ORIGINAL ARTICLE

Ischemia-Modified Albumin Levels in Children with Chronic Liver Disease

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Background/Aims: Ischemia-modified albumin (IMA) levels have been shown to correlate with the severity of liver failure in adults. However, the role of IMA levels has not been evaluated in children with chronic liver disease (CLD). We analyzed the clinical significance of IMA levels in children with CLD. **Methods:** Thirty-three children with CLD and 33 healthy children were included in the study. Blood was collected to analyze biochemical parameters, oxidant status, and IMA. Liver biopsies were re-evaluated for liver fibrosis; severe fibrosis (SF) was defined as fibrosis stage ≥ 4. **Results:** The IMA and and IMA to albumin ratios (IMARs) were significantly higher in children with CLD than in those without (IMA: 0.545±0.095 vs 0.481±0.062, p=0.003; IMAR: 0.152±0.046 vs 0.126±0.018, p=0.04). The IMAR was positively correlated with the pediatric end-stage liver disease score (p=0.03, r=0.503) and fibrosis score (p=0.021, r=0.400). Patients with SF had higher IMARs compared to patients with mild fibrosis (0.181±0.056 vs 0.134±0.025, p=0.003). The area under the receiver operation curve (AUROC) for predicting SF was 0.78 (p=0.006). Using a cutoff ratio value of 0.140, the sensitivity and specificity were 84% and 70%, respectively. The AUROC for predicting the need for liver transplantation and/or death was 0.82 (p=0.013). With a cutoff value of 0.156, the sensitivity and specificity was 83% and 82%, respectively. Kaplan-Meier analysis revealed increased morbidity and/or mortality in the group with an IMAR>0.156 (50% vs 4.3%, p=0.005). **Conclusions:** IMARs have been shown to provide important clues in predicting the fibrosis stage of the disease and determining the outcome in children with CLD. **(Gut Liver 2012;6:92-97)**

Key Words: Ischemia modified albumin; Chronic liver disease; Children

INTRODUCTION

Albumin is the major plasma protein that is primarily synthesized in the liver.[1] Its concentration is reduced in patients with chronic liver failure, and with the advanced cirrhosis the effective plasma volume decreased as a result of hypoalbuminemia and splenic vasodilatation. Albumin is mainly used in patients with chronic liver diseases (CLD) to replenish the circulating volume, to prevent the post-paracenthesis circulatory disturbance and to reduce mortality associated with spontaneous bacterial peritonitis.[2,3]

The amino terminal end of albumin molecule appears to be the primary site for binding transition metal ions, cobalt and nickel. This site of the albumin is susceptible to biochemical degradation, and is less stable under environmental changes.[4] Under various conditions such as ischemia, hypoxia, acidosis and superoxide-radical injury, the binding capacity of albumin for metals is decreased and generates a metabolic variant of protein with reduced transition metal binding. This change is quantifiable and commonly known as ischemia modified albumin (IMA).[4-7]

High serum IMA level has been proposed as a sensitive marker for the diagnosis of myocardial ischemia before the onset of irreversible cardiac injury in patients presenting with typical acute chest pain.[7] However, IMA level is also known to increase in other ischemic conditions, and in diseases such as advanced cancer, systemic sclerosis, intrauterine disorders and end-stage renal disease due to free radical productions.[7,8] Elevated IMA level has been used to assess the subclinical vascular disease in patients with type 2 diabetes mellitus.[9]

Recently, albumin functions have been studied in patients with decompensated cirrhosis in an adult study; and found that albumin functions are compromised in advanced cirrhosis. Ad-

ditionally, they claimed that serum IMA level (expressed as an albumin ratio and used as a marker of albumin function) correlate with the disease severity and may have prognostic use in acute-on-chronic liver failure.[10] However, the role of IMA has not been evaluated in children with CLD. Therefore, we aimed to analyze 1) serum IMA level, 2) their clinical significance, 3) correlation with laboratory findings including liver function tests and oxidant status, 4) association with liver histology, and 5) impact on outcome in children with CLD.

MATERIALS AND METHODS

Thirty-nine consecutive children with newly diagnosed CLD were enrolled into the study. The diagnosis of CLD was based on clinical, laboratory, radiological, and histopathological findings. Patients were excluded if they received albumin infusion within the 1 month prior to entry in the study, had clinical or laboratory findings of infection and hepatorenal syndrome, had hepatic/extrahepatic malignancy and had vascular disease or thrombosis (n=6). Finally 33 children (mean age, 9.4±5.4 years and 17 F, 16 M) with chronic liver disease were included in the study, and 33 age and sex matched healthy children were (mean age, 9.3±5.5 years and 17 F, 16 M) served as control group. Twenty-one children (63.6%) had metabolic liver diseases including Wilson's disease in 11, glycogen storage disease in 5, tyrosinemia in 2 and other metabolic liver diseases in 3. Six patients (18.1%) had cholestatic liver diseases, 3 (9.1%) had autoimmune liver diseases and 3 (9.1%) had cryptogenic cirrhosis (Table 1). Three patients had acute-on-chronic liver failure due to acute variceal bleeding in two and large volume paracenthesis in the other.

Peripheral venous blood of the participants was collected from the subjects at the time of inclusion into the study and used for routine biochemical parameters, oxidant status, and IMA. Routine biochemical parameters including total protein, albumin, creatinine, liver enzymes, bilirubin and international normalized ratio (INR) levels were studied enrolment. The rest of the plasma stored at -80°C for studying to IMA levels and oxidant status at the end of the study. Clinical findings of the patients including presence of malnutrition, portal hypertension, ascites and encephalopathy were recorded, and Child-Pugh score and pediatric end-stage liver disease (PELD) score were calculated.[11,12]

IMA level (reduced cobalt to albumin binding capacity) was analyzed using the rapid and colorimetric method developed by Bar-Or et al.[5] Two hundred microliter (μL) of patient serum was placed into glass tubes and 50 μL of 0.1% CoCl$_2$+6H$_2$O (Sigma) added. After gentle shaking, the mixture was left undisturbed for 10 minutes to ensure sufficient cobalt albumin binding. Then, 50 μL of 1.5 mg/mL dithiothreitol (DTT) was added as a coloring agent. After 2 minutes, 1 mL of 0.9% NaCl was added to quench the reaction. A control sample was prepared for every sample. At the DTT addition stage, 50 μL of distilled water was used instead of 50 μL of 1.5 mg/mL DTT to obtain a control sample without DTT. Sample absorbencies were analyzed at 470 nm using a spectrophotometry (Shimadzu UV1601; Shimadzu, Tokyo, Japan). Color formation in specimens with DTT was compared with color formation in the control tubes, and the results were expressed as absorbance units.

Total oxidant status (TOS) of serum was determined using a novel automated measurement method as previously described.[13] Serum TOS levels were calculated in μmol H$_2$O$_2$ equivalent/L. Total antioxidant status (TAS) of the serum was determined using a novel-automated measurement method, developed by Erel.[14] Serum TAS levels were calculated in mmol Trolox equivalent/L. The TOS/TAS ratio was used as the oxidative stress index (OSI). To perform the calculation, the units of TAC, mmol Trolox equivalent/L, was converted to mmol Trolox equivalent/L, and OSI was calculated as follows: OSI=[(TOS, μmol H$_2$O$_2$ equivalent/L)/(TAS, μmol Trolox equivalent/L)×100].

Table 1. Demographic, Clinical, and Laboratory Findings in Patients with CLD and in the Control Group

Parameter	Patients with CLD (n=33)	Control group (n=33)
Age, yr	9.4±5.4	9.3±5.5
Gender, F/M	17/16	17/16
Primary diagnosis		
Metabolic liver disease	21 (63.6)	
Cholestatic liver disease	6 (18.1)	
Autoimmune hepatitis	3 (9.1)	
Cryptogenic cirrhosis	3 (9.1)	
Encephalopathy	5 (15.1)	
Ascites	4 (12.1)	
Portal hypertension	6 (18.1)	
Malnutrition	8 (24.2)	
Child-Pugh score (A, B, C)	22, 6, 5 (66.7, 18.1, 15.2)	
PELD score (range)	6.6±12.1 (-8 to 43)	
Fibrosis score	2.7±1.8	
Severe fibrosis	13 (39.4)	
Fatty changes	12 (36.3)	
ALT, IU/L	240.5±340.6	N/A
Albumin, mg/dL	3.6±0.5*	3.8±0.4[†]
Hypoalbuminemia, <3.5 mg/dL	8 (24.2)[‡]	1 (3)[§]
Bilirubin, mg/dL	2.7±4.5	N/A
TAS, mmol Trolox equiv./L	19.6±12.2	N/A
TOS, μmol H$_2$O$_2$ equiv./L	7.7±6.9	N/A
OSI, arbitrary unit	0.7±1	B/A

Data are presented as mean±SD (range) or number (%).
CLD, chronic liver disease; PELD, pediatric end-stage liver disease; ALT, alanine aminotransferase; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; N/A: non available.
*,[†] p=0.039; [‡,§]p=0.013.

Liver biopsies of the patients were re-evaluated for the liver fibrosis by the pathologist (U.C) according to modified ISHAK score (0 to 6) as defined by Standish et al.[15] Severe fibrosis was defined as fibrosis stage ≥4. Fatty changes were classified according Schwimmer et al.[16] In all patients with CLD, percutaneous liver biopsy was done.

Serum IMA and IMA/albumin ratio (IMAR) were compared between the patients and control group. Then, clinical and laboratory factors that may affect the serum IMA and IMAR levels were evaluated in children with CLD. IMAR is used in order to eliminate the effect of reduced albumin concentrations, common in children with CLD, on serum IMA level. We found out if there was correlation between histopathological findings and serum IMAR levels. Additionally, we also analyzed the effect of serum IMAR level on the outcome of the patients.

Informed consent for participating in the study was obtained from parents of all cases and the ethics committee of the Karadeniz Technical University Faculty of Medicine, approved this study.

All the data were described as mean and standard deviation. Differences between groups were calculated using an independent samples t-test for the normally distributed data and the Mann-Whitney test for data not normally distributed. Correlations between variables were calculated using linear regression. The area beneath the receiver operating characteristics curves was used to discriminative power of the IMA, IMAR in the diagnosis of severe fibrosis. Values of p<0.05 were considered significant.

RESULTS

Serum IMA and IMAR levels were significantly high in children with CLD compared to healthy controls (0.545±0.095 vs 0.481±0.062, p=0.003; and 0.152±0.046 vs 0.126±0.018,

p=0.04, respectively) (Table 2). No correlation was determined between serum IMA level and age (p=0.845, r=0.341), and no difference was established between the genders. Additionally, no correlation was determined between albumin and IMA level (p=0.339, r=-0.123).

Clinical findings and their relation with serum IMAR level were evaluated and found that patients with portal hypertension (n=6), encephalopathy (n=5), cholestasis (n=11), and malnutrition (n=8) had higher IMAR level than the others (0.230±0.044 vs 0.135±0.023, p=0.0001; 0.229±0.049 vs 0.139±0.029, p=0.0001; 0.183±0.061 vs 0.137±0.026, p=0.004; 0.197±0.059 vs 0.136±0.026, p=0.003, respectively).

Correlation of serum IMAR level with the laboratory findings is shown in Table 3. No correlation was found between serum alanine aminotransferase (ALT), INR, TAS, TOS, and OSI levels and serum IMAR level. Only bilirubin level was significantly correlated with serum IMAR level (p=0.003, r=0.503).

Serum IMAR level was significantly high in patients with Child-Pugh score B and C compared to patients with Child-Pugh score A (0.185±0.059 vs 0.136±0.027, p=0.002). IMAR level was also positively correlated with PELD score (p=0.03, r=0.503) (Fig. 1).

Table 3. The Correlation of IMARs with the Laboratory Findings

Parameter	IMAR level	
	r	p-value
ALT	-0.121	0.502
Bilirubin	0.503	0.003
INR	0.561	0.413
TAS	-0.065	0.744
TOS	-0.155	0.390
OSI	-0.008	0.966

IMAR, ischemia modified albumin ratio; ALT, alanine aminotransferase; INR, international normalized ratio; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; r, correlation coefficient.
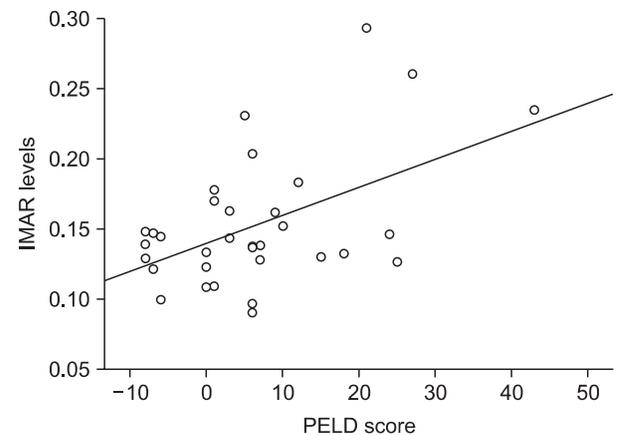
Table 2. The Serum IMA Levels and IMARs in Children with CLD and in the Control Group

Parameter	Patients with CLD (n=33)	Control group (n=33)	p-value
IMA (ABSU)			
Mean	0.545	0.481	
Median	0.550	0.480	0.003
SD	0.095	0.062	
Range	0.330-0.755	0.323-0.595	
IMAR			
Mean	0.152	0.126	
Median	0.139	0.127	0.004
SD	0.046	0.018	
Range	0.09-0.290	0.09-0.170	

CLD, chronic liver disease; IMA, ischemia modified albumin; ABSU, absorbance unit; SD, standard deviation; IMAR, ischemia modified albumin ratio.

Fig. 1. The correlation of serum ischemia modified albumin ratios (IMARs) with the pediatric end-stage liver disease (PELD) score (p=0.03, r=0.503).

IMAR level was positively correlated with fibrosis score (p=0.021, r=0.400), and patients with severe fibrosis (n=13) had higher IMAR level compared to patient with mild fibrosis (n=20) (0.181±0.056 vs 0.134±0.025, p=0.003). The area under the receiver operation curve (AUROC) for predicting severe fibrosis was 0.78 (p=0.006; 95% confidence interval [CI], 0.61 to 0.95) (Fig. 2). With a cutoff ratio value of 0.140, the sensitivity and specificity were 84% and 70%, respectively. Other biochemical parameters such as serum ALT, bilirubin, TAS, and TOS levels did not showed any significantly difference between the patients with severe and mild fibrosis. On the other hand, no significant difference was determined in serum IMAR level between the patients with (n=12, 36.3%) and without (n=21, 63.7%) fatty changes (0.165±0.066 vs 0.145±0.029, p=0.245).

In long term follow-up (14.2±7.2 months); 5 (15.1%) of the 33 patients underwent liver transplantation, and 1 patient (3%) died without liver transplantation. Comparison of these 6 patients with the other patients revealed higher IMAR level (0.209±0.063 vs 0.140±0.031; p=0.011). The AUROC for predicting need for liver transplantation and/or death was 0.82 (p=0.013; 95% CI, 0.60 to 1.04) (Fig. 3). With a cutoff value of 0.156, the sensitivity and specificity were 83% and 82%, respectively. Kaplan-Meier analysis revealed increased morbidity and/or mortality in the group with an IMAR>0.156 (50% vs 4.3%, p=0.005) (Fig. 4). The AUROC for predicting need for liver transplantation and/or death for PELD score and Child-Pugh score was 0.84 and 0.62, respectively.

DISCUSSION

This is the first study that evaluates the serum IMA and IMAR levels in patients with pediatric CLD, and revealed that IMA and IMAR levels were higher in these patients compared to healthy subjects. Elevation of IMAR, a marker of albumin function, and impairment of liver functions in patients with CLD not only decreases the effective circulation volume in association with hypoalbuminemia, but also declines the removal of toxic metabolites due to impairment in albumin functions.

Higher level of IMAR in decompensated cirrhotic children (with cholestasis and portal hypertension) may contribute to accumulation of toxic metabolites (tryptophan, endogenous benzodiazepines, bile acids, and fatty acids) in these patients, and may aggravate the development of hepatic encephalopathy. In addition, despite the low number of cases, IMAR level was higher in patients with encephalopathy compared to the other patients. Treatment regimens that aim to improve the albumin functions in patients with hepatic encephalopathy may be effective on the clinical improvement and outcome. However, Jalan et al.[10] demonstrated that the application of Molecular Adsorbents Recirculating System had no effect on albumin functions
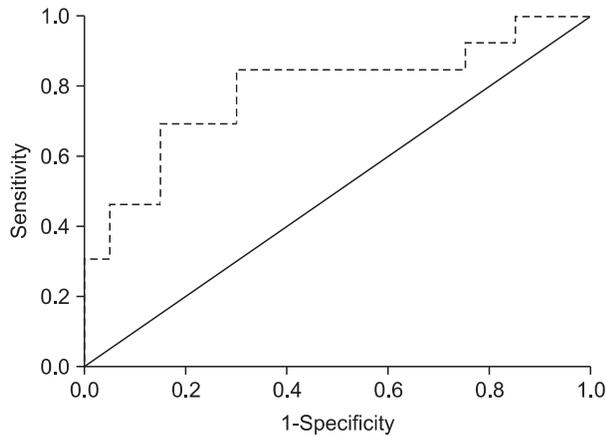

Fig. 2. The receiver operation curve of serum ischemia modified albumin ratio (IMAR) to examine its predictive use for assessing severe fibrosis (area under the receiver operation curve [AUROC], 0.78; p=0.006; 95% confidence interval, 0.61-0.95; best cutoff value, 0.140).
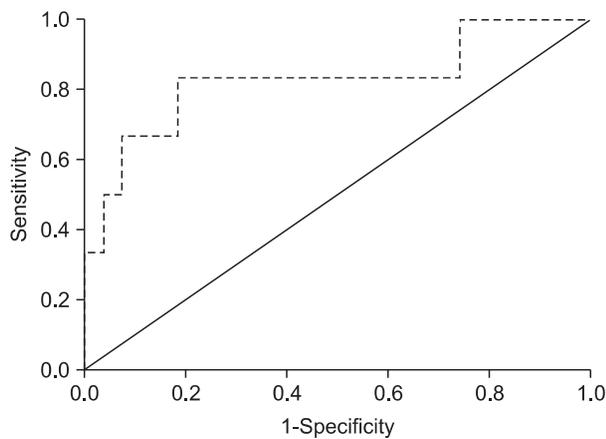

Fig. 3. The receiver operation curve of serum ischemia modified albumin ratio (IMAR) to examine its predictive use for assessing the need for liver transplantation and/or mortality (area under the receiver operation curve [AUROC], 0.82; p=0.013; 95% confidence interval, 0.60-1.04; best cutoff value, 0.156).
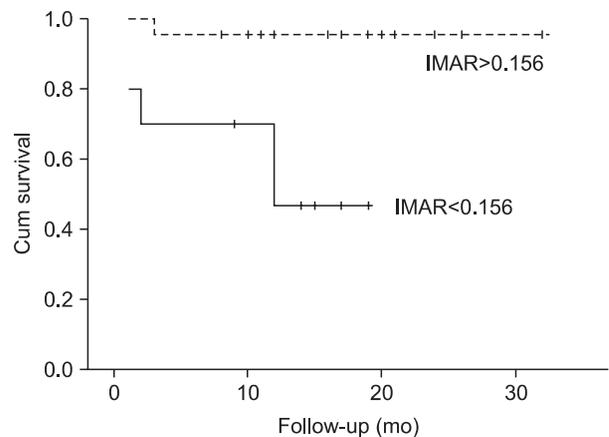

Fig. 4. The Kaplan-Meier analysis of outcomes based on a cutoff value of 0.156. Morbidity and/or mortality were significantly different between the children with chronic liver disease and the control group (50% vs 4.3%, p=0.005).

in patients with advanced cirrhosis and suggested that the impairment in albumin functions in such patients was irreversible.

IMAR level was observed to be correlated with Child-Pugh score and PELD score. Similar results were reported by Jalan et al.[10] and Klammt et al.[17] Apart from this, no correlation was determined between INR and serum IMAR level. Moreover, other clinical studies about IMA have suggested that elevated IMA is more associated with superoxide radicals forming as a result of increasing oxidizing capacity and decreasing antioxidizing capacity; but no correlation was determined between IMAR level and TAS and TOS in our study.[18] This shows that elevated IMAR level in CLD is more associated with primary liver function than micro-environmental changes.

One of the interesting results of our study is that there is a correlation between liver fibrosis and serum IMAR level. In addition, an IMAR level above 0.140 has been shown to be a good marker of severe fibrosis (fibrosis stage ≥ 4) (AUROC, 0.78; sensitivity and specificity were 84% and 70%, respectively). In recent years, the usefulness of non-invasive markers for determining liver fibrosis has been investigated because of complications, albeit rarely, following liver biopsy and difficulties in determining whole fibrosis with a little biopsy material. These have included radiological methods (FibroScan, Echosens, Paris, France; and MR elastography), indirect serological markers (AST/ALT ratio, AST/platelet ratio, Prothrombin, γGT, and Apolipoprotein A1 (PGA) index, FibroIndex, FIB-4 index, Fibrotest, and ActiTest) and direct serological markers (matrix deposition and matrix degradation markers).[19] IMAR is correlated with hepatic disease severity, not affected by other parameters, cost-effective and measured easily made it acceptable as a fibrosis marker. But this usefulness will become clearer with further studies with larger numbers of patient group with homogeneous etiology.

Another result of our study is that serum IMAR level is a good predictor of need for liver transplantation and/or death even in metabolic liver diseases. The most important parameter on this subject is PELD score, but there are some problems about the usefulness of PELD especially in patients with liver tumors, metabolic liver diseases and patients with malnutrition.[20] The usefulness of IMAR on these subjects needs to be shown with wider studies using multiple serial measurements rather than a single measurement.

Limitations in our study are that 1) our patient group was small, 2) the primary etiology for the CLD was heterogeneous, 3) most of our study group had very early stage patients (Child-Pugh score A), and 4) a single measurement was used instead of serial measurements.

In conclusion, IMAR level is investigated for the first time in children with CLD, and has been shown to provide important clues in predicting the fibrosis stage of the disease, and determining the outcome. Our study suggests that albumin therapy in liver failure can be used not only as a volume replacement agent but also in the removal of toxic metabolites. Additionally, further prospective studies are needed with larger patient numbers to analyze the effect of liver transplantation on albumin functions in children with CLD.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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