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Original Research Article

Correlation of albumin concentration and ischemia modified albumin in the diagnosis of acute myocardial infarction

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Abstract

Objectives: To find out if there is any relationship between serum level of albumin and ischemia modified albumin **(**IMA) estimated by albumin cobalt binding assay. The effectiveness of albumin adjusted IMA index in the diagnosis of Acute Myocardial Infarction (AMI) was also studied.

Material and methods: We analyzed serum levels of IMA and albumin adjusted IMA index in 120 patients each with AMI and apparently healthy subjects belonging to the control group. Further, both control and AMI groups were separately divided based on serum albumin concentration in to four groups of <3.5g/dl, 3.51-4.0g/dl, 4.01-4.5 g/dl and >4.51g/dl (n =30 each). Sensitivity, specificity, positive predictive value and negative predictive value (NPV) of IMA was calculated and compared with that of Albumin adjusted IMA index.

Results: There was a significant negative correlation (r = -0.473 and p value = <0.001) seen between the IMA level and serum albumin. Serum levels of IMA were significantly higher in AMI patients than in healthy controls (p value = <0.001), where as albumin adjusted IMA index values did not show any significant difference between the groups. Comparison of AMI and control groups showed higher values of Albumin adjusted IMA index than IMA in all the parameters used for comparison except sensitivity.

Conclusion: Except sensitivity and NPV at very low concentration of albumin, albumin adjusted IMA has better clinical utility as it nullifies the interference of albumin concentration on IMA at all levels of serum albumin concentration.

Keywords: ischemia modified albumin, myocardial infarction, Serum albumin index

1. Introduction

Cardiovascular diseases (CVD) have a broad categorization and this would include any disease which comprises the heart and blood vessels. In industrialized countries, CVDs were the leading cause of death and the same is expected to happen in the emerging countries by 2020[1]. Adding to this, it is also reported that CVDs would probably be the biggest cause of deaths in the world, which could account for as much as a third of all the deaths which occur [2].

Among the CVDs, the most prevalent condition is the coronary heart disease (CHD) and is associated with high rates of mortality and morbidity. Coronary heart disease is due to narrowing of the epicardial coronary arteries supplying the heart muscle. Atherosclerosis is the most common cause of coronary heart diseases and is caused due to formation of plaques and plugs in the walls of the arteries. This build up progressively narrows the arteries, making it harder for blood to flow through.

Rupture of atherosclerotic plaque causes occlusion of the coronary artery, which results in the interruption of the oxygen and blood supply to the myocardium, leading to myocardial infarction (MI).

Early diagnosis of MI is very important because early treatment may reduce the extent of injury to the myocardium. Currently electrocardiography (ECG) and blood investigations are used for the diagnosis of MI. As per the current guidelines for the diagnosis of MI, biomarkers of myocardial necrosis used are Creatine-Kinase-MB isoform mass assay and Cardiac Troponin (cTn). Of these two biomarkers, cardiac troponin is preferred, but when it is not available, CK MB mass assay is an acceptable alternative for the diagnosis of MI. Increased cardiac troponin above the reference range at least on one occasion in the first 24 hour after the clinical event or in case of CK MB mass assay increased values above reference range on two separate occasions are indicative of myocardial necrosis.

In the diagnosis of MI, troponins are used as a marker of myocardial necrosis, but their low sensitivity, especially in the first few hours after MI, forms a limiting factor in the standard cTn assays. This delay is seen during the first presentation of the patient as the circulating levels of cTns have a delayed increase. During this period, continuous monitoring and blood sampling could take 06-12 hours and this may delay diagnosis, which probably could be a reason for increased morbidity and mortality [3]. In addition, specificity of troponins for Acute Coronary Syndrome (ACS) has been questioned, because in various other clinical situations, such as sepsis, hypovolemia, renal failure, acute massive or submassive pulmonary embolism, after prolonged episodes of supraventricular tachyarrhythmia (SVT) etc., an increase in troponin level is observed [4-7]. The high rates of death and readmission of patients with ACS with reason being MI could probably be due to the lack of a biomarker, which increases in the initial six hours of MI.

This leads to the requirement of an appropriate biomarker for MI in the first six hours. In this context, IMA, a derivative of albumin, has been found to serve the purpose, as its levels in serum raise well before necrosis of myocardial tissues. The N-terminal portion of serum Albumin made up of aspartate–alanine–histidine–lysine sequence is known to be a binding site for transition metal ions, binding cobalt, copper and nickel in their (II) forms. Free radicals generated during myocardial ischemia, modify N-terminal amino acid residues of Albumin and change the ability of albumin to bind transition metal ions to form IMA[8,9].

Some studies on analytical assessment of IMA and albumin concentration indicated that IMA results reflect albumin concentrations rather than myocardial ischemia when albumin concentration is less than 3.4g/dL [10]. Therefore, it is recommended that IMA concentration determined in patients with albumin concentrations less than 3.4g/dL should be interpreted with some caution[11]. In order to overcome the effect of serum albumin concentration on IMA another marker called albumin-adjusted ischemia-modified albumin index (IMA index) is proposed. It is calculated by using IMA, albumin level and mean albumin level of population. Comparative study in patients suffering from stroke observed that sensitivity and accuracy of IMA index is better than ischemia-modified albumin (IMA) as early detection marker of ischemic stroke [12]. The objectives of the study are to find out if there is any relationship between serum level of Albumin and IMA and also to find out if Albumin adjusted IMA index has better diagnostic value than IMA in the diagnosis of acute myocardial infarction.

2. Materials and Method

This case control study including 120 cases with acute myocardial infarction and 120 apparently healthy control subjects was carried out in Fr. Muller Medical College Hospital, Mangalore, Karnataka. The study design and protocol has been approved by the institutional ethical committee.

2.1. Inclusion criteria

After an informed consent blood samples were collected from patients (belonging to both sexes) with complaints of chest pain admitted to hospital, in red top vacuum tubes within one hour of admission. Similarly, fasting blood samples were collected from apparently healthy normal subjects who visit the hospital for routine health check-up in plain and in sodium fluoride tube (n=120). In serum samples, lipid profile and Albumin levels were estimated, glucose was measured in sodium fluoride sample. The serum samples were stored at -20^0C till analysis of IMA. From the chest pain group serum samples, patients with myocardial infarction were selected based on clinical diagnosis mentioned in the case sheet (n =120). In all the samples IMA was estimated by using Albumin Cobalt Binding assay and Albumin adjusted ischemia modified albumin index was calculated by using the formula proposed by Lippi *et al*.

Both control and AMI groups were separately divided based on serum albumin concentration in to four groups each of <3.5g/dl,

3.51- 4.0g/dl, 4.01-4.5 g/dl and >4.51g/dl (n =30 each). Sensitivity, specificity, positive predictive value and negative predictive value of IMA was calculated and compared with that of Albumin adjusted IMA index.

2.2. Exclusion criteria

Based on clinical details and biochemical investigations the following patients were excluded from the study.

a. Patients with chest pain due to non cardiac etiology.
b. Patients with serum creatinine concentration more than 1.5 mg/dl.
c. Pregnant women.
d. Patients admitted after 8 hours of onset of chest pain.

2.3. Methods

Albumin cobalt binding assay is used in spectrophotometric estimation of IMA. The reduction in the exogenous cobalt binding due to the changes caused in human serum albumin by myocardial ischemia, lies as the principle behind this assay [8].A known amount of Co(II) is added to the serum specimen and the unbound Co(II) is measured by colorimetry using dithiothreitol (DTT). This assay is based on the inverse relationship between the intensity of the colour formation and the quantity of albumin bound cobalt present.

In the estimation of serum IMA, 200 µl of patient serum added to 50 µl of 1gm/l cobalt chloride solution. This mixture is then mixed vigorously and incubated for 10 min. Following incubation, 50 µl of 1.5 g/l Dithiothreitol solution is added and mixed. After incubating for 2 min., 1.0 ml of a 9.0 g/l solution of NaCl was added and the absorbance of this mixture was read at 470 nm using a Shimadzu Spectrophotometer. The blank was prepared alongside similarly with the exclusion of Dithiothreitol. The results are expressed as absorbance units (ABSUs).

The calculation of albumin adjusted IMA levels, the formula suggested by Lippi *et al*. was used [13]. Albumin adjusted IMA index is calculated by using patient's serum IMA value, albumin concentration and median value of albumin of the control group.

The formula used for calculation expressed albumin-adjusted IMA levels is individual serum albumin concentration/median albumin concentration of the population × IMA value[14].

2.4. Statistical analysis

The results were statistically analysed using software SPSS (Version 16). Continuous variables were presented as mean and standard deviation. Karl Pearson correlation test was used to explore the relationship of serum IMA levels between control subjects and MI patients group as well as with albumin. Sensitivity, Specificity, positive and negative predictive value of IMA and Albumin adjusted IMA for ACS was calculated together as well as separately for different groups made based on albumin concentration.

3. Results

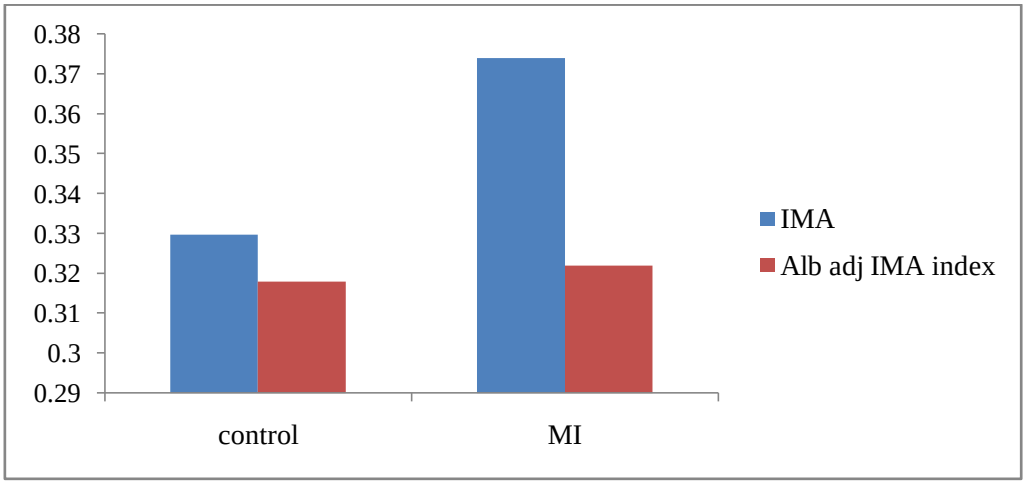
We estimated IMA in both acute myocardial infarction (AMI) group and in the control group containing 120 patients each. The serum levels of lipid parameters, IMA and albumin adjusted IMA index was measured in these groups and are given in Table 1.

Serum levels of IMA were significantly higher in AMI patients than in healthy controls (p value = 0.000, significance <0.001), where as albumin adjusted IMA index values did not show significant difference between the groups (p value = 0.557).

Table 1: Comparison of glucose, lipids, IMA and Albumin adjusted IMA index between control and AMI patients.

	Normal healthy Controls (n-120)	Myocardial Infarction (n=120)
Age (in years)	46	52
Glucose (in mg/dl)	94.25 ± 7.40	222 ± 71.00
Total Cholesterol (in mg/dl)	183.5 ± 45.70	206.63 ± 39.30
HDL Cholesterol (in mg/dl)	46.6 ± 10.20	42.7±12.70
LDL Cholesterol (in mg/dl)	137.4 ± 34.90	139±38.90
Triglycerides (in mg/dl)	100.5 ± 41.40	104±36.40
IMA (in ABSU)	0.329 ± 0.066	0.375 ± 0.086
Albumin Adjusted IMA (in ABSU)	0.318 ± 0.069	0.322 ± 0.087

Fig 1: Comparison of mean value of IMA and Albumin adjusted IMA index between control and Myocardial infarction patients



As IMA is formed by oxidative modification of albumin, its level may be dependent on serum albumin level. To find out this, correlation of serum level of albumin and IMA was studied. This showed that IMA level is negatively correlated with serum albumin (r = -0.473 and p value 0.000). To remove the effect of serum albumin level on IMA, albumin adjusted IMA index proposed by Lippi *et al* was studied in patients with AMI and the values were compared with the values of IMA. In this study, specificity, sensitivity, positive predictive value and negative predictive value of IMA was compared that of Albumin adjusted IMA index in patients with AMI. Specificity, PPV, NPV of Albumin adjusted IMA index was higher than IMA. In contrast, sensitivity of albumin adjusted IMA index was lower compared to IMA. The values of sensitivity, specificity, PPV and NPV are given in the table 2.

Table 2: Comparison of Sensitivity, specificity, PPV and NPV of IMA and Albumin adjusted IMA index between AMI and control group

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
IMA	69.17	66.67	68.19	68.65
Albumin adjusted IMA index	67.5	71.67	70.97	68.67

[PPV= Positive predictive value and NPV =Negative predictive value]

Further, both control and AMI patient group were divided in to 4 groups based on the serum albumin level to find out if there is difference in diagnostic values at different concentration of albumin. The values obtained are showed in the table 3.

Table 3: Comparison of Sensitivity, specificity, PPV and NPV of IMA and Albumin adjusted IMA index at different concentration of Albumin

	Groups based on Albumin concentration (in g/dl)							
	< 3.5 g/dl		**3.51 – 4.0 g/dl**		**4.01 – 4.5g/dl**		**> 4.51g/dl**	
	IMA	**Alb.adj. IMA index**	**IMA**	**Alb.adj. IMA index**	**IMA**	**Alb.adj. IMA index**	**IMA**	**Alb.adj. IMA index**
Sensitivity	80.0	73.33	70	63.33	63.33	66.67	63.33	66.67
Specificity	56.67	66.67	56.67	60	76.67	80	76.67	80
Positive predictive value (PPV)	64.86	68.75	61.76	61.29	73.07	76.92	73.08	76.92
Negative predictive value (NPV)	73.91	71.43	65.38	62.06	67.65	70.59	67.65	70.59

4. Discussion

Serum IMA level is elevated significantly in ACS and is a marker of myocardial ischemia[15]. The Albumin Cobalt Binding test used for the estimation of IMA was the first US FDA-cleared assay. For the formation of IMA early after cardiac ischemia-reperfusion, different mechanisms including free radical generation have been postulated. Modifications of N- terminal residues of albumin, due to hypoxia as a result of lack of blood supply, decrease its metal binding ability to form Ischemia modified albumin. Oxidative stress induced

by free radical generation is suggested to be involved in causing oxidative damage to amino terminal amino acid residues of albumin [16]. Further, it has also been hypothesized that formation of IMA and reduced metal binding ability is due to conformational change in the albumin caused by binding of fatty acids released in myocardial ischemia[17]. In addition, some studies on analytical assessment of IMA indicated that IMA results reflect albumin concentrations rather than myocardial ischemia[10]. Our study also demonstrates that IMA level is dependent on serum albumin concentration and there is a negative correlation between serum ischemia modified albumin and serum albumin concentration. Albumin cobalt binding assay used to measure IMA gives falsely higher values especially at lower serum albumin concentration. This finding is in agreement with the study of Hakligor *et al* which recommended that ischemia modified albumin concentration determined in patients with albumin concentrations less than 3.4g/L should be interpreted with some caution[11].

To overcome the effect of serum albumin concentration on IMA estimation and to improve the diagnostic value in ACS, Lippy *et al* proposed albumin adjusted IMA index calculated using serum IMA and albumin concentration. Our data on comparison of albumin adjusted IMA index between AMI and control showed that in acute myocardial infarction, albumin adjusted IMA index is increased but, the difference in the mean values of albumin adjusted IMA index in the control group and AMI patients group was not significant. Albumin adjusted IMA index values of patients with serum albumin value lower than median value of albumin are decreased as part of correction. On the other hand, IMA index values of patients with albumin value higher than median value of albumin are increased. Because the AMI group contained almost equal number of patients with serum albumin concentration less than median albumin and patients with serum albumin concentration more than median, there was no significant difference between the groups. In contrast to albumin adjusted IMA index, values of IMA in AMI group were significantly higher than control group. In this study, comparison of IMA and albumin adjusted IMA index in the diagnosis of AMI showed that IMA index has better specificity, PPV and NPV than IMA. On the other hand, sensitivity was higher in the case of IMA which could be because of higher false negative values in albumin adjusted IMA index due to over correction for samples with lower albumin concentration.

Further, diagnostic value of albumin adjusted IMA index was measured separately in four groups of patients containing different concentrations of serum albumin. Our findings demonstrated that albumin adjusted IMA index was better than IMA separately in all groups. Specificity and PPV of IMA index was better than IMA in all the groups. Over all, false positive values are reduced when IMA values are corrected based on the amount of serum albumin concentration. However, in the group of patients with serum albumin level less than 3.5g/dl, sensitivity and NPV of albumin adjusted IMA index were lower than that of IMA. Probably, this was due to more number of false negative values at lower serum albumin concentration which could be because of over correction. While calculating albumin adjusted IMA index, median value of serum albumin of control group was used for correction which might be a reason for over correction at serum albumin concentration below the median value. This was supported by decreased sensitivity of albumin adjusted IMA index observed even in the second group containing patients with albumin concentration from 3.5g/dl to 4.0g/dl. On the other hand, in the third and fourth group with higher serum albumin concentration sensitivity is better than IMA. Our results suggest that sensitivity of albumin adjusted IMA index is better than IMA when serum albumin concentration is higher than median value. Even though specificity, NPV and PPV of IMA index calculated using median value of albumin are higher than IMA, sensitivity is lower in patients having albumin concentration below the median value. Higher specificity and PPV of albumin adjusted IMA index are related to lower false positive values than IMA due to albumin correction.

This study has several potential limitations which must be taken into account when interpreting the results. First, our sample size in each group is small and this might have reduced the accuracy of sensitivity, specificity, NPV and PPV used to compare diagnostic value. Even though, the control and AMI group contained both patients with lower as well as higher serum albumin levels than the median value, in our study, the number of patients with lower albumin value than median was slightly more than that with higher serum albumin values. A larger study with equal number of patients with albumin concentration lower and higher than median value would also have sufficient power to accurately determine the diagnostic value of IMA index and its performance over IMA in the diagnosis of AMI. Secondly, we included apparently healthy individuals

in control group which might contain patients with various non-cardiac conditions associated with increased serum IMA level. Therefore, further studies are required to confirm the clinical significance of albumin adjusted IMA in AMI by excluding various other conditions in which IMA is found to be elevated.

5. Conclusion

Our study demonstrated that there was a negative correlation between serum IMA level estimated by albumin cobalt binding assay and albumin concentration. Albumin adjusted IMA index calculated using serum albumin and IMA was found to be a better marker of AMI than IMA as it showed higher specificity, NPV and PPV at higher levels of albumin. However, sensitivity of IMA is better than IMA index at lower concentrations of serum albumin. Therefore, except sensitivity and NPV at very low concentration of albumin, albumin adjusted IMA has better clinical utility as it nullifies the interference of albumin concentration on IMA.

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