Journal of Clinical Laboratory Analysis 24 : 273–277 (2010)

Ischemia-Modified Albumin Levels in Patients With End-Stage Renal Disease Patients on Hemodialysis: Does Albumin Analysis Method Affect Albumin-Adjusted Ischemia-Modified Albumin Levels?

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Ischemia-Modified albumin (IMA) has been used as an early marker in the evaluation of the patients with acute coronary syndrome. We aimed to evaluate IMA in end-stage renal disease (ESRD) patients on hemodialysis and the effect of albumin methods on albumin-adjusted IMA levels. A total of 30 ESRD patients were included in this study. Serum IMA and albumin levels were measured before and after a hemodialysis session. Albumin concentrations were determined with bromocresol green and bromocresol purple methods. Postdialysis IMA and albumin-adjusted IMA levels with two different albumin methods were significantly increased compared with the predialysis levels ($P<0.05$). However, we did not find any difference in albumin-adjusted IMA levels in either at the beginning or at the end of the dialysis session. IMA levels increase after hemodialysis, whereas albumin method has no effect on albumin-adjusted IMA levels. J. Clin. Lab. Anal. 24:273–277, 2010.

Key words: Ischemia-Modified albumin; Bromocresol green; Bromocresol purple; end stage renal disease; hemodialysis

INTRODUCTION

Ischemia-Modified albumin (IMA) was introduced as a novel serum biomarker of myocardial ischemia in the early 2000s by Bar-Or et al. (1). Albumin cobalt binding test (ACB), which was developed for measurement of serum IMA concentrations, is based on the principle that the *N*-terminal region of human serum albumin and its affinity for the metal ion, Co(II). Serum albumin of patients with myocardial ischemia exhibited reduced binding to Co (II) compared with serum albumin of nonischemic ones. More free cobalt exists in the reaction mixture to react with dithiothreitol (DTT) because of the ischemic modification in the albumin molecule and consequently a darker color is formed. Suggested underlying mechanisms of this modification in albumin structure are free radical damage, hypoxia, acidosis, sodium and calcium pump disruptions, and free iron and copper ion exposures. However, the exact biochemical mechanism is still unclear. But all of these events involve in myocardial ischemia—reperfusion conditions (2,3). The value of the ACB test in acute coronary syndromes was further validated in a meta-analysis (4). However, afterwards serum IMA levels were found to be increased in ischemic situations other than myocardial ischemia. Skeletal muscle ischemia, stroke, and pulmonary thromboembolism are examples of these nonmyocardial ischemic conditions (5–7). Recently IMA was considered to be related with increased oxidative stress and as a potential cardiovascular risk factor; therefore, the researchers tended to demonstrate a possible relation between IMA and the other cardiovascular risk factors and some diseases with higher incidences of vascular events (8–12).

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Received 25 March 2010; Accepted 1 June 2010
DOI 10.1002/jcla.20399
Published online in Wiley InterScience (www.interscience.wiley.com).

It was previously reported that patients with chronic renal failure were more prone to coronary artery disease and cardiovascular events are the leading cause of deaths in this population. However, these events are more frequent in end-stage renal disease (ESRD) patients on hemodialysis (13,14).

In recent studies some investigators concluded that IMA results should be interpreted considering albumin concentrations of the samples used (15–19). There are also reports about discrepancies between albumin results of dye-binding methods in ESRD patients (20–24). Therefore, it can be thought that if the adjustment due to albumin concentration is necessary while interpretation of IMA results, the method used for determination of albumin levels may affect these values, especially in ESRD patients. So, we aimed to evaluate IMA levels in ESRD patients on hemodialysis and the impact of albumin analysis method on albumin-adjusted IMA levels.

METHODS

Patients

A total of 30 ESRD patients who were on maintenance hemodialysis programme for at least 2 years were included in this study. Patients with positive history of cardiovascular events, other acute or chronic ischemic conditions such as stroke, transient ischemic attack, claudication, peripheral vascular disease, shock, and pulmonary diseases were excluded. Diabetes mellitus and smoking were the other exclusion criteria. Patients received no other medications except vitamins, phosphate binders, and calcitriol. In hypertensive hemodialysis patients, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, or combined therapy were given; all patients were on erythropoietin treatment. Hemodialysis session duration was approximately four hours and it was applied three times a week using unfractionated heparin for anticoagulation. The study protocol was approved by the Local Ethics Committee, and informed consent was given by all the subjects.

Blood Collection

Fasting blood samples were drawn immediately before (pre-D) and after the end of the hemodialysis session (post-D) by antecubital venipuncture. Blood samples were collected directly into serum separator tubes (Becton-Dickinson, Oxford, UK). After coagulation, centrifugation process at 1,500 g for 10 min was performed. Sera were separated, stored in aliquots, and kept frozen at –70°C until analysis.

Biochemical Analysis

Urea, creatinine, and high sensitive C-reactive protein levels were measured on Synchron LX20 system (Beckman Coulter, Fullerton, CA, USA) with the original Beckman reagents. IMA levels were determined by a colorimetric assay described by Bar-Or et al. previously (1). Albumin analysis was performed by bromocresol green (BCG) (Trace, ThermoScientific, Fremont, CA, USA) and bromocresol purple (BCP) (Beckman Coulter, Fullerton, CA, USA) methods simultaneously.

The formula suggested by Lippi et al. was applied to correct IMA values for serum albumin. It is as follows: [(individual serum albumin concentration/median albumin concentration of the population) × IMA value] (19).

Statistical Analysis

SPSS (version 13.0) was used for the statistical evaluation of the data. Distribution characteristics of the variables were tested with Shapiro Wilk test. Paired *t*-test was used for comparison of predialysis and postdialysis albumin, IMA, and adjusted IMA levels. Results were given as mean ± SD. Pearson correlation analysis was performed for correlations between variables. $P < 0.05$ was considered as significant.

RESULTS

Demographic characteristics and biochemical results of the subjects were presented in Table 1.

Predialysis albumin concentrations determined by both methods were significantly lower than the postdialysis concentrations ($P < 0.05$). Predialysis albumin levels by BCP method were significantly lower than those of BCG ($P < 0.05$). However, there was no significant difference between BCG and BCP methods in postdialysis albumin measurements ($P > 0.05$) (Fig. 1).

Postdialysis IMA levels were significantly higher than those of predialysis ($P < 0.05$) (Fig. 2).

IMA values were corrected due to albumin concentrations by BCG or BCP methods and adjusted IMA values with both methods increased after hemodialysis ($P < 0.05$). But there was no significant difference between adjusted IMA levels due to albumin corrections by BCG or BCP methods in both predialysis and postdialysis measurements ($P > 0.05$)(Fig. 3).

DISCUSSION

A few numbers of researches was performed on chronic renal failure patients, concerning IMA as an oxidative stress marker or a cardiovascular risk factor. IMA was evaluated as a myocardial ischemia and prognostic marker in patients with ESRD. A total of

TABLE 1. Demographic Characteristics, Serum Albumin and Ischemia-Modified Albumin Levels of the Subjects

Parameter		Predialysis	Postdialysis
Age (years)		57.33±14.57	
Gender		Male 15	
		Female 15	
Albumin(g/dl)	BCG	3.9±0.27[a]	4.2±0.72[b]
	BCP	3.6±0.32[c]	4.2±0.51[d]
IMA (ABSU)		0.283±0.087	0.357±0.083[e]
Adjusted IMA (ABSU)	BCG	0.276±0.076[f]	0.349±0.125[g]
	BCP	0.274±0.074[h]	0.349±0.113[i]

[a] $P = 0.013$, pre-D vs. post-D albumin concentrations with BCG method.
[b] $P > 0.05$, post-D albumin concentrations with BCG vs. BCP.
[c] $P = 0.0001$, pre-D albumin concentrations with BCG vs. BCP.
[d] $P = 0.0001$, pre-D vs. post-D albumin concentrations with BCP method.
[e] $P = 0.004$, pre-D vs. post-D IMA levels.
[f] $P = 0.003$, pre-D vs. post-D albumin adjusted IMA levels with BCG.
[g] $P > 0.05$, post-D albumin adjusted IMA levels with BCG vs. BCP.
[h] $P > 0.05$, pre-D albumin adjusted IMA levels with BCG vs. BCP.
[i] $P = 0.003$, pre-D vs. post-D albumin adjusted IMA levels with BCP.

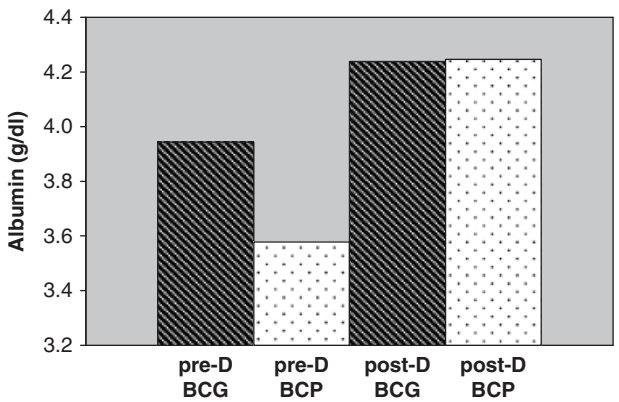

Fig. 1. Predialysis and postdialysis albumin concentrations with BCG or BCP methods.

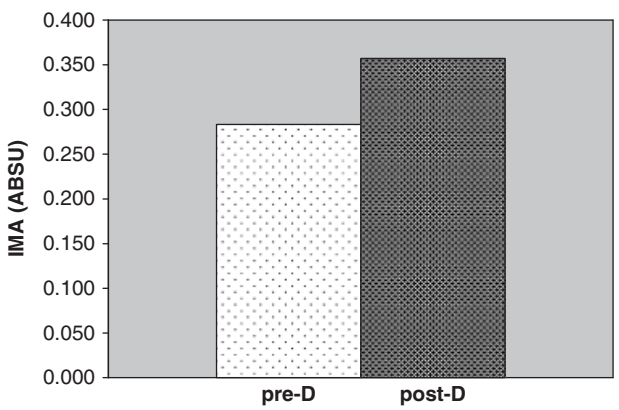

Fig. 2. Predialysis and postdialysis ischemia-modified albumin concentrations.

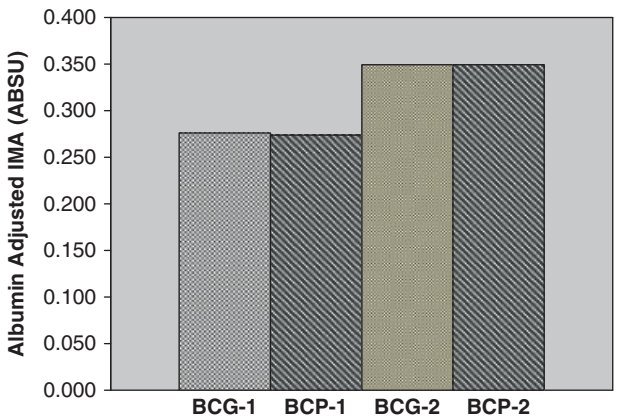

Fig. 3. Predialysis and postdialysis albumin-adjusted ischemia-modified albumin concentrations with BCG or BCP methods.

114 renal transplant candidates were studied prospectively. They concluded that IMA was a moderately accurate marker of myocardial ischemia in ESRD (25,26). In another study, IMA levels were determined in patients with anemia due to chronic kidney disease and healthy controls. Increased IMA levels were observed in chronic kidney disease group compared with controls and IMA showed strong correlations with hemoglobin, creatinine, and lactate levels (27). Conversely, Carrega et al. concluded that under basal conditions, the IMA to Alb ratio was not significantly different in patients with ESRD on hemodialysis and controls and hemodialysis did not significantly modify this ratio (28). Another research group reported that IMA levels were above the diagnostic cut-off value

(85 kU/l), during and at the end of the HD session (29). It was demonstrated by Montagnana et al. that predialysis levels of serum IMA were above the diagnostic threshold and significant increase was observed after the dialysis session (30).

In our study, postdialysis IMA levels were significantly higher than the predialysis levels, similarly. Our results are in agreement with the data of Montagnana et al. Although IMA levels show a negative correlation with albumin concentrations and predialysis albumin levels were significantly lower than they were in postdialysis, we think that this elevation in IMA levels of postdialysis phase resulted from the oxidative stress induced by hemodialysis process rather than low albumin concentrations. Because our results indicated that albumin-adjusted IMA levels were also significantly higher in postdialysis measurements than in predialysis.

Serum albumin concentration is used as a prognostic marker in hospitalized patients especially in ESRD patients on hemodialysis (31). It was reported in 1980s that the major problem of BCG methods is nonspecificity, especially in samples with low albumin/globulin ratio; then it was suggested that this nonspecific binding would be minimized by decreasing the reaction time (32,33). A toxic substance, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), present in the plasma of the uremic patients and which can not be cleared from the plasma with dialysis was suggested as the inhibitor of BCP binding to serum albumin and responsible for underestimation of albumin concentrations (21). Later it was concluded that the problem was hemodialysis procedure itself but not the ESRD. The researchers reported that BCP method quantified accurately the albumin concentration in serum of patients with chronic renal failure being treated with continuous ambulatory peritoneal dialysis or in serum of patients showing increased serum creatinine concentrations but not in plasma of patients with ESRD being treated by hemodialysis (22). However, Carfray et al. concluded that BCP method showed a better correlation with immunoturbidimetric method in hemodialysis patients than BCG (23). In another study, BCG, BCP, and nephelometric methods were used for determination of albumin concentrations in chronic renal failure patients on hemodialysis and peritoneal dialysis and also in healthy individuals. They claimed that BCP method was in good agreement with nephelometry in healthy subjects; however, albumin results of BCG method were closer to nephelometric measurements in both HD and PD patients (24).

We also demonstrated that albumin concentrations were significantly lower with BCP compared with BCG. But this difference was seen only in predialysis measurements. So our results dealing with albumin levels in patients on hemodialysis are again in agreement with the previous findings (20,21). However, we believe that the underlying mechanism of the disagreements between albumin concentrations measured by BCP or BCG methods in these patients is still not clear. We observed a significant difference between albumin concentrations determined by these two methods in predialysis phase but not in postdialysis. So we propose that this might be resulted from an interference of uremic toxins to BCP binding as suggested by Mabuchi, but reversely we suggest that they are removed by dialysis. Our other hypothesis is that BCG binds nonspecifically to other small plasma proteins which are also removed by dialysis. This can be fully proved by measuring these plasma proteins in either predialysis or post dialysis samples.

In previous reports, strong negative correlation was demonstrated between IMA levels and albumin concentrations (15–17). Therefore, Lee et al. suggested an 'albumin-adjusted IMA index' for ACB test which uses the U/l unit for assignment of IMA results (18). Lippi et al. proposed another formula which is suitable for albumin adjustment of IMA results with absorbance units (ABSU) (19). We used the formula of Lippi et al. and albumin-adjusted IMA levels with both of the albumin methods were also higher in postdialysis than the predialysis measurements. Although, our albumin concentrations were underestimated with BCP method compared with the BCG method in predialysis samples, there was no significant difference between adjusted IMA values with BCG or BCP in either predialysis or postdialysis samples.

CONCLUSION

We can suggest that both IMA and albumin-adjusted IMA levels increase after hemodialysis process; however, albumin-adjusted IMA levels do not change with the albumin method used whether it is BCG or BCP.

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