Correlation between Ischemia modified albumin with pH in acidosis and alkalosis: A possible link

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Abstract
Ischemia modified albumin (IMA), a recently developed marker for myocardial ischemia is measured by using albumin cobalt binding (ACB) assay. The correlation between the formation of IMA and alterations in the pH levels are of considerable importance and the same was not clear in the available literature. Based on our literature search, the study was designed to check and compare the levels of IMA and the pH in acidosis and alkalosis patients with those in normal patients. The mean of IMA in patient with acidosis (0.832±0.16) was higher when compared with the normal pH patients (mean 0.71±0.21). The IMA levels in patients of alkalosis were also found higher (mean 0.804±0.19) compared to that of the normal control. It was also observed that the mean of IMA was lower in the patients with alkalosis than the patients with acidosis. When the IMA levels and the pH levels in acidosis and alkalosis patients were correlated, there was a weak negative correlation between pH levels in acidosis and IMA levels. In alkalosis, the correlation was positive but there was no significance in these changes. In conditions of acidosis and alkalosis, where the pH levels are altered which could be one of the factors responsible for increase in the IMA levels. However, the correlation was weak and an increase in the sample size could provide us with a better relation between the levels of pH and IMA.

Keywords: Acidosis, Alkalosis, Ischemia modified albumin (IMA), Albumin cobalt binding assay (ACB).

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Introduction
Recently there has been an increased interest in the detection of markers for myocardial ischemia or the acute coronary syndrome. Looking at all these improvements and the various reports linking to the developments in this area, there are no well-defined biomarkers for detecting them in the early stages[(1,2)]. The decreased capacity of albumin for its binding capacity to metals on exposure to ischemic tissue leads to the formation of Ischemia modified albumin (IMA). This IMA is a well-developed marker for transient myocardial ischemia and its levels are seen to increase in Acute Coronary Syndrome[(3,4)]. Such modification of albumin is reported to be likely a result of reduction in oxygen tension and an increase in free radical generation. This decreased ability of albumin to bind to metals is used in albumin cobalt binding assay, which is used in the diagnostic assay for quantification of albumin. In addition to these reactive species, some physiological compounds like glucose also are found to cause modifications of albumin[(5)].

Albumin is one of the important transport proteins involved in transport of many ligands including fatty acids, unconjugated bilirubin, small drugs and certain metals like cobalt, zinc, nickel etc.[(6-8)] Decreased supply of oxygen to myocardial cells due to occlusion of coronary artery leads to biochemical and metabolic changes in myocardial tissue. Increased production of lactate as a result of metabolism of glucose in the absence of oxygen results in reduced intracellular pH[(9)].

Increased amounts of IMA result in less cobalt binding and more residual unbound cobalt available for complex with a chromogen (dithiothreitol), which can be measured photometrically. During ischemia, the N-terminus of albumin is altered, possibly as the result of hypoxia, acidosis, free-radical injury and energy-dependent membrane disruption, decreasing its binding capacity for metals[(10)]. Four metal-binding sites with different specificities have been described in HSA: (i) the N-terminal site provided by Asp1, Ala2, and His3, (ii) the site at the reduced Cys34, (iii) site A, including His67 as a ligand, and (iv) the nonlocalized site B. HSA can bind CoII, and HSA was proposed to be involved in CoII transport. Although the binding of CoII to HSA is important, the binding of CoII to HSA is not well-characterized. Study conducted to determine the sites of binding of CoII to HSA revealed that the first two equivalents of CoII bind to sites A and B. Only the third may be bound to the N-terminal site.[(11)]

Under normal conditions of humans, the levels of IMA range from 1% to 2% of the total concentration of albumin, whereas it rises from 6% - 8% in case of ischemia patients. The rise in the levels of IMA could

initially be positive within 6-10 min post ischemia and remain identifiable for up to six hours[12].

Modification of binding site of albumin is likely to be the result of decrease in pH, reduced oxygen tension, and increased generation of free radicals[13]. It is also postulated that the generation of free radicals, development of acidosis, anaerobic metabolism of glucose to lactate and release of free iron and copper ions in ischemia, would result in a change in the ability of the N terminus of albumin to bind transition metal ions[10]. Based on the above literature, our study was designed to look into the relation between levels of pH and levels of IMA in acidosis and alkalosis patients.

Materials and Methods

This study was conducted at Father Muller Medical College Hospital Laboratory, Clinical biochemistry, Mangalore for duration of 6 months from January to June 2015.

Collection of samples:

Arterial blood sample was collected from in-patients in heparinised tubes and ABG analysis was done immediately in the clinical lab. Plasma was then separated by centrifugation and stored at -20^0 C until used for analysis of IMA.

Inclusion criteria: The samples were separated under different groups as

Group A- Normal pH (7.35-7.45),

Group B- Acidosis (<7.35 pH),

Group C- Alkalosis (>7.45 pH).

Exclusion criteria: Venous blood samples, Haemolysed sample, inadequate sample, pregnant women, ischemic cardiac disease, liver cirrhosis.

Procedure to Estimate Ischemia Modified Albumin: (Albumin Cobalt binding method)

The Ischemia modified albumin was estimated by using Albumin Cobalt binding procedure. 200µL patient sample transferred into glass tubes and 50µL 0.1%CoCl2 added. After gentle shaking, the mixture then incubated for 10 minutes to ensure that sufficient cobalt albumin binding occurred. To this, 50µL 1.5mg/mL dithiothreitol (DTT) added as a colouring agent. After exactly 2 minutes, 1mL 0.9% NaCl was added to halt binding between the cobalt and albumin. Similarly blank was even prepared for every specimen: at the DTT-addition step, 50µL distilled water was added to obtain a blank without DTT. The absorbance was recorded at 470 nm with Shimadzu 1600 spectrophotometer. Colour formation in the specimens with DTT was compared with the colour formed in blank tube, and the results are expressed as absorbance units (ABSU).

Levels of Lactate, pH, and Ionised calcium were measured by the Gem Premiere 3000 blood gas analyser. The serum levels of albumin, LDL-C, HDL-C, TG were estimated in Hitachi COBAS-6000 auto-analyser.

Statistical analysis: The statistical analysis was done using SPSS version 16. ANOVA study was used for the group mean comparison and deriving the significance and the correlation between the groups was done.

Results

The study consisted of total 201 participants, including 100 men (49.75%) and 101 women (50.25%). Age of the subjects included in the study ranged from 1 to 88 years with the mean age of 49 years. Categorization of Study Subjects is given in Table 1.

Table 1: Table showing categories of patients based on sex and the mean age within each of the groups

Group	No. of subjects	Males	Females	Age group in years
Normal pH (7.35 – 7.45)	88	45	43	47±21 (1 – 81)
Acidosis (< 7.35)	70	35	35	46±26 (1 – 88)
Alkalosis (> 7.45)	43	20	23	51±18 (1 – 77)

Table 2: Correlation between IMA levels and pH levels in acidosis patients

Correlations			
		Acidosis pH	**Acidosis IMA**
Acidosis pH	Pearson Correlation	1	-.096
	Sig. (2-tailed)		.430
	N	70	70
Acidosis IMA	Pearson Correlation	-.096	1
	Sig. (2-tailed)	.430	
	N	70	70

Table 3: Correlation between IMA levels and pH levels in alkalosis patients

Correlations			
		Alkalosis IMA	**Alkalosis pH**
Alkalosis IMA	Pearson Correlation	1	.234
	Sig. (2-tailed)		.141
	N	41	41
Alkalosis pH	Pearson Correlation	.234	1
	Sig. (2-tailed)	.141	
	N	41	41

In this study, IMA level was found increased in patients with pH values outside the normal range. Albumin is a protein and its conformation depends on pH of the medium. Alteration in pH brings about conformational changes in the Albumin structure which might alter metal binding ability of Albumin.[14]

For the formation of IMA early after cardiac ischemia-reperfusion, different mechanisms 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. Decreased supply of oxygen to myocardial cells due to occlusion of coronary artery leads to biochemical and metabolic changes in myocardial tissue. In the absence of oxygen, production of lactate is increased due to metabolism of glucose resulting in reduction in intracellular pH.

Discussion

Albumin is one of the important transport proteins involved in transport of many ligands including fatty acids, unconjugated bilirubin, small drugs and certain metals like cobalt, zinc, nickel etc.[6-8] The diagnostic albumin Co2+ binding (ACB) test used for IMA is based on the observation that the affinity of serum albumin for Co2+ is reduced due to N terminal modifications. In addition to these reactive species some physiological compounds like glucose also are found to cause modifications of albumin.[5]

Decreased supply of oxygen to myocardial cells due to occlusion of coronary artery leads to biochemical and metabolic changes in myocardial tissue. Production of lactate is increased due to metabolism of glucose in the absence of oxygen resulting in reduction in intracellular pH.[9]

It is therefore postulated that in ischemia, generation of free radicals development of acidosis, anaerobic metabolism of glucose to lactate and release of free iron and copper ions would result in a change in the ability of the N terminus to bind transition metal ions.[10]

Though pH is reduced during hypoxia, it is not clear whether it has any direct role to play in the formation of IMA or not. Increased amounts of IMA result in less cobalt binding and more residual unbound cobalt available for complex with a chromogen (dithiothreitol), which can be measured photometrically. This is the basis of the albumin cobalt-binding (ACB) test. During ischemia, the N-terminus of albumin is altered, possibly as the result of hypoxia, acidosis, free-radical injury and energy-dependent membrane disruption, decreasing its binding capacity for metals[10].

There are studies which demonstrate the link between changes in pH levels and various changes happening due to these changes in the levels of pH. In a study by kurtin and group, it was suggested that there is a change in the intracellular pH levels which is also linked to alterations in the ion transport in the intestines[15]. It is also shown that in respiratory acidosis and alkalosis, there is a change observed in the intracellular pH of brain[16]. Variation in the levels of pH is also shown to have its effects at the intracellular levels in organs such as pancreas[17].

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Conclusion

There is a correlation seen between the pH levels in acidosis and alkalosis with that of the levels of IMA. Even though there is no significance observed with this correlation, it could possibly be attributed to the sample size itself and other minor variations in the normal, alkalosis and acidosis patients.

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