

Ischemia Modified Albumin: A Recent Biomarker in the Diagnosis of Acute Coronary Syndrome

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Original Research Article

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Article History

Received: 03.07.2018

Accepted: 12.07.2018

Published: 30.07.2018

DOI:

10.36347/sjams.2018.v06i07.016



Abstract: To identify the role of Ischemia Modified Albumin (IMA) as a cardiac biomarker in the early diagnosis of Acute Coronary Syndrome (ACS). A study was done including 40 Acute Coronary Syndrome patients (Group 2) and 40 age sex matched controls (Group 1). Blood samples were estimated for Ischemia Modified Albumin, Troponin T, CK-MB, glucose, urea, creatinine and albumin. Stastical analysis was done using SPSS version 16. Serum IMA levels and CK-MB levels were significantly increased in ACS patients when compared to the controls and they were positively correlated. No significant difference was observed in serum Albumin levels among the groups. Sensitivity of IMA was increased when used in combination with other markers like CK-MB and cTnT in the diagnosis of ACS. From our study, Ischemia Modified Albumin is a sensitive cardiac biomarker to detect myocardial ischemia and is more helpful in identifying patients with unstable angina when compared to other routine biomarkers. The serum levels of IMA and CK-MB are significantly elevated in ACS patients, when compared to the healthy controls. The combination of these markers had better sensitivity in identifying patients with ACS. Cardiovascular diseases are the leading causes of morbidity and mortality. Newer technologies have been developed in the past few decades to monitor cardiovascular disease more efficiently. Discovery of biochemical markers such as troponin I, troponin T etc help to measure myocardial damage. The majority of the markers detect only the end stage of the disease. Serum IMA, a marker of ischemia can be used for the diagnosis of early stage of ACS, that in turn could help in designing treatment modalities to reduce the morbidity and mortality associated with myocardial infarction (MI).

Keywords: Acute coronary syndrome, Ischemia Modified Albumin, biomarker, Myocardial Infarction, autoanalyser, reinfarction.

INTRODUCTION

Worldwide cardiovascular diseases (CVD) are found to be the leading cause of death [1]. Coronary artery disease (CAD) continues to be the leading cause of death in developed countries. CAD can result in Acute Coronary syndrome (ACS) characterized by signs and symptoms of sudden myocardial ischemia most commonly due to the sudden reduction in blood flow to the heart. ACS presents a spectrum of conditions that includes unstable angina (UA), non ST elevation Myocardial Infarction (NSTEMI) and ST elevation Myocardial Infarction (STEMI). Cardiac ischemia is the most common mechanism underlying ACS which when prolonged may lead to myocardial damage and necrosis resulting in Myocardial Infarction (MI) [2,3].

Cardiac biomarkers are proteins or enzymes released from damaged myocardial cells into the circulation. Cardiac biomarkers help to diagnose whether a patient is having or has recently had myocardial ischemia or infarction. In a patient presenting with acute chest pain biomarkers help to differentiate patient with acute MI from unstable angina in a timely fashion [4]. Diagnosis of ACS is difficult in unpredictable ECGs[5]. The common biomarkers used for the diagnosis of ACS are cardiac troponins (cTn), Creatinine Kinase MB (CK-MB), Myoglobin and natriuretic peptides[6].

Cardiac troponins are considered to be the gold standard for the diagnosis of ACS. They are sensitive and specific markers of myocardial damage but it takes 4-6 hours to be detected in peripheral circulation[7]. CK-MB, a cardiac specific biomarker is also elevated in

serum, 4-6 hours after the onset of ischemia. It is useful in the early diagnosis of ACS and to detect reinfarction[8,9].

IMA is regarded as a new sensitive marker of myocardial ischemia in contrast to that of other cardiac markers which were released when myocardial necrosis occurred. During an ischemic event structural changes occur in the amino terminus of Albumin, rapidly reducing its capacity to bind transition metal ions possibly as a result of acidosis, free radicals injury and energy dependent membrane disruption. The metabolic variant of albumin generated is referred to as Ischemia Modified Albumin (IMA)[10-12]. Normal IMA is 1-2% of total albumin concentration which increases to 6-8% in patients experiencing ischemia. IMA increases within 6-8 minutes and remains elevated during an ischemic event[13].

According to the study conducted by Xavier and his colleagues, the mean age of the patients with ACS in India is 57.5. STEMI occur at much younger age when compared to non-STEMI or unstable angina^[14] Many studies have been conducted all over the world, to identify the role played by IMA in the early diagnosis of ACS. IMA has also been compared with other biomarkers like troponin T, troponin I and CK-MB. IMA has also been found to be a sensitive biomarker in the diagnosis of Myocardial Infarction and Unstable Angina[15-17]

We have undertaken this study with the view of identifying the utility of IMA as a better cardiac biomarker for early diagnosis of ACS in comparison with CK-MB and cardiac Troponin-T (cTnT).

AIM AND OBJECTIVES

- To assess the changes in the levels of Ischemia Modified Albumin (IMA), CK-MB and cardiac Troponin T in patients with Acute Coronary Syndrome compared to the normal healthy controls.
- To compare the sensitivity of IMA with other cardiac biomarkers like CK-MB and TroponinT.

MATERIALS AND METHODS

A cross sectional study was conducted for 80 individuals in the age group of 40 to 75 years. 40 patients with complaints of chest pain less than 6 hour's duration were taken up for the study and the formed Group 2. They were clinically diagnosed to have Acute Coronary Syndrome based on ECG findings. 40 number of age sex matched volunteers formed the control Group 1. Institutional Ethical Committee clearance was obtained. Standard proforma was used to collect data from the subjects. Informed consent was obtained after explaining the study protocol to the patients and their attenders.

Exclusion criteria

- Past history of cerebrovascular accidents or cardiac disorders
- Renal disease (serum creatinine >3mg/dL)
- Ischaemia in other tissues like GIT, peripheral vascular disease.
- Severe hypoalbuminaemia with serum albumin less than 2 gms/dl.
- Neoplasm.

Venous blood was collected in fluoride tube for blood glucose measurements and 4ml of venous blood was collected in a clot activator tube from all the participants taking part in the study. Serum sample was used to assess Ischemia Modified Albumin using Albumin Cobalt Binding Assay [18,19] in a colorimeter, CK MB, urea, creatinine and serum albumin were measured in a semi autoanalyser and Troponin T using a card test.

Analysis of blood samples

Ischemia Modified Albumin- Albumin Cobalt Binding Assay

CK-MB - immuno inhibition method.

Troponin T - Roche card test

Random Blood glucose- Glucose oxidase peroxidase method

Urea -Modified Berthelot method

Creatinine- Jaffe's method

Serum Albumin - Bromocresol Green method

Albumin Cobalt Binding Assay

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)[20]. It is measured by colorimetric assay at 470nm using dithiothreitol (DTT). Free cobalt gives a brown coloured complex with the chromogen dithiothreitol. An inverse relationship exists between the level of albumin bound cobalt and the intensity of the color formation.

200 µl of patient's serum was added to 50 µl of 1gm/l cobalt chloride solution. After 10 minutes incubation, dithiothreitol (50 µl of a 1.5 g/l solution) was added and mixed. 1.0 ml of 9g/l solution of NaCl was added after 2 min incubation,. The absorbance of the assay mixture was read at 470 nm using colorimeter. The blank was prepared similarly with the exclusion of DTT[17,15].

RESULTS

40 patients diagnosed to have ACS were taken up for the study. Among the ACS patients 11 patients

had unstable angina (UA), 9 had non ST elevation myocardial infarction (NSTEMI) and 20 had ST elevation myocardial infarction (STEMI).

Table-1: Mean and standard error for ima, ck-mb and serum albumin among the 4 groups

| PARAMETERS | Group 1 (controls) | Group 2 (Unstable Angina) | Group 3 (NSTEMI) | Group 4 (STEMI) |
|----------------------------------|---------------------------|----------------------------|------------------------------|----------------------------|
| IMA** in U/mL | 84.61 ± 1.41 ^a | 107.50 ± 7.30 ^b | 110.01 ± 5.07 ^b | 110.87 ± 4.64 ^b |
| CK-MB**in IU/L | 17.25 ± .60 ^a | 22.4 ± 0 .66 ^b | 50.23 ± 18.23 ^{b,c} | 68.35 ± 15.13 ^c |
| Sr.ALBUMIN ^{NS} in g/dl | 3.77 ± .07 | 3.93 ± .15 | 4.12 ± .09 | 3.91 ± .08 |

^{abc}Mean bearing different superscript in a column differ significantly (NS- Non significant; **P<0.01)

As per table 1, the mean serum IMA levels in patients with acute coronary syndrome were significantly higher than the healthy controls (p<0.01). There is no significant difference in the IMA values among the groups 2, 3&4 but they are all significantly higher than that of the control group1. CK-MB levels

were significantly higher in group 2 (22.4 IU/L ± 0 .66), group 3 (50.23 IU/L ± 18.23) and group 4 (68.35 IU/L ± 15.13) patients, when compared to healthy individuals (17.25 IU/L ± .60) in group 1 (p<0.01). There was no significant difference in serum Albumin levels in all the four groups.

Table-2: Correlation of CK-MB with IMA

| PARAMETERS | Correlation Coefficient | Sig. (2-tailed) |
|---------------|-------------------------|-----------------|
| IMA AND CK-MB | 0.418** | .000 |

** . Correlation is significant at the 0.01 level (2-tailed)

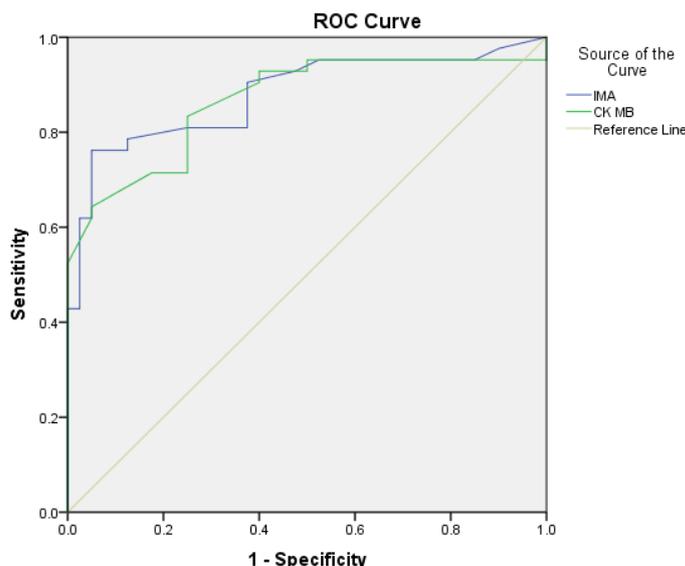


Fig-1: Receiver operating characteristic curve (ROC) of IMA and CK-MB

Table-3: ROC- Area under the curve (AUC)

| TEST RESULT VARIABLE(S) | AREA UNDER THE CURVE (AUC) |
|-------------------------|----------------------------|
| IMA | 0.879 |
| CK MB | 0.866 |

There was also a positive significant correlation between serum IMA and CK-MB (p<0.01) as depicted in table 2. We have done a ROC (Receiver Operator Characteristic) curve (figure 1) analysis to identify the role of IMA as a cardiac biomarker. ROC

analysis was also done for CK-MB. As per table 3, the area under the curve (AUC) for IMA was 0.879, which is quite significant, recommending its utility as a cardiac biomarker. This AUC is higher than that for CK-MB (0.866). We have identified various cut off

points for IMA and CK-MB from the ROC curve and have tabulated the sensitivities and specificities of IMA

and CK-MB.

Table-4: Presentation of diagnostic tests (both individual & combined) - diagnosis of ACS

| TEST | SENSITIVITY % | SPECIFICITY % | PPV % | NPV % |
|-------------------|---------------|---------------|-------|-------|
| IMA | 81 | 75 | 76.19 | 78.94 |
| CK MB | 90.5 | 60 | 70.37 | 92.31 |
| cTn T | 55 | 100 | 100 | 68.9 |
| IMA+ CK MB | 95 | 57.5 | 69.09 | 92 |
| IMA+ cTn T | 92.5 | 75 | 78.72 | 90.90 |
| CK MB+ cTn T | 95 | 75 | 79.17 | 93.75 |
| IMA+ CK MB+ cTn T | 100 | 57.5 | 70.18 | 100 |

Table 4 shows the sensitivity and specificity of the biomarkers when taken separately and in combination in the diagnosis of ACS. When the biomarkers IMA and CK-MB were used together, the sensitivity increased to 95% but the specificity was 57.5%. The sensitivity and specificity of IMA and cTnT taken together was found to be 92.5% and 75% respectively. CK MB and cTnT when taken together the sensitivity was found to be 95% and specificity was 75%. When all the three markers namely IMA, CK-MB and cTnT were used in combination as shown in table 4, the sensitivity increased to 100% while the specificity fell to 57.5%.

DISCUSSION

In 1990, it was first identified that myocardial ischemia produces changes in circulating albumin and IMA was produced[13,11]. IMA are proteins that are modified as a result of oxidative stress induced protein modification and they are used in the diagnosis of myocardial infarction[21]. Binding site for transition metal ions such as cobalt, copper and nickel is present in the NH₂ terminal end amino acid residues of albumin. When there is a period of ischemia albumin loses its capacity to bind to cobalt and this property is used in albumin cobalt binding test (ACB test) for IMA. It has been extensively studied and U.S. Food and Drug Administration cleared test for the detection of myocardial ischemia[21,22,18].

Studies have shown that IMA rapidly rises following an ischemia. It starts to fall at 6 hrs and returns to normal at 24 hours. IMA rises immediately after the onset of plaque rupture when compared to cardiac troponins and natriuretic peptides [23]. Skeletal muscle, cerebral, pulmonary and gastrointestinal ischemia also cause a rise in IMA and in diseases which are potent producers of free radicals, such as liver cirrhosis, infections and advanced neoplasms. Hence the specificity of IMA in the detection of cardiac ischemia is limited[24,25].

CK-MB levels have been found to be significantly increased in patients with ACS compared to the normal individuals. There was also a significant

increase in the STEMI group compared to the NSTEMI group. CK-MB and used for the diagnosis of myocardial necrosis[26]. Some studies have suggested serial measurement of CK-MB to qualitatively estimate the infarct size. Many studies have revealed that a single measurement of plasma cTn can be used as a convenient, cost-effective, and non-invasive method[27,28].

Myocardial ischemia is identified at a very early stage by IMA, while CK-MB is a good marker of myocardial necrosis caused by ischemia. When all the 3 markers -IMA, CK-MB and Troponin T are used in combination the sensitivity reached 100% which is the maximum attainable limit. But the specificity still did not improve (55%), which could be due to our low sample size.

During single/ double/ triple vessel block in the cardiac muscle, the myocardial cells will be in different stages of the spectrum ranging from minimal cell injury to ischemia to complete necrosis. So when different markers pertaining to different stages of progression of ischemia are used in combination they could accurately predict the onset of acute coronary Syndrome rather than being used alone.

CONCLUSION

Ischemia Modified Albumin (IMA), a biochemical by product of albumin appears to be a sensitive cardiac biomarker in patients presenting with Acute Coronary Syndrome. Its ability to detect myocardial ischemia before the onset of myocardial necrosis allows for implementation of earlier and more accurate management strategies and in turn can prevent adverse cardiac outcomes. IMA could be more useful in identifying patients with unstable angina, with otherwise inconclusive ECG findings.

LIMITATIONS OF THE STUDY

- In our study, the major limitation is the relatively small sample size.
- Compared to the qualitative bedside TroponinT test, which we have done, quantitative estimation

of Troponin T could have been more sensitive in the detection of micro infarcts.

- A positive ACB test which detects IMA but does not discriminate patients between Unstable Angina and Myocardial infarction, when other markers of myocardial necrosis are not elevated.

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