exclusion criteria

- Severe skeletal muscle damage or trauma as CK is deranged in skeletal muscle damage.
- Cardiac resuscitation
- Patients undergone cardiac surgery
- Patients suffering from re-infarction

Data Collection Procedure: Serum samples were drawn from one of peripheral vein of arm, and serum was analyzed. Age and sex matched individuals presenting for a routine, general health check-up and diseases like hypertension, diabetes mellitus with ECG not suggestive of myocardial infarction were taken as the control group. Blood specimen were collected from each participant after admission suffering from acute symptoms. Serum levels of CK-NAC, CKMB and LDH was analyzed using chemistry analyzer Micro lab.

Principle: Assay method was based on the reverse reaction (ATP formation). Hexokinase and glucose-6phosphate dehydrogenase were used as coupling enzymes. CK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP and Glucose-6-phosphate by Hexokinase (HK). Glucose-6-phosphate dehydrogenase (G-6-PDH) oxidizes at the glucose-6phosphate and reduces the nicotinamide adenine dinucleotide (NAD). The rate of NADH formation measured at 340 nm is directly proportional to the serum CK activity.

CK

Creatine phosphate + ADP → Creatine + ATP HK

Glucose + ATP → Glucose-6-P + ADP

Glucose-6-P + NADP → Glucose-6-P + NADPH+ H

Procedure: 1.0 ml. of substrate reagent was put into each cuvette and incubated at 37 C for 3 minutes with absorbance of 340nm. 0.05 ml (50 µL) of specimen was added into appropriate cuvette. Mixed gently and placed into the temperature controlled cell compartment of the instrument. Immediately timer was started. After 2 minutes, initial absorbance was recorded (A1). Two readings were recorded at 1 minute intervals after A1. Decrease in absorbance per minute

ΔA/min. was recorded from the linear path of the assay with greater slope. ΔA/min. was multiplied by the Factor 3376 to calculate U/L of CK.

B) Lactate Dehydrogenase

Principle: It utilizes the principle of spectro-photometer carried out at 340 nm. In the reaction, the LD catalyzed the reversible oxidation of L-Lactate to Pyruvate with the concurrent reduction of ß-Nicotinamide Adenine Dinucleotide (NAD) to ß-Nicotinamide Adenine Dinucleotide (reduced form) (NADH). The system monitored the rate of change in absorbance at 340 nm over a fixed-time interval. The rate of change in absorbance was directly proportional to the activity of LD in the sample.

Pyruvate + NADH + H → L- lactate +NAD

Procedure: 0.05ml of Sample of serum was obtained and was mixed with 3 ml of reagent (phosphate buffer

Timer was started simultaneously and reading was obtained after 1, 2 and 3 minutes.

Decrease in absorbance per minute ΔA/min. was recorded from the linear path of the assay with greater

slope. ΔA/min. was multiplied by the Factor 9683 to calculate U/L of LDH.

Data Analysis: Data entry and analysis was done by using SPSS 20. Quantitative variables were presented by using mean± SD. Study variables were analyzed by using frequency table and percentages. Participants were divided into 3 age groups: 21-39, 40-59 and 60 years and above for statistical analysis. Association between study variables in between the groups and within the groups was assessed by applying ANOVA to see the level of CK-NAC and LDH. P<0.005 was taken as significant.

RESULTS

The cardiac enzymes used as biomarkers of myocardial infarction (MI) have been assayed in MI affected and compatible non MI control subjects. The pertinent cardiac enzymes in related to MI included CK-NAC and LDH. The responses of the cardiac enzymes have been analyzed in relation to age.

Cardiac enzymes in relation to myocardial infarction status & non-infarction: Collectively, in all the subjects of study, mean cardiac enzyme concentrations were increased in MI patients as compared to non MI subjects. CK-NAC was 54% higher(p= 0.000) in MI patients, with observed mean 364.48±39.11 as compared to observed mean 167.10±3.11 in non-MI subjects. LDH was 28% higher (p= 0.000) in MI patients, with observed mean 482.96±27 as compared to observed mean 346.70±6.2 in non-MI subjects as shown in Table 1.

Table No.1: Cardiac Enzymes in Cardiovascular Diseases In Relation To MI & non-MI

	CK-NAC	P	LDH
MI	364.48±39.1	0.00	482.96±26.7
Non-MI	167.10±3.11		346.70±6.2
% difference	54		28

* Significant p value< 0.05

AGE-WISE ANALYSIS IN BOTH GENDERS

CK-NAC and LDH were assessed in age groups of 21-39, 40 -59, and 60 years and more, in patients of myocardial infarction and unaffected subjects.

MYOCARDIAL INFARCTED SUBJECTS

CREATININE KINASE-NAC:

The mean CK-NAC value was 596±120U/L in the age group 21-39 years in MI subjects. The concentrations of the enzyme were 425±69 and 245±30U/L in MI affected 40-59 years and 60 years and above subjects respectively. In the comparisons in the enzyme concentrations p between different age groups, values were significantly 59% lower (P= .044) in 60 years and above as compared to $21_{0.000}$-39 years of age group. In the comparisons between 40-59 and 60 year and above groups, the enzyme concentration was 43% lower in the older group (P=.067). The difference between these groups had been non-significant at 5% confidence interval; however, it was statistically significant at 10% confidence interval.

The concentrations of CK-NAC in MI affected subjects were the highest in the younger age groups that was found lower gradually in middle age group and the older age group of the study.

LACTATE DEHYDROGENASE:

CARDIAC ENZYMES ANALYSES

A) CREATINE KINASE –NAC Pyruvate and reconstituted NADH).

Both were mixed and initial absorbance at 340nm was obtained after 0.5 min.

In the comparison between the groups, the enzyme concentrations between 40-59 years and 60 years and above age were significantly (P=.004) different and was about 13% greater in the older than the younger group. The comparisons among the other groups were however non-significant.

The concentrations of CK-NAC in non-MI affected subjects were the highest in the 60 years and above age group and were found gradually lower in the younger as compared to older groups.

LACTATE DEHYDROGENASE:

The average concentrations of LDH in non-MI affected subjects were 312±3, 323±9 and 365±7 U/L in 21-39, 40-59 and 60 years and above age groups respectively. In 21-39 years of age group the enzyme concentration was lower than both the 40-59 years (3.4%) as well as the older age (14%) group. However, the difference between the middle and the older age groups was significant statistically (P=.003).

The enzyme LDH remained in lowest concentration in 21-39 years of age as compared to other groups in non-MI subjects.

NON- MYOCARDIAL INFARCTED SUBJECTS

CREATININE KINASE- NAC:

Table No.2: Cardiac Enzymes (U/L) in different age groups following M.I.

Enzymes	Age Group	Concen-tration U/L	F	Signifi-cance
CK-NAC	21-39	596.30±120	4.211	0.017
	40-59	425.10±68		
	60&above	245.38±30		
LDH	21-39	410.60±54	0.334	0.717
	40-59	487.15±39		
	60&above	492.40±41		

Table No.3: Cardiac Enzymes (U/L) in different age groups in non- M.I.

LDH	21-39	312±3	6.247	0.003
	40-59	323.76±9		
	60& above	365.55±7		

The CK-NAC value was 146±16U/L in the group of 21-39 years of age in non -myocardial infracted (MI) subjects. The concentrations of the enzyme were 156±3 and 176±4 U/L in non-MI affected 40-59 years and 60 years and above subjects respectively.

DISCUSSION

Coronary artery disease is the leading cause of morbidity and mortality. Approximately about 25% of patients presenting with chest pain are diagnosed as suffering from MI (Kumar et al., 2008).[13] In terms of increase in life expectancy, age has been related to increased prevalence rates of coronary artery disease (Yazdanyar, 2009).[14] Onset of coronary artery disease may proceed due to aging with the additional factors, of nutrition, activity, genetics etc. It develops without significant indicators for it and if there appear certain are mixed for multiple disorders. Generally it appears with the episode of MI that has been characterized with the specific responses of cardiac enzymes. Although cardiac enzymes have been assessed for diagnostic purposes otherwise the cardiac enzymes may have specific patterns varying in the different states of subject inflicted with MI. Thus the present had been conducted in the population of Mirpur, AJK in order to analyze the age related changes in cardiac enzymes in subjects with and without MI presenting to Kashmir Institute of Cardiology with the history of chest pain from June 2013 to May 2014.In present study, the mean age of male cases was 51.4 years while it was 56.6 years for females. The female cases were 5.1 year older than males. This observation is in accordance with (Joshi et al., 2007)[15] who reported female cases are 5.6 years older than male cases.

In the present study, CK-MB levels were 45% higher i youngest age group of males with MI as compared to oldest age group. In females with MI, it was observed that CK-MB was highest in middle age group a compared to younger and older age groups.

This finding is not in accordance with (Teryet al., 2002)[16] who reported 86% rise in CK-MB level in older age group.

This might indicate the severity of involvement of myocardium in MI of the younger age subjects. This observation is of significance and further such studies on this age group may reveal importance results.

It was observed in current study that cardiac enzyme CK-NAC was 64% higher in youngest age males than the oldest males. In females with MI, CK-NAC was 68% higher in 40-59 years age group as compared to youngest age group. (Haseebet al; 2013)[17] showed that there were no differences in both genders with MI in different age groups. LDH levels were lowest in youngest age group with MI in both genders as compared to other age groups and were insignificant ($p<0.05$). In current study in non-MI affected males and females, CK-NAC, CK-MB and LDH showed statistically significant differences for different age groups.

CONCLUSION

It is concluded in this study that, in all the subjects of study, main cardiac enzymes CKNAC and LDH concentrations were increased in MI patients as compared non-MI subjects. This study also concludes this significant rise of CKNAC in younger group and that of LDH in oldest age group.

Author's Contribution:

Concept & Design of Study:	Khuram Shazad
Drafting:	Rashid Mahmood, Abdullah Mashori
Data Analysis:	Syeda Asya Parveen, Farooq Muanfat
Revisiting Critically:	Khuram Shazad, Rashid Mahmood
Final Approval of version:	Khuram Shazad

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Jafar T, Jafary F, Jessani S.et al Heart disease epidemic in Pakistan: Women and men at equal risk. Am Heart J. 2009;150 (2) : 221-226.
2. Munir TA and Afzal MN. C-reactive protein and acute coronary syndrome: comparison of conservative and interventional management. J Ayub Med Coll. 2007;19(2):26-31.
3. Meier MA,Al-BadrWH, Cooper JV.et al. The new definition of myocardial infarction: diagnostic and prognostic implications in patients with acute coronary syndromes. Arch Int Med.2002; 162: 1585-1589.
4. Johnson PA , Goldman L ,Sacks DB et al. Cardiac Troponin T as a marker for myocardial ischemia in patients seen at the emergency department of acute chest pain Am Heart J. 1999; 137: 1137-44.
5. Swap CJ, Nagurney JT. Value and limitations of chest pain history in the evaluation of patients with suspected acute coronary syndromes. JAMA 2005;294(20):2623-9.

6. Apple FS,Christenson RH,Valdes R Jr. et al. Simultaneous rapid measurement of whole blood myoglobin, creatine kinase MB, and cardiac troponin I by the triage cardiac panel for detection of myocardial infarction. Clin Chem 1999; 45(2):199-205.
7. White WB. Defining the problem of treating the patient with hypertension and arthritis pain. Am J Med 2009;122(5): S3–9.
8. Layland J, Solaro RJ, Shah AM. Regulation of cardiac contractile function by troponin I phosphorylation. J Cardio Res 2005; 66(1): 12-21.
9. Fontes JP, Gonçalves M, Ribeiro VG.Serum markers for ischemic myocardial damage. Rev Port Cardiol 1999; 18:1129-1136.
10. Andrieu N, Prevost T, Rohan TE, et al. Variation in the interaction between familial and reproductive factors on the risk of breast cancer according to age, menopausal status, and degree of familiality. Int J Epidemiol 2000; 29 (2): 214-23.
11. Pfeffer M, McMurray J, Leizorovicz A, et al. Valsartan in Acute Myocardial Infarction Trial: rationale and design. Am Heart J 2000;140: 727-750.
12. Merz CN, Shaw LJ, Reis SE, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part II: gender differences in presentation, diagnosis, and outcome with regard to gender-based pathophysiology of atherosclerosis and macrovascular and microvascular coronary disease. J Am CollCardiol 2006; 47(3): 21–29.
13. Kumar A, Sivakanesan R, Gunasekera S. Ischemia modified albumin: A potent marker in acute myocardial infarction in normolipidemic. Pak J Med Sci 2008;24(3) :364-67.
14. Yazdanyar A, Newman AB. The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. Clin Geriatr Med 2009; 25(4):563-77.
15. Joshi P, Islam S, Pais P, et al Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA 2007; 297:286-94.
16. Terry MW, Gary DK, Lynn M. Differences of Creatine Kinase MB and Cardiac Troponin I Concentrations in Normal and Diseased Human Myocardium. Ann Clin Lab Sci 2002;32:(1).
17. Haseeb AK, Abdullah SA, Samia HS. et al. Serum markers of tissue damage and oxidative stress in patients with acute myocardial infarction. Biomed Res 2013; 24 (1): 15-20.