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ISCHEMIA MODIFIED ALBUMIN (IMA): A NOVEL BIOMARKER FOR THE DIAGNOSIS OF ACUTE CORONARY SYNDROME

Aula Abo Saleh[1*], Bassem Marouf[2] and Afraa Zrieki[3]

[1]*Biochemistry and Microbiology Department, Faculty of Pharmacy, Tishreen University, Lattakia, Syria*
[2]*Internal Medicine Department, Faculty of Medicine, Tishreen University, Lattakia, Syria*
[3]*Pharmaceutics and Pharmaceutical Technology Department, Faculty of Pharmacy, Tishreen University, Lattakia, Syria*

Due to the high mortality rate related to Acute Coronary Syndrome (ACS), early detection is critical. In the emergency room, ischemia modified albumin (IMA) has been proposed as a helpful indicator for the diagnosis of ACS. In this study, we aimed to investigate the role of IMA for its potential in the early diagnosis of ACS in patients experiencing sudden and severe chest pain, and the influence of some variables (gender, age, hypertension, diabetes, hypertriglyceridemia, hypercholesterolemia and smoking) on its level in ACS patients. A total of 127 patients with acute chest pain were included in the study. Patients were divided into three groups: those with acute myocardial infarction (N=45), unstable angina (N=52), and non-ischemic chest pain (NICP) (N=30). Patients with ACS had a higher level of IMA compared to those with NICP. To achieve the best sensitivity (86.5%) and specificity (76.7%), the cutoff for IMA was determined to be 71.3 u/ml. Based on our results, IMA is a sensitive marker for the early diagnosis of ACS in patients presenting with symptoms of acute chest pain.

Keywords: *Ischemia modified albumin, unstable angina, myocardial infarction, Acute chest pain*

INTRODUCTION

Annually, about 7 million people are hospitalized with severe chest pain. Rupture of the atherosclerotic plaque is responsible for causing ACS in about 17% of these patients[1&2]. ACS is a term that describes a group of life-threatening conditions ranging from Unstable Angina (UA), linked with reversible heart damage, to Myocardial Infarction (MI), associated with irreversible heart cell injury[3]. Diagnosis of ACS is typically depending on clinical history, cardiac biomarkers levels, and electrocardiogram (ECG) findings[4]. However, early detection of ACS is a challenge for physicians since current diagnostic approaches have some limitations. Creatine kinase-MB (CK-MB) and other cardiac biomarkers are only released in response to cardiomyocyte injury and can be detected in circulation within 3 to 6 hrs of myocardial necrosis[5]. ECG is a fast diagnostic tool but it may be normal at the time of admission[6]. Therefore, it is important to find early and sensitive indicators for detecting ACS even without the presence of necrosis. Here, ischemia-modified albumin (IMA) is one of many potential indicators being studied.

The N-terminal region of human serum albumin includes the amino acid sequence, N-Aspartate - Alanine - Histidine - Lysin, which is the binding site for transition metals such as cobalt (Co^{+2}), copper (Cu^{+2}) and nickel (Ni^{+2})[7]. Ischemia causes the production of reactive oxygen species (ROS), which in turn generate highly reactive hydroxyl free radicals that harm the N-terminal site of albumin. Therefore, albumin's capacity to bind transition metals, particularly cobalt, undergoes a significant change. Ischemia-modified albumin (IMA) is the name given to this albumin variant caused by ischemia[8&9]. It has been shown that IMA levels

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*Corresponding author: Aula Abo Saleh, E-mail: ola.abo.salih@gmail.com

rise within 2 mins from the onset of ischemia, remain increased until 12 hrs, and then return to their normal level after 24 hrs[10]. Therefore, we aimed to study the diagnostic performance of presentation IMA among patients referred to the Emergency Department (ED) within 3 hrs of signs of acute myocardial ischemia and its significance in conjunction with cardiac kinase-MB (CK-MB) and electrocardiogram (ECG). In addition, we studied the relationship between IMA levels and some variables (gender, age, hypertension, diabetes, hypertriglyceridemia, hypercholesterolemia and smoking) in ACS patients.

MATERIAL AND METHODS

This research was conducted at Albassel's Hospital, Lattakia, Syria, and was approved by Tishreen University Board, written consent was obtained from all participants.

Ethical Approval

All procedures were approved by the Institutional Review Board of Tishreen University. The decision involved Ethical Approval (Decision Number: **2808** in September 2020).

Patients

127 individuals with severe chest pain suggestive of ACS were enrolled in the study. All included patients underwent an electrocardiogram (ECG) and serum CK-MB measured on admission as part of usual hospital treatment and patient management. Of 127 patients admitted, 97 were given a final diagnosis of ACS, and 30 were given a diagnosis of NICP. Among the ACS patients, 45 had MI and 52 had UA. Patients with liver disorders, renal disorders, and pregnant women were excluded.

Analytical Methods and Instrumentation

IMA was measured by the Albumin Cobalt Binding test (ACB Test) which is a quick colorimetric method developed by Bar-Or et al[11]. the principle of ACB test is based on the premise that myocardial ischemia causes changes in human serum albumin that are demonstrated by reduced exogenous cobalt. The concentration of IMA can be determined by addition of a known amount of cobalt to a serum sample and measurment of unbound cobalt by adding dithiothreitol (DTT). The procedure involved adding 200 µl of patient serum to 50 µl of 1 g/L cobalt chloride solution (Phytotecholab, CoCl2.6H20), mixing vigorously, and incubating the mixture for 10 min. During incubation, the cobalt binds to the N-terminus of unaltered albumin in the sample. After incubation, 50 µl of 1.5 g/L dithiothreitol (Titan Biotech Ltd, DTT) was added and mixed, DTT forms a coloured complex with cobalt that is not bound at the N-terminus of albumin. Following a 2-min incubation period, 1.0 mL of 9.0 g/L NaCl solution was added. A spectrophotometer was used to measure the absorbance of the mixture at 470 nm. The blank was made in the same way with the exclusion of DTT. The standard curve of $CoCl_2$ was prepared in the range of 10 to 40 µg/ml. One IMA unit was defined as one µg of free Co^{+2} in the reaction mixture per ml of serum samples[12]. The activity of serum CK-MB was evaluated using a Biosystem kit based on the kinetic principle.

Statistical Analaysis

SPSS was used for the statistical analysis (version 24). For continuous variables, data are shown as mean ± standard deviation (SD), while for categorical variables, data are presented as a percentage. For the evaluation of normality, Shapiro Wilk test was used. The correlation between the normally distributed variables was analyzed using ANOVA test. Mann-Whitney and Kruskal- Wallis tests were applied for variables that didn't follow the normal distribution. To find the best IMA threshold, a receiver operating characteristic (ROC) curve analysis was conducted. McNemar test was used to compare the diagnostic efficacy of CK-MB, ECG, and the combined variables. Statistical significance was considered for P values greater than 0.05.

RESULTS AND DISCUSSION

Results

Subject characteristic

Table 1 shows participant baseline characteristics. No significant difference was observed between the two groups with regard to age, gender, diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, and smoking ($p > 0.05$). The mean CK-MB levels in MI group were significantly higher than UA and NICP groups ($p < 0.05$)

Ischemia modified albumin levels between groups

The mean IMA value was substantially greater in ACS group compared to NICP group, either in MI group compared to NICP (P<0.001), or in UA group compared to NICP (p<0.001). However, there was no statistically significant difference in IMA levels between UA and MI patients (p = 0.2) (Table 2)

Diagnostic performance of IMA comparing to other parameters

Based on the ROC curve of IMA, the optimal cutoff value was found to be 71.3 u/ml, with a sensitivity of 86.5%, specificity of 76.7%, and area under the curve (AUC) of 0.92 (95% CI: 0.87, 0.96) (figure 1). Thus, we considered 71.3 u/ml as an optimal diagnostic cutoff value of IMA in our study.

Table 1: Characteristics of the study population.

	UA $(n = 52)$	MI $(n = 45)$	NICP $(n = 30)$	P value
Age (mean ± SD)	58 ± 11.7	56.9 ± 9	55.2 ± 11.5	0.33
Male %	64.5	77.8	63.3	0.12
Diabetes mellitus %	32.7	46.4	30	0.24
Hypertension %	59.6	68.9	56.7	0.49
Hypercholesterolemia %	38.1	40	26.6	0.5
Hypertriglyceridemia %	34.5	44	36.7	0.31
Smoking %	63.5	66.7	63.3	0.93
CK-MB (mean ± SD)	14.4 ± 4.5	21.4 ± 8.2	13.2 ± 4.8	**0.001**

Kruskal-Wallis test was used to compare Age between groups, ANOVA test was used to compare levels of CK-MB between groups

Table 2: Mean IMA values in study groups (UA, MI, and, NICP).

	UA	**MI**	**NICP**	**P value**
IMA (u/ml)	75.5 ± 6	77.1 ± 5.6	58.6 ±11.4	NICP vs UA, p<0.001 NICP vs MI, p<0.001 UA vs MI, p= 0.2

IMA: ischemia modified albumin, **UA: un stable angina, MI: myocardial infarction, NICP: non ischemic chest pain.** Kruskal- Wallis test was applied to compare IMA levels between the three groups, Mann-Whitney test was used to compare IMA levels between each of two groups.

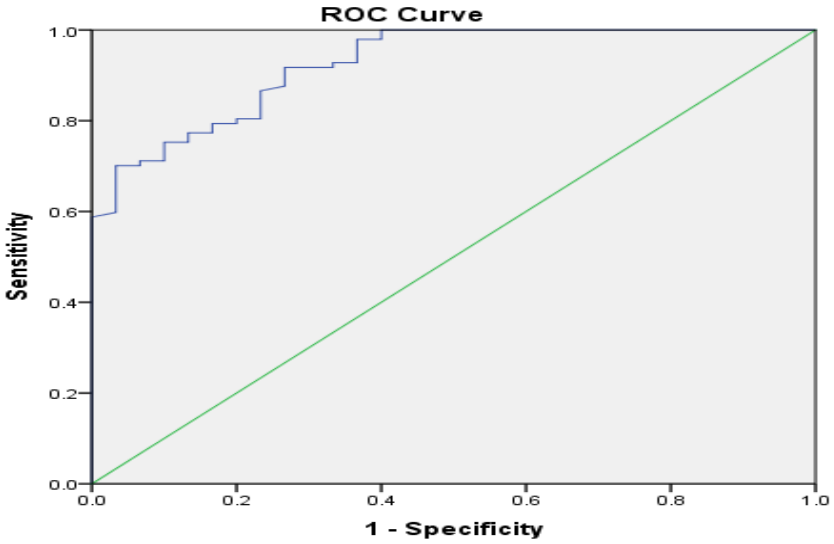

Fig. 1: ROC curve for the ability of IMA to diagnose ACS.

At presentation to Emergency Department (ED), IMA identified 84 of 97 ACS patients (sensitivity 86.5 %) compared to 53 of 97 (sensitivity 54.6%) by admission ECG, and 11 of 97 (sensitivity 11.3%) by admission CK-MB. When ECG and CK-MB were used in combination (standard practice in the hospital), only 59.8% of patients with ACS were identified. Regarding the specificity, we observe a decrease in the specificity of IMA (76.7%) compared to CK-MB and ECG, when used each alone (93.3%, and 90% respectively) or combined (83.3%). The positive predictive value (PPV) of IMA was (92.3 %) which was relatively comparable to those observed with ECG or CK-MB alone or combined. However, although the negative predictive value (NPV) for IMA did not exceed (63.9 %), it was better than NPVs observed with ECG or CK-MB each alone or combined as shown in table 3.

Diagnostic performance of IMA combining with other parameters

Table 4 displays the results of the combined use of IMA, ECG, and CK-MB in the diagnosis of ACS. When IMA was used with ECG, the ECG sensitivity significantly increased to 90.7% and this was statistically similar to that when IMA was used with CK-MB, 88.7% (p=0.8). All three tests together had a sensitivity of 95.9%, which was considerably higher than the sensitivity of IMA + CK-MB (p= 0.03) and IMA + ECG (p= 0.031). However, the sensitivity of IMA was not significantly improved when IMA was used in combination neither with ECG nor with CK-MB. Only the combination of the three tests showed a significantly increased sensitivity (95.9%) compared to IMA alone. The effects of the various combinations of IMA with the other parameters on the specificity, NPV and PPV are summarized in table 4.

Table 3: Comparison of presentation diagnostic tests for diagnosis of ACS.

TEST	Sensitivity for ACS %	Specificity for ACS %	PPV for ACS %	NPV for ACS %
IMA	**86.5**	**76.7**	**92.3**	**63.9**
ECG	**54.6**	**90**	**94.7**	**38.6**
CKMB	**11.3**	**93.3**	**86.7**	**24.6**
ECG+CK-MB	59.8	83.3	92.1	39.1

[a] sigificantly different from the **sensetivity** of ECG, CK-MB, ECG+CK-MB; [b] significantly different from the **specificity** of ECG, CK-MB, ECG+CK-MB; [c] significantly different the **PPV** of CK-MB; [d] sigificantly different from the **NPV** of ECG, CK-MB, ECG+CK-MB.

Table 4: Diagnostic performance of IMA in conjunction with other parameters (ECG and CK-MB.

TEST	Sensitivity %	Specificity %	PPV %	NPV %
IMA	**86.5** [a]	**76.7** [a]	**92.3** [a]	**63.9** [a]
CK-MB	**11.3** [b]	**93.3** [b]	**86.7** [b]	**24.6** [b]
IMA+CK-MB	**88.7** [a]	**73.3** [a]	**91.8** [a]	**66.7** [a]
ECG	**54.6** [c]	**90** [b]	**94.7** [a]	**38.6** [c]
IMA+ECG	**90.7** [a]	**73.3** [a]	**91.6** [a]	**71** [d]
IMA+ECG+CK-MB	**95.9** [d]	**70** [a]	**91.2** [a]	**84** [e]

Values with different letters (a-d) within the same column are significantly different (P<0.05).

The relationship between IMA and other variables in ACS patients

To study the relationship between IMA and age, we divided ACS patients into 6 age groups of 10 years and we calculated the mean of IMA in each group as presented in table 5. No significant difference was noticed in IMA means among age groups (P=0.6). Concerning other variables, there was no significant difference in IMA means between males and females (p= 0.6), hypertensive and non-hypertensive AS patients (p=0.7). In addition, there were no statistically significant differences in the means of IMA levels between hypertriglyceridemic and non-hypertriglyceridemic (p= 0.38), and between smokers and non-smokers ACS patients (p=0.16).

However, our study showed a statistically significant difference in IMA mean between diabetics and non-diabetics (p= 0.03), and between hypercholesterolemic and non-hypercholesterolemic ACS patients (p= 0.004) (table 6).

Table 5: Relationship between IMA and variables in ACS patients (n=97).

Age groubs (years)	[34 – 43]	[44 – 53]	[54 – 63]	[64 – 73]	[74 – 83]	[84 – 93]	**P value**
Number of patients	7	33	28	19	6	4	
Mean IMA (u/ml) ± SD	76.9 ±5.8	75.1 ±5.2	77.8 ± 6.6	75.8 ±6.8	76 ± 2.9	74.9 ±6.8	0.6

Table 6: Relationship between IMA and some variables in ACS patients (n=97).

Characteristics	**N**	**Mean IMA ± SD**	**P value**
Males	68	76.3 ± 5.3	0.6
Females	29	75.8 ± 6.7	
Diabetic patients	38	77.8 ± 6.6	**0.03**
Non-diabetic patients	59	75.2 ± 4.9	
Hypertensive patients	62	76 ± 6.2	0.7
Non-hypertensive patients	35	76.5 ± 4.9	
Hypercholesterolemic patients	37	78.3 ± 5.4	**0.004**
Non- hypercholesterolemic patients	60	74.8 ± 5.6	
Hypertriglyceridemic patients	44	75.6 ± 6.1	0.38
Non- hypertriglyceridemic patients	53	76.6 ± 5.4	
Smoker patients	63	76.8 ± 5.1	0.16
Non-smoker patients	34	75.1 ± 6.3	

Mann-Whitney test was used to compare IMA levels between each of two groups.

Discussion

Many acute chest pain patients, who attend ED, may present with myocardial ischemia in the absence of myonecrosis. Most of the biomarkers are products of myocardial necrosis and thus typically discovered at a later stage of cardiac injury. Furthermore, extended ischemia may lead to cardiac cell death and it is a pre-condition to infarction. Therefore, the identification of myocardial ischemia at the earliest phase is substantial to ban the destructive results of the disease. In this study, we assessed the role of IMA in early diagnosis of ACS among patients with acute chest pain.

In this study, we showed that the serum IMA levels of UA and MI patients were substantially greater than those of NICP group. Several studies have reported the same result[13&14&15&16]. However, previous studies did not take into account the time of onset of acute chest pain[13&14], other studies showed this result within six hrs of symptoms[15&16]. Our finding suggest that IMA levels increase early in ACS patients during three hrs of symptoms. In addtion, elavated IMA levels which have been showed in UA and MI gives support the concept that cobalt binding to serum albumin is reduced in patients with myocardial ischemia. The mechanism which has been proposed for the generation of IMA is that cardiac ischemia may induce hypoxia (which in turn results, in an increase, in reactive oxygen radicals), acidosis, membrane energy-dependent sodium and calcium pump disruptions, free iron and copper ion exposure, all of which involves damage of the amino terminal of human serum albunin and removal of two or three N-terminal amino acids which is the binding site of transition metal especially to cobalt, as a result the capacity of albumin to bind cobalt is reduced.

Furthermore, our study revealed that IMA is a poor discriminator between ACS categories, contrary to the study done by Mojibi et al, which reported that IMA levels were significantly higher in patients with UA compared with MI[17]. They supposed that in myocardial necrosis, albumin would be less damaged by free radicals than in non-necrotic injury. Therefore, IMA production will decrease. Furthermore, they supposed that myocardial necrosis causes limiting IMA entrance to the bloodstream. However, in line with our results, the study done by Reddy et al. revealed that there was no significant difference in IMA values between UA and MI groups[16]. In a similar way, Etli et al. studied the association between IMA levels and Gensini scores; which classify ACS patients depending on the severity of coronary disease, they found that IMA level was not associated with the severity of ACS[18].

The sensitivity and specificity of IMA, at a cutoff point of 71.3 u/ml, were found to be 85.5% and 76%, respectively, using ROC curve analysis. In our population, IMA had higher sensitivity than either ECG or CK-MB by itself or in combination.

Although several studies have reported high sensitivity of IMA, different results related to specificity have been shown. In Sinha et al study, which included 208 patients presenting to the ED within three hrs of symptoms of acute chest pain, the sensitivity of IMA was 82%, and the specificity was 46% with a cutoff point of 85 u/ml[19]. In Lee et al study, with 413 patients who have visited ED with symptoms of ACS, the sensitivity and specificity of IMA were 93% and 35.6% respectively, and the cutoff point was 83 u/ml[20], while the study of Reddy et al showed that the sensitivity and specificity of IMA were 92% and 87% respectively. This study involved 89 patients with acute chest pain and used a cutoff point of 80 u/ml[16]. When comparing the three mentioned studies with our study, the disparity seen in the specificity of IMA may reflect the diversity in sample size, baseline population characteristics, and the ideal cutoff value used. Indeed, it is suggested that each study should determine its reference value and the best IMA cutoff point, which may change according to factors including location, food, and environmental conditions.

For ruling out ACS, the NPV is the most important. False negatives are undesirable results. In our study, we found that NPV was only 63.9%, which means that a negative IMA cannot be used to exclude ACS by itself., contrary to the study of Lee et al and Christenson et al, both studies have reported high NPV for the test, 91.8% and 96% respectively[17&21]

For efficient management in ED, we studied the diagnostic performance of IMA in conjunction with ECG and CK-MB, the sensitivity of the three tests combined was 95.9%, which was significantly higher than using each test alone or two tests combined. In addition, the NPV increased to 80%. This finding was in support of the study of Sinha et al.[19]. Anwaruddin et al found that using IMA in conjunction with myoglobin, CK-MB, and cTnI (cardiac troponin I) boosted the sensitivity of

IMA from 80% to 97% for diagnosing cardiac ischemia[22]. According to research conducted by Takhshid et al.[23], combining IMA with cTnI and ECG significantly increased IMA's sensitivity from 84% to 96%. However, Mishra et al have shown that the sensitivity of IMA alone (92%) was significantly equal to the sensitivity of combination of IMA, cTnI, CK-MB and ECG[13].

Our study revealed for the first time that IMA levels are not influenced by age, gender, hypertension, hypertriglyceridemia, and smoking. However, higher levels of IMA were observed in diabetic ACS patients compared to nondiabetic ACS patients. Similarly, Hypercholesterolemic ACS patients had higher IMA levels compared to non hypercholesterolemic patients. This finding suggests the presence of oxidative stress associated with diabetes mellitus and hypercholesterolemia. Oxidative stress itself means an increased production of reactive oxygen species (ROS), which might damage the N-terminal site of albumin.

Our study had some limitations. First, the work was performed in a single center, which may restrict the generalization of our findings. Second, this study did not consider all cases that cause acute chest pain because of the relatively small sample size.

Conclusion

This study found that IMA is a sensitive marker for the detection ACS during three hrs of symptoms. In addition, using IMA improved the current diagnostic procedures in the emergency department to make safer decision for ruling in or ruling out ACS.

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نشرة العلوم الصيدليـــــة
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تقييم دور الألبومين المعدّل بنقص التّروية (IMA) في تشخيص الحوادث الإقفاريّة القلبيّة لدى مرضى الألم الصدري الحاد

علا أبو صالح [*1] – باسم معروف [2] – عفراء زريقى [3]

[1] قسم الكيمياء الحيوية والميكروبيولوجيا ، كلية الصيدلة، جامعة تشرين ، اللاذقية ، سوريا

[2] قسم الطب الباطني ، كلية الطب ، جامعة تشرين، اللاذقية ، سوريا

[3] قسم الصيدلانيات والتكنولوجيا الصيدلية ، كلية الصيدلة ، جامعة تشرين، اللاذقية ، سوريا

يُعدُّ التّ شخيص المبكر للمتلازمة الإكليليّة الحادّة أمراً بالغ الأهميّة، نظراً لارتفاع معدّل الوفيّات المرتبطة بها. تمَّ اقتراح الألبومين المعدَّل بنقص التروية كمشـــعر مفيد لتشـــخيص المتلازمة الإكليليّة الحادّة في قسم الإسعاف. قيَّمت هذه الدراسة فائدة الألبومين المعدَّل بنقص التروية في التّشخيص المبكر للمتلازمة الإكليليّة الحادّة عند مر ضى الألم الـصدري الحاد. تمَّ اجراءُ هذه الدّرا سة على ١٢٧ مريـضاً ممن قُدِموا الى قسم الإسعاف خلال ثلاث ساعات من بدء الألم الصّدري الحاد. اعتماداً على التّشخيص النّهائي، تم تـ صنيف المر ضى الى مجموعة مر ضى احتـ شاء عـ ضلة القلب (n=45)، مجموعة مر ضى الذبحة غير المســـتقرة (n=52)، ومجموعة مرضـــى الألم الصّـــدري غير الإقفاري .(n=30) كانت قيم الألبومين المعدَّل بنقص التَّروية أعلى بشكل هام احصائياً عند مر ضى المتلازمة الإكليليّة الحادّة مقارنة بمجموعة مرضـــى الألم الصّـــدري غير الإقفاري، بلغت القيمة الحديّة المُثلى للألبومين المعدَّل بنقص التروية ٧١,٣ (وحدة/مل)، ترافقت تلك القيمة مع حساسيّة ٨٦,٥%، ونوعيّة ٧٦,٧%. تفوَّقت حساسيّة الألبومين المُعدَّل بنقص التَّروية بشكل هام احصائياً على حساسيّة (٥٤,٦%) ECG و(١١,٣ %) CK-MB أظهرت النّتائج التي تو صلنا إليها أن الألبومين المُعدَّل بنقص التَّروية هو مـ شعر حـ سّاس لكـ شف المتلازمة الإكليليّة الحادّة عند المرضى المُراجعين لقسم الإسعاف بشكوى ألم صدري حاد.