

Comparative Study of Potential Diagnostic Biomarkers in Myocardial Infarction with Survival and Myocardial Infarction without Survival

¹Sangita M Patil, ²Mangesh Bankar, ³Ramchandra Padalkar, ⁴Abhijit Phatak

ABSTRACT

Introduction: Because of the varied presentation and associated high mortality, the identification of patients with acute myocardial infarction (MI) is very critical for patient management and has a bearing on the prognosis. The goal of present study was to correlate the diagnostic value of cardiac biomarkers in MI with survival and MI without survival.

Materials and methods: Diagnostic case-control study was conducted on 110 MI patients presenting to the Emergency Department within 12 hours of acute chest pain, and 120 healthy age- and sex-matched volunteers formed the control group. Serum ischemia-modified albumin (IMA), troponin I (TnI), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and aspartate transaminase (AST) were measured. Statistical software SYSTAT version 12 was used to analyze the data. The results were expressed in mean \pm standard deviation. Comparisons of study groups and study groups with control groups were done by applying Z test. Correlation was tested by Student's t-test at 5% ($p = 0.05$) and 1% ($p = 0.01$) level of significance.

Results: Mean levels of serum IMA, TnI, CK-MB, LDH, and AST levels were significantly higher ($p < 0.01$) in patients with MI as compared with healthy controls. Serum levels of cardiac biomarkers were significantly elevated ($p < 0.01$) in MI patients without survival as compared with MI with survival.

Conclusion: The serum levels of biomarkers were increased in MI without survival as compared with MI with survival. These study data prove that these changes might be helpful to obtain a comprehensive view of the infarct size and severity of vascular stenotic lesions.

Keywords: Acute myocardial infarction, Creatine kinase-MB, Ischemia-modified albumin, Myocardial infarction, Troponin I.

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INTRODUCTION

Coronary heart disease (CHD) is defined as an acute or chronic cardiac disability arising from imbalance between the myocardial supply and demand for oxygenated blood.¹ It is multifactorial in etiology and has a spectrum of presentations ranging from stable angina, acute coronary syndrome to completely asymptomatic disease.²

It is the largest killer disease in developed countries, and is rapidly assuming a similar role in developing countries. The World Health Organization (WHO) has drawn attention to the fact that CHD is our modern epidemic, not an unavoidable attribute of aging.³ The burden of CHD is rising in India. According to WHO,⁴ 7.2 million deaths (i.e., 12.8% of total deaths) of CHD occurred in 2008.

According to a previous study, it was concluded that the cascade of thrombotic events following atherosclerotic plaque rupture cause occlusion of the coronary artery, which interrupts blood supply and oxygen to myocardium.⁵ Myocardial necrosis with subsequent infarction is followed by heart failure, myocardial rupture, or arrhythmias. Myocardial infarction is the main pathophysiological characteristic of CHD. It occurs due to a lack of nutrients and oxygen reaching the heart muscle by reduction of blood supply to one area of the heart.⁶ Early treatment like fibrinolysis, coronary artery bypass grafting, and percutaneous coronary intervention of MI help to prevent necrosis. However, for well-timed diagnosis, biomarkers play important roles to help us improve our diagnostic of the MI.⁷

The manifestations of the MI are varied and multiple like chest pain, epigastric or arm discomfort, breathlessness, nausea, and vomiting. However, these symptoms may be subtle and are not recognized. Because of the varied presentations and associated high mortality, the identification of MI and early diagnosis of CHD are one of the bottlenecks in medical practice of cardiology.

¹Assistant Professor, ²Associate Professor, ³Professor and Head, ⁴Cardiologist

^{1,3}Department of Biochemistry, Dr. Vitthalrao Vikhe Patil Foundation's Medical College, Ahmednagar, Maharashtra, India

²Department of Biochemistry, Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals, Pune Maharashtra, India

⁴Department of Cardiology, Swasthya Hospital Medical Research Centre, Ahmednagar, Maharashtra, India

Corresponding Author: Sangita M Patil, Assistant Professor Department of Biochemistry, Dr. Vitthalrao Vikhe Patil Foundation's Medical College, Ahmednagar, Maharashtra India, Phone: +919823873928, e-mail: vsrk_om@rediffmail.com

Only about 22% patients admitted to cardiac care centers with chest pain actually suffer from MI, which is the acute clinical pattern of CHD.⁸

The blood test is a noninvasive method. The use and interpretation of cardiac markers are fundamental for the diagnosis of MI. The classic biomarkers of myocardial ischemic damage are creatine kinase isoenzymes (CK-MB) and cardiac TnI and TnT.

The CK-MB is the most universal marker of myocardial necrosis, but specificity of CK-MB for diagnosing MI is within limits. It is not sole to the myocardial, but also high in the setting of muscle trauma. Troponins (I, C, and T) are complex proteins that modulate the calcium-mediated interaction between actin and myosin in myocardial tissue. Elevated levels of troponins provide self-governing prognostic information. They offer plentiful advantages over CK-MB for evaluating patients with possible MI.⁹

The CK-MB and troponins become elevated in circulation within 3 to 6 hours after symptomatic onset of MI.⁹ They do not provide reliable information when measurable in the first 1 to 2 hours. Following an ischemic heart, IMA has been recently introduced as a marker of MI.¹⁰ Previous studies have shown that IMA levels rise within minutes after cardiac ischemia. The IMA is a form of human serum albumin (HSA) in which the N-terminal amino acids have been modified by ischemia. The diagnostic albumin cobalt binding test for IMA is based on the observation that the affinity of serum albumin for cobalt is reduced after N-terminal modification. The test has already been licensed by the US Food and Drug Administration for diagnosis of suspected MI.^{10,11} Takshid et al have shown increased IMA levels in patients with acute coronary syndrome.¹¹

Thus, in view of above information and several risks of complication, it is worthwhile to study the various biomarkers in MI. Very few studies have been reported from serum IMA testing and its application in the Indian context. Thus, the aim of our study was to determine serum level of cardiac markers for early diagnosis of MI in patients presenting with symptoms of acute chest pain. The study also tried to correlate the serum level of biomarker in MI with survival and MI without survival.

MATERIALS AND METHODS

The present study was conducted at the Department of Biochemistry of Dr. Vithalrao Vikhe Patil Foundation's Medical College, Ahmednagar and Swasthya Hospital Medical Research Centre, Ahmednagar Maharashtra, India, in collaboration with the Department of Biochemistry, Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals, Pune, Maharashtra, India.

The study was approved by the Ethics Committee of Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals, Pune, Maharashtra, India with all participants providing informed consent, and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1975.

Study Design

Type: Analytical case control study.

Population: Totally, 230 subjects were enrolled in the present study of which MI patients were 110 and controls were 120.

Sampling: Simple random sampling. In the present study, population was not universal. The study was carried out on available individuals that served as the accessible population.

Sample Size Calculation

The present study was a quantitative study. Thus, the sample size was calculated by using the following formula

$$\text{Sample size } n = 4 \times \sigma^2/E^2$$

n = sample size, σ = Standard deviation in population, E = Allowable error.

Control Group

Totally, 120 healthy age- and sex-matched individuals without any evidence of MI as per clinical examinations were taken as control subjects.

Patients Group

The study included totally 110 patients between the age group 26 to 75 years of MI, and had been taken from the intensive cardiac care unit (ICCU) having chest pain. The patients were diagnosed by physicians, blinded to the results of markers; data included history, physical examination, serial 12-lead electrocardiogram, and cardiac markers measurement.

Inclusion Criteria

The diagnosis of all patients of MI was made by physicians. Patients who had typical symptoms of MI like chest pain, sweating, breathlessness, etc., and specific abnormalities for MI on electrocardiogram, and elevated cardiac markers were included in the present study.

Exclusion Criteria

All patients having heart diseases like congenital heart disease, diseases of heart valves, and myocardium. Confounding factors that could interfere in the biochemical analyses of study subjects and alter the results were

diabetes mellitus, renal insufficiency, hypertension, hepatic disease, inflammatory disease, history of recent infection, and febrile disorders.

After taking informed consent, all subjects were screened for inclusion and exclusion criteria. All the subjects were categorized into different groups as follows.

Collection of Specimen

Criteria for blood collection were different for different groups:

- For control, 5 mL blood was collected between 9.00 and 11.00 am after fasting from 10.00 pm from previous day by using 20G disposable needle from cubital vein with aseptic precaution.
- For MI, 5 mL blood was collected within 12 hours after admission in the ICCU.

Plain vacutainer (Yucca Diagnostic) was used for estimation of CK-MB, TnI, IMA, AST, and LDH. After an hour, the samples were centrifuged at 3000 rpm for 10 minutes to separate serum. The separated serum was collected in polythene tube with cork and stored at 20°C (precautions were taken to avoid the hemolysis) and used for analysis of respective parameters.

Methods

Determination of CK-MB by Liquid Stable Optimized UV Method/immunoinhibition Method

The procedure included the measurements of CK activity in the presence of antibody to CK-MB monomer.¹² This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB, while not affecting the B-subunit activity of CK-MB and CK-BB monomers. The CK-MB activity was obtained by multiplying the CK-B activity by two.

Determination of TnI by Two-site Immunoenzymometric Assay with Fluorescence Detection

Principle: Cardiac TnI in serum was bound with alkaline phosphatase (ALP)-conjugated monoclonal anticardiac TnI antibody, directed to amino acids 87 to 91 of the cardiac TnI molecule, and magnetic beads coated with another monoclonal anticardiac TnI antibody, directed to amino acids 41 to 49.¹³ After incubation, unbound sample and excess antibodies were washed away; cardiac TnI sandwiched between the two antibodies was bound to the solid phase in a magnetic field; a fluorogenic substrate, 4-methylumbelliferyl phosphate, reacts with the ALP antigen-antibody complex. Intensity of fluorescence generated by the product, 4-methylumbelliferone, was directly proportional to cardiac TnI in the sample.

Estimation of IMA by Albumin Cobalt Binding Method

The assay was based on the premise that MI causes changes in HSA that were demonstrated by reduced exogenous cobalt II binding. The concentration of ischemia-modified serum albumin was determined by addition of a known amount of cobalt (II) to a serum specimen and measurement of the unbound cobalt (II) by spectrophotometric assay using dithiothreitol. An inverse relationship thus exists between the level of albumin-bound cobalt and the intensity of the color formation.¹⁴

Estimation of Aspartate Aminotransferase by Modified UV Kinetic Assay¹⁵

Aspartate aminotransferase catalyses the transamination of L-aspartate and α -keto glutarate to form L-glutamate and oxaloacetate. In subsequent reaction, Malate Dehydrogenase (MDH) reduces oxaloacetate to malate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide (reduced) NADH to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH was measured kinetically by monitoring the decrease in absorbance at 340 nm and was directly proportional to AST activity in the sample. Lactate Dehydrogenase is added to enzyme system to prevent endogenous pyruvate interference which is normally present in the serum

Estimation of LDH by Optimized German Society for Clinical Chemistry, Kinetic Assay.¹⁶

Lactate dehydrogenase in serum catalyses the conversion of pyruvate to lactate and NADH to NAD. Lactate dehydrogenase activity was directly proportional to the rate of decrease in the absorbance of NADH at 340 nm.

Statistical Analysis

Statistical software SYSTAT version 12 (by Cranes software, Bengaluru) was used to analyze the data. The results were expressed in mean \pm standard deviation (mean \pm SD). Data were analyzed by descriptive statistics as mean, SD, percentage, etc. Comparisons of study groups and study groups with control groups were done by applying Z test of difference between two sample means at 5% ($p = 0.05$) and 1% ($p = 0.01$) levels of significance.

RESULTS

Table 1 show highly significant ($p < 0.01$) mean levels of CK-MB, TnI, IMA, LDH, and AST in MI as compared with healthy controls. In the same way, Table 2 illustrates that mean levels of CK-MB, TnI, IMA, LDH, and AST were significantly ($p < 0.01$) increased in MI without survival when compared with MI with survival.

Table 1: Biochemical changes in CHD and controls

Variable	Controls (n = 120) mean ± SD	MI (n = 110) mean ± SD
CK-MB (U/L)	0.088 ± 0.17	57.68 ± 25.98**
TnI (ng/mL)	0.028 ± 0.014	14.83 ± 37.17**
IMA (ABSU)	0.497 ± 0.058	0.76 ± 0.21**
AST (IU/L)	13.16 ± 4.91	53.41 ± 21.89**
LDH (IU/L)	280.55 ± 78.56	694.13 ± 279.27**

Values were expressed as mean ± SD; **p < 0.01 considered as highly significant

DISCUSSION

Cardiovascular diseases, generally coronary artery disease, have the potential of causing sudden death, which is defined as a natural death due to cardiac causes through abrupt loss of consciousness within one hour of the onset of acute symptoms.¹

The CHD is invariably caused by disease affecting the coronary arteries. Atherosclerosis is the main cause of CHD, having a prevalence of more than 90%, while other causes are responsible for less than 10% cases of CHD. The MI is due to an acute or subacute primary reduction of myocardial oxygen supply provoked by destruction of an atherosclerotic plaque associated with inflammation, thrombosis, vasoconstriction, and microembolization. Myocardial infarction is the main pathophysiological characteristic of CHD. It mainly occurs due to lack of nutrients and oxygen reaching the heart muscle by reduction of blood supply to one area of the heart.⁶ Detection of MI is extremely important for the diagnosis, treatment, and prognosis of CHD.⁸ Hence, in clinical practice, more attention has been paid to the determination of myocardial markers in the diagnosis of acute MI, stratification of acute coronary syndrome risk, and differential diagnosis of reversible *vs* irreversible MI and acute chest pain.

The present study showed highly significant ($p < 0.01$) mean levels of CK-MB, TnI, IMA, LDH, and AST in MI as compared with healthy controls. In the current study, we also tried to compare the MI with survival and MI without survival. The mean levels of CK-MB (43.40% increased), TnI (45.19% increased), and IMA were significantly ($p < 0.01$) increased in MI without survival as compared with MI with survival.

Pasupathi et al¹⁷ have shown that biochemical marker "CK-MB" is significantly altered with MI patients. The

CK-MB enzyme normally exists in cellular compartment and leaks out into the plasma during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum.¹⁸

Our observation (increased serum TnI in MI due to myocardial necrosis) also supported the study by other researchers.^{17,19} The TnI and TnT are proteins of troponin regulatory complex involved in cardiac contractibility. Both have high myocardial tissue specificity and offer an improved sensitivity and specificity for MI *vs* combination of electrocardiogram and traditional biomarkers.¹⁸ Keller et al¹⁹ have suggested that initial use of sensitive TnI assay substantially improves the early diagnosis of MI and helps to safely rule out or rule in coronary causes of acute chest pain.

In the current study, our findings confirm and expand upon previous reports,^{20,21} which have shown higher levels of IMA in MI. This is because of an ischemic event that may cause more damage to serum albumin and the surrounding tissue as ischemia itself.

It has also been hypothesized that in MI, release of fatty acids results in binding of fatty acid to albumin, which leads to conformational changes in the albumin and reduces the ability of albumin to bind to cobalt. Hence, it accounts for generation of IMA.²²

Albumin is a hemodilating agent and increases cerebral blood flow to both the normal and ischemic brain by decreasing blood viscosity and by vasodilation in response to diminished oxygen delivery. Thus, it serves as an endogenous neuroprotectant. Protective function of albumin is associated with the N-terminal of the albumin, which gets modified by the reactive oxygen species generated in response to ischemia event. This leads to a loss in its protective ability and, thereby, an increase in the IMA in acute ischemic stroke is observed.²³

In the current study, serum levels of LDH and AST were significantly increased in MI. Peppes et al²⁴ have suggested that increased serum levels of myocardial enzymes with coronary artery disease in Greek patients. Our results were precisely matched to this outcome. Abdullah²⁵ had demonstrated in his study that prolonged ischemia originates in the accumulation of nonesterified fatty acids intra and extracellularly, which might change

Table 2: Biochemical changes in MI with survival and MI without survival

Variable	MI with survival (n = 97)	MI without survival (n = 13)	p-value	% increased
CK-MB (U/L)	55.19 ± 23.91	79.14 ± 30.47	p < 0.01, HS	43.40
TnI (ng/mL)	10.29 ± 30.77	56.82 ± 60.88	p < 0.01, HS	45.19
IMA (ABSU)	0.743 ± 0.202	0.93 ± 0.27	p < 0.01, HS	25.17
AST (IU/L)	53.32 ± 20.75	61.45 ± 32.18	p < 0.01, HS	15.25
LDH (IU/L)	681.69 ± 75.48	821.02 ± 297.71	p < 0.01, HS	20.44

HS: Highly significant

the permeability of plasma membrane of heart leading to the leakage of cellular substance and enzyme outside the cells.

CONCLUSION

The CHD is invariably caused by diseases affecting the coronary arteries. The MI is mainly due to an acute or subacute primary reduction of myocardial oxygen supply provoked by destruction of an atherosclerotic plaque associated with inflammation, thrombosis, vasoconstriction, and microembolization. Myocardial infarction is the main pathophysiological characteristic. The biochemical marker of MI should have the properties like a considerable concentration in the myocardium, absence from non-myocardial tissue and normal serum, rapid release into the blood at the time of ischemia, relationship to extent of injury, and persistence in the blood for a sufficient length of time to provide a diagnostic window.

The core of the present study takes into consideration that measurement of serum cardiac markers IMA, TnI, and CK-MB levels might make a diagnosis of MI in patients with ongoing ischemic pain presenting to the emergency department. The serum levels of biomarkers are increased in MI without survival as compared with MI with survival. These study data may prove that these changes might be helpful to obtain a comprehensive view of the infarct size and severity of vascular stenotic lesions.

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REFERENCES

- Mohan, H. Textbook of pathology. Foreword by Ivan Danjanov. 5th ed. Tunbridge Wells: Anshan Publishers; 2005. pp. 305-326.
- Kaul A, Sethi R, Misra MK. Erythrocyte adenosine deaminase in post myocardial infarction angina patients. *Indian J Clin Biochem* 2009 Jan;24(1):49-51.
- Reddy KK, Rao AP, Reddy TP. Socioeconomic status and the prevalence of coronary heart disease risk factors. *Asia Pac J Clin Nutr* 2002 Jun;11(2):98-103.
- World Health Organization. Disease and injury, regional estimates, cause specific estimates for 2008. Geneva: World Health Organization; 2011.
- Tunstall-Pedoe H, Vanuzzo D, Hobbs M, Mähönen M, Cepaitis Z, Kuulasmaa K, Keil U. Estimation of contribution of changes in coronary care to improving survival, event rates and coronary heart disease mortality across the WHO MONICA project population. *Lancet* 2000 Feb;335(9205):688-700.
- Singh K, Singh A. Changes in plasma fibrinogen, homocysteine and lipid profile in coronary artery disease patients of north Indian (Punjab) population. *Biomed Res* 2008 May;19(2):125-128.
- Chan D, Ng LL. Biomarkers in acute myocardial infarction. *BMC Med* 2010 Jun;8:34.
- Zhu Z, Yan Y, Wang Q, Qian J, Ge J. Analysis of serum cardiac biomarkers and treadmill exercise test electrocardiogram for the diagnosis of coronary heart disease in suspected patients. *Acta Biochem Biophys Sin (Shanghai)* 2010 Jan;42(1):39-44.
- de Lemos JA. Biomarkers for the evaluation of patients with ischemic heart disease. Biomarkers in heart disease. 1st ed. Malden (MA): Blackwell Publishing; 2008. pp. 3-19.
- Pan SM, Tong CY, Lin Q, Yao CL, Zhao J, Deng Z. Ischemia modified albumin measured with ultra-filtration assay in early diagnosis of acute coronary syndrome. *World J Emerg Med* 2010;1(1):37-40.
- Takhshid MA, Kojuri J, Tabei SMB, Tavasouli AR, Heidary S, Tabanbeh M. Early diagnosis of acute coronary syndrome with sensitive troponin I and ischemia modified albumin. *Iranian Cardiovascular Research Journal* 2010;4(4):144-151.
- IFCC. IFCC methods for the measurement of catalytic concentration of enzymes. *JIFCC* 1989;1:130.
- Haug C, Bachem MG, Woehrle H, Martin H, Gruenert A. Evaluation of two modified cardiac troponin I enzyme immunoassays. *Clin Chem Lab Med* 2002 Aug;40(8):837-992.
- Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. *J Emerg Med* 2000 Nov;19(4):311-315.
- Moss, DW.; Henderson, AK. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz textbook clinical chemistry. 3rd ed. Philadelphia (PA): W B Saunders; 1994. pp. 617-721.
- Young, D. In effect of preanalytical variables on clinical laboratory tests. 2nd ed. Washington: AACC Press; 1997. p. 499.
- Pasupathi P, Rao YY, Farook J, Saravanan G, Bakthavathsalam G. Oxidative stress and cardiac biomarkers in patients with acute myocardial infarction. *Eur J Sci Res* 2009 Jan;27(2): 275-285.
- Kasap S, Gonenc A, Sener DE, Hisar I. Serum cardiac markers in patients with acute myocardial infarction: oxidative stress, C-reactive protein and N-terminal probrain natriuretic peptide. *J Clin Biochem Nutr* 2007 Jul;41(1):50-57.
- Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyn E, Bickel C, Baldus S, Warnholtz A, Fröhlich M, et al. Sensitive Troponin I assay in early diagnosis of acute myocardial infarction. *N Engl J Med* 2009 Aug;361(9):867-877.
- Anwaruddin S, Januzzi JL Jr, Baggish AL, Lewandrowski EL, Lewandrowski KB. Ischemia modified albumin improves the usefulness of standard cardiac biomarkers for the diagnosis of myocardial ischemia in the emergency department setting. *Am J Clin Pathol* 2005 Jan;123(1):140-145.
- Collinson PO, Gaze DC, Bainbridge K, Morris F, Morris B, Price A, Goodacre S. Utility of admission cardiac troponin and ischemia modified albumin measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. *Emerg Med J* 2006 Apr;23(4): 256-261.
- Mothes E, Faller P. Evidence that the principal Coll-binding site in human serum albumin is not at the N terminus: implication on the albumin cobalt binding test for detecting myocardial ischemia. *Biochemistry* 2007 Feb;46(8):2267-2274.

23. Nayak AR, Kashyap RS, Kabra D, Purohit HJ, Taori GM, Dagainawala HF. Prognostic significance of ischemia modified albumin in acute ischemic stroke patients: a preliminary study. *Ann Neurosci* 2011 Jan;18(1):5-7.
24. Peppes V, Rammos G, Manios E, Koroboki E, Rokas S, Zakopolos N. Correlation between myocardial enzyme serum levels and markers of inflammation with severity of coronary artery disease and genisini score: a hospital based, prospective study in greek patients. *Clin Interv Aging* 2008 Dec;3(4):699-710.
25. Abdullah MR. Comparative study of serum lactic acid, lactate Dehydrogenase and lipid profile in ischemic heart disease patients and healthy control. *IBN Al-Haitham J Pure Appl Sci* 2010;23(1):171-179.