Original Article

Early Diagnosis of Acute Coronary Syndrome with Sensitive Troponin I and Ischemia Modified Albumin

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Background: In this study we compared the diagnostic performance of serum ischemia modified albumin (IMA) and sensitive cardiac troponin I (cTnI) assay for the detection of acute coronary syndrome (ACS) in patients presenting to the emergency department (ED) with acute chest pain.
Methods: A prospective study was conducted on 123 patients presenting to the ED within three hours of acute chest pain. A 12-lead ECG was recorded and IMA and cTnI were measured on arrival at the ED. After diagnostic testing, the patients were classified as either ACS (n=70) or non- ACS (n= 53). The results of IMA, ECG, and cTnI, alone and in combination, were correlated with final diagnoses.
Results: IMA showed higher sensitivity (84%) and negative predictive value (NPV, 88%) compared to cTnI (sensitivity 42%, NPV 66%) and ECG (sensitivity 58%, NPV 74%). Combined use of IMA, cTnI and ECG significantly improved the sensitivity (96%, $P<0.05$) and NPV (96%) of IMA. The diagnostic performance of IMA was similar in the case of non-ST-segment-elevation ACS (sensitivity 80%, NPV 80%). The sensitivity and specificity of IMA for diagnosis of acute myocardial infarction (AMI) were 88% and 48%, respectively.
Conclusion: Measuring IMA at the time of ED admission improves the early diagnosis of ACS and non-ST-segment-elevation ACS in patients with acute chest pain. However, the test is not an effective tool for diagnosis of AMI in patients with chest pain presenting to ED.

Keywords: Ischemia modified albumin; cardiac troponin; acute coronary syndrome

Introduction

Early identification and confirmation of acute coronary syndrome (ACS) in patients presenting with acute chest pain to emergency department (ED) are essential for correct medical treatment and management of the patients.[1] Electrocardiography (ECG) and cardiac troponins are conventional ED tests in the diagnosis of ACS.[2] ECG is a simple, fast and reliable method for early diagnosis of ACS. However approximately half of the ACS patients have no diagnostic ECGs at the time of presentation to ED.[3] Cardiac troponins are considered as the gold standard for diagnosis of ACS.[4,5] However, approximately 40 -60 % of patients with ACS present with an initial troponin concentration below the clinical decision limit for the assay.[2] Some patients present early after an acute myocardial infarction (AMI) for which cardiac troponin is not yet detectable by serum/plasma testing and some others present with acute myocardial ischemia without necrosis (i.e. unstable angina).

International cardiology and laboratory medicine guidelines have suggested the use of the 99th percentile of a healthy population, with an assay imprecision of 10% or less, as the cutoff for the diagnosis of ACS.[4,5] However, most of the commercial assays do not have sufficient analytical sensitivity and precision to reliably detect troponin at the 99th percentile of a healthy population. This limits the ability of the commercial troponin assays in detecting very low increases of troponin at the time of

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the patient's presentation. Recently, improvements in analytical methods for cTn monitoring have led to troponin assays with higher sensitivity than the previous methods. These assays have a lower limit of detection, i.e. below the 99th percentile in a normal reference population.[6,7] Improved sensitivity which coincides with lowering the diagnostic cutoff to the 99th percentile values has led to substantial improvement in clinical sensitivity for the early detection of AMI,[8] and diagnosing a greater number of patients with AMI.[9]

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 cardiac troponins increase to clinical decision limit for the assay. In this connection, schemia modified albumin (IMA) is among the several biomarkers under investigation.[10] IMA is a form of human serum albumin in which the N-terminal amino acids have been modified by ischemia. This modification reduces the affinity of plasma albumin to bind heavy metal ions such as cobalt.[11] Bhagavan et al.[12] and others[11,13] have shown increased IMA levels in patients with spontaneous coronary ischemia, with abnormal values detectable before subsequent increases in cardiac troponin.[13]

IMA is measured indirectly by albumin cobalt binding (ACB) test. The concentration of albumin has been shown to influence albumin cobalt binding and IMA values.[14,15] Different methods have been proposed to eliminate the effect of albumin concentration on IMA values .Lippi et al.[16] suggested adjusting the results by the use of median albumin values of the population by the following formula: (individual serum albumin concentration/median albumin concentration of the population) ×IMA value.

No study has been reported from Iran regarding IMA testing and its application in Iranian context. Therefore, the aim of our study was to compare the clinical performance of IMA, albumin adjusted IMA and cardiac troponin I measured by high sensitive troponin I assay for early diagnosis of ACS in patients presenting with symptoms of acute chest pain.

Patients and Methods

This study was conducted at Kosar Hospital in Shiraz, Iran between August 2008 and September 2009.The study was approved by the ethics committee of Shiraz University of Medical Sciences with all the participants providing informed consent.

A total of 123 patients who arrived at the ED within 3 h of clinical signs suggestive of ACS were enrolled in this prospective double blind study. The exclusion criteria were acute or chronic renal diseases, hepatic diseases and pregnancies. Data included history, physical examination, serial 12-lead ECG and cardiac marker measurement.

Diagnosis of ACS was made by two independent physicians blinded to the results of markers. The IMA results were not available at the time of diagnosis. Diagnosis of ACS was made with reference to the ACC/AHA 2007 guidelines for the diagnosis of unstable angina and acute myocardial ischemia[17] and ESC/ACC 2007 guidelines for the redefinition of AMI.[18]

from 123 patients admitted to ED, 70 patients had ACS (25 and 45 patients with AMI and unstable angina respectively). Of the 70 ACS patients, 54 had no ST-segment-elevation and were classified as non-ST-segment-elevation ACS. The remaining 53 were diagnosed as non-ischemic chest pain (NICP).

Fifty healthy volunteers (54% males) with no clinical evidence of heart disease, and mean age of 51±16 served as controls.

Blood sample collection

Blood samples were taken from patients within 1 h of admission to ED and 6-9 h after the first collection. For IMA measurement, blood samples were centrifuged to separate the serum which was stored at −20 °C.

IMA measurements

IMA level was determined by the rapid and colorimetric method developed by Bar-Or et al.[11] In brief, the protocol involved the addition of 200 µL of patients' serum to 50 µl of 1 g/L cobalt chloride solution, followed by vigorous mixing, and 10-min incubation. Fifty µl of dithiothreitol (DDT; 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 Hewlett Packard 8452A Diode Array Spectrophotometer. The blank was prepared similarly with the exclusion of DTT. The values are expressed in U/ml. IMA assay was standardized in Laboratory Sciences and Technology Research Center and a standard curve was prepared in the range of 6.0-60.0 µg CoCl2/ml. One IMA unit was defined as µg of free Co (II) in the reaction mixture per ml of serum sample. All chemicals, including cobalt chloride and DTT, were purchased from Sigma-Aldrich. Duplicate IMA values were expressed

Table 1. Clinical characteristics of the study group

	NICP (n=53)	ACS (n=70)	Control (n=50)	P value
Age (years)	54±15	58±19	51±16	NS
Male (%)	58	60	54	NS
Albumin (gr/dl)	4.4±0.4	4.2±0.4	4.5±0.3	NS
IMA(U/ml)	63.4±15.1	112.1±24.5	64.7±16.3	<0.0001
IMA index	63.7±16.9	110.5±27.8	68.0±19.4	<0.0001

Data are expressed as mean±2SD or percent; NICP: Non ischemic chest pain; ACS: Acute coronary syndrome; NS: not significant; IMA: ischemia-modified albumin

as the mean obtained from the result of the assay. In our laboratory, the ACB test within run duplicate CV% of patients samples averaged 5.97% (range 0.8% - 8.5%). Serum albumin was measured by bromocresol green binding method.[19]

cTnI assay

cTnI was measured in heparin plasma, following manufacturer's guidelines, using the VIDAS Troponin I Ultra assay (bioMérieux, Marcy L'Etoile, France). This assay is calibrated against the National Institute of Standards and Technology International standard. Analytical characteristics for this cTnI assay, established by the manufacturer, were as follows: limit of detection<0.01 µg/l; 10% total imprecision concentration determined over 20 days using 2 kit lots and 2 calibrations per lot in 3 systems was 0.11 µg/l; and total imprecision (n=244) for quality control materials with concentrations at 0.58 µg/l and 3.5 µg/l were 3.3% and 3.4%, respectively. A cTnI value≥0.01 µg/l was suggestive of AMI.

Statistical Analysis

Receiver operator characteristic curves (ROC curve) analysis and calculation of the area under the curve (AUC) was done for the IMA and IMA index in 123 patients enrolled in the study. The optimum cutoff for the IMA and IMA index was selected from the ROC analysis to minimize the number of false-positive and false-negative results in this study population. This optimum cutoff was used

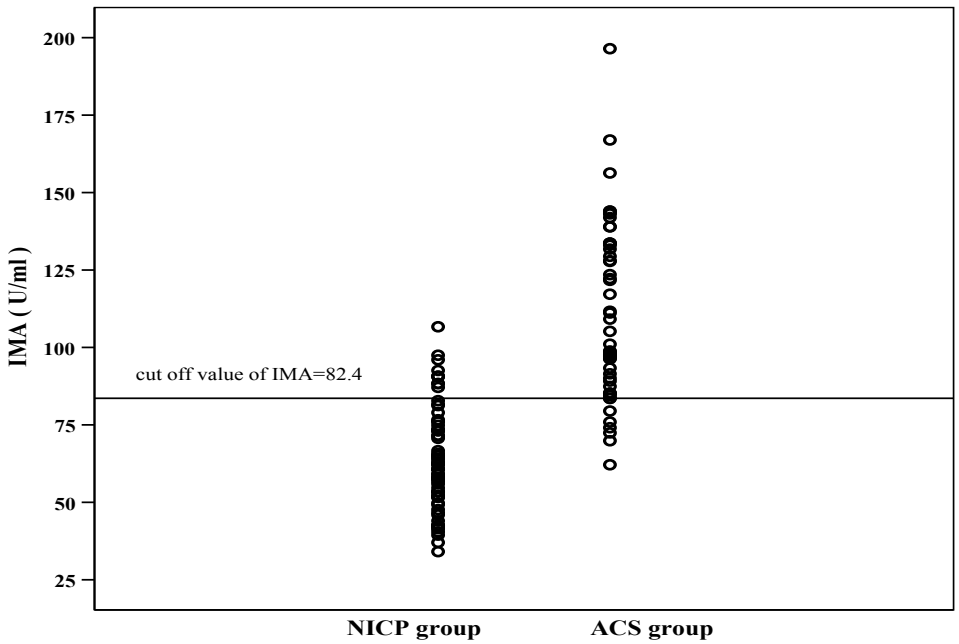

Figure 1. The scatter plot of the serum IMA values in patients with non-ischemic chest pain (NICP group) and patients group with acute coronary syndrome (ACS).

Table 2. Clinical characteristics of diagnostic tests for detection of ACS

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IMA	84 (81-93)	85 (75-92)	81 (68-90)	88 (77-94)
IMA index	89 (76-95)	84 (73-91)	81 (68-89)	91 (80-96)
CTnI	42 (28-55)	100 (92-100)	100 (81-100)	66 (55-75)
ECG	58 (44-71)	92 (83-97)	86 (69-94)	74 (64-83)
CTnI and ECG	66 (51-78)	90 (79-95)	83 (68-92	77 (66-85)
IMA and CTnI and ECG	96 (85-99)	78 (66-87)	77 (65-86)	96 (86-99)

to dichotomously classify each patient as IMA test positive or IMA test negative. The results of IMA, IMA index, cTnI and ECG were analyzed for clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), alone and in combination. The two and three test combinations of IMA, cTnI and ECG were considered positive if any one of the tests was positive, and negative if all were negative. The performance estimates were compared using McNemar's test. The comparison of the mean of IMA and IMA index values among different groups was performed using one - way ANOVA. In all tests, a two-tailed p-value < 0.05 was considered significant. SPSS version 14.0 (SPSS, Inc., Chicago, IL) was used for data analysis.

Results

Baseline demographic and clinical characteristics of the patients and healthy control groups are given in Table 1.

IMA results

As shown in Table 1, the IMA levels were significantly (P<0.05) higher in the ACS group (112.1±24.5 U/ml) as compared to NICP group (63.4±15.1 U/ml) and the control group (64.7±16.3 U/ml). Figure 1 shows a scatter plot distribution of the results for ACS and NICP groups. There was no significant difference between IMA levels in the AMI (111.2±23.9 U/ml) and UA groups (113.9±26.1 U/ml). The ROC curve of IMA for diagnosis of ACS on admission is shown in Figure 2.The AUC for IMA was 0.96 (95% CI 0.94% 0.99%).The optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 82.4 U/ml, with a sensitivity of 84% and specificity of 85%. The corresponding PPV and NPV levels were 81% and 88%, respectively.

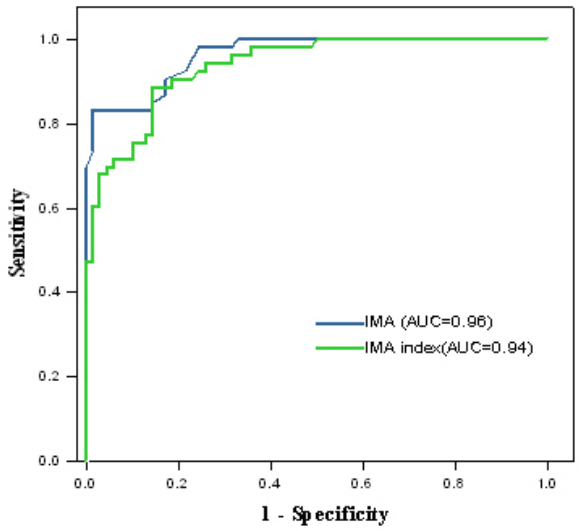

Figure 2. Receiver operator characteristic curves comparing the performance of IMA (AUC=0.96) and IMA index (AUC=0.94) in diagnosis of acute coronary syndrome.

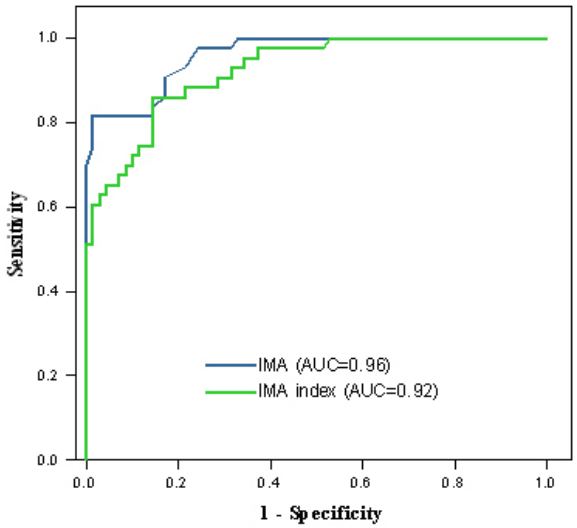

Figure 3. Receiver operator characteristic curves comparing the performance of IMA (AUC=0.96) and IMA index (AUC=0.92) in diagnosis of non-ST segment elevation ACS

Table 3. Clinical characteristics of diagnostic tests for identifying non-ST-segment-elevation ACS

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IMA	80 (66-89)	85 (72-93)	84(71-93)	80 (67-89)
IMA index	83 (70-92)	85 (72-93)	85 (72-93)	83 (70-92)
CTnI at patient admission	10 (4-22)	99 (92-100)	99(46-100)	54 (44-64)
CTnI at 6 h after patients admission	17 (8-30)	99 (92-100)	99(63-100)	55 (44-64)

Adjustment of IMA for serum albumin

The median albumin concentration in the control group was 4.34 gr/L (range 3.7 to5.3).We observed an inverse but not significant correlation between serum albumin and IMA(r=-0.111, P=0.14). After adjusting IMA for serum albumin by Lippi formula[16] (IMA index= IMA×(individual serum albumin concentration/median albumin concentration of the population)), the mean of IMA index in the control group was 68.0±19.4 that remained unchanged compared to the mean of IMA (64.7±16.3**)**. In the ROC analysis of the IMA index for diagnosis of ACS the AUC was 0.94. The optimum diagnostic cut off point of IMA index for the ACS diagnosis was determined to be 82.8 with respective sensitivity and specificity of 89% and 84. Also, the corresponding PPV and NPV levels were 81% and 91%, respectively.

Comparison of diagnostic tests for detection of ACS

The performance of IMA, IMA index, cTnT and ECG used alone and in combination, for diagnosis of ACS is presented in Table 2. Comparisons of sensitivities for statistically significant differences are illustrated in Figure 4. Sensitivity of IMA index (89%, 95% CI 76 to 95) was statistically equivalent to that of IMA (84%, 95% CI 81% to 93%). Sensitivity of IMA and IMA index on admission for diagnosis of ACS were significantly higher (P<0.05) compared with corresponding ECG (58%, 95% CI 44% to 71%), cTnI (42%, 95% CI 28% to 55%) and combined ECG and cTnI (66%, 95% CI 51% to 78%). The sensitivity of the combined IMA, ECG and cTnI was 96% (95% CI 86% to 99%), which was significantly greater than that of IMA alone.

Comparison of diagnostic tests for detection of non-ST-segment-elevation ACS:

The ROC curve of IMA and IMA index for diagnosis of non-ST segment elevation ACS on admission is shown in Figure 2.The ROC curve supports the assay as a good discriminator (AUC=0.96) between non-ST-segment elevation ACS and NICP. The sensitivities of IMA, IMA index, and cTnI for diagnosis of non-ST segment elevation ACS are presented in Table 3. IMA identified more non-ST segment elevation ACS patients (80%) compared with cTnI. For cTnI, clinical sensitivity for admission sample was 10% (95% CI 4% to 22%) and increased to 17% (95% CI 8% to 30%) for the follow-up specimen. Clinical specificities of cTnI at baseline and follow-up were 99% (95% CI 92% to100%).

Comparison of diagnostic tests for detection of AMI

ROC curve analysis was used to determine performance characteristics for identifying individuals with AMI from non-AMI ischemic subjects. As shown in Figure 3, ROC curve analysis revealed an area of 0.56 (IMA) and 0.55 (IMA index) for non-AMI vs AMI ischemic individuals**,** suggesting a poor

Table 4. Clinical characteristics of diagnostic tests for detection of AMI

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IMA	88 (67-97)	48 (38-58)	31(21-43)	94 (82-98)
IMA index	92 (72-99)	45 (35-55)	30 (21-42)	95 (83-99)
CTnI at patient admission	28 (13-50)	98 (92-100)	78(40-96)	84(75-90)
CTnI at 6 h after patients admission	80 (59-92)	98 (92-100)	92(69-98)	95 (88-98)

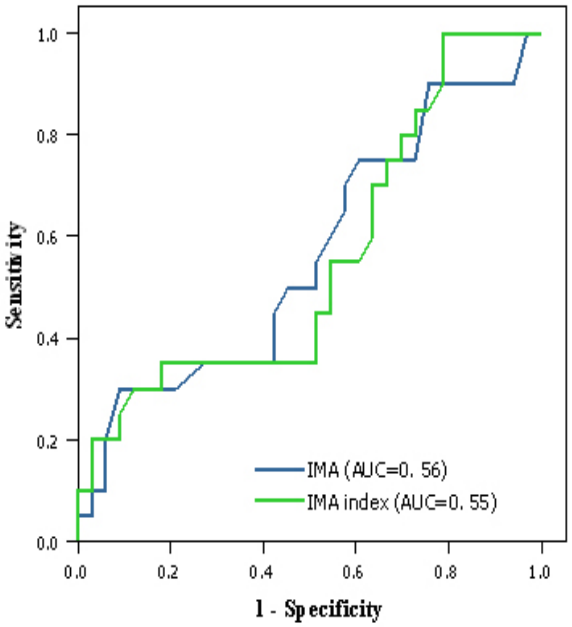

Figure 4. Receiver operator characteristic curves comparing the performance of IMA (AUC=0.56) and IMA index (AUC=0.55) in diagnosis of AMI.

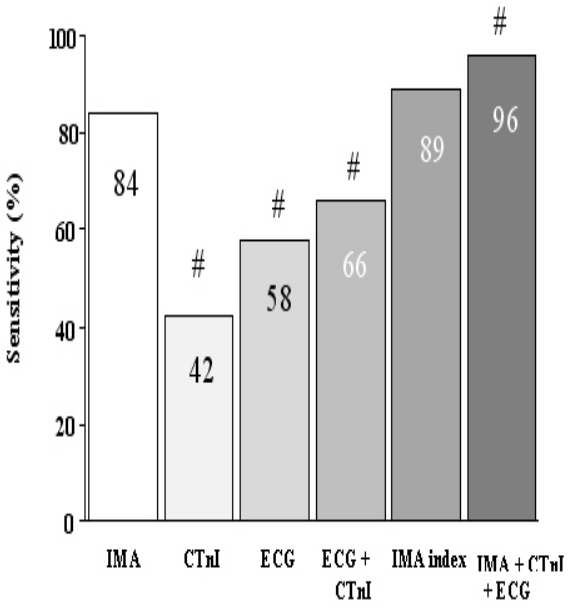

Figure 5. Comparison of sensitivities of diagnostic test for detecting ACS
Significant differences (P<0.05) compared to IMA

discrimination between the two groups. The performance of IMA, IMA index, and cTnI, for diagnosis of ACS is presented in Table 4. The sensitivity and specificity of IMA for diagnosis of AMI were 88% and 48%, respectively.

Discussion

In this prospective study, we examined the diagnostic performance of IMA assay, a high sensitive cTnI assay and ECG for the early diagnosis of ACS at the time of the patient's presentation to the ED. In agreement with the study by others, the present study is in agreement with previous reports[12,15,20,21] in demonstrating that IMA measurement might contribute to detection of ACS in patients presenting with ongoing chest pain. Our data highlighted four major findings with the potential to improve the ability to rule out ACS.

First, IMA values were significantly higher in ACS patients as compared with NICP and normal control groups; although the values overlapped between the groups. Different mechanisms have been postulated for generation of IMA early after cardiac ischemia. Increased generation of free radicals has been suggested to be involved in damage to amino terminal of albumin and generation of IMA.[22] It has also been hypothesized that in myocardial ischemia the release of fatty acids results in binding of fatty acids to albumin, producing a conformation change in the albumin and reducing the ability of albumin to bind to cobalt and hence account for generation of IMA.[23] Second, in this study we observed a slight but not significant negative correlation between IMA levels and serum albumin concentration. IMA correction for albumin by using median albumin values in normal population as proposed by Lippi et al.[16] leads to little change in the results. A negative correlation has been demonstrated between IMA values and serum albumin levels in some studies although this is much less marked within the reference interval for albumin.[14,15] The difference between our results and other studies may be related to exclusion of some conditions. That may affect albumin concentration in this study, so that serum albumin concentration in the patients and control group were in the normal intervals.

Third, ROC curve analysis clearly showed that IMA measurement yields diagnostic information on identifying patients with acute myocardial ischemia. The optimum cut- off point of 82.4 (U/ml) that was derived from ROC curve analysis was approximately equivalent to 85 (U/ml) as reported by others.[15,21] However, some studies have used higher values of 90 U/ml[20] and 93.5 U/ml.[24] This may reflect differences in baseline characteristics of our study population compared to those of the aforementioned studies. Such a low cut-off point, in view of its high negative predictive value, may be particularly use-

ful for the exclusion of the disease, although limited by low specificity and positive predictive value. IMA index had a higher sensitivity and NPV in comparison to IMA; however, the differences were small and were not deemed to be clinically relevant.

Fourth, in this study the diagnostic performance of IMA levels in the admission sample was superior to the conventional ED testing in the diagnosis of ACS. We used VIDAS Troponin I Ultra assay with a cutoff value of 0.01 (µg/l) in this study. In a recent study, Apple et al.[25] demonstrated that VIDAS Troponin I assay in admission sample improved the sensitivity for early diagnosis of MI to 88 % that is a substantial improvement compared to the first generation of cTnI assay demonstrating clinical sensitivities of 3 to 33%. In our study, the sensitivity of VIDAS Troponin I assay for diagnosis of ACS in the admission sample was 42%. Although, this is an improvement compared to conventional cTn assay which demonstrated clinical sensitivities of 3% to 33 %,[26,27] it is much lower compared to the sensitivity of IMA for prediction of ACS. The sensitivity of IMA was significantly greater than those of VIDAS Troponin I assay and ECG used alone or combined. The combination of IMA, ECG and cTnI results improved sensitivity of the detection of ACS to 96%. Therefore, despite the high performance of IMA, it should be used in conjunction with ECG and cTnI assay for the early diagnosis of ACS. The high NPV demonstrated by IMA test results together with those of ECG and cTnI for samples collected at the time of admission to ED may substantially reduce the inappropriate admission of low-risk patients.

The clinical sensitivity IMA for detection of non-ST segment elevation in patients with ACS, who presented within 3 hours after the onset of chest pain, was 80%. This is a substantial improvement compared to cTnI assays which demonstrated clinical sensitivities of 10% for admission and 17% for the follow-up specimen. This early prediction by a biochemical marker of ischemia is important so that appropriate medical management can be initiated.[28] In addition, cTnI assays had high specificity compared to IMA. One of the reasons for this may well be that IMA detects myocardial ischemia, while cTnI, which are structural proteins unique to the heart, are sensitive and specific biochemical markers of myocardial damage.

The diagnostic accuracy of IMA in this study does not support its use as an effective tool for diagnosis of AMI in patients with chest pain presenting to ED. The reported sensitivities are high, and the large number of false positives generates a low specificity. Higher cut-off values used to improve specificity would have an unacceptable effect on the test's sensitivity. Therefore, there is no IMA cut-off that will give high values for both sensitivity and specificity.

Several potential limitations are noted for this study that must be taken into account when interpreting the results. First, the major limitation of our study involves the relatively small number of patients under study with acute myocardial infarction and unstable angina, mainly because of the selective criteria for inclusion into the study. Second, there was no consistent ''gold standard'' test for myocardial ischemia; therefore, in our study the diagnostic performance of IMA levels was calculated against the final diagnosis**,** which was done by the interpretation of the results of appropriate tests. Furthermore, including both UA and AMI patients into the myocardial ischemia group might have affected the overall results and precluded any final distinction between transient ischemia and myocyte necrosis. Third, this study was also limited by strict exclusion criteria used for the selection of patients. The patients enrolled in the present study were carefully chosen to exclude diseases that could affect the albumin concentration in the serum. As a consequence, the results presented may not necessarily be applicable to all patients with chest pain presenting to ED.

The strengths of the study, on the other hand, include the homogeneity of the study group with respect to exclusion of possible confounding clinical conditions that may affect our ability to interpret the results, as well as the proper timing of blood collection, which precluded confounding clinical conditions that might affect our ability to interpret the results.

In conclusion, measurement of IMA levels could diagnose ACS in patients with ongoing ischemic pain presenting to the ED. IMA measurement was superior for diagnosis of ACS compared to the initial high sensitivity cTnI assay and ECG. Measuring IMA along with high sensitivity cTnI assay and ECG at the time of hospital admission improves early diagnosis of ACS in patients with acute chest pain. However, the test is a poor discriminator between ischemic patients with and without MI.

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