

## ISCHEMIA MODIFIED ALBUMIN: A POTENT MARKER IN ACUTE MYOCARDIAL INFARCTION IN NORMOLIPIDAEMIC

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### ABSTRACT

**Objectives:** Currently, there are no well-defined biochemical markers for identification of myocardial ischemia in normolipidaemic AMI individuals. Biochemical markers such as CK-MB, cTnl and myoglobin, used in assessing cellular necrosis, are not suitable for assessing myocardial ischemia. The introduction of the Ischemia Modified Albumin (IMA) assay for the first time provides emergency physicians with an objective diagnostic study to determine the presence of myocardial ischemia completely within the control of the emergency department. The present study was planned to evaluate IMA concentration in myocardial infarction patients with normal lipid profile.

**Methodology:** The serum IMA levels were determined in 165 normolipidemic MI patients and 165 age-sexes matched healthy volunteers served as control. The levels of IMA were determined by addition of known amount of Cobalt (II) to a serum specimen and measurement of the unbound cobalt (II) by colorimetric assay using dithiothreitol (DTT). Lipid profile was also analyzed enzymatically in these subjects. The values were expressed as means  $\pm$  standard deviation (SD) and data from patients and control was compared using student's 't'-test.

**Results:** Serum IMA levels were significantly increased in MI patients as compared to control ( $p < 0.001$ ). Also total cholesterol, TC: HDL-C ratio, triglycerides, LDL-cholesterol, LDL-C: HDL-C ratio and TG: HDL-C ratio were higher in AMI subjects ( $p < 0.001$ ) and HDL-cholesterol were lower in MI patients ( $p < 0.001$ ).

**Conclusions:** IMA can be used as an alternative biochemical parameter to aid clinical diagnosis which is cost effective.

**KEY WORDS:** Acute Myocardial Infarction, Normal Lipid Profile, Ischemia Modified Albumin.

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## INTRODUCTION

Coronary artery disease (CAD) is a leading cause of mortality and morbidity.<sup>1</sup> Approximately 20-23% of patients presenting at emergency cardiology care with chest pain have coronary disease. Most death occurs outside the hospital or during first few hours of Acute Cardiac event. Myocardial ischemia results due to inadequate blood perfusion to the myocytes, leading to a deficiency of oxygen and nutrients, eventually compromising their vital

functions. In a clinical setting, myocardial ischemia is assessed by an individual's symptoms and electrocardiographic (ECG) studies.<sup>2</sup> The ECG changes may include ST-T segment wave alterations.<sup>2</sup> Myocardial ischemic manifestations are vague and multiple. Symptoms may include chest pain (angina), epigastric and arm discomfort with exertion or at rest, shortness of breath, nausea, and vomiting.<sup>2</sup> However, these symptoms may be subtle and are not easily recognized. Prolonged ischemia can lead to myocardial cell death (necrosis), which is known as myocardial infarction (MI). Acute MI (AMI) or an evolving MI is diagnosed by measuring myocardial proteins in the serum [e.g., creatine kinase MB (CK-MB), troponin I or T] along with ECG studies and imaging procedures. Ideally, it is essential to identify myocardial ischemia before the onset of irreparable myocardial cell damage. Thus, identification of a biochemical marker that is sensitive and specific for myocardial ischemia and can be rapidly measured in serum would be clinically valuable. Recently, a serum-based biochemical test has been found to be useful in the diagnosis of acute myocardial ischemia.<sup>3</sup> The basic principle of this test involves the N-terminal region of human serum albumin (HSA) and its inherent affinity for the metal ion, Co(II). Serum albumin of individuals with myocardial ischemia exhibits reduced binding to Co(II) compared with serum albumin of non-ischemic individuals. The present study was undertaken to evaluate whether the Ischemia Modified Albumin assay could be clinically useful in patients with suspected myocardial injury in the setting of an emergency department of a health maintenance organization so that prospective measure could be taken to avoid acute coronary complications.

## METHODOLOGY

One hundred sixty five patients (males 123; females 42) with AMI and 165 age-sex matched healthy volunteers were taken for this study. The study was conducted for a period of four and half years from April 2002 to August 2006. Informed consent was taken. Smoking habits,

systolic and diastolic blood pressure and family history of coronary heart diseases were recorded after clinical confirmation of AMI.

*Diagnostic Criteria of patients:* All the patients had their first episode of MI with diagnostic criteria: typical chest pain, specific abnormalities for MI on electrocardiogram, elevated serum creatine phosphokinase (CP-MB) and aspartate aminotransferase enzyme levels.

*Exclusion Criteria:* Patients with diabetes mellitus, renal insufficiency, hypertension, current smokers, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

*Criteria for Normolipaedemics:* Normal lipid profile was defined if LDL was <160mg/dl, HDL  $\geq$ 35mg/dl, Total cholesterol (TC) <200 mg/dl and Triglycerides (TG) <150mg/dl.<sup>4</sup>

Venous blood was collected after fasting overnight and EDTA was added and samples were processed for lipid profiles. For IMA studies blood samples were collected within an hour once the patients was admitted in Intensive Care Unit.

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 serum albumin can be determined by addition of 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. Patient serum 200 $\mu$ l was mixed with 50 $\mu$ l of a solution of one gram/litre cobalt chloride, followed by 10 minutes vigorous mixing. Dithiothreitol (50 $\mu$ l of a 1.5g/l solution) was then added and mixed. After two minutes of incubation 1.0ml of 9.0g/l solution of NaCl was added. The absorbance of the assay mixture was read at 470. The blank was prepared similarly with the exclusion of DTT.

Lipid profile (Total cholesterol, triglycerides, and HDL-cholesterol) were analyzed enzymatically using kit obtained from (Randox Labora-

Table-I: Ischemia modified albumin concentration and lipid profile in MI patients and controls (mean  $\pm$ SD)

Variables	Controls (n=165)	Patients (n=165)	P value (95%CI)
Age	60.55 $\pm$ 3.98	61.84 $\pm$ 3.80	0.0037( 61.26-62.42)
Total Cholesterol †	168.58 $\pm$ 12.16	186.44 $\pm$ 13.95	<0.001(184.31-188.56)
HDL-Cholesterol †	50.51 $\pm$ 6.78	41.27 $\pm$ 4.62	<0.001(40.56-41.97)
TC:HDL-C*	3.39 $\pm$ 0.36	4.57 $\pm$ 0.58	<0.001(4.48-4.65)
Triglycerides †	107.84 $\pm$ 11.51	128.96 $\pm$ 12.19	<0.001(127.10-130.82)
LDL-Cholesterol †	83.59 $\pm$ 11.95	119.37 $\pm$ 14.05	<0.001(17.22-21.51)
LDL:HDL-C*	1.90 $\pm$ 0.31	2.93 $\pm$ 0.51	<0.001(2.85-3.00)
TG:HDL-C*	2.17 $\pm$ 0.35	3.16 $\pm$ 0.49	0.3149(3.086-3.234)
Ischemia modified albumin (U/ml)	81.88 $\pm$ 3.92	97.50 $\pm$ 11.71	<0.001(95.71-99.28)

\* ratio † (mg %)

tories Limited, Crumlin, UK). Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the following formulae:

$$\text{LDL-cholesterol} = \frac{\text{Total cholesterol} - \text{Triglycerides} - \text{HDL-cholesterol (mg/dl)}}{5}$$

**Statistical analysis:** Data on lipid profile and IMA activity was entered in Microsoft excel for windows 2000. The mean  $\pm$  SD was obtained using excel software. The two-sample-t-test value was obtained between the patients and the control. The distribution of 't'- Probability was calculated depending on 'n' and significance of test was obtained. For p value <0.001 was considered as highly significant.

## RESULTS

IMA concentration was significantly increased in MI patients than in controls [Table-I], [Table-II] and [Table-III] (p<0.001). Total cholesterol, TC: HDL-C ratio, triglycerides, LDL-cholesterol, LDL: HDL-C ratio were

higher in MI patients compared to control [Table-I], (p<0.001). Also, significant differences were seen in HDL-C levels between MI patients and controls (p<0.001). Total cholesterol, TC: HDL-C ratio, triglycerides were higher in both genders of MI patients as compared to control [Table-II] and [Table-III] (p<0.001). Significant differences were seen in HDL-C levels between MI patients and control (p<0.001). LDL-cholesterol, LDL: HDL-C ratio were higher in male AMI subjects compared to control (p<0.001).

## DISCUSSION

Currently, there are no well-defined biochemical markers for identification of myocardial ischemia in normolipidaemic AMI individuals. Biochemical markers such as CK-MB, cTnI and myoglobin, used in assessing cellular necrosis, are not suitable for assessing myocardial ischemia. The Co (II)-albumin binding assay has been reported to be an early marker for myocardial ischemia.<sup>3,5,6</sup> The results of the present study confirm the findings of the

Table-II: Ischemia modified albumin concentration and Lipid Profile in Male patients and controls (mean  $\pm$ SD)

Variables	Control Male (n=123)	Male Patients (n=123)	P value (95%CI)
Age	60.68 $\pm$ 4.14	61.53 $\pm$ 3.28	0.0366(60.95-62.10)
Total Cholesterol †	168.09 $\pm$ 12.10	183.84 $\pm$ 13.65	<0.001(182.41-186.25)
HDL-Cholesterol †	49.90 $\pm$ 7.30	41.78 $\pm$ 4.88	0.0801(40.91-42.64)
TC:HDL-C*	3.42 $\pm$ 0.30	4.45 $\pm$ 0.58	<0.001(4.34-4.55)
Triglycerides†	105.02 $\pm$ 10.31	126.22 $\pm$ 11.74	<0.001(124.14-128.29)
LDL-Cholesterol †	79.88 $\pm$ 7.98	116.82 $\pm$ 13.76	<0.001(114.38-119.25)
LDL:HDL-C*	1.92 $\pm$ 0.25	2.84 $\pm$ 0.52	<0.001(2.74-2.93)
TG:HDL-C*	2.15 $\pm$ 0.37	3.06 $\pm$ 0.47	0.0123(2.97-3.14)
Ischemia modified albumin (U/ml)	81.70 $\pm$ 3.87	97.87 $\pm$ 12.21	<0.001(96.01-99.72)

\*ratio † (mg %)

Table-III: Ischemia modified albumin concentration &amp; Lipid Profile in Female patients and controls (mean±SD)

Variables	Control Female (n=42)	Patients Female (n=42)	P value (95%CI)
Age	60.52 ± 2.93	62.73 ± 4.97	0.0356(61.22-64.23)
Total Cholesterol †	170.00 ± 12.35	194.03 ± 13.03	<0.001(190.08-197.97)
HDL-Cholesterol †	52.31 ± 4.58	39.77 ± 3.37	<0.001(38.75-40.78)
TC:HDL-C*	3.28 ± 0.47	4.96 ± 0.44	<0.001(4.82-5.09)
Triglycerides †	116.11 ± 10.96	136.99 ± 9.81	<0.001(134.02-139.95)
LDL-Cholesterol †	94.47 ± 14.81	126.86 ± 12.22	0.2044(123.16-130.55)
LDL:HDL-C*	1.83 ± 0.44	3.21 ± 0.40	0.3066(3.08-3.33)
TG:HDL-C*	2.23 ± 0.28	3.47 ± 0.41	<0.001(3.34-3.59)
Ischemia modified albumin (U/ml)	82.43 ± 4.06	96.41 ± 10.18	<0.001(94.86-97.95)

\*ratio † (mg %)

previous studies<sup>3,5,7</sup> that reported that the Co(II)-albumin colorimetric assay distinguishes myocardial ischemic patients from nonischemic patients ( $P < 0.0001$ ). The introduction of the IMA assay for the first time provides emergency physicians with an objective diagnostic study to determine the presence of myocardial ischaemia completely within the control of the emergency department. The IMA assay presents a quantitative accurate laboratory determination of the occurrence of an ischaemic myocardial event, angina. Unlike previous serum studies that identify myocardial damage after the fact, this test allows emergency physicians to determine which patients have potential coronary artery lesions before occlusion occurs. Considering the potential consequences the introduction of IMA assay is a welcome aid to even the most confident clinician. More important than the existence of this diagnostic test itself is the decision making capabilities it provides emergency physicians. The test can be performed and returned to the ordering physician in about 30 minutes providing a disposition point well within the limits of the typical ED visit. This compares very favourably with the 9–12 hour periods for chest pain unit stress testing or the multiple day admissions for inpatient evaluations. For as promising as the IMA assay appears some degree of caution is still indicated. Before we relinquish all those acute coronary syndrome beds and renovate the cardiac stress laboratory there are a number of questions on IMA testing that need to be established. Specifics on the timing of blood sampling in relation to the onset of chest pain

must be established as well as other conditions that may cause false positive or negative results. In conclusion, IMA can be used as an alternative biochemical parameter to aid clinical diagnosis which is cheaper than other markers like TnT.

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