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Original Research

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Assessment of ischemia modified albumin in unstable angina and its correlation with selected acute phase reactants

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ABSTRACT

Objective: There is evidence that ischemia modified albumin increases (IMA) in acute coronary syndrome but enough work has not been done in assessing the correlation between IMA and inflammatory markers in unstable angina (UA). The aim of the study was to observe whether IMA would significantly be altered in UA patients and also whether the same would be correlated with selected acute phase reactants. **Method:** The present hospital-based, non-interventional, cross-sectional study was undertaken in Medical College and Hospital, Kolkata. 47 cases of UA and 50 suitable controls were enrolled for the study. Serum IMA, serum albumin, serum total cholesterol, serum high-sensitivity C-reactive protein (hsCRP), serum ceruloplasmin and serum transferrin were measured in blood from the study population. **Results:** Serum IMA and hsCRP ($P < 0.001$) were significantly higher in UA cases whereas serum transferrin ($P < 0.001$) and albumin ($P < 0.001$) were significantly lower in cases as compared to controls. IMA was not correlated with acute phase reactants. Serum IMA ($P - 0.06$) was not considered as an important inflammatory marker in UA. However, serum hsCRP and serum transferrin play a significant role ($P < 0.05$) in assessing the severity of inflammation. **Conclusions:** Though there was a significant increase in IMA, but it was not correlated with common markers of inflammation in UA. IMA is not superior to other markers in identifying inflammation in UA.

KEY WORDS: Albumin, high-sensitivity C-reactive protein, inflammation, ischemia modified albumin, transferrin, unstable angina

INTRODUCTION

In the majority of patients presenting with acute coronary syndromes (ACS) [1], the thrombus is partially obstructive, or only transiently occlusive, resulting in coronary ischemia without persistent ST-segment elevation (unstable angina [UA] or non-ST-segment elevation myocardial infarction) [2]. The contribution of inflammation to the pathogenesis of ACS [2] has attained increasing recognition as evident from changes in the level of acute-phase reactants, the nonspecific markers of inflammation [3]. Furthermore, increased concentrations of the acute-phase reactant high-sensitivity C-reactive protein (hsCRP) appear to be predictive of higher risk for long-term cardiovascular morbidity/mortality in patients with unstable and stable angina [4]. This potential predictive capacity of hsCRP warrants further evaluation alone and in conjunction with other positive acute phase reactant ceruloplasminas well as the negative acute phase reactants (transferrin, albumin and total cholesterol) [5,6].

Previous studies have shown that [7,8] ischemia modified albumin (IMA) which is considered for use in conjunction with

electrocardiography (ECG) and cardiac troponins for exclusion of ACS [9], increases in stable as well as UA, but enough work has not been done in assessing the correlation between IMA and inflammatory markers in UA. The aim of the present study was to observe whether IMA would significantly be altered in UA patients and also whether it would be correlated with a positive (serum hsCRP and serum ceruloplasmin) and the negative (serum albumin, serum transferrin and serum total cholesterol) acute phase reactants.

Materials and Methods

The present study was a hospital-based, non-interventional, cross-sectional case-control study. This work was undertaken in the Department of Biochemistry of Medical College and Hospital, Kolkata in collaboration with the Department of Cardiology, Medical College and Hospitals, Kolkata, West Bengal from May 2009 to May 2010.

Subjects

A total of 47 cases (the mean standard deviation [SD] for age is 53.64 [10.75] years) including 25 women attending the cardiology and emergency department diagnosed to have UA were selected on the basis of clinical features and ECG changes. Clinical features included any of the three features: (1) pain occurring at rest (or with minimal exertion) usually lasting for >10 min (2) severe in intensity and of new onset, and/or (3) occurring with a crescendo pattern. ECG changes [10] include:

1. Transient ST-segment elevation of ≥1 mm in 2 contiguous leads
2. ST-segment depression of ≥1 mm
3. New T-wave inversion of ≥1 mm; or
4. Pseudonormalization of previously inverted T waves.

Similarly, 50 age and sex matched apparently healthy individuals (The mean [SD] for age is 53.21 [10.69] years) including 23 women were selected from the relatives of patients attending the cardiology department.

The cases and controls were both selected by a simple random method. All the cases and controls were informed about the purpose of the study and written consent for inclusion in the study and for the publication of the study report was obtained. The study was approved by a properly constituted institutional Ethical Committee.

A volume of 5 ml venous blood was collected from the median anti-cubital vein of the subjects within 3 h of their admission and from controls using standard aseptic technique and the collected clotted blood was centrifuged at 1500 rpm speed for 3-5 min. All the tests were done with serum harvested from clotted blood. The assays were performed on fresh samples.

IMA was measured by adding a known amount of cobalt (II) to serum the sample and the unbound cobalt (II) was measured by intensity of the colored complex formed by adding mercaptoethanol by colorimeter at 470 nm. One unit of IMA was defined as "milligram of free cobalt (II) in the reaction mixture/ml of serum sample." The IMA was measured by the method described by Christenson, *et al*. [8] and modified in Biochemistry Department of Medical College and Hospital. (Intra-assay coefficient of variation [CV] was 4.81% [0.0151/0.32]). CV% was calculated by measuring cobalt binding activity of albumin of 20 samples, dividing the SD by mean value and expressed in percentage.

Serum albumin was measured by bromo cresol green dye (BCG) binding method [11] by XL-600 autoanalyzer (transasia). (Intra-assay CV was 1.55% [0.067/4.33]).

Serum total cholesterol was estimated by cholesterol oxidase and peroxidase method [12]. (Intra-assay CV was 2.21% [3.1/139.90]).

Serum hsCRP, (intra-assay CV, was 4.77% [0.13/2.72]) was estimated by standard kits utilizing the immunoturbidimetric method [5].

Serum ceruloplasmin (Intra-assay CV was 2.5% [0.011/0.44]) and serum transferrin (intra-assay CV was 2.6% [0.0702/2.70]) were measured by standard kits utilizing the nephelometric method [5] in BN ProSpec nephelometer.

Statistical Analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) statistical analysis software (SPSS version 17.0, Chicago IL, USA). Statistically, a significant difference was determined by the Student's *t*-test. All *P* values are two-sided, with values <0.05 considered to be significant. Correlation coefficients were calculated according to the Brave-Pearson function. The six parameters measured were examined whether they exhibit any bivariate and partial correlation.

Results

Table 1 displays all the results of the two groups. Results are displayed in the form of mean (± SD) and standard error of the mean. It displays the results of unpaired *t*-test for equality of means of the control population and diagnosed cases of UA. It was observed that serum IMA ($P < 0.001$) and serum hsCRP ($P < 0.001$) were significantly higher in UA cases in comparison to controls whereas serum transferrin ($P < 0.001$) and serum albumin ($P < 0.001$) were significantly lower in cases. However, serum ceruloplasmin ($P = 0.323$) and total cholesterol ($P = 0.175$) showed no significant difference between cases and controls.

Table 2 shows the results of bivariate correlation analysis among different parameters of cases and controls. IMA was not significantly correlated with serum ceruloplasmin ($r = -0.031$, $P = 0.838$), serum hsCRP ($r = -0.048$, $P = 0.751$), serum albumin ($r = 0.063$, $P = 0.676$), serum cholesterol ($r = -0.029$, $P = 0.847$) as well as transferrin ($r = 0.278$, $P = 0.059$) in the UA patients. These are summarized in Table 2 controls also showed the same result as cases.

Table 1: Group statistics and test of significance (independent samples test) of different parameters between unstable angina patients (cases) and age and sex-matched healthy individuals (controls)

Parameters	Mean±SD		Standard error mean		*t* value	*P* value (level of significance)
	Cases (*n*=47)	Controls (*n*=50)	Cases	Controls		
Serum IMA (U/ml)	37.14±11.30	22.64±4.12	1.648	0.583	−8.297	0.000*
Serum ceruloplasmin (g/L)	0.299±0.058)	0.29±0.033	0.008	0.004	−0.994	0.323
Serum hsCRP (mg/dL)	3.028±2.838	0.309±0.163	0.414	0.023	−6.555	0.000*
Serum albumin (g/dL)	3.735±0.395	4.041±0.472	0.057	0.066	3.447	0.001*
Serum cholesterol (mg/dL)	182.70±31.432	173.86±32.183	4.585	4.551	−1.368	0.175
Serum transferrin (g/L)	2.546±0.517	2.963±0.395	0.075	0.055	4.479	0.000*

**P* value significant at 0.05 level, SD: Standard deviation, IMA: Ischemia modified albumin, hsCRP: high-sensitivity C-reactive protein

Table 2: Bivariate correlation analysis among different parameters in unstable angina (cases) and healthy individuals (controls)

	r_{12}	r_{13}	r_{14}	r_{15}	r_{16}
Cases (*n*=47)					
Pearson's correlation, *r*	−0.031	0.048	0.063	−0.029	0.278
Level of significance	0.838	0.751	0.676	0.847	0.059
Controls (*n*=50)					
Pearson's correlation, *r*	−0.097	0.193	0.160	0.041	−0.076
Level of significance	0.505	0.179	0.268	0.776	0.602

**P* value significant at 0.05 level, X_1=Serum IMA (U/ml), X_2=Serum ceruloplasmin (g/L), X_3=Serum hsCRP (mg/dL), X_4=Serum albumin (g/dL), X_5=Serum cholesterol (mg/dL), X_6=Serum transferrin (g/L), IMA: Ischemia modified albumin, hsCRP: High-sensitivity C-reactive protein

Table 3: Partial correlations of ischemia modified albumin with others one eliminating the effect of remaining parameters in cases and controls

	Controlled variables	Parameters with which IMA is correlated	Pearson's correlation (*r*)	Level of significance (*P*)
Cases (*n*=47)	X_3, X_4, X_5, X_6	Ceruloplasmin	−0.117	0.456
	X_2, X_4, X_5, X_6	hsCRP	−0.027	0.865
	X_2, X_3, X_5, X_6	Albumin	−0.020	0.897
	X_2, X_3, X_4, X_6	Cholesterol	0.006	0.971
	X_2, X_3, X_4, X_6	Transferrin	0.289	0.060
Controls (*n*=50)	X_3, X_4, X_5, X_6	Ceruloplasmin	−0.117	0.456
	X_2, X_4, X_5, X_6	hsCRP	0.205	0.172
	X_2, X_3, X_5, X_6	Albumin	0.125	0.409
	X_2, X_3, X_4, X_6	Cholesterol	−0.027	0.861
	X_2, X_3, X_4, X_6	Transferrin	−0.083	0.585

**P* value significant at 0.05 level, X_1=Serum IMA (U/ml), X_2=Serum ceruloplasmin (g/L), X_3=Serum hsCRP (mg/dL), X_4=Serum albumin (g/dL), X_5=Serum cholesterol (mg/dL), X_6=Serum transferrin (g/L), hsCRP: high-sensitivity C-reactive protein, IMA: Ischaemia modified albumin

Table 4: Multinomial logistic regression through multivariate approach

Parameter	β-values	Significant (*P* value)
IMA	0.288	0.060
Ceruloplasmin	6.183	0.730
hsCRP	27.734	0.049*
Transferrin	−8.237	0.047*
Albumin	−1.149	0.388
Cholesterol	0.009	0.720

**P* value significant at 0.05 level, hsCRP: high-sensitivity C-reactive protein, IMA: Ischemia modified albumin, CTRL=0/UA=1, UA: Unstable angina

Tables 3 shows partial correlation analyses between each individual parameter and IMA keeping the other variables constant, i.e., eliminating the effects of other variables. In the case of both cases and controls, no significant partial correlation can be demonstrated between IMA and other parameters.

Table 4 displays the parameter estimates, which have been obtained through multinomial logistic regression through multivariate approach using the SPSS Software. In UA, serum IMA (β value 0.288) may not be considered as an important marker of inflammation. However, serum hsCRP and serum transferrin having corresponding β-values of 27.734 and −8.237 play significant role in assessing inflammation.

Discussion

ACS encompass a spectrum of unstable coronary artery disease from UA to transmural myocardial infarction in which inflammation and oxidative stress are the major contributors [3,4,13]. Thus in this present work, it was our objective to find out whether suspected inflammatory process in patients of UA modifies the cobalt binding property of albumin and whether this change correlates with the other markers of inflammation, which had been included in this study. That inflammation participates in atherosclerosis from its inception and development to its ultimate endpoint, i.e., thrombotic complications, is evident from the data in Table 1 the study demonstrates significant elevation of serum hsCRP ($P < 0.001$) in case of UA in comparison to controls. Various other studies have shown a rise in acute phase reactants in UA [14-16]. A study by Sharma *et al.* showed that CRP level is significantly higher in UA compared with controls ($P = 0.01098$) favoring the idea that there are some underlying process related to inflammation that are relevant to coronary artery disease [17]. These findings were further corroborated by the work of Schiele *et al.*, in 2009 [18] who emphasized that elevated CRP level is one of the most powerful predictors of atherosclerosis and independent risk factor in ACS patients, again supported by present study [vide Table 4]. Therefore, that CRP has emerged as one of the most important novel inflammatory markers in UA suggests the role of inflammation in the causation of UA.

In the case of UA, serum IMA is significantly elevated in comparison to controls ($P < 0.001$), thus suggesting altered cobalt binding activity of albumin. Similar findings have been described by different workers that IMA is increased in ACS [8,19,20-22]. Bivariate analysis revealed that cobalt binding activity was not significantly correlated with acute phase reactants in the UA patients [Table 2]. Even after performance of the test for partial correlation (keeping four variables fixed), IMA was not significantly correlated with individual marker of inflammation [Table 3]. This finding that IMA had no significant correlation with inflammatory markers in cases may stand in apparent contradiction to observations by several workers. Studies by Borderie *et al.* [22] in 2004 had shown that IMA reflected oxidative stress in patients of systemic sclerosis, and high IMA levels had correlated well with other markers of oxidative stress.

The results from a randomized control study conducted by Kutlu, *et al.* [23]determined a high level of ischemia-modified albumin in testicular torsion of mature male Wistar rats, indicating its potential value in diagnosis of testicular torsion. However, they failed to observe any significant correlation between IMA and malondialdehyde level, which is in agreement with the observation of the present study.

Kumar, *et al*. (2008) [20] have described that IMA in normolipidemic patients may provide an index of oxidative stress and ischemia in acute myocardial infarction. However, this is in contrast to the finding of the present study as evident from multinomial logistic regression analysis. [Table 4] Therefore, it can be concluded from this result that though IMA distinguishes ischemia from non-ischemic individuals, but it cannot be used as a predictor of the inflammatory process in UA.

Further, serum albumin ($P = 0.001$) had been found to be significantly lower in cases as compared to controls. These data were also in accordance with the findings in the works of Kaysen *et al*. in 2001 and 2002 who had shown that ceruloplasmin and α_1 acid glycoprotein (two long-lived acute-phase proteins) predict future albumin concentration [24] and correlate with fractional catabolic rate of albumin [24,25]. They had also shown that S-albumin correlated inversely with CRP level for a CRP level >1.3 mg/dL in hemodialysis (HD) patients [26]. In addition, Zimmermann *et al.* [27] reported that inflammation is associated with hypoalbuminemia and increased mortality in HD patients. It may be possible that N-terminus modification of albumin due to ongoing inflammatory process in UA may not react with BCG dye, and this is the cause of reduced albumin in the case.

Now coming to another negative acute phase reactant serum transferrin, analysis had revealed that there had been a significant decrease in its value in cases in comparison to controls ($P < 0.001$). In a study by San Miguel, *et al.* [28] it had been found out that serum transferrin had significantly decreased in case of monoclonal gammopathy, a myeloproliferative disorder confirming its role as a negative acute phase reactant. This was in agreement with Imhof, *et al.* [29] who in 2001 in their study of the effect of alcohol consumption on systemic markers of inflammation had considered serum transferrin as a negative acute phase reactant. In a further study by Altamura, *et al.* [30] in 2009 it had been concluded that serum ceruloplasmin and serum transferring levels were representative of clinical status severity in acute stroke patients play a pathogenic role in stroke progression.

The present study however suffers from some limitations such as its small sample size and the heterogeneous nature of the patient population being studied. Coronary angiography could not be performed in all the study subjects due to ethical, socio-economic constraints; hence the diagnosis of UA was only clinical.

Thus, it can be concluded from the present study that though ongoing inflammation modifies the cobalt binding activity of albumin in UA but this parameter (IMA) cannot be considered as a predictor of inflammation in those patients. However, serum hsCRP and serum transferrin having corresponding β-values of 27.734 and −8.237 play significant role in assessing the severity of ischemia in UA and encourage future studies probing the role of inflammation in the causation of UA.

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