Estimation of Ischemia Modified Albumin (IMA) Levels in Patients with Acute Coronary Syndrome

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Abstract Myocardial ischemia produces free radicals that catalyze a series of oxidative reactions that damage healthy tissues. The N-terminal sequence of albumin is one of the proteins modified by these highly reactive oxygen species and forms the ischemia modified albumin (IMA). This study involves investigations undertaken in different study groups to assess the levels of IMA. Mean serum IMA levels (U/mL) in patients with ST-segment elevated myocardial infarction (92.1 ± 10.6), non-ST-segment elevated myocardial infarction (87.3 ± 5.95) and unstable angina (UA) (88.9 ± 6.16) were significantly higher than non-cardiac chest pain (77.9 ± 6.69) and also healthy subjects (54.7 ± 17.2) ($p < 0.001$). IMA is a highly sensitive marker and has a high predictive value, which might prove the usefulness of this biomarker for early detection of myocardial ischemia. These data indicate a possible role of the IMA test in the early triage of patients with chest pain.

Keywords Acute coronary syndrome · Biomarker · Ischemia · Ischemia modified albumin · Reactive oxygen species

Introduction

Acute coronary syndrome (ACS) represent a continuum of diseases ranging from unstable angina, associated with reversible myocardial cell injury to frank ST-segment elevation myocardial infarction (STEMI) with large areas of necrosis [1]. Among patients with definite ACS, early treatment may reduce the extent of myocardial injury. Therefore, rapid diagnosis and initiation of therapy is also a central tenet of management. Biomarkers play a pivotal role in the diagnosis and management of patients with ACS [2]. ACS is typically diagnosed by assessing the patient's clinical history, blood cardiac troponin I (cTnI) levels and electrocardiographic findings. In the past, creatine kinase MB (CK-MB) levels were routinely examined in patients presenting with chest pain. However, this is becoming less common as CK-MB has been shown to be a less reliable diagnostic biomarker of ACS [3]. However, patients with suspected ACS, cTnI concentrations and electrocardiograms (ECG) may be normal [4]. Indeed, cTnI is only released when cardiomyocytes are damaged and attains measurable levels in the bloodstream 3–6 h after myocardial injury [5]. As a specific marker of myocardial damage, cTnI would therefore appear to be more suitable for use in the evaluation of myocardial necrosis rather than myocardial ischemia. ECG is a simple, fast and reliable method for early diagnosis of ACS. Approximately half of the ACS patients have no diagnostic ECGs at the time of presentation to emergency department (ED) [6]. Myocardial ischemia is the most common underlying cause of ACS. Therefore, increasing attention is paid to find a sensitive biomarker for detection of myocardial ischemia even in the absence of necrosis or before cTnI increase to clinical decision limit for the assay. In this connection, ischemia modified albumin (IMA) is among the several biomarkers

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under investigation [7]. The precise mechanism for IMA generation is not yet known, though it appears that reactive oxygen species (ROS), produced during ischemia and ischemia–reperfusion, generate highly reactive hydroxyl free radicals resulting in site-specific modification of the N-terminus of the albumin moiety, especially of the *N* Asp–Ala–His–Lys sequence [8, 9], which result in a significant change in the ability of albumin to bind transition metals, notably, cobalt [10]. It has been shown that the concentration of IMA increases within a couple of minutes from the onset of ischemia and remains elevated until 6–12 h and returns to its normal range after 24 h [11]. Recently, in vitro studies have shown that ROS, specifically the hydroxyl radical, may chemically modify human serum albumin, resulting in IMA formation [12].

Therefore, if rapid and reliable, an assay measuring IMA might represent a promising marker for the identification of patients with myocardial ischemia, which, together with standard markers of myocyte necrosis, might be a superior screening method for patient evaluation to rule out acute myocardial ischemia. Accordingly, we studied the role of IMA together with CK-MB, and cTnI among patients admitted to an ED with symptoms suggestive of acute myocardial ischemia.

Patients and Methods

The study was carried out at the Department of Biochemistry, Frontier Lifeline Hospital and Dr K.M. Cherian Heart Foundation, Chennai, India, over a period of 3 years. The patients were enrolled in the study on arrival at the ED with chest pain. Written informed consent was obtained from the study subjects, and the study was approved by the Institutional Ethics Committee. The study group included 675 subjects of whom 540 were patients admitted with chest pain with or without radiation, palpitations, shortness of breath, lower jaw pain, left arm pain, epigastric pain, hypotension, and other symptoms suggestive of acute myocardial ischemia. A 12-lead electrocardiogram and all demographic details of the patients were recorded. Of the 540 patients admitted, there were 135 patients with ST-segment elevation myocardial infarction (STEMI), 135 with non-ST segment elevation myocardial infarction (NSTEMI), and 135 with unstable angina (UA). The other 135 were noncardiac chest pain patients (NCCP). A group of 135 healthy volunteers who came for a health checkup, with no clinical evidence of heart disease, were included as a control group. Patients with a malignant tumor, liver disorders and renal disorders, chronic inflammatory disorders, chronic rheumatoid arthritis, brain ischemia, acute mesenteric ischemia and pregnant women were excluded from the study. All patients underwent routine electrocardiography

and measurements of serum CK-MB and cTnI on admission. Some also underwent angiography and echocardiography. Myocardial infarction included STEMI and NSTEMI with typical chest pain persisting for at least 30 min, ST-segment elevation >0.2 mV in at least 2 contiguous leads, and increased serum troponin I (>0.3 ng mL⁻¹). UA was defined as ischemic chest pain at rest within the preceding 48 h or within the past month (Braunwald class II and III).

Blood samples from all subjects were collected by venipuncture in plain tubes, and immediately centrifuged at $3,000\times g$ for 10 min at 4 °C. The serum samples were stored at –20 °C until analysis.

IMA levels were determined by the rapid colorimetric method developed by Bar-Or et al. [13]. In brief, the methodology involved the addition of 200 µL of patient's serum to 50 µL of 1 g/L cobalt chloride solution, followed by vigorous mixing, and 10-min dark incubation. Fifty µL of dithiothreitol (DTT; 1.5 g/L) was then added and mixed. After 2 min of incubation, 1.0 mL of a 9.0 g/L solution of NaCl was added. The absorbance of assay mixtures was read at 470 nm with a Shimadzu UV-1800 Spectrophotometer. The blank was prepared similarly with the exclusion of DTT. The values are expressed in U/mL.

The standardized procedures employed by the central laboratory of our institution were used to determine plasma concentrations of total cholesterol, high density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and glucose. cTnI was estimated using Microparticle Enzyme Immunoassay kits (Abbott AxSYM) and CK-MB by the International Federation of Clinical Chemistry method, using Randox kits and an RX Daytona analyzer (Randox, UK).

Statistical Analysis

Statistical evaluations were performed using SPSS version 9.0 software. Receiver operating characteristic curve (ROC) analysis was carried out using Med Calc version 9.6 (filecrop.com). Data are expressed as Mean $\pm$ standard deviation for continuous variables and percentage for categorical variables. The student *t* test was used to compare continuous variables in patients and controls. Significance between subgroups was analyzed using the Kruskal-Wallis test. *p* value < 0.05 was considered statistically significant.

Results

The clinical characteristics and biochemical parameters are listed in Table 1. The cut-off points for cTnI (>0.01 ng/mL) and CK-MB (>25 U/L) were predetermined clinically, and were used to classify the patients. The cTnI and CK-MB

Table 1 Basic characteristic parameters of controls and patients with acute coronary syndrome

Variables	Controls N = 135	NCCP N = 135	STEMI N = 135	NSTEMI N = 135	UA N = 135	p value
Angiogram	0	0	85 %	100 %	100 %	0.001
Electrocardiogram	0	0	98 %	66 %	22 %	0.001
Echo	0	0	82.9 %	97.7 %	31.8 %	0.001
Pulse rate (beat/min)	82.75 ± 8.58	84.37 ± 15.78	85.12 ± 13.55	74.88 ± 12.46	81.53 ± 13.44	0.001
Diabetes mellitus (mg/dL)	21 %	31 %	51 %	58 %	54 %	0.001
Hypertension (mmHg)	22 %	22 %	82 %	58 %	57 %	0.001
Family history of CAD	0.6 %	11 %	82 %	20 %	32 %	0.001
Smoking	16 %	31 %	72 %	30 %	22 %	0.001
Food habits (non-veg)	63 %	73 %	79 %	57 %	68 %	NS
BMI (kg/m ²)	25.96 ± 3.97	26.42 ± 3.10	27.14 ± 5.19	25.76 ± 3.29	27.41 ± 3.62	0.001
Serum glucose (mg/dL)	117.22 ± 38.17	143.00 ± 48.35	193.13 ± 91.96	181.64 ± 74.93	179.39 ± 89.98	0.001
Serum creatinine (mg/dL)	0.84 ± 0.13	0.80 ± 0.12	0.84 ± 0.14	0.84 ± 0.11	0.79 ± 0.10	NS
Serum total protein (g/dL)	6.03 ± 0.84	5.96 ± 1.00	5.99 ± 0.74	6.15 ± 0.86	5.98 ± 0.83	NS
Hb (g %)	13.82 ± 1.36	13.57 ± 1.46	14.06 ± 1.74	13.54 ± 1.54	13.55 ± 1.43	NS
TWBC (cells/cmm)	8,047 ± 2,150	7,734 ± 1,331	11,863 ± 3,835	10,128 ± 3,179	9,462 ± 2,435	0.001
Total cholesterol (mg/dL)	194.79 ± 32.87	136.08 ± 39.63	201.52 ± 42.43	192.77 ± 44.03	183.59 ± 45.09	0.001
Triglycerides (mg/dL)	136.08 ± 39.63	146.83 ± 45.56	166.51 ± 45.58	137.19 ± 51.03	158.76 ± 58.13	NS
LDL (mg/dL)	91.39 ± 29.52	110.33 ± 28.94	131.55 ± 35.95	124.19 ± 34.66	133.46 ± 42.14	0.001
HDL (mg/dL)	45.17 ± 8.04	38.61 ± 6.38	38.73 ± 5.13	36.78 ± 4.10	37.40 ± 3.70	0.001

Continuous variables are presented as mean ± standard deviation (range); categorical variables are presented as number (%)

Kruskal-Wallis one-way ANOVA was used to arrive at the *p*-value

CK-MB creatine kinase MB, NCCP non cardiac chest pain, NSTEMI non-ST segment elevation myocardial infarction, STEMI ST-segment elevation myocardial infarction, UA unstable angina, IMA ischemia modified albumin

concentrations were recorded serially for the efficient diagnosis of patients. The mean Cardiac Troponin I levels in STEMI (6.20 ± 7.32), NSTEMI (2.98 ± 6.70), and UA patients (0.08 ± 0.19) were significantly higher than NCCP (0.10 ± 0.54) and healthy volunteers (0.04 ± 0.21) (Table 2). In ROC curve, the area under the curve is found to be 0.771 with 95 % CI (0.736–0.805). The sensitivity and specificity are found to be 52 and 95 % respectively. The mean CK-MB levels in STEMI (84.63 ± 79.50), NSTEMI (60.91 ± 76.19), and UA patients (28.39 ± 1.77) were significantly higher than NCCP (21.42 ± 10.40) and healthy volunteers (21.66 ± 10.76) (Table 2). In ROC curve, the area under the curve is found to be 0.745 with 95 % CI (0.708–0.781). The sensitivity and specificity are found to be 56 and 88 % respectively.

The mean serum IMA levels in STEMI (92.10 ± 10.60), NSTEMI (87.31 ± 5.95), and UA patients (88.90 ± 6.16) were significantly higher than in noncardiac chest pain patients (77.94 ± 6.69) and healthy volunteers (54.70 ± 17.29 ; $p < 0.001$; Table 2; Fig. 1). ROC curve analysis of IMA in serum revealed that the cut-off value above which IMA can be considered positive was 84.4 U/L. The area under the curve is found to be 0.933 with 95 % CI

(0.911–0.954) ($p < 0.0001$). The sensitivity and specificity are found to be 88 and 89 % respectively (Fig. 1).

Discussion

In the present study, we assessed IMA, cTnI and CK-MB concentrations in a cohort of patients with ACS who presented to the ED. Our aim was to determine the significance of measuring IMA concentrations to identify acute myocardial ischemia as an underlying mediator of the symptoms of ACS. We found that assessment of concentrations of IMA were vastly superior to the assessment of cTnI and CK-MB concentrations within the same time-frame for early diagnosis of ACS.

Many patients have myocardial ischemia in the absence of myonecrosis, and markers such as CK-MB, and the cTnI although mainstays for the diagnosis of acute cardiac myonecrosis, thus are limited for the confident exclusion of coronary ischemia. Furthermore, the release of these markers is time-dependent; an initially negative result does not exclude the presence of myocardial ischemia. Therefore, a rapidly detectable, highly sensitive marker for

Table 2 Comparison of standard biomarkers (troponin I and CK-MB) with IMA

Parameter	Control N = 135	NCCP N = 135	STEMI N = 135	NSTEMI N = 135	UA N = 135	p value
Troponin I (ng/mL)	0.04 ± 0.21	0.10 ± 0.54	6.20 ± 7.32	2.98 ± 6.70	0.08 ± 0.19	0.001
CK-MB (U/L)	21.66 ± 10.76	21.42 ± 10.40	84.63 ± 79.50	60.91 ± 76.19	28.39 ± 1.77	0.001
IMA (U/mL)	54.70 ± 17.29	77.94 ± 6.69	92.10 ± 10.60	87.31 ± 5.95	88.90 ± 6.16	0.001

CK-MB creatine kinase MB, NCCP noncardiac chest pain, NSTEMI non-ST segment elevation myocardial infarction, STEMI ST-segment elevation myocardial infarction, IMA ischemia modified albumin, UA unstable angina

Kruskal-Wallis one-way ANOVA was used to arrive at the *p*-value

Fig. 1 Receiver operator characteristic curve (ROC) of ischemia modified albumin (IMA). The area under the ROC = 0.933 (95 % confidence intervals (CI) 0.911–0.954). IMA cut-off point 84.4 U/mL; sensitivity 88 %; specificity 89 %

myocardial ischemia would be desirable to identify patients with only ischemia and those early in the course of an ACS without evidence of myocardial necrosis. The results of IMA testing were additive to those of ECG and useful when used in conjunction with markers of myocardial necrosis. Consistent with the findings of previous studies, we found that standard biomarkers of cardiac myonecrosis had low sensitivity and negative predictive value for the confident diagnosis or exclusion of coronary ischemia at the time of initial evaluation in the ED [14, 15].

The basis of the IMA assay is that, serum albumin of individuals with myocardial ischemia exhibits reduced binding to Co^{2+} compared with serum albumin of non ischemic individuals [16]. It is well known that within minutes of onset of myocardial ischemia, there is hypoxia, free radical damage and acidosis followed by disruption of membrane sodium/calcium ion pumps. This, in turn, causes alteration in the NH_2 terminus of albumin which has a high affinity for transition metals like Cu^{2+} and Ni^{2+} . This leads to reduced binding capacity of cobalt ions to albumin.

Studies have indicated that mechanisms involved in ischemia/reperfusion are induced changes in albumin and may include exposure to endothelial and extracellular hypoxia, acidosis, free radical damage, ATP dependant sodium and calcium pump disruption resulting in exposure to free iron and copper ions [17–19].

ROC curve analysis clearly showed that IMA measurement yields diagnostic information on identifying patients with acute myocardial ischemia. The area under the curve is found to be 0.933 with 95 % CI (0.911–0.954) ($p < 0.0001$). The sensitivity and specificity are found to be 88 and 89 % respectively. The optimum cut- off point of 84.4 (U/mL) that was derived from ROC curve analysis was approximately equivalent to 85 (U/mL) as reported by others [20, 21]. However, some studies have used higher values of 90 U/mL [22] and 93.5 U/mL [23]. This may reflect differences in baseline characteristics of our study population compared to those of the aforementioned studies. Previous studies have shown that IMA levels rise within minutes after ischemia and return to baseline within

6 h [24]. Unfortunately, we did not carry out serial sampling measurements that may have given additional information about marker kinetics and may have increased assay sensitivity for ACS.

In conclusion, IMA appears to be a sensitive biomarker of myocardial ischemia in MI patients presenting to the emergency department. Its ability to detect ischemia before myocyte destruction would allow for earlier and accurate management decisions as well as its role in a definitive biochemical ruling out strategy. While the specific molecular alterations and events that induce albumin modification have been only partially elucidated, better understanding of ischemic and reperfusion events at sub-cellular level needs further research in future.

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