Study of Ischemia Modified Albumin as New Potential Diagnostic Biomarker In Acute Myocardial Infarction.

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Abstract :

Background: Because of the varied presentation and associated high mortality the identification of patients with acute myocardial infarction is very critical for the patient management and has a bearing on the prognosis. Only about 22% patients admitted to cardiac care centers with chest pain having truly myocardial infarction. **Aim:** The goal of present study was to assess diagnostic value of serum ischemia modified albumin and compare it with sensitive cardiac troponin I and Creatine Kinase-MB in acute myocardial infarction. **Methods:** A diagnostic case control study was conducted on 102 patients presenting to the Emergency Department within 6 hrs of acute chest pain and 115 healthy age and sex matched volunteers formed the control group. Serum ischemia modified albumin level was estimated by albumin cobalt binding test using digital spectrophotometer, while troponin I was measured by immunofluroscence assay and creatine Kinase-MB was determined by immunoinhibition method. The sensitivity and specificity of ischemia modified albumin, troponin I and creatine kinase-MB for detection of acute myocardial infarction were analyzed. The results of ischemia modified albumin, troponin I and creatine kinase-MB alone and in combination were correlated. **Results:** Ischemia modified albumin ($p<0.05$) and troponin I ($p<0.001$) concentrations were significantly higher in acute myocardial infarction than healthy controls. Sensitivity, specificity, positive predictive value and negative predictive value of ischemia modified albumin for detection of acute myocardial infarction was 88.24%, 93.91%, 92.78% and 90.00% compared to 86.27%, 93.04%, 91.67% and 88.43% respectively for the troponin I and 78.43%, 100%, 100%, and 83.94% for creatine kinase-MB. Combined use of ischemia modified albumin, troponin I, creatine kinase-MB significantly enhanced the sensitivity to 96%. The area under the receiver operating characteristic curve of ischemia modified albumin in acute myocardial infarction was 0.90. **Conclusion:** Ischemia modified albumin is a new potential diagnostic biomarker used together with other gold standard cardiac biomarkers can improve early diagnosis of acute myocardial infarction.

Key words: Acute myocardial infarction, Ischemia modified albumin, Troponin I, creatine kinase-MB, Myocardial Ischemia.

Introduction:

Coronary heart disease (CHD) is defined as acute or chronic cardiac disability arising from imbalance between the myocardial supply and demand for oxygenated blood.[(1)] In the etiology, CHD is caused by disease affecting the coronary arteries. More than 90% cases caused by coronary atherosclerosis and 10% by other causes like Vasospasm, Stenosis of coronary Ostia, Arteritis, embolism, thrombic diseases like shock, polycythaemia, Trauma.[(2)]

In today's world about 17 million deaths occurred due to Cardiovascular Disease. In India, Number of deaths due to Ischemic heart disease increased from 1.17 million in 1990 to 1.59 million in 2000 and 2.03 million by 2010.[(3)] The diagnostic approach and the clinical management of patients presenting with suspected acute myocardial infarction or cardiac dysfunction are challenging.[(4)]

The manifestation of the myocardial ischemia is varied and multiple like chest pain, epigastric discomfort, breathlessness, nausea and vomiting. However these symptoms may be subtle and are not easily recognized. Because of varied presentation and associated with high mortality, the early identification of patients with acute myocardial infarction is very critical.[(5)]

The Various Biomarkers like creatine kinase-MB, lactate dehydrogenase along with echocardiogram (ECG) is used in diagnosis of CHD. They are not providing reliable information when measured in the first 2-6 hrs. Moreover, the usual biomarkers may not rise during reversible myocardial ischemia and other diagnostic tools such as stress testing, echocardiology are not routinely available.[6] Following an ischemic heart, Ischemia modified Albumin has been recently introduced as a marker of Myocardial Ischemia. Initially the test was named as albumin cobalt binding assay (ACB assay) since it is based on the human serum albumin for metal ions (cobalt COII) in patients with ischemia.[7]

Recent research has found that Ischemia Modified Albumin (IMA) is an ideal biomarker for ischemia. IMA is a form of human serum albumin in which the N-terminal amino acids have been modified by ischemia. This modification reduces the affinity of plasma albumin to bind heavy metal ion such as cobalt.[8] Bhagwan et al and others have shown increased IMA levels in patients with spontaneous coronary ischemia with abnormal values detectable before subsequent increases in cardiac Troponin I.[9]

Very few studies have been reported from serum IMA testing and its application in Indian context. The aim of our study was to compare the clinical performance of IMA with cardiac TnI and CK-MB for early diagnosis of myocardial infarction in patients presenting with symptoms of acute chest pain.

Material & method:

The present diagnostic case-control study was conducted at Department of Biochemistry in DVVPF's Medical College Ahmednagar & Swasthya Hospital & Research Centre Ahmednagar (Maharashtra) in collaboration with Department of Biochemistry, B. J. Medical College & Sassoon General Hospital Pune. The study was approved by Ethics Committee of B. J. Medical College & Sassoon General Hospital Pune with all participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Patients: Total 102 patients between age group 26 to 70 years admitted in the Intensive Cardiac Care Unit (ICCU) chest pain were taken for the study. 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.

Control subjects: 115 healthy age and sex matched individuals who didn't have any evidence of acute myocardial infarction as per clinical examination were taken as control subjects.

Exclusion criteria: The exclusion criteria were acute or chronic renal diseases, hepatic diseases, diabetes & heart disease like congenital heart disease, diseases of heart valves & myocardium.

Approximately 5 ml blood was collected by venipuncture from anticubital vein of the forearm of each subject in plain vaccutainer (yucca diagnostic) under aseptic conditions within 6 hrs after admission in ICCU and centrifuged for serum collection.

Method:

1) Spectrophotometrically determination of serum IMA by albumin cobalt binding assay:- The assay is based on the premise that myocardial ischemia causes change in human serum albumin that is demonstrated by reduced exogenous cobalt (II) binding. The concentration of ischemia modified serum albumin can be determined by addition of a known amount of cobalt (II) to a serum and measurement of the unbound cobalt (II) by colorimetric assay using Dithiothereitol (DTT). Color development with DTT was compared to a serum-cobalt blank without DTT and results were reported in absorbance units (ABSU).[10]
2) Determination of troponin I:- TnI was estimated by kinetic Immunofluroscence assay using Tosoh AIA-360 Immunoassay analyzer.[11]
3) Determination of CK-MB:- CK-MB estimated by a Liquid stable optimized UV method / Immunoinhibition Assay using semiautoanalyser. (Pathozyme kit).[12,13]

Statistical analysis: The statistical analysis was carried out by using the SYSTAT software package for window version 12. The students "t" test was applied for the statistical analysis and the results were expressed in mean± Standard Deviation (mean ±SD).p values $p<0.05$ for IMA and $p<0.001$ for TnI and CK-MB were considered to be statistically significant and highly significant respectively. The Receiver Operating Characteristic (ROC) curve analysis and the area under the curve were performed for determination of diagnostic performance of serum IMA and TnI in the all patients included in the study. The optimum cutoff values for determination of serum IMA and TnI were selected from ROC analysis. This optimum cutoff was used to dichotomously classify positive or negative serum IMA and TnI level, and used for calculating of diagnostic sensitivity and specificity by using MEDCALC. software version12.3.

Results:

Baseline demographic and clinical characterization of the patients and healthy controls groups are given in table1. As shown in **table 1** IMA levels were increased significantly ($p<0.05$) in the AMI Group (0.704±0.155) as compared to controls group (0.492±0.054). The mean values of serum TnI were significantly ($P<0.001$) higher in AMI (15.60±38.32) as compared to healthy controls (0.029±0.016). The mean values of serum CK-MB were significantly ($P<0.001$) higher in AMI (60.29±24.72) as compared to healthy controls (0.870±0.160).

Figure 1 shows a scatter plot distribution of the results of serum IMA in controls and AMI groups. The optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 0.551 Absorption Units. (ABSU) with a sensitivity of 88.24 % and specificity 93.91% the area under curve for IMA was 0.90.

Figure 2 shows a scatter plot distribution of the results of serum TnI in controls and AMI groups. The optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 0.04ng/ml with a sensitivity of 86.27% and specificity of 93.04.

The performance of IMA, TnI and CK-MB alone and in combination for diagnosis of AMI is presented in **table 2.** Sensitivity, specificity, positive predictive value and negative predictive value were analyzed. Combined use of IMA, TnI and CK-MB significantly improve the sensitivity specificity, positive predictive value and negative predictive value to 96%, 93%, 93.33% and 96.26% respectively.

Table-1 Baseline characteristics of all subjects

Variables	Controls (n=115)	Myocardial Infarction(n=102)
Age in years	40.1± 12.34	48.8±14.56
Gender (Men/Women)	67/48	70/32
Body Mass Index	20.23±1.88	22.9±2.89
IMA (ABSU)	0.495±0.054	0.704±0.155*
TnI (ng/ml)	0.029±0.016	15.60±38.32**
CK-MB (U/L)	0.870±0.160	60.29±24.72**

Values were expressed in mean with Standard Deviation (Mean±SD),

* Statistically significant ($P<0.05$), ** Statistically highly significant,($p<0.001$)

n =numbers

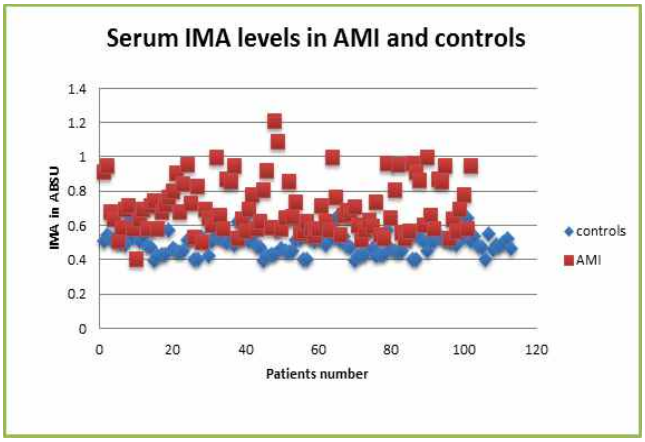

Figure -1

Table-2 Clinical characteristics of diagnostic test for detection of AMI

Variables	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
IMA	88.24	93.91	92.78	90.00
TnI	86.27	93.04	91.67	88.43
CK-MB	78.43	100	100	83.94
IMA ,TnI,CK-MB	96	93	93.33	96.26

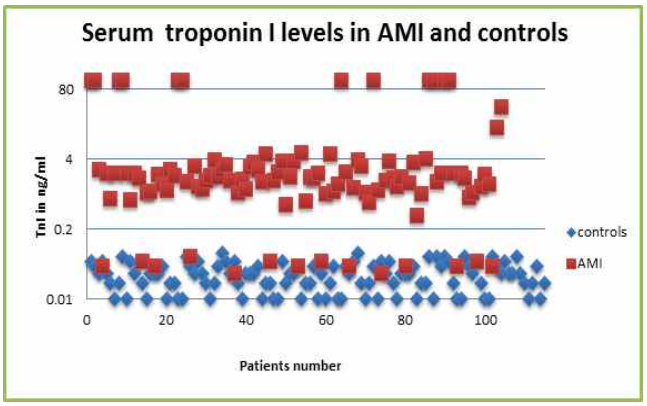

Figure -2

Discussion:

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the world.[14] The most common cause of an AMI is atherosclerotic coronary artery with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion. Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable. Several risk factors for coronary heart disease have been well recognized, including hypertension, hyperlipidemia, diabetes, a positive family story, smoking, obesity.[15]

Annually, several million patients seek care in the emergency department because of chest pain or other symptoms suggesting an acute coronary syndrome but only 10% are subsequently confirmed to have AMI.[16] The criteria for the diagnosis of myocardial infarction have been redefined recently, as reported in a consensus document of the European Society of cardiology (ESC) and the American College of Cardiology (ACC) and require at least 2 of the 3 following characteristics: typical symptoms, characteristic rise and fall pattern of a cardiac marker and typical electrocardiogram (ECG) pattern involving the development of Q waves.[17]

In clinical practice, more attention has been paid to the determination of myocardial markers in the diagnostic of acute myocardial Ischemia, Stratification of acute coronary syndrome risk and differential diagnosis of reversible verses irreversible myocardial ischemia and acute chest pain.

Prolonged ischemia can lead to myocardial cell death and is a pre-condition to infarction. Therefore identification of myocardial ischemia at the earliest stage is must to prevent the devastating consequences of the disease.

Ischemia Modified Albumin has recently been evaluated as new sensitive serum biomarker of cardiac ischemia in contrast to cardiac enzymes, which are released when cardiac necrosis occurs. During an acute ischemic event structural changes occur in the amino terminal of albumin, rapidly reducing its capacity to bind transition metal ions and generate a metabolic variant of the albumin referred as IMA[18-20] IMA has already been licensed by the US food and drug administration for diagnosis of suspected myocardial ischemia.[21] Therefore in our study, we examined the diagnostic performance of IMA assay, high sensitive TnI assay and CK-MB for the early diagnosis of AMI at the time of patient presenting to the Emergency Department.

The result of our study showed that, the serum IMA level was significantly higher in MI than the healthy controls. Diagnostic appearance of serum IMA in MI was Maximum. The sensitivity and specificity were 88.24% and 93.91% respectively. Our observations also support the study by others [18, 21, 22] because ischemic events may cause as much or more damage to serum albumin and the surrounding tissue as ischemia itself. Different mechanisms have been postulated for generation of IMA early after cardiac ischemia. Either ischemia or reperfusion may include hypoxia, acidosis, free radical damage, membrane energy-dependent sodium and calcium pump disruptions, and free iron and cupper ion exposure.[22] Most of these conditions occur in vivo within minutes after the onset of acute myocardial ischemia.

It has also been hypothesized that in myocardial ischemia the release of fatty acids results in binding of fatty acid to albumin which leads to conformational changes in the albumin and reduce the ability of albumin to bind to cobalt and hence account for generation of IMA.[23] Creatine Kinase (CK) isoenzymes catalyze the phosphorylation reaction of Creatine to Creatine phosphate. CK-MM is the predominant CK isoenzymes in adult skeletal muscle and cardiac tissue.CK-MB is mainly present in cardiac muscle.

CK-activity in serum is proportional to muscle mass and therefore serum CK activity characteristically decreases as a patient's age and muscle mass diminish. Troponin I is polypeptide which binds to actin and inhibits the activity of actinomyosin ATPase. Troponin plays a vital role in the diagnosis and risk stratification of acute coronary syndrome.[24] Inflammation of the myocardium lead to release of Creatine Kinase-MB (CK-MB) and Troponin I. Determination of CK-MB and TnI should be recommended in case of suspected myocarditis because elevation of this markers support the diagnosis.[25]

Mean values of serum TnI and CK-MB were significantly higher ($P<0.001$) in all AMI patients as compared to healthy controls. The sensitivity and specificity of troponin I were 86.27% and 93.04% where sensitivity and specificity of CK-MB were 78.43% and 100% for detection of AMI. Aiki kaul have found that significantly elevated serum levels of CK-MB and Tn I ($P<0.01$) have found in post myocardial infarction angina patients.[26] Till keller, *et al* have shown that with the use of TnI assay on admission (cut off value 0.04 ng/ml) the clinical sensitivity was 90.7% and the specificity was 90.2.[27] The cardiac isoforms of troponins T and I have been used to evaluate myocardial cell damage associated AMI. Measurement of the cardiac specific contractile proteins TnT or TnI were superior to other golden markers and were valid predictors of adverse events in patients with ACS.[28]

In present study, the diagnostic performance of IMA level in AMI patients was greater as compared to TnI and CK-MB assay. The sensitivity and specificity of IMA was significantly greater than those of TnI. The combination of IMA, TnI and CK-MB improved sensitivity of the detection of AMI to 96%. The results are in complete conformity with previous report,[29] where combination of IMA, ECG and TnI results improved sensitive to 96% for detection of ACS has been reported. Saif Anwaruddin et al have found that, the combination of IMA, Myoglobin, CK-MB,TnI increased the sensitivity for detecting ischemia to 97% with a negative predictive value of 92%.[30] Bhagwan et al showed the sensitivity of 88% and a specificity of 94% for the IMA assay which is quite close to our result. They also reported an AUC under ROC plot of 0.95.[9] Collinson P et al have recommended that distinct advantage of measuring the combination of IMA and cardiac TroponinI on admission, in population with a non-diagnostic electrocardiogram is to rule out a final diagnosis of AMI.[31]

Conclusion:

The core of this study lie in fact that measurement of serum IMA levels could make a diagnosis of AMI in patients with ongoing ischemic pain presenting to the emergency department. IMA can be used as an independent biomarker or an additional parameter along with TnI and CK-MB to boost the confidence of the clinicians in ruling out the cardiac ischemia. The combination seems to have a clear potential to save time, early intervention and shorten stay in the emergency department.

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