ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Relationship Between Ischemic Modified Albumin (IMA) and Chronic Hepatitis B

İskemik Modifiye Albumin (İMA) ile Kronik Hepatit B Arasındaki İlişki

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ABSTRACT

Aim: *Ischemic Modified Albumin (IMA) is a molecule that is found to be elevated in liver diseases with liver damage. In our study, the relationship between IMA and chronic hepatitis B (CHB) was investigated.*

Material and Method: *Patients with CHB who were admitted to the Infectious Diseases polyclinic in between April 2016 and September 2016 and the healthy control group were included in the study. Fifty-three of the patients (24 female, 29 male) with CHB and 51 (21 female, 30 male) were the healthy control group. Blood samples were taken and centrifuged and serums were stored at -80°C. IMA levels were measured by spectrophotometric method. IMA/albumin ratio (IMAR) was calculated.*

Results: *IMA level was measured as 1.08±0.13 in CHB patients and 0.96±0.11 in control group (r=0.42, p<0.000). And, also, IMAR level was measured as 0.23±0.03 in CHB and 0.20±0.03 in control group (r=0.43, p<0.000). According to the control group, the IMA and IMAR level elevation was statistically very significant in CHB. However, there was no statistically significant difference between IMA and IMAR levels in CHB.*

Conclusion: *In our study, IMA and IMAR levels were found to be high in patients with CHB. This significant elevation should be investigated in terms of reflecting liver damage in large studies with other noninvasive parameters followed in patients with CHB.*

Key words: *ischemic modified albumin; chronic hepatitis B; noninvasive parameter*

ÖZET

Amaç: *İskemik Modifiye Albumin (İMA) kronik karaciğer hastalıklarında, karaciğerdeki hasarla birlikte yükseldiği saptanan bir moleküldür. Bizim çalışmamızda İMA'nın kronik hepatit B (KHB) ile ilişkisi irdelendi.*

Materyal ve Metot: *Nisan 2016 – Eylül 2016 tarihleri arasında Enfeksiyon Hastalıkları polikliniğine başvuran KHB hastaları ve sağlıklı kontrol grubu çalışmaya dâhil edildi. Hastaların 53'ü (24 kadın, 29 erkek) KHB ve 51'i (21 kadın, 30 erkek) sağlıklı kontrol grubunu oluşturuyordu. Kanları alınıp santrifüj edildi ve serumlar -80°C derecede saklandı. Bu serumlardan daha sonra, spektrofotometrik yöntemle, İMA düzeyleri ölçüldü. İMA/albumin oranı (İMAR) hesaplandı.*

Bulgular: *İMA düzeyi KHB hastalarında 1,08±0,13, kontrol grubunda ise ise 0,96±0,11 ölçüldü (r=0,42, p<0,000). İMAR düzeyi ise KHB'de 0,23±0,03 kontrol grubunda 0,20±0,03 olarak ölçüldü (r=0,43, p<0,000). KHB'de kontrol grubuna göre İMA ve İMAR düzeyindeki yükseklik istatistiksel olarak çok anlamlıydı. Ancak KHB'de İMA ve İMAR düzeyleri arasında istatiksel olarak anlamlı fark saptanmadı.*

Sonuç: *Çalışmamızda serum İMA ve İMAR düzeyleri KHB'li hastalarda yüksek olarak tespit edildi. Bu anlamlı yükseklik KHB'li hastalarda takip edilen diğer noninvazif parametreler ile birlikte yapılacak geniş çalışmalarda karaciğer hasarını yansıtması açısından irdelenmelidir.*

Anahtar kelimeler: *iskemik modifiye albumin; kronik hepatit B; noninvaziv parametre*

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Introduction

Hepatitis B virus (HBV) infection is clinically observed as; acute infection, chronic infection or primary hepatocellular carcinoma (PHC). Chronic hepatitis is observed in 5–10% of patients; cirrhosis and liver failure may develop in 10% of chronic hepatitis patients[1]. Chronic and progressive liver diseases result in extensive damage to the liver parenchyma, which is replaced by collagenous scar tissue. Useful biochemical tests and researches on this subject are still ongoing[2,3]. Albumin is a protein, synthesized by liver[4]. It constitutes 60% of plasma proteins[5]. The main functions of albumin are adjusting osmotic pressure and transporting some metabolites in the blood. In many studies, it has been shown that metals such as cobalt, copper, and nickel can bound directly to albumin by amino terminal end[6]. Metal ion-binding properties of albumin are reduced in cases of ischemia, hypoxia, increased free radicals and acidosis.

This newly formed albumin is called Ischemic Modified Albumin (IMA)[7]. The literature reported that increased serum IMA concentrations and IMAR in chronic liver diseases of various etiologies also in several diseases such as myocardial ischemia, acute stroke, muscle ischemia, and bowel ischemia[8].

The aim of our study is to investigate the relationship between IMA and IMAR levels with hepatitis B in CHB patients and to suggest a new laboratory parameter for future studies.

Material and Method

This study was conducted between May 2016 and July 2016 at the Erzurum Regional Training and Research Hospital Infectious Diseases Clinic. The study population included a total of 104 participants; 53 CHB (24 women and 29 men) patients, and 51 healthy volunteers (21 women and 30 men) with no history of chronic illness or regular drug use.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Erzurum Regional Training and Research Hospital Ethics Research Committee (2018/05-34).

All subjects had given a written informed consent prior to participation for the study. The patients who were clinically and serologically diagnosed and followed for at least 6 months as chronic hepatitis B were added to our study. Blood samples were taken as a routine control. Control group was selected from patients referred to our clinic with a diagnosis other than hepatitis B. Patients who were under systemic steroid treatment and diagnosed with cerebrovascular disease, chronic and acute kidney disease, additional liver disease, acute and chronic systemic disease like malignancy excluded from our study. Chronic usage of drugs such as antioxidant, antilipid, vitamin drugs, smokers and alcoholics were also excluded from the study.

After blood sample was taken, plasma and serum were separated by centrifuging at 1500 revolutions per minute (rpm) for ten seconds. Serum was kept in -80°C in ependorf tubes. Afterwards IMA was studied from the same blood sample. Patients' complete blood count (CBC), biochemical parameters and hepatitis markers, HBV– DNA levels were also recorded.

IMA values were measured using the cobalt binding test. According to this method, a protein called cobalt (Co) and dithiothreitol (DTT) was added to the serum to measure the binding capacity of albumin. The reaction of the cobalt was measured spectrophotometrically. This amount of free Co indicates the IMA level[9]. In our study, 50 uL of 1 g/L cobalt chloride solution was added in 200 uL of serum and kept at room temperature for 10 minutes. Then 50 uL of 1.5 g/L DTT solution was added and mixed. The color change of the sample was evaluated at 470 nm and the values were measured and recorded in the absorbance units (AbsU). Using IMA/serum albumin concentration (g/dL) formula IMAR was calculated.

Total protein (TP), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamil transferase (GGT), alkaline phosphatase (ALP) was measured with the autoanalyzer. (Architect c16000, Abbott, USA). All CBC analysis was performed in the hematology laboratory of our hospital with the use of an autoanalyzer. (Cell-Dyn Ruby, Abbott, USA). Hepatitis B markers were performed by autoanalyzer. (Architect i2000 sr, HBV-DNA RT PCR Rotorgene (Giasymphny), Abbott, USA).

Statistical Analyses

With an alpha error of 0.05 and significance level of 0.05; and when the incidence of hepatitis B is regarded as 4% in our region, the study power was calculated as 98%. We performed all statistical analyses using Statistical Package for the Social Sciences (SPSS Inc. for Windows, version 17.0, USA). Continuous variables were expressed as means ± Standard Derivation (SD). To test the normality of variable distributions,

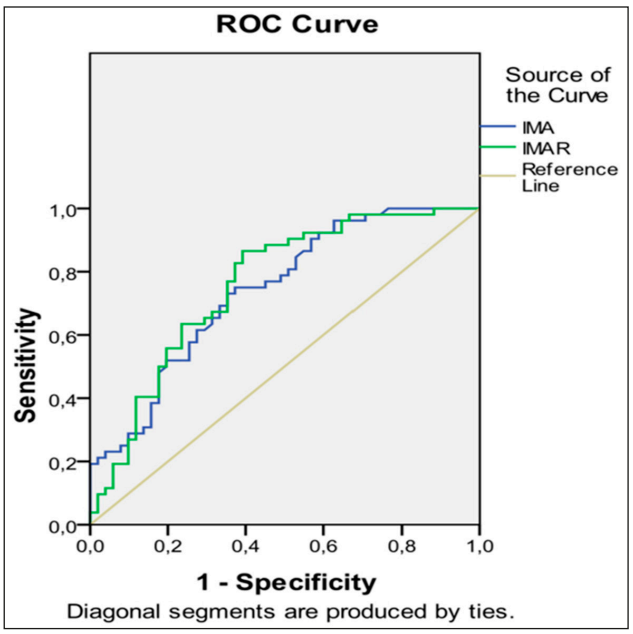

Figure 1. *IMA (ischemic modified albumin) and IMAR (ischemic modified albumin to albumin ratio) level ROC (receiver operating characteristic) curve in chronic hepatitis B patients: 78% specificity and 59% sensitivity for IMA >0.97 predicts CHB (AUC=74%; p=0.000); 89% specificity and 59% sensitivity for IMA >0.2 predicts CHB (AUC=75%; p=0.000) (AUC, area under curve).*

a one-sample Kolmogorov-Smirnov test was used. Unless otherwise stated, results were expressed as mean ± SD. We used the Mann-Whitney U test or independent sample t test between two subject groups, and used the Pearson correlation test or Spearman correlation test, as appropriate. Categorical data were analyzed by Chi-square test. The receiver operating characteristic (ROC) curve analysis assessed the cut-off IMA/IMAR levels the best diagnostic accuracy for detecting differentiated CHB. p<0.05 was considered statistically significant.

Results

In this cross-sectional study, 53 patients with CHB (24 women and 29 men), and 51 control (21 women and 30 men) subjects were evaluated (p<0.55). Mean age ± SD of subjects were 39.79±13.11 in the hepatitis group and 34.54±13.39 in the control group (p=0.05). Age and sex matched between patient and control groups.

IMA level was calculated as 1.08±0.13 in CHB patients and 0.96±0.11 in control group (r=0.42, p<0.000). Also, IMAR level was measured as 0.23±0.03 in CHB and 0.20±0.03 in control group (r=0.43, p<0.000). According to the control group in CHB, the elevation in IMA and IMAR level was statistically significant (Figure 1). However, there was no significant difference between IMA and IMAR levels in CHB.

In addition, there was a positive correlation between IMA level and age, ALP, and a negative correlation between Albumin, direct bilirubin (DB), ALT, GGT and alpha feto-protein (AFP) in CHB patients. There was a positive correlation between IMAR and age, Body mass index (BMI), ALP and a negative correlation between DB, ALT, GGT, AFP and Albumin.

There was no correlation between IMA and IMAR levels and HBV-DNA, Total protein, AST, thrombocytes in CHB. IMA and IMAR levels, demographic and laboratory values were compared between CHB and healthy control group (Table 1).

In addition, all parameters studied in patients with CHB were compared with IMA and IMAR levels (Table 2).

Discussion

In this study, 78% specificity and 59% sensitivity were found for IMA >0.97 in CHB (Area Under Curve (AUC)=74%, p=0.000). 89% specificity and 59% sensitivity for IMAR >0.2 were observed for the prediction of CHB (AUC=% 75, p=0.000). IMA and IMAR levels were significantly higher in CHB patients than healthy control group p<0.000.

The numbers of studies that evaluate the association of serum IMA concentration and IMAR with disease progression in chronic liver diseases are limited. In these limited studies, there is a correlation between progression of the disease and elevation of IMA and IMAR[10–13].

HBV is one of the most common causes of chronic liver diseases in the World[14]. Synthesis and functions of albumin deteriorate in patients with liver failure[15]. Human serum albumin is the most abundant circulating protein in the plasma and shows important antioxidant activities[16].

Studies have shown that new isoforms emerged as a result of changes in albumin structure due to different reasons[15,17]. IMA is the most important isoform of albumin[8]. Several studies have been performed on IMA, which has been shown to increase in cases such as ischemia, hypoxia, increased free radicals, and acidosis[7]. In another study, IMA levels were significantly higher in 4 groups of patients with brain infarction,

Table 1. *Demographics and laboratory findings of study population*

	Control n (51)	Chronic Hepatitis B (CHB) n (53)	p value
Sex (male) (n)	51 (30)	53 (29)	0.55
Age (years)	34.54±13.39	39.79±13.11	0.051
BMI (kg/m^2)	26.53±4.14	27.08±3.51	0.47
Ischemic Modified Albumin (IMA)	0.96±0.11	1.08±0.13	0.000*
IMA/albumin ratio (IMAR)	0.20±0.03	0.23±0.03	0.000*
WBC (10^3/µL)	7880.4±1674.6	7308.2±1931.6	0.11
Platelet	257.34±56	277.06±86	0.19
HBV-DNA		903275±4.1	0.000*
ALT (U/L)	20.90±10.19	27.63±23.20	0.000*
AST (U/L)	21.37±9.27	23.14±11.91	0.287
GGT (U/L)	26.05±40.15	18.06±7.73	0.094
Albumin (g/dL)	4.84±0.39	4.60±0.44	0.004*
AFP	4.42±0.43	2.43±1.87	0.000*
TP (g/dL)	7.03±0.42	7.07±0.84	0.129
ALP	20.26±9.17	89.46±38.17	0.000*
IB	0.24±0.20	0.65±1.71	0.000*
DB	2.30±0.98	0.21±0.11	0.000*

BMI, body mass index; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamil transferase; TP, total protein; ALP, alkaline phosphatase; AFP, alfa feto protein; IB, indirect bilirubin; DB, direct bilirubin; HBV-DNA, hepatitis B virus deoxyribonucleic acid.
* $p<0.05$

Table 2. *Bivariate correlation between IMA, IMAR parameters and other variables in CHB*

	IMA		IMAR	
Variables	r	p	r	p
Group	0.417	0.000	0.447	0.000
Age	0.205	0.041	0.318	0.001
BMI	0.062	0.537	0.219	0.029
HBV-DNA	0.153	0.320	0.039	0.805
Albumin	-0.297	0.002	-0.755	0.000
TP	-0.131	0.202	-0.107	0.300
AFP	-0.479	0.000	-0.503	0.000
GGT	-0.279	0.006	-0.245	0.016
ALP	0.328	0.001	0.370	0.000
ALT	-0.311	0.002	-0.349	0.000
AST	0.026	0.794	0.027	0.789
PLT	0.013	0.905	0.100	0.348
BK	-0.043	0.669	-0.031	0.761
I. Bilirubin	0.034	0.744	0.033	0.751
D. Bilirubin	-0.440	0.000	-0.452	0.000

BMI, body mass index; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, Platelet; GGT, gamma glutamil transferase; TP, total protein; ALP, alkaline phosphatase; AFP, alfa feto protein; IB, indirect bilirubin; DB, direct bilirubin; HBV-DNA, hepatitis B virus deoxyribonucleic acid; IMA, ischemic modified albumin; IMAR, ischemic modified albumin to albumin ratio.
$p<0.05$ was accepted as statistically significant.

epileptic seizure, transient ischemic attack (TIA) and intracerebral hemorrhage than control group[18]. Sinha et al.[19] showed elevated IMA during the acute coronary episode without developing necrosis. In other studies, it has been shown that IMA increases in conditions such as cirrhosis, bacterial-viral infections, advanced cancers, stroke, pulmonary embolism and end-stage renal failure[13,20]. When the serum albumin concentration is lower, less cobalt bounds to albumin molecule and a more intense response occurs between cobalt ions and dithiothreitol (DTT). Consequently, lower albumin concentrations may cause proportionally higher IMA levels in the same patients. Because of the loss of albumin level, there is also loss of synthesis capacity in chronic liver patiens. In addition to IMA, IMAR is also measure which is believed to be more valuable. IMAR is a calculated with the ratio of IMA to serum albumin level[11,12]. In some studies, IMAR level has been shown to be useful as a liver function test[10]. Therefore, IMA/albumin ratio (IMAR) was included in the study.

In our CHB patient group, there was a negative correlation between serum albumin levels and serum IMA and IMAR levels. To support this fact, in another study, negative correlation was observed between serum albumin and serum IMA and IMAR levels[8].

In addition, there is negative correlation between DB, ALT, GGT, AFP and IMA, IMAR levels, but found a positive correlation between ALP, age and IMAR, IMA. Also in Yavuz et al.'s[8] study there was also positive correlation between IMA, IMAR and ALT, AST levels and negative correlation between IMA, IMAR and thrombocyte and white blood cell count. In our study, there was no correlation between HBV DNA, Total protein, AST, thrombocyte, white blood cell count and IMA, IMAR levels.

In this study, Yavuz et al.[8] found that serum IMA and IMAR levels were increased as the degree of liver fibrosis in HBV-related chronic liver diseases. In patients with advanced fibrosis, IMAR serum level was found to be higher than IMA. In another study, Cakir et al.[12] reported that serum IMA concentration and IMAR were higher in pediatric patients with chronic liver diseases with various etiologies than in healthy group. It was observed that IMA and IMAR levels were increased in children with the chronic liver disease; IMAR was positively correlated with end-stage liver disease. Higher amount of IMAR levels were found especially in patients with advanced stage fibrosis compared to those with moderate fibrosis. In our study, level of fibrosis and IMA and IMAR levels could not be compared in patients as there were no biopsy results. There was no significant difference between IMA and IMAR levels in patients with CHB. This is due to the fact that our patients with CHB have laboratory and clinical findings that do not require biopsy and consisted of patients are not accepted as an advanced stage.

Chronic and progressive liver disease results in widespread damage in the liver parenchyma and replaces it with collagenous scar tissue. According to current clinical practice, liver biopsy is necessary for the diagnosis and treatment of progressive chronic liver disease. However, nowadays, the need for biochemical tests that can be easily repeated and is thought to show advanced stage liver fibrosis is increasing[2,3]. Since biopsy was not performed in our study, only non-cirrhotic chronic hepatitis B patients were included in the study and their fibrous levels were not determined. However, serum IMA and IMAR levels were significantly higher in CHB than healthy patients. In the present study of noninvasive diagnostic methods, this significant elevation should be examined in terms of reflecting liver damage in more comprehensive studies with other parameters in patients with CHB.

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