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ISCHEMIA MODIFIED ALBUMIN : A NOVEL MARKER FOR ACUTE CORONARY SYNDROME

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ABSTRACT

Early identification of patients with acute myocardial infarction is of prime importance due to the associated very high mortality. Only 22% of the patients presenting at emergency cardiology care with chest pain have coronary disease. A number of biochemical tests like CKMB and Troponin-T/I have been introduced for early detection of the coronary syndrome (ACS). Ischemia modified albumin (IMA) has been recently introduced as a marker of myocardial ischemia. We estimated serum IMA in four sequential samples from 25 patients admitted to ICCU. Twenty five healthy volunteers formed the control group from which the normal range was derived. IMA was significantly raised in ischemia patients than in controls as well as compared to the patients who did not have cardiac ischemia. IMA demonstrated good discrimination between the ischemic and the non-ischemic patients with an Odds Ratio of 16.9 (6.29 - 46.87) than CKMB which showed an Odds Ratio of 2.07 (1.18 - 6.08). Sensitivity and specificity of IMA for the detection of ACS was 78.0% and 82.7% compared to 58.0% and 60.0%, respectively for the CK-MB assay. The area under the ROC curve of IMA for ischemic v/s non-ischemic patients was 0.834. IMA appears to be developing into a new and very potent marker of cardiac ischemia.

KEY WORDS

Ischemia Modified Albumin, Albumin Cobalt Binding, Cardiac Ischemia

INTRODUCTION

Myocardial ischemia results from the lack of adequate blood perfusion of the myocytes, leading to a deficiency of oxygen and nutrients, thus compromising their vital functions. The manifestations of the myocardial ischemia are varied and multiple like chest pain, epigastric or arm discomfort, breathlessness, nausea and vomiting. However, these symptoms may be subtle and are not easily recognized. Prolonged ischemia can lead to myocardial cell death known as acute myocardial infarction (AMI). Because of the varied presentation and associated high mortality, the early identification of patients with AMI is very critical for the patient management and has a bearing on the prognosis. Only about 22% of the patients admitted to cardiac care centers with chest pain actually develop AMI (1). Biochemically, AMI is diagnosed with the help of myocardial proteins in the serum viz. creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH), aspartate transaminase (AST) and Troponins (T & I) along with ECG and other imaging modalities. Ideally, we should be able to identify myocardial ischemia before it progresses to the irreparable myocardial cell damage. Thus identification of a biochemical marker that is sensitive and specific for myocardial ischemia and can be rapidly measured is of great importance.

Cardiac markers of cell necrosis such as myoglobin, CK-MB and troponins are highly sensitive and exhibit good specificity. They do not, however, detect myocardial ischemia in the absence of necrosis and provide no reliable information when measured in the first 2-6 hours following an ischemic event (2,3). Recently, a new parameter, Ischemia Modified Albumin (IMA) has been developed and found to be very useful for the detection of acute myocardial ischemia. Initially the test was named as Albumin Cobalt Binding (ACB) assay since it is based on the reduced binding affinity of the human serum albumin for metal ions (Cobalt, CoII) in patients with ischemia (4). The reduction in the binding affinity of albumin has been attributed to the free radical damage to the N-terminal of albumin molecule in patients with myocardial ischemia. Preliminary results with myocardial ischemia patients (5) and angioplasty patients transiently made ischemic with balloon

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inflation (6) proved very encouraging. The Food and Drug Adminstration (FDA) of USA approved the IMA test in 2003 for detection/ruling out of AMI in patients presenting with chest pain in emergency room.

IMA testing is promising to be a major breakthrough in cardiac testing; a negative IMA result may help in moving the patients into a low risk category by initial evaluation based on the clinical presentation, ECG and IMA study thereby providing a major cost saving (7). No study has been reported from India regarding IMA testing and its application in indian context. The present study was thus undertaken to standardize the procedures and for the evaluation of the analytical performance and the clinical validation of IMA assay.

MATERIALS AND METHODS

The study was carried out at the Department of Biochemistry in collaboration with Department of Cardiology of Christian Medical College & Hospital, Ludhiana.

Patients

Twenty-five patients, between the age group of 30 to 60 years, admitted in the Intensive Cardiac Care Unit (ICCU) with chest pain were taken up for the study. The patients were diagnosed and classified as Ischemic and Non-Ischemic depending upon the assessment as per the clinical signs & symptoms and the ECG findings at the time of admission.

Control subjects : Twenty-five healthy individuals, who didn't have any evidence of coronary artery disease per se clinical examination, were taken as control subjects and the blood samples from this group were utilized to determine the 95th percentile of a control reference population for the IMA test.

Blood samples drawn from all the subjects into vacutainer tubes by venipuncture, were brought to Biochemistry laboratory within an hour of admission; were processed for the cardiac enzymes (CK, CK-MB, LDH) and stored at -20^{0}C till IMA testing. The samples were also collected from all the study subjects at 24 hr, 48 hr after admission and at discharge. Electrocardiography was also done at the above time points.

Exclusion Criteria : Since the normal serum albumin level is a prerequisite for the IMA estimation, all the patients with renal disease were excluded from the study. Serum creatinine more than 3.0 mg/dl was used as the exclusion criteria.

Methods

a) Albumin Cobalt Binding Assay (IMA)

Principle : The assay is based on the premise that myocardial ischemia causes changes in human serum albumin (HSA) that are 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 specimen and measurement of the unbound cobalt (II) by colorimetric assay using dithiothreitol (DTT). An inverse relationship thus exists between the level of albumin bound cobalt and the intensity of the color formation.

Procedure : Preparations for the Co (II) albumin binding protocol involved the addition of 200 l of patient serum to 50 l of a solution of 1gm/l cobalt chloride, followed by vigorous mixing and 10-min incubation. Dithiothreitol (50 l of a 1.5 g/l solution) was then added and mixed. After 2 min. incubation, 1.0 ml of a 9.0 g/l solution of NaCl was added. The absorbance of the assay mixture was read at 470 nm using a Gilford Spectrophotometer. The blank was prepared similarly with the exclusion of DTT.

b) Creatine Kinase (CK) : The method of Oliver modified by Rosalki (8) was used for CK estimation on an Autoanalyser (Hitachi 902).

c) CK-MB: The MB isozyme of Creatinine Kinase was estimated by an Immunoinhibition assay where a specific antibody inhibits the CK-M sub-unit. The CK-MB is then calculated by estimating the total CK activity (9).

d) Lactate Dehydrogenase (LDH) : LDH was measured by using lactate as the substrate and NAD as coenzyme on the Hitachi 902 autoanalyser IFCC (10) method.

Statistical Analysis

The data generated were analyzed with the help of computer statistical software packages namely EPI-INFO (Ver 6.0) and SPSS (Ver 11.0). The association of IMA and CK-MB levels with the clinical findings was compared by Chi-Square and ANOVA.

RESULTS

IMA assay was standardized in the Department of Biochemistry and a standard curve was prepared in the range 6.0-60.0 g $CoCl_2$/ml. One IMA unit was defined as " g of free Co (II) in the reaction mixture per ml of serum sample". The assay was found to maintain linearity within this range. Interassay coefficient of variance (CV) was calculated by repeating a patient sample (IMA 15.6 Units) in six assays in duplicate and was found to be 13.8%, which was slightly on the higher side but within acceptable levels.

Twenty-five patients admitted to ICCU of CMC were selected for the study in the age group of 32 to 60 years. Among these subjects, 18 (72%) were male and

7 (28.0%) were female. Majority of the patients (60%) were between 50-60 years of age. The samples were tested anonymously in the laboratory and the study was double blinded. The clinical data were noted down from the patient's hospital files and correlated with laboratory data at the end of the study.

The control group subjects showed very low IMA levels; all of them being below 20 IMA Units. The mean±SEM levels in the control group were found to be 9.78±1.04 IMA Units with a median of 8.50. The reference range of 0.0-20.0 IMA Units was thus established and a cut-off of 20 IMA Units was defined to identify the IMA positive results.

The study subjects had serum IMA levels between 3.6 to 144.6 IMA units with a median of 21.1 and Mean ± SEM as 22.70±1.96 units. The IMA levels in the study group were significantly (p-value = 0.0014) higher than those in the control group (Mean ± SE = 9.78±1.14, Median = 8.50). The comparative results are presented in Table 1. IMA level in the study group was found to be raised in 52 (52%) of the samples as compared to none (0%) in the control group (Fig. 1).

Table 1. Mean ± SE levels (IMA Units) of Ischemia Modified Albumin in the different groups

Subjects	Mean ± SE	Median
Study	22.70*±1.96	21.10
Controls	9.78*±1.04	8.50
Ischemic	28.76$^{\varepsilon}$±3.44	23.65
Non-Ischemic	16.64$^{\varepsilon}$±1.47	14.10

* p-Value : 0.00147; $^{\varepsilon}$ p-Value : 0.00165.

The samples were divided into ischemic and non-ischemic groups based on the clinical indices i.e. ST-segment depression or elevation on ECG, chest pain, epigastric and arm discomfort with exertion at rest, shortness of breath, nausea and vomiting at the time of sampling. In the ischemic group, 39 (78%) samples were found to have increased IMA levels as compared to 13 (26%) samples in the non-ischemic group (Fig. 1).

Normal IMA levels were found to be present in 11 (22%) of the ischemic and 37 (74%) samples of the non-ischemic group. For the ischemic group the IMA levels (mean±SE) were 28.76±3.44 IMA Units (Median 23.65); in comparison the non-ischemic group showed a Mean of 16.64±1.47 IMA Units with a Median of 14.10 (Table 1). The difference between IMA levels between the two groups were statistically significant (p = 0.0016).

The sequential samples from the patients showed that IMA levels were raised in 53.8 % of the patients in

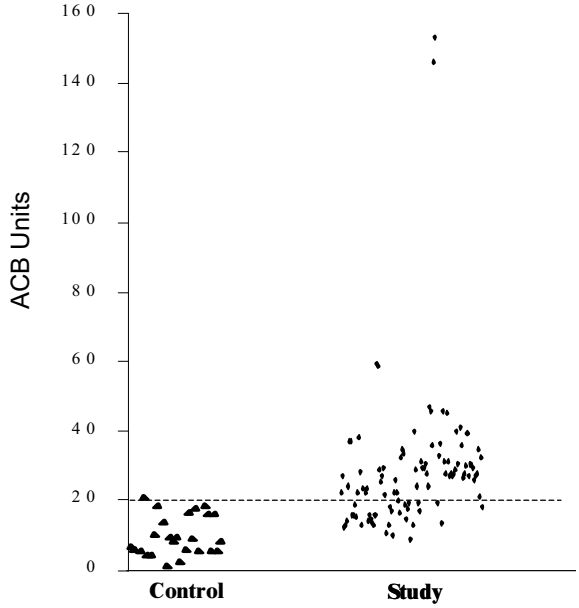

Fig. 1. Serum IMA levels in the two groups

ischemic group at the time of admission (Fig. 2). The incidence of IMA positive samples increased to 100% in the ischemic group by the third day after admission. At the time of discharge, IMA levels in a number of subjects came within the normal limits, thus reducing the proportion of the abnormal IMA levels to 70.0% in the ischemic group. The corresponding results for the CK-MB were - 53.8%, 57.1%, 69.2% and 50% at admission, 24 h, 48 h and at discharge respectively.

IMA test was able to detect 78.0 % of the patients with cardiac ischemia (with or without overt infarction) with a specificity of 82.7 % (Table 2, Fig. 3). The positive predictive value for the test was 75.0% and the negative predictive value was 84.9%. CK-MB assay, on the other hand, was not as good as IMA as the indicator of myocardial ischemia. The sensitivity of 58.0% with 60.0% specificity was significantly

Table 2. Efficiency of Serum IMA and CK-MB levels as marker of Cardiac Ischemia

	Serum IMA	Serum CK-MB
Sensitivity	78.0	58.0
Specificity	82.7	60.0
Predictive Value +Ve	75.0	49.2
Predictive Value -Ve	84.9	62.2
Odds Ratio	16.91 (6.29-46.87)	2.07 (1.18-6.08)

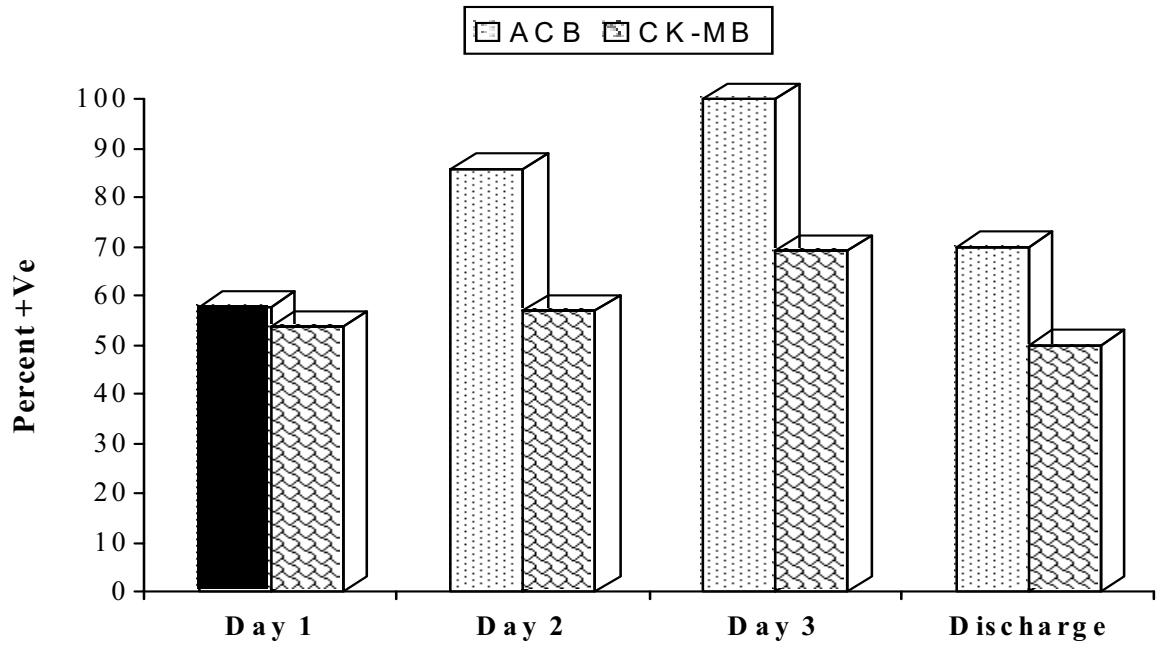

Fig. 2. Per cent of positive IMA and CK-MB results in ischemia patients during their stay in ICCU

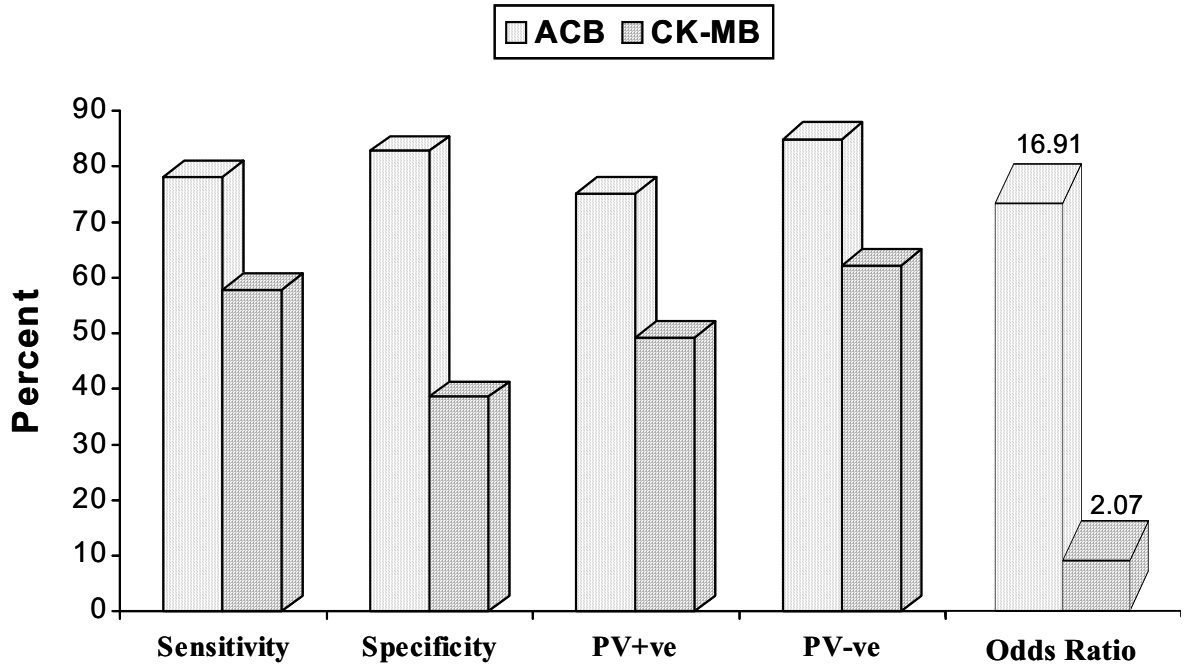

Fig. 3. Efficiency of ACB and CK-MB as marker of cadiac ischemia

(p=0.024) lower than that in IMA assay. The positive predictive value of CK-MB was 49.20% and the negative predictive value was 62.2% only. Odds Ratio of a positive IMA test for Ischemic against Non-Ischemic samples was 16.91 (95% CL 6.27-46.87) whereas for CK-MB the corresponding value was 2.07 (95% CL 1.18-6.08).

DISCUSSION

This paper reports the characterization of the IMA test for its utility in the early identification of ischemic patients and its comparison with CK-MB, the most common biochemical marker of coronary artery disease. 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 a must to prevent the devastating consequences of the disease. Currently available biochemical markers viz. CK, CK-MB and LDH don't seem to serve this requirement, because for these markers to become positive for identification, these proteins have to leak out of the myocytes, which becomes possible only when sufficient amount of cell death has already taken place (11). Therefore the need of a cardiac marker that can serve the above purpose is well felt all over the world and IMA, as a potential candidate for the purpose, is being studied with great enthusiasm.

In the absence of a reproducible universal unit for IMA, we had to define our own laboratory unit for the assay since the absorbance (optical density) units used by some other workers appeared to be rather unscientific and the absorbance was noted to change if the Dithiothreitol reagent was not fresh at the time of estimation.

HSA is the most abundant multifunctional protein in blood, consists of 585 amino acid residues (66.5 kDa), is synthesized in the liver and has a half-life of ~19 days. Co(II) binding to albumin occurs at the N-terminal region of HSA (6, 12). The mechanism that causes altered Co(II) binding to albumin during ischemia is not understood, but it appears to be reversible as shown by Bar-Or 2001 (6). They reported high IMA levels during transient ischemia that returned to baseline values by 6 hr after percutaneous transluminal angioplasty. In our study, altered Co (II) binding as measured by IMA was raised in about half of ischemic subjects at admission, subsequently more and more cases became IMA positive till third day when all the samples from ischemic individuals were positive. It appears that the N-terminal oxidative damage to albumin is cumulative and the repair is slow. IMA in eight ischemic patients returned to the normal range as their condition stabilized. Since cardiac ischemia in clinical setting is not one-event phenomenon and does not cease immediately on commencement of the treatment in ICCU, we do not expect all the patients to be IMA negative at discharge.

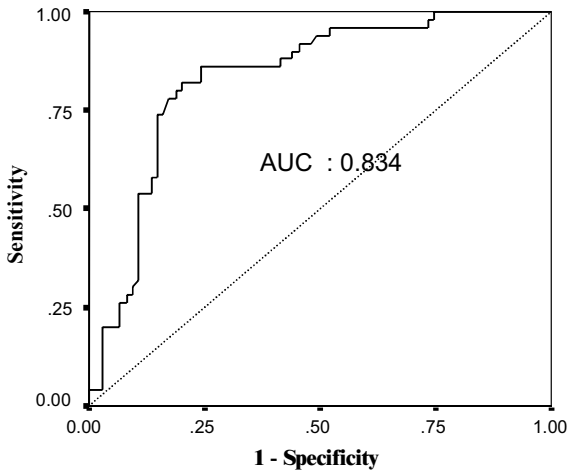

Fig. 4. Receiver Operative Curve (ROC) plot serum IMA levels as discriminator between ischemia and non-ischemia

A higher specificity and sensitivity along with a higher positive and negative predictive value for IMA as compared to the CK-MB for the detection of ischemic heart condition reinforces the postulate that the two tests have different roles to play in the management of ICCU patients. A very high (~85%) negative predictive value of IMA can help the cardiac emergency room to identify the non-cardiac chest pain cases, who can then be shifted to low risk areas. Almost similar observations have also been reported by Sue Auxter (7). Our results are in complete agreement with those in literature (6, 13, 14), where the sensitivity of 82% for the IMA assay has been reported. IMA appears to establish itself as the marker for cardiac ischemia whereas CK-MB should be used to follow up the cases with overt infarction. The receiver operative curve (ROC) of the IMA test for ischemic/non-ischemic dichotomy (Fig. 4) also supports the assay as a good discriminator (Area under the curve, AUC : 0.834) between the two conditions. A very high Odds Ratio (16.9) also confirms this interpretation. Bhagwan *et al*. (15) showed the sensitivity of 88% and a specificity of 94% for the IMA assay which is quite close to our results. They also reported an AUC under ROC plot of 0.95.

In view of the above results, it appears that IMA can serve to be a better detector for myocardial ischemia than CK-MB. However the results can only be referred as a prelude to what might prove to be significant change in the approach to hospital admissions and their subsequent treatment plan of ACS subjects. An extensive study with a larger set of patients is thus

required which should compare the IMA test with other markers such as the troponins and myoglobin etc. Whether IMA develops as an independent point-of-care test or an additional parameter along with troponins to boost the confidence of the clinicians in ruling out the cardiac ischemia is yet to be seen.

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