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Serum ischemic modified albumin (IMA) concentration and IMA/albumin ratio in patients with hepatitis B-related chronic liver diseases

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Background/aim: Albumin is the most important protein synthesized by the liver. Posttranscriptional changes occur in the molecular structure of albumin due to various factors and isoforms arise. Ischemic modified albumin (IMA) is one such isoform. This study was conducted to evaluate serum IMA concentrations in patients with hepatitis B virus (HBV)-related chronic liver diseases.

Materials and methods: This study included 74 treatment-naive chronic hepatitis B patients, 25 patients with HBV-related cirrhosis, and 49 healthy controls. Serum IMA concentration was measured spectrophotometrically using the albumin cobalt binding test.

Results: The mean IMA concentrations in the chronic hepatitis B group and healthy controls were 0.33 ± 0.11 ABSU and 0.27 ± 0.70 ABSU, respectively, and the difference was statistically significant ($P < 0.001$). Mean IMA/albumin ratios (IMAR) in the chronic hepatitis B and control groups were 0.08 ± 0.04 and 0.06 ± 0.17 , respectively, and the difference was also statistically significant ($P < 0.001$). Higher serum IMA concentrations and IMAR were detected in patients with advanced fibrosis.

Conclusion: Serum IMA concentration and IMAR are increased in patients with HBV-related chronic liver diseases and IMA and IMAR are associated with the degree of liver fibrosis. IMA and IMAR may have potential use as noninvasive markers of fibrosis in chronic hepatitis B patients.

Key words: Chronic hepatitis B infection, fibrosis, ischemia-modified albumin, IMAR

1. Introduction

Hepatitis B virus (HBV) infection is an important health problem. The seroprevalence of hepatitis B surface antigen (HBsAg) was previously reported to be 3.61% globally (1). Approximately 600,000 people are estimated to die each year due to HBV-related diseases and their complications. Liver cirrhosis and hepatocellular carcinoma are the most important HBV-related complications (2). Assessment of the degree of necroinflammation and fibrosis in the liver is crucial for treatment decisions and follow-up of patients with HBV-related liver diseases. Hepatic fibrosis was formerly considered as an irreversible course, but data from recent studies suggest that fibrosis is a dynamic process and it may regress if the underlying insult is treated (3). As a consequence, the need for elucidating the changes in fibrosis during disease course in patients with chronic HBV infection has emerged, and this yielded studies focused on noninvasive methods to evaluate liver fibrosis

(4,5). Unfortunately, there is no optimal noninvasive marker to determine liver inflammation and fibrosis today, and liver biopsy still remains the gold-standard method (6). Although liver biopsy is generally considered to be safe, it is an invasive intervention and it may cause several life threatening complications like bleeding, pain, infection, and even death.

Albumin is the most important protein synthesized by the liver, and it is the most secreted protein in extracellular fluids, which constitutes 70% of plasma colloidal osmotic pressure. For this reason, albumin has a key role in fluid balance and distribution in the body (7). Moreover, albumin binds to many molecules (metals, drugs, fatty acids, etc.) in the circulation by various functional regions in its structure, taking roles in transportation, detoxification, and elimination of them from the body. The amino-terminal end of albumin has the capacity to bind metals like cobalt and nickel. Ischemia, hypoxia, increased

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free radicals, and acidosis associated with various diseases result in changes in the molecular structure of albumin, decreasing this metal ion-binding capacity. This new isoform of albumin is called ischemic modified albumin (IMA), and its serum levels can be measured (8–10). Serum IMA levels and IMA/albumin ratio (IMAR) were shown to increase in several diseases such as myocardial ischemia, acute stroke, muscle ischemia, and bowel ischemia (11–14). There are also studies in the literature reporting increased serum IMA concentrations and IMAR in chronic liver diseases of various etiologies (15–18).

In this study, we aimed to investigate serum IMA concentration and IMAR in cirrhotic and noncirrhotic chronic hepatitis B patients. Associations of IMA and IMAR with liver biopsy findings were also evaluated in order to determine the usability of IMA and IMAR as noninvasive markers of fibrosis levels in HBV-related liver diseases.

2. Materials and methods

Seventy-four chronic hepatitis B patients, 25 patients with HBV-related liver cirrhosis, and 49 healthy controls were enrolled in the study. Ethical approval of the study protocol was obtained from the local ethics committee of Necmettin Erbakan University, Meram Faculty of Medicine, and written informed consent was obtained from all participants. The chronic hepatitis B group consisted of treatment-naïve patients who were HBsAg-positive for at least 6 months with active viral replication (HBV DNA: >2000 IU/mL). Liver biopsy was performed for all patients in the chronic hepatitis B group and the degree of inflammation and/or fibrosis was graded using the Ishak scoring system. On the other hand, liver biopsy was not a must in the HBV-related cirrhosis group. HBsAg-positive patients for whom cirrhosis was diagnosed with clinical, laboratory, and radiological findings and patients with Ishak stage 5/6 fibrosis in liver biopsies were included in this group. Forty-nine age- and sex-matched healthy subjects were included in the control group. Complete blood count, serum aspartate and alanine aminotransferases, HBsAg, anti-HBc IgG, and anti-HCV values were obtained for each person in the control group to exclude any possible liver disease. All of the participants were informed prior to the study and provided signed informed consent. Patients with known chronic diseases (malignancies, diabetes mellitus, coronary artery disease, etc.), patients with chronic liver diseases of any other etiology, and patients with considerable alcohol consumption (>20 g/day for males and >10 g/day for females) were not included in the study. Patients with a history of albumin transfusion within 30 days were also excluded from the study as it might affect the IMAR.

Blood samples from patients were obtained early in the morning after overnight fasting. Biochemical

analyses of fasting blood glucose, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and albumin were performed in our hospital's laboratory with automated analyzers. Blood samples for IMA were centrifuged for 5 min at 4 °C and 4000 rpm and serum samples were immediately transferred to a freezer to be stored at –80 °C until spectrophotometric measurements were done.

IMA measurements were done by albumin cobalt binding (ACB) test. In this test, cobalt (Co) is added to the serum sample to measure the binding capacity of albumin. Free cobalt is stained with a protein named dithiothreitol (DTT) and spectrophotometric measurements are done. DTT cannot react with the albumin that binds to Co. As a consequence, the free Co amount also reflects the IMA level (19). Serum IMA measurements were performed as follows: 50 µL of 1 g/L cobalt chloride solution was added to 200 µL of patient serum and kept at room temperature for 10 min after a gentle shake. Then 50 µL of 1.5 g/L DTT solution was added and mixed. After 2 min, 1 mL of 9.0 g/L NaCl solution was added. Absorbances of test mixtures were measured at 470 nm by a spectrophotometer and calorimetric method. Results were obtained in approximately 30 min and reported as absorbance units (ABSU). IMAR was calculated using the following formula: $IMAR = IMA / \text{serum albumin concentration (g/dL)}$.

Chronic hepatitis B patients were further classified into mild (group 1), moderate (group 2), and advanced fibrosis (group 3) groups on the basis of liver histological findings; patients with Ishak stage 1–2 fibrosis were included in group 1 and patients with Ishak stage 3–4 fibrosis were included in group 2. Patients with Ishak stage 5–6 fibrosis and also patients for whom cirrhosis was diagnosed with clinical, laboratory, and radiological findings were included in group 3.

SPSS 16.0 was used for statistical analyses. Continuous variables were presented as mean \pm standard deviation. Significance of the difference in more than two groups was tested using one-way ANOVA tests for normally distributed parameters and by Kruskal–Wallis test for nonnormally distributed parameters. For comparisons between two groups, the independent samples t-test and the Mann–Whitney U test were used for normally and nonnormally distributed parameters, respectively. Pearson and Spearman rho tests were used for correlations between numerical variables. $P < 0.05$ was considered as significant.

3. Results

The study group consisted of 56 (56.6%) male and 43 (43.4%) female subjects with a mean age of 47.9 ± 13.0 years (range: 16–73 years) and the control group included 49 patients with a mean age of 43.9 ± 9.8 years (range: 21–

70 years), of whom 26 (53.1%) were males and 23 (46.9%) were females. There were no significant differences between the study and the control groups regarding age and sex ($P = 0.186$ and $P = 0.519$, respectively).

Mean serum IMA concentrations in the chronic hepatitis B group and healthy controls were 0.33 ± 0.11 ABSU and 0.27 ± 0.70 ABSU, respectively, and the difference between the groups was statistically significant ($P < 0.001$). Patients in the chronic hepatitis B group were subclassified in three groups according to the degree of fibrosis in liver biopsy specimens as previously described to search for any possible association between mean serum IMA concentration and the degree of liver fibrosis. Mean IMA concentrations in group 1 (mild fibrosis), group 2 (moderate fibrosis), and group 3 (advanced fibrosis) were 0.29 ± 0.12 ABSU, 0.34 ± 0.10 ABSU, and 0.39 ± 0.13 ABSU, respectively, and the difference between the groups was statistically significant ($P < 0.001$) (Figure 1). Intergroup comparisons revealed that the differences between the control group and group 2 ($P = 0.002$), control group and group 3 ($P < 0.001$), and group 1 and group 3 ($P = 0.044$) were statistically significant.

Mean IMAR values in the chronic hepatitis B group and healthy controls were 0.08 ± 0.04 and 0.06 ± 0.17 , respectively, and the difference between the groups was statistically significant ($P = 0.017$). Mean IMAR in the mild (group 1), moderate (group 2), and advanced fibrosis (group 3) groups was 0.06 ± 0.03 , 0.08 ± 0.03 , and 0.13 ± 0.06 , respectively, and the difference between the groups

was also statistically significant ($P < 0.001$) (Figure 2). Intergroup comparisons revealed that the differences between the control group and group 2 ($P = 0.004$), control group and group 3 ($P < 0.001$), group 1 and group 2 ($P = 0.043$), and group 1 and group 3 ($P < 0.001$) were statistically significant.

Demographic characteristics and laboratory data in groups 1, 2, and 3 and the control group are summarized in the Table.

Receiver operating characteristic (ROC) curves were obtained in the chronic hepatitis B group for both IMA and IMAR to differentiate patients with advanced fibrosis from patients with mild and moderate fibrosis. The computed area under the curve (AUC) was 0.692 (95% CI: 0.578–0.806) for IMA and the specified cut-off value of 0.35 revealed 60.6% sensitivity and 72.4% specificity. On the other hand, ROC analyses for IMAR revealed that the computed AUC was 0.797 (95% CI: 0.693–0.902), and for the specified cut-off value of 0.082 the calculated sensitivity and specificity were 60.6% and 86.0%, respectively. ROC curves for IMA and IMAR are summarized in Figure 3.

4. Discussion

Serum albumin is the most abundant protein in systemic circulation, and it has antioxidant and immune modulatory functions in addition to its primary oncotic function (20). Several studies have shown that posttranscriptional structural and subsequent functional alterations might occur in the structure of albumin due to several factors

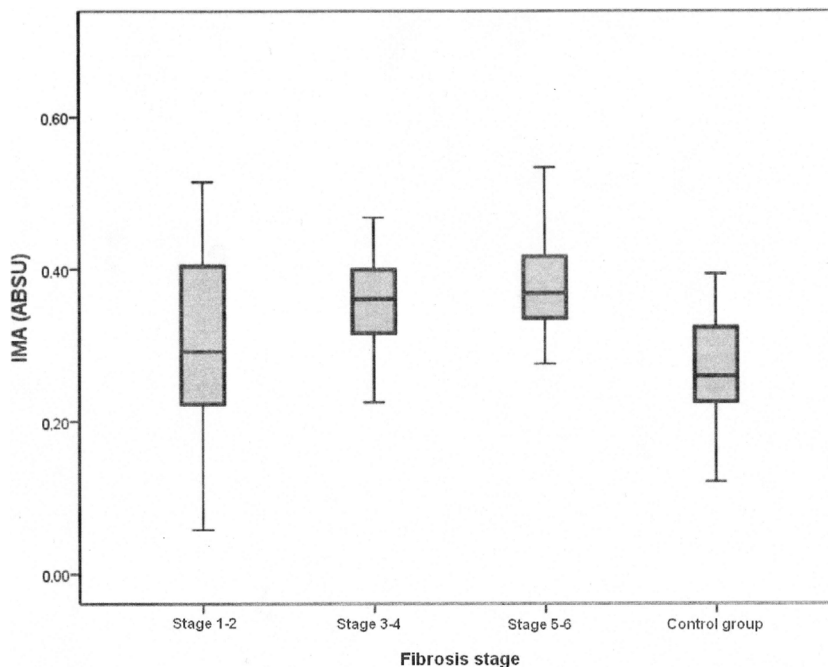


Figure 1. Serum IMA concentrations in mild, moderate, and advanced fibrosis groups and the control group.

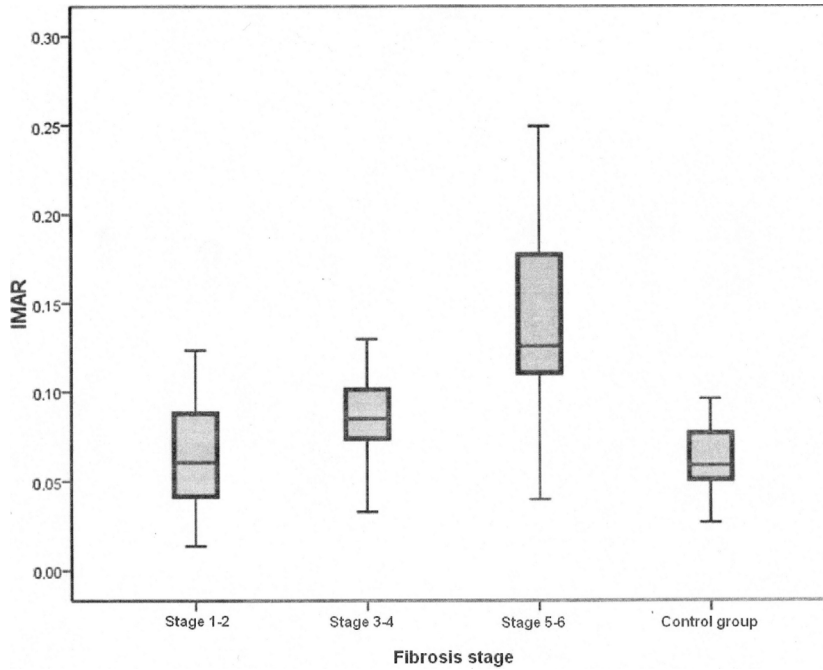


Figure 2. IMAR in mild, moderate, and advanced fibrosis groups and the control group.

Table. Demographic characteristics and laboratory data of the study groups.

	Group 1 (n = 48) (mild fibrosis)	Group 2 (n = 28) (moderate fibrosis)	Group 3 (n = 31) (advanced fibrosis)	Control (n = 49)	P
Age (years)	47.44 ± 12.75	51.96 ± 11.89	52.35 ± 8.84	46.63 ± 10.16	0.051
IMA (ABSU)	0.29 ± 0.12 a, d	0.34 ± 0.10 a, b	0.39 ± 0.13 b	0.27 ± 0.07 c, d	<0.001
IMAR	0.06 ± 0.01 a	0.08 ± 0.03 b	0.13 ± 0.06 c	0.06 ± 0.02 a, d	<0.001
Glucose (mg/dL)	107.77 ± 44.26	102.18 ± 30.23	100.60 ± 16.12	92.65 ± 18.78	0.246
Creatinine (mg/dL)	0.77 ± 0.14 a	0.73 ± 0.12	0.76 ± 0.24	0.74 ± 0.12	0.777
AST (U/L)	31.81 ± 25.01 a	41.70 ± 33.59 a, b	48.69 ± 37.54 b	19.82 ± 4.50 c	0.003
ALT (U/L)	47.48 ± 60.07 a	52.96 ± 54.95 a, b	36.17 ± 19.65 b	22.22 ± 15.33 c	0.022
GGT (U/L)	27.42 ± 21.75 a	40.65 ± 3.66 a	83.46 ± 85.37 b	19.50 ± 5.89 a	<0.001
ALP (U/L)	84.89 ± 25.49 a	84.18 ± 35.00 a	112.38 ± 39.30 b	83.00 ± 23.51 a	0.004
Total bilirubin (mg/dL)	0.91 ± 0.62 a	0.92 ± 0.33 a	2.22 ± 2.01 b	0.44 ± 0.20 c	<0.001
Direct bilirubin (mg/dL)	0.27 ± 0.16 a, c	0.33 ± 0.11 a	0.87 ± 0.91 b	0.17 ± 0.08 c	<0.001
Albumin (g/dL)	4.38 ± 0.33 a	4.12 ± 0.38 b	3.40 ± 0.66 c	4.38 ± 0.35 a	<0.001
Leukocyte (mm ³)	7.10 ± 2.05 a	7.25 ± 1.97 a	5.04 ± 2.02 b	7.48 ± 1.60 a	<0.001
Hemoglobin (g/dL)	14.35 ± 1.88 a	14.38 ± 1.95 a	12.60 ± 2.57 b	14.14 ± 1.53 a	0.001
Thrombocyte (mm ³)	254.50 ± 75.30 a, d	226.00 ± 57.98 a	97.87 ± 47.32 b	270.20 ± 52.04 c, d	<0.001

IMA: Ischemic modified albumin, IMAR: ischemic modified albumin ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, ALP: alkaline phosphatase. Values in rows followed by different letters are significantly different.

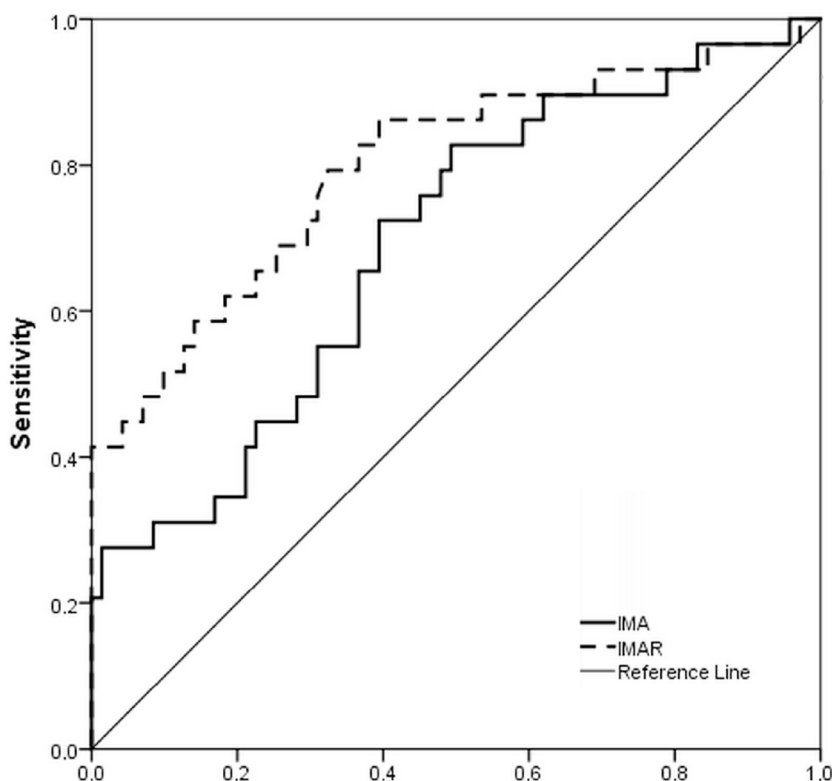


Figure 3. ROC curves for IMA and IMAR to differentiate patients with advanced fibrosis from patients with mild and moderate fibrosis.

and new isoforms arise (21,22). These changes are minimal under physiologic conditions. However, factors like ischemia and oxidative stress cause increases in serum concentrations of these isoforms. In chronic liver diseases, in addition to the decreased synthesis of albumin by the liver, concentrations of different albumin isoforms also increase due to alteration of the microenvironment within the liver or effects of several prooxidants. Some of these albumin isoforms were shown to be related to ascites, renal dysfunction, and bacterial infections in patients with chronic liver diseases (21). On the other hand, it was shown that serum concentration of naturally protected native albumin was much lower than total serum albumin concentration in cirrhotic patients and more importantly this undamaged native albumin concentration was found to be related to survival in patients with chronic liver diseases (15). IMA is the most important isoform of albumin.

The findings of the current study can be summarized as follows: serum IMA concentration and IMAR were higher in patients with chronic hepatitis B and HBV-associated liver cirrhosis when compared to healthy controls; both serum IMA concentration and IMAR were higher in patients with advanced fibrosis in the chronic hepatitis B group; and IMAR predicted liver fibrosis better than serum IMA concentration.

It is well known that the synthesis and functions of albumin deteriorate in patients with liver failure (22). The number of studies evaluating the association of serum IMA concentration and IMAR with disease progression in patients with chronic liver diseases is limited. In a study conducted by Chen et al. (18) it was shown that serum IMA concentration and IMAR were correlated with serum bilirubin concentration and international normalized ratio, both of which are well-known indicators of synthetic dysfunction of the liver, in patients with chronic liver diseases. They also reported that IMAR was associated with Child-Pugh and MELD scores in cirrhotic patients. In another study, Cakir et al. (16) reported that serum IMA concentration and IMAR were higher in pediatric patients with chronic liver diseases of various etiologies than in healthy controls. It is also remarkable that both studies suggested using IMAR rather than IMA in patients with advanced liver diseases. We also agree that using IMAR would be more appropriate in patients with advanced liver diseases. Parallel to liver parenchymal failure, albumin synthesis is decreased and serum albumin concentration is low in these patients. As a consequence of low serum albumin concentration, the cobalt binding capacity of albumin also decreases. In this setting, the measured serum IMA concentration would be higher than the actual

serum concentration (15). Therefore, using the IMAR would more accurately reflect the actual serum IMA levels in patients with advanced-stage liver diseases.

Another important finding of this study was the association among IMA, IMAR, and the degree of fibrosis in liver biopsy samples in patients with chronic hepatitis B. Serum IMA and IMAR levels were significantly higher in patients with advanced-stage fibrosis than the ones with lower grades of fibrosis. Although the studies mentioned above also suggested the association of IMA and IMAR with disease progression in patients with chronic liver diseases of various etiologies, to our knowledge, there is no other study in the literature showing the association among serum IMA concentration, IMAR, and the degree of liver fibrosis in liver biopsy samples in patients with chronic hepatitis B. The results of this study also showed that IMAR was more valuable than serum IMA concentration to discriminate patients with mild and advanced fibrosis and cirrhosis.

We think that one of the strong points of this study is that it includes patients with various stages of liver diseases of a common etiology. The etiological agent in

both the chronic hepatitis group and the cirrhosis patients was chronic hepatitis B infection. On the other hand, the major weakness of this study is that it is a cross-sectional study and therefore it lacks serial measurements of IMA in patient groups. Therefore, we suggest that further prospective studies with adequate numbers of patients that evaluate IMA measurements at different time points in the course of chronic HBV infection would more clearly demonstrate the association of serum IMA levels and IMAR with disease progression and prognosis.

In conclusion, during the course of chronic liver diseases several factors result in posttranscriptional structural changes in albumin, and isoforms with unique biological characteristics are produced. IMA is one of these isoforms. Serum IMA concentration and IMAR increase in HBV-associated chronic liver diseases and correlate with the degree of liver fibrosis. For this reason, we think that IMA and IMAR may have the potential to be used as noninvasive indicators of fibrosis. Nevertheless, further studies are needed to better elucidate the roles and associations of IMA and other isoforms with different clinical and laboratory parameters during the course of chronic liver diseases.

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