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Original Article

Biochemistry Section

Evaluation of Ischaemia-modified Albumin by Cobalt Chloride Binding Assay in Coronary Artery Disease: A Cross-sectional Study

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ABSTRACT

Introduction: Ischaemia-modified Albumin (IMA) is an altered form of human serum albumin in which its N-terminal amino acids are modified due to ischaemia. IMA is produced when serum albumin is modified while circulating through ischaemic cardiac tissue, thereby reducing its binding capacity to heavy metal ions such as cobalt or nickel. This ability of albumin to bind to cobalt is diminished in patients with myocardial ischaemia, providing the basis for the albumin cobalt binding test for detecting IMA.

Aim: To determine the level of IMA in Coronary Artery Disease (CAD) after standardising the parameter using the cobalt chloride binding method.

Materials and Methods: This cross-sectional study was carried out from June 2018 to December 2019 at a tertiary care centre in the Department of Biochemistry and the Cardiology Department, NRS Medical College, Kolkata, West Bengal, India. A total of 100 CAD patients were included in the study, along with 100 age- and gender-matched healthy individuals (controls). IMA levels were measured in all study subjects by adding a known amount of cobalt (II) to serum samples and the unbound cobalt (II) was measured by the intensity of the coloured complex formed by adding mercaptoethanol, using a colorimeter at 470 nm. The difference in mean values between cases and controls was determined using the Student's t-test. The precision of the assay was expressed as CV% {Standard Deviation (SD)/mean 100%}. To determine the cut-off value for serum IMA, an Receiver-operating Characteristic Curve (ROC) was plotted.

Results: Among the 100 CAD patients, 49 were male and 51 were female. The mean age of the case group was 60.90±11.02 years, with a Body Mass Index (BMI) of 27.6±4.32 (kg/m^2). The mean serum IMA level in cases was found to be significantly elevated in comparison to controls (89.77±10.73 and 49.35±10.68 u/mL, respectively). The standard curve was found to be linear up to 120 U/mL. The precision of the assay was expressed as Coefficient of Variation (CV%) (within run 4.23%, between runs 4.61%). The cut-off value for Serum IMA level was determined to be 74.23 u/mL. The diagnostic value of this cut-off level was calculated using standard formulas, which yielded diagnostic accuracy (87.00%), sensitivity (80.00%), specificity (94.00%), positive predictive value (93.02%) and negative predictive value (82.46%).

Conclusion: The IMA can be used as an important biomarker to detect ischaemia in patients with CAD.

Keywords: Cardiovascular disease, Ischaemic cardiac tissue, Serum albumin, Unbound cobalt

INTRODUCTION

The CAD is the most common of all cardiovascular diseases, where the heart muscle receives a reduced amount of blood, leading to either stable angina, unstable angina, or myocardial infarction. CAD has become a significant cause of mortality and morbidity worldwide, including in India [1]. In fact, India has witnessed an epidemiological transition over the past two decades, with a decrease in communicable diseases and an increase in Non Communicable Diseases (NCDs), of which CAD contributes the most significant burden. It has also been reported that CAD affects Indians at a comparatively younger age group [2].

The diagnosis of cardiac ischaemia depends on cardiac imaging or stress testing. Both are expensive and require special setup. Diagnosing through serum biomarkers remains a challenge [3]. There are several biomarkers, such as Troponin T and N-terminal pro-brain Natriuretic Peptide (NT-proBNP), which can diagnose acute myocardial infarction when ischaemia occurs [4]. Dyslipidemia can predict atherosclerotic plaque formation, but early diagnosis of heart ischaemia continues to be a challenge [5].

The N-terminal site of albumin has the strongest affinity for cobalt, copper and nickel ions. The N-terminal sequence of Human Serum Albumin (HSA)-Asp1-Ala2-His3-Lys4-is very susceptible to biochemical modifications and degradation induced by oxidative stress. Consequently, the affinity of the N-terminal site to transition metals, especially cobalt, is reduced. This variant of albumin is referred to as Ischaemia-modified Albumin (IMA). Thus, IMA is an altered form of human serum albumin in which its N-terminal amino acids are modified due to ischaemia [6]. This property of IMA is utilised for its estimation through the albumin cobalt binding test [7].

Since 1990, it has been known that IMA is formed by human serum albumin following ischaemia or acidosis. Subsequent studies have shown that the blood level of IMA increases within minutes of the onset of ischaemia and lasts for 6 to 12 hours [8,9]. However, IMA levels are also found to be elevated in conditions other than chest pain, such as cerebral ischaemia, vertebral basilar artery stenosis [10] and acute aortic dissection [11]. Therefore, its ineffectiveness raises doubts about its specificity. While IMA assessment remains the sole clinical biomarker for myocardial ischaemia, the process of IMA generation and the precise unit being evaluated remain unknown [12].

Another important aspect regarding IMA measurement is that no commercialised kit is available. As a result, all laboratories need to establish their own methods, reference ranges and precision to report the IMA levels of patients [13]. In this context, the present study was undertaken with the objective of determining the level of IMA in Coronary Artery Disease (CAD) after standardising the parameter using the cobalt chloride binding method.

MATERIALS AND METHODS

The present cross-sectional study was carried out at tertiary care centre in the Department of Biochemistry and the Cardiology Department, NRS Medical College, Kolkata, West Bengal, India, from June 2018 to December 2019. The study commenced after obtaining clearance from the Institutional Ethics Committee (No./NMC/7497 dated 13.11.2017).

Inclusion criteria: A total of 100 patients who were admitted to the Department of Cardiology as cases of CAD {presenting with complaints of chest pain, shortness of breath and sweating and confirmed by clinical examination and Elecrocardiogram (ECG)} were included as cases. A similar number of age- and gender-matched healthy individuals were taken as controls.

Exclusion criteria: Patients with a Post-percutaneous Transluminal Coronary Angioplasty (PTCA) status and those who had undergone Coronary Artery Bypass Grafting (CABG) were excluded. Additionally, patients with diabetes mellitus, known malignancy, renal disorders and liver cirrhosis were also excluded.

Sample size calculation: Based on a previous study by Mandal S et al., with a prevalence of 6.4%, a confidence level of 95% and a margin of error of 5%, the obtained sample size was 93 [14]. Since all cases in the present study were symptomatic, the authors considered this prevalence, confidence level of 95% and margin of error of 5% to determine the sample size, which was rounded up to 100 samples in each arm for both case and control groups.

Study Procedure

Data collection: Baseline information, including age, gender, smoking/alcohol consumption, height and weight, was tabulated in a preformed data sheet. BMI was calculated from height and weight. Approximately 3 mL of blood was collected from the left antecubital vein in a vial containing no anticoagulant. The blood was allowed to clot and serum was separated, which was then stored at -80°C. Serum IMA levels were measured using the cobalt chloride method. The serum level of albumin was estimated using a standard kit method (Transasia Bio Medicals Ltd.).

Principle of the assay: The albumin cobalt binding assay measures the ability of the amino terminus (N-terminus) of human albumin to bind exogenous cobalt. Under physiological conditions, transition metals can bind tightly to the exposed N-terminus of albumin. In the presence of ischaemic conditions, structural changes occur in the N-terminus of the protein, which reduce its binding capacity. IMA is measured by adding a known amount of cobalt (II) to the serum sample and the unbound cobalt (II) is measured by the intensity of the coloured complex formed after adding mercaptoethanol, using a colorimeter at 470 nm [15].

Reagents: Cobalt chloride solution (1 gm/litre), mercaptoethanol solution (1.5 gm/litre), sodium chloride solution (9 gm/litre).

Procedure of the test: A 200 µL sample of the patient's serum was mixed with 50 µL of cobalt chloride solution, which was mixed vigorously using a vortex and then incubated for 10 minutes. Following this, 50 µL of mercaptoethanol solution was added and the mixture was incubated for an additional two minutes. Next, 1 mL of sodium chloride solution was added and the wavelength of the developed colour was read at 470 nm. A blank solution was prepared similarly, excluding the mercaptoethanol. The values obtained are expressed in U/mL. A standard curve was prepared in the range of 12-120 mg of $CoCl_2$/mL with six calibrators having assigned values of 12-120 U/mL. One unit of IMA is defined as mg of free Co (II) in the reaction mixture per mL of serum sample. The graph was plotted with concentration on the abscissa and absorbance on the ordinate. The precision of the method was evaluated by assessing within-run and between-run assay precision. Within-run precision, also known as repeatability, is defined as the closeness of agreement between results of successive measurements obtained under identical conditions. In contrast, reproducibility refers to the closeness of agreement between results of successive measurements obtained under changed conditions (including time, operators, calibrators, reagents and laboratory) [16].

STATISTICAL ANALYSIS

The MedCalc statistical software package was used for statistical calculations. The values of IMA and serum albumin in cases and controls were expressed as mean±SD. The significance of the difference in means was tested using the Student's t-test, with $p<0.05$ considered significant. For within-run precision, 20 consecutive replicates were assayed in a single run. The Coefficient of Variation (CV%) was calculated using the formula: (standard deviation/mean)* 100. Between-run precision was determined by running two different concentrations of samples once daily for 10 days. The CV (%) was calculated using the same formula. A Receiver Operating Characteristic (ROC) curve was plotted to find the Area Under the Curve (AUC), followed by the Youden method to determine the cut-off value of the parameter for diagnosing cases. Based on that cut-off value, diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value were calculated using the following formulas:

Diagnostic Accuracy=(TP+TN)/Total Cases×100, Sensitivity=TP/(TP+FN)×100

Specificity=TN/(TN+FP)×100; Positive predictive value=TP/(TP+FP)×100

Negative Predictive Value=TN/(TN+FN)×100

(TP-True Positive, TN-True Negative, FN- False Negative, FP- False Positive)

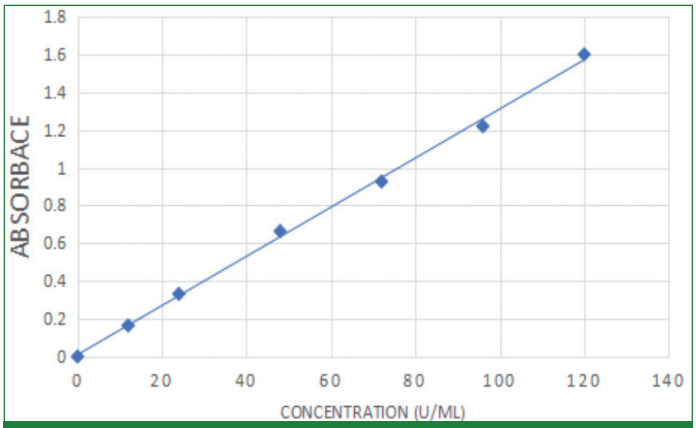
RESULTS

In the present study, the mean age of 100 cases was found to be 60.90±11.02 years, with 49 males and 51 females. The mean BMI of the cases was 27.6±4.32 kg/m^2 and 37 among them had some form of addiction (drinking, smoking, or both) [Table/Fig-1].

Parameters	Case (n=100)	Control (n=100)	p-value
Mean age±SD	60.90±11.02 years	59.41±10.40 years	0.3266
Gender distribution	Male=49, female=51	Male=48, female=52	0.8878
BMI (kg/m^2)	27.6±4.32	22.3±6.54	0.0001
Addiction (either smoking or drinking or both)	37	32	0.4582

[Table/Fig-1]: Baseline data of study population (N=200).

Absorbance against each calibrator was plotted to estimate IMA using the cobalt binding assay, generating the standard curve presented in [Table/Fig-2]. It was found to be linear up to 120 U/mL, indicating that the concentration of IMA is directly proportional to the absorbance at least up to 120 U/mL. The coefficient of variation



[Table/Fig-2]: Standard curve for ischaemia-modified albumin by cobalt chloride binding assay.

(CV%) for within-run and between-run measurements was found to be 4.23% and 4.63%, respectively [Table/Fig-3].

Precision assay	No. of replicates	CV %
Within run	20	4.23%
In between run	20	4.61%

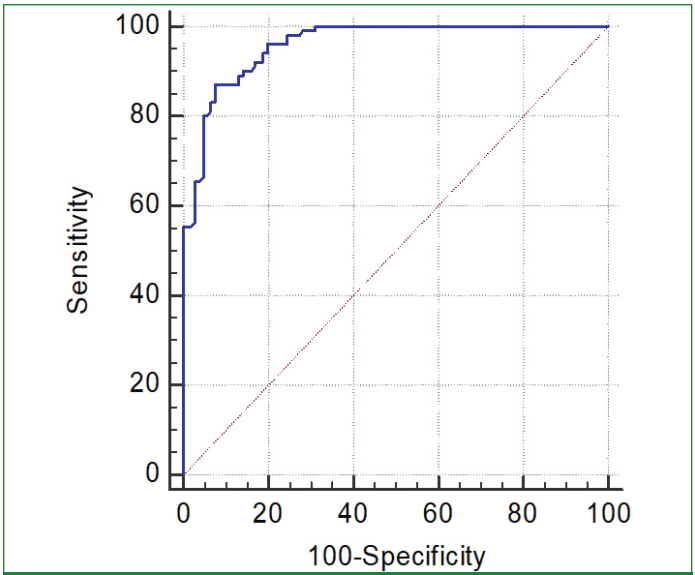
[Table/Fig-3]: Precision assessment of ischaemia-modified albumin by cobalt chloride binding assay.

The levels of albumin and Ischaemia-modified Albumin in the study population (a total of 100 cases of CAD and 100 healthy controls) were compared. The mean value of IMA was found to be 89.77±10.73 U/mL in cases, in contrast to 49.35±10.68 U/mL in controls. This difference was found to be statistically significant. On the other hand, there was no significant difference in the level of albumin, implying that the alteration in the level of IMA is due to the disease process [Table/Fig-4].

Biochemical parameters	Case (n=100)	Control (n=100)	p-value
Albumin (gm/dL)	3.93±0.29	3.98±0.29	T=1.2191 p=0.2242
Ischaemia-modified Albumin (IMA) (u/mL)	89.77±10.73	49.35±10.68	T=26.7 p<0.01

[Table/Fig-4]: Level of albumin and ischaemia-modified albumin in study population (N=200).
Result expressed in mean±SD; NS: Not significant; S: Significant at 0.05

The area under the ROC curve for IMA in detecting CAD was found to be 0.934. The ROC curve for IMA is presented in [Table/Fig-5]. The optimal cut-off value of IMA for discriminating CAD from non CAD was found to be 74.23 using the Youden Method. The diagnostic value of IMA at 74.23 U/mL as the cut-off value is presented in [Table/Fig-6].



[Table/Fig-5]: ROC curve for ischaemia-modified albumin by cobalt chloride binding assay.

Diagnostic value	Result
Diagnostic accuracy	87.00%
Sensitivity	80.00%
Specificity	94.00%
Positive predictive value	93.02%
Negative predictive value	82.46%

[Table/Fig-6]: Diagnostic value of ischaemia-modified albumin by cobalt chloride binding assay taking 74.23 as cut-off value.

DISCUSSION

In the present study, the authors standardised the method for estimating IMA using the cobalt chloride method in our setup, along with a precision assay. The authors then measured the levels in 100 patients suffering from CAD and compared the results with healthy controls. The diagnostic value of IMA for detecting CAD was also evaluated.

The IMA is gaining importance as a biomarker to detect myocardial ischaemia and has currently been approved by the US Food and Drug Administration [15]. Its level is found to be increased within minutes and it continues to rise for 6 to 12 hours [16]. Studies have shown that IMA levels are related to the left ventricular ejection fraction in patients with ST-segment Elevation Myocardial Infarction (STEMI) treated with primary percutaneous coronary intervention, as well as in those who develop acute Heart Failure (HF) after STEMI. IMA demonstrates high sensitivity and negative predictive value for the diagnosis of HF [17,18]. Further studies have shown that IMA is not only a promising biomarker for diagnosing acute HF but is also useful for assessing the effect of inotropic therapy for acute HF [19-21]. However, Etli M reported that although IMA can predict ischaemia, it cannot be correlated with the severity of disease in patients with CAD [22]. This study supports reports that IMA is a sensitive and early biochemical marker for diagnosing myocardial ischaemia in patients presenting with symptoms of acute chest pain. Christenson RL and Ouh SH determined the optimum decision level of IMA to be 75 units/mL, which is similar to the present study. However, the sensitivity, specificity, negative predictive value and positive predictive value were different from those of the present study [23]. A common factor responsible for false negatives in IMA is lactic acidosis secondary to tissue ischaemia. Increased lactate displaces cobalt from albumin, increasing the binding sites of albumin for cobalt and resulting in a falsely low quantity of IMA [24]. To decrease the fatality from Acute Coronary Syndrome (ACS), early diagnosis and initiation of treatment are very important. Cardiac Troponin I and Creatine Kinase-myocardial Band (CK-MB), the two well-known biomarkers, rise three hours after the onset of symptoms. Jawade P et al., compared different biomarkers and inferred that the diagnostic accuracy of IMA was the highest, better than that of cardiac Troponin I and CK-MB [25]. According to their study, the diagnostic accuracy of IMA, CK-MB and Troponin I were 82%, 65% and 68%, respectively. The sensitivities were 64%, 30% and 36%, respectively and the specificities were 100% for all three parameters.

Limitation(s)

The present study has some limitations. The patients were selected from a single tertiary care centre and other known biomarkers were not compared with IMA for diagnostic accuracy.

CONCLUSION(S)

The IMA can be considered an important biomarker for detecting ischaemia, as it is found to be significantly increased in patients with CAD. This test can be easily standardised in the laboratory with acceptable accuracy and precision. The cut-off value for this parameter was found to be 74.23 µg/mL in this study, which had a diagnostic accuracy of 87.00%, sensitivity of 80.00% and specificity of 94%. However, more studies in this regard are necessary to determine a consensus cut-off value for IMA.

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REFERENCES

[1] Sreeniwas Kumar A, Sinha N. Cardiovascular disease in India: A 360-degree overview. Med J Armed Forces India. 2020;76(1):01-03.
[2] Prabhakaran D, Jeemon P, Roy A. Cardiovascular diseases in India: Current epidemiology and future directions. Circulation. 2016;133(16):1605-20.
[3] Chacko S, Haseeb S, Glover BM, Wallbridge D, Harper A. The role of biomarkers in the diagnosis and risk stratification of acute coronary syndrome. Future Sci OA. 2017;4(1):FSO251.

[4] Eggers KM, Oldgren J, Nordenskjöld A, Lindahl B. Diagnostic value of serial measurement of cardiac markers in patients with chest pain: Limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. Am Heart J. 2004;148(4):574-81.
[5] Mitchell RS, Williams H, Earle G. Diagnostic markers of acute myocardial infarction and their limitations. Am J Med. 1989;82:623-31.
[6] Barter S, Chapman M, Rader SJ, Bergman C. Ischemia modified albumin and its role in diagnosis of ischemia. Arterioscler Thromb Vasc Biol. 2003;36(5):187-94.
[7] Corella P, Hedayati LS, Louis R. Ischemia modified albumin; its chemistry and formation. J Lipid Res. 2006;24(6):249-56.
[8] Myszka W, Wierzchowiecki M, Torliński L, Dziegielewska S. Ischemia modified albumin- A new biochemical marker of myocardial ischemia. Pol Arch Med Wewn. 2005;114(6):1252-59.
[9] Worster A, Devereaux PJ, Heels-Ansdell D, Guyatt GH, Opie J, Mookadam F, et al. Capability of ischemia-modified albumin to predict serious cardiac outcomes in the short term among patients with potential acute coronary syndrome. CMAJ. 2005;172:1685-90.
[10] Zhong C, Chen T, Shen Y, Zhang Y, Liu Y, Li N. The effects of serum ischemia modified albumin on diagnosis of cerebral infarction and vertebral basilar artery stenosis. Brain Injury. 2021;35(11):1457-61.
[11] Xiang J, He L, Pen T, Wei S. Ischemia modifed albumin can act as an independent predictor of in hospital mortality in patients with acute aortic dissection. Scientific Reports. 2023;13:343.
[12] Sokhanvar S, Mellati AO, Mousavinasab SN, Taran L, Vahdani B, Golmmohamadi Z. Ischemia-modified albumin (IMA) in differential diagnosis of transient myocardial ischemia from non-ischemic chest pain. Bratisl Lek Listy. 2012;113:612-15.
[13] Abadie JM, Blassingame CL, Bankson DD. Albumin cobalt binding assay to rule out acute coronary syndrome. Ann Clin Lab Sci. 2005;35(1):66.
[14] Mandal S, Saha JB, Mandal SC, Bhattacharya RN, Chakraborty M, Pal PP. Prevalence of ischemic heart disease among urban population of siliguri, west bengal. Indian J Community Med. 2009;34(1):19-23.
[15] Pan SM, Tong CY, Lin Q, Yao CL, Zhao J, Deng Z. Ischemia-modified albumin measured with ultra-filtration assay in early diagnosis of acute coronary syndrome. World J Emerg Med. 2010;1(1):37-40.
[16] Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt–albumin binding and its potential as a marker for myocardial ischemia- a preliminary report. J Emerg Med. 2000;19:311-15.
[17] Gurumurthy P, Borra SK, Reddy RK, Victor D, Babu S, Cherian KM. Estimation of Ischemia Modified Albumin (IMA) levels in patients with acute coronary syndrome. Indian J Clin Biochem. 2014;29(3):367-71.
[18] Lippi G, Montagnana M, Salvagno GL, Guidi GC. Potential value for new diagnostic markers in the early recognition of acute coronary syndromes. CJEM. 2006;8:27-31.
[19] Tien RJ, Chang RL, Louis CK. Ischemia modified albumin and its superiority in diagnosis of ischemic modified albumin. Acta Cardiol. 2011;86:246-31.
[20] deFillippi C, Yoon S, Bounds C, Romar L, Ro A, Herzog W, et al. Early detection of myocardial ischemia by a novel blood-based biomarker: The kinetics of ischemia modified albumin. J Am Coll Cardiol. 2003;41(6_Supplement_2):340-41.
[21] Çavuşoğlu Y, Korkmaz Ş, Demirtaş S, Gencer E, Şaşmaz H, Mutlu F, et al. Ischemia-modified albumin levels in patients with acute decompensated heart failure treated with dobutamine or levosimendan: IMA-HF study. Anatol J Cardiol. 2015;15:611-17.
[22] Etli M. Investigation of serum ischemia-modified albumin levels in coronary artery disease patients. Indian J Thorac Cardiovasc Surg. 2021;37(2):147-52.
[23] Christenson RL, Ouh SH. Characteristics of an ACB test for assessment of ACS patient. Can Med J. 2001;132:64-78.
[24] Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of "Ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J. 2004;21(1):29-34.
[25] Jawade P, Khillare KM, Mangudkar S, Palange A, Dhadwad J, Deshmukh M. A comparative study of ischemia-modified albumin: A promising biomarker for early detection of Acute Coronary Syndrome (ACS). Cureus. 2023;15(8):e44357.

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