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Is serum high-mobility group box 1 (HMGB-1) level correlated with liver fibrosis in chronic hepatitis B?

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Abstract

Background: High-mobility group box 1 (HMGB1), identified as an alarmin molecule, was shown to have a role in virus-triggered liver injury. We aimed to evaluate the association between serum levels of HMGB1 and liver fibrosis.

Method: This cross-sectional case-control study included 189 chronic hepatitis B (CHB) patients and 51 healthy controls. All patients underwent liver biopsy and modified Knodell scoring system used to determine the fibrosis level in CHB patients. Serum HMGB1 levels were determined with enzyme-linked immunosorbent assay (ELISA).

Results: Mean serum HMGB1 levels of patients (58.1 ± 54.7) were found to be higher than those of the control group (7.1 ± 4.3) ($P = .001$). HMGB1 levels of patients with advanced-stage fibrosis (stage 4 and 5) were detected to be higher than those of patients with early-stage fibrosis (stage 1–3). However, this difference was not statistically significant ($P > .05$). Albumin levels of fibrosis 3 and 4 patients were lower than fibrosis 1 and 2 patients. ALT, HBV DNA, and AFP levels of fibrosis 5 patients were significantly higher than fibrosis 1 and 2 patients, and their platelet and albumin levels are lower than fibrosis 1 and 2 patients ($P < .001$). In a logistic regression model, fibrosis levels were correlated with ALT values and inversely correlated with albumin levels.

Conclusion: In this study, we demonstrated that serum HMGB1 levels increase in the early course of liver injury and this increase is not correlated with severity of the liver damage.

Abbreviations: ALT = alanine aminotransferase, CHB = chronic hepatitis B, ELISA = enzyme-linked immunosorbent assay, HBV DNA = hepatitis B virus DNA, HMGB1 = high-mobility group box 1.

Keywords: alarmin molecules, chronic hepatitis B, high-mobility group box 1, liver fibrosis

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ACI, NAD, SK, and SS participated in obtaining kits used in this study, collecting data, interpreting findings, and writing paper. ACI, NAD, SK, and OU made biopsies, collected serum samples, stored samples, and obtained serum samples of controls. LSD made statistical evaluations. MA and AA studied the required biochemical parameters from sera of patient and control groups. FSP evaluated liver biopsy samples.

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1. Introduction

Hepatitis B virus (HBV) infection is worldwide public health problem. Nearly one-third of people have serological evidence of past or present HBV infection and 350 million people are chronically infected.[1] Decay of hepatitis B virus DNA (HBV DNA) is largely accomplished (up to 90%) by antiviral cytokines that are produced by cells of the innate and adaptive immune systems—including tumor necrosis factor alpha, interferon alpha, or interferon beta. If the immune response fails to eradicate HBV in the acute term, low-grade and dismodulated chronic inflammation develops which may lead to chronic liver inflammation and liver injury. Cirrhosis as well as hepatocellular carcinoma may develop through the natural course of the disease.[2]

In chronic HBV infection, fibrosis progression is mediated by proinflammatory cytokines.[2,3] Alarmin class molecules are immune activators. Alarmins, upon binding their respective receptors, mobilize and activate immune cells which will further take part in host defense and tissue repair.[4] High mobility group box 1 (HMGB1) is one of the alarmin molecules with an array of functions. HMGB1 is a nonhistone nuclear protein that supports nucleosome stabilization. HMGB1 also eases nuclear transactions and gene transcription.[5] Furthermore, HMGB1 has immunomodulatory activities which led to classification as an "endokine."[6] HMGB1 takes part in the response against tissue damage and stress. HMGB1 plays role in innate immunity against viral and bacterial invaders. HMGB1 may contribute to the response against tissue damage like in atherosclerosis, stroke,

and autoimmune conditions.[7] HMGB1 can be passively or actively secreted from the cell in response to inflammation and tissue regeneration.[4–6,8] Recently, it is shown that HMGB1 can act as a late mediator of systemic inflammatory condition. HMGB1 itself, or in conjunction with other proinflammatory cytokines (eg, interleukin-1β, interferon-γ, and tumor necrosis factor-α), amplifies inflammatory cascade by stimulating the production of certain cytokines.[9,10]

In this study, we aimed to determine whether serum HMGB-1 levels are correlated with liver injury in chronic hepatitis B (CHB) patients.

2. Methods

2.1. Patients and ethics

This study was carried out at Infectious Disease and Clinical Microbiology Clinics of Adiyaman Education and Research Hospital between June 1, 2011 and December 31, 2012. One hundred eighty-nine CHB patients and 51 healthy controls were included in this study after obtaining informed consent. The local ethics committee reviewed and approved the study protocol (Adiyaman University IRB Decision No: 2011/03–4.3). CHB was diagnosed as defined by American Association for the Study of Liver Disease (AASLD) practice guidelines.[11] All patients were over 18 years old, nonpregnant at diagnosis, and also were screened for hepatitis C virus (HCV), hepatitis D virus (HDV), and human immunodeficiency virus (HIV) coinfections and found to be negative. All eligible patients fulfilling requirements had undergone liver biopsy to determine liver injury. The control group consisted of healthy volunteers who do not have any serological signs of HBV, HCV, or HIV infections.

2.2. Laboratory tests

Peripheral blood samples from the patients were obtained simultaneously with a liver biopsy and kept on dry ice until centrifugation. Serum was extracted from blood samples by centrifugation (5000 rpm for 3 minutes). Serum samples were kept in a freezer at −80°C until assays. Serum alanine aminotransferase (ALT), alpha fetoprotein (AFP), platelet, albumin, and HBV DNA levels were analyzed at a central laboratory. Markers for HBsAg, anti-HBs, HBeAg, and anti-HBe were measured by Makro ELISA (Abbott AXSYM SYSTEM, Germany). HBV DNA testing was conducted using the real-time PCR (ICycler IQ Real-Time PCR; Biorad Sciences, CA) method. Biochemical parameters such as ALT, aspartate aminotransferase (AST), and albumin levels were measured using an Abbott Architect plus c16000 device. Sysmex XT 2000i (Roche) was used to measure hemograms. Alpha fetoprotein (AFP) levels were measured using a Modular E170 (Roche) device.

2.3. Liver biopsy

The liver biopsy was performed with 14G or 16G single use liver biopsy needles. Biopsy samples longer than 1.5 cm were eligible for further analysis. All samples were sent to the pathology laboratory in 5% formaldehyde solution. Liver biopsy samples were evaluated by an experienced pathologist (FSP) according to the modified Knodell scoring system.[12] Samples with more than 6 portal tracts were taken into consideration. Hematoxylin eosin, reticulin, and Masson trichrome dyes were used for analysis.

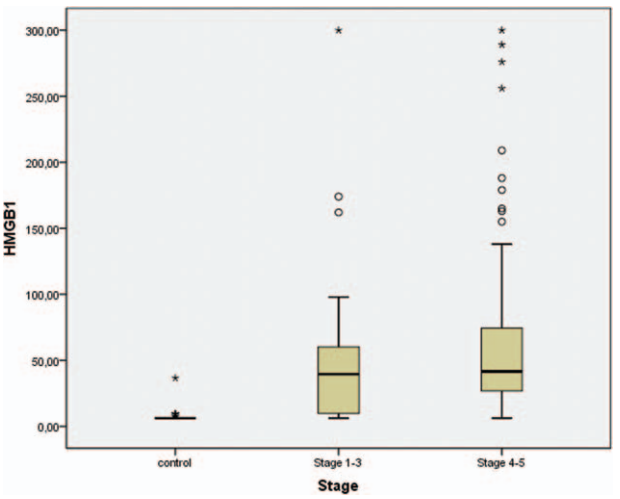

Figure 1. Schematic description of serum HMGB1 levels in patients and controls.

2.4. Determination of HMGB1

Serum HMGB1 levels were measured with ELISA assay csb-e08223h© kit (Cusabio Biotech Co Ltd, Wuhan, Hubei Province, P.R. China). Samples were incubated for 2 hours after adding 100 μL serum or water at 37°C. Incubation was continued for 1 more hour after adding study solution containing 100 μL biotin-antibody. After an hour of incubation, wells were taken back to room temperature and shaken gently and washed 3 times. HRP-avidin solution measuring 100 μL was added and incubated for an extra hour at 37°C. Wells were washed 4 times after incubation and TMB substrate was added and incubated for 20 minutes at 37°C. Stop solution was added and reading was performed at 450 nm.

2.5. Statistical analysis

Data was analyzed with SPSS©v16.0. Descriptive statistics were expressed as mean ± standard deviation and percentage distribution. Kruskal-Wallis variance analysis tests and Mann-Whitney *U* test with Bonferroni Correction were utilized in statistical analyses. Correlation with HMGB1 was tested with Spearman correlation analysis test. Stepwise logistic regression was done to determine factors associated with fibrosis. *P* values <.05 were accepted as statistically significant.

3. Results

Among the CHB patients 120 were male (63.5%) and 69 were female (36.5%). There were 25 male (49.0%) and 26 (51.0%) female volunteers in the control group. Mean age of the study group and control group was 34.6 ± 10.8 and 33.8 ± 9.3 years, respectively ($P > .05$).

Serum HMGB1 values of the patients were (58.1 ± 54.7 ng/mL) found to be higher than the control group (7.1 ± 4.3 ng/mL)

Table 1

Serum HMGB1 values of the patients and control group.

	CHB patients, n = 189	Control group, n = 51	*P*
HMGB1, ng/mL	58.1 ± 54.7	7.1 ± 4.3	.001

CHB = chronic hepatitis B, HMGB1 = High-mobility group box 1.

Table 2

Serum HMGB1 values of the patients are elevated compared to control group.

	Control group	Early stage (stage 1–3)	Advanced stage (stage 4–5)	*P* (patients vs control)
HMGB1, ng/mL	7.0 ± 4.3	53.0 ± 63.0	59.1 ± 53.2	.001

HMGB1 = High-mobility group box 1.

($P = .001$) (Fig. 1 and Table 1). HMGB1 levels of patients from various fibrosis stages were found to be higher than the control group (Table 2). However, HMGB1 values were not statistically different between fibrosis stages. HMGB1 levels of patients with advanced-stage fibrosis (stage 4 and 5 patients) were not different from those of patients with early-stage fibrosis (stage 1, 2, and 3 patients). HMGB1 levels did not differ with age, gender, Knodell histological activity score, serum AST, ALT, HBV DNA, albumin levels, and platelet count.

We compared plasma HBV DNA, platelet, and serum ALT, AFP, and albumin levels according to different fibrosis stages. Patients with stage 1 fibrosis did not differ from patients with fibrosis 2 in terms of HBV DNA, ALT, AFP, albumin, and platelet levels (Table 2). However, HBV DNA, ALT, and albumin levels of stage 4 fibrosis differed from stage 1 patients significantly. Platelet and albumin levels of stage 4 and 5 patients were lower than the patients stage 1 to 3 patients ($P < .001$) (Table 2). To determine the factors correlated with fibrosis level stepwise regression analysis was performed including age, gender, serum HMGB1, albumin, ALT, AFP, HBV DNA values, and platelet count. Fibrosis level was found to be correlated with ALT values and inversely correlated with albumin levels (Table 3).

4. Discussion

In recent years, there has been an increase in the number of studies performed on molecules associated with inflammation in order to determine the presence and progression of fibrosis in chronic hepatitis. One of the molecules studied is HMGB1.[13–21] However, the debate on the role of HMGB1 levels in CHB patients is not over yet. The results of the present study suggest that HMGB1 can be implemented to identify the liver injury but not discriminate between levels of liver fibrosis.

HMGB1 was upregulated during liver fibrosis and its expression was closely correlated with the deposition of collagen in an experimental model of liver fibrosis.[13,22] HMGB1 is elevated after reperfusion of liver graft and this graft tissue is found to be the main source of HMGB1.[23–25] Oshima et al[17] demonstrated that serum HMGB1 levels were elevated in liver damage. Authors stressed the importance of future studies in order to determine whether serum HMGB1 levels may predict disease severity in acute liver failure patients.[17] Wang et al[19] found that elevated HMGB1 levels in CHB patients were associated with liver injury. In another study, Albayrak et al[3] demonstrated that serum levels of HMGB1 were higher in CHB patients when compared with healthy controls. In accordance with previous findings, we showed that serum HMGB1 levels were higher in CHB patients when compared with controls.

In the follow-up of chronic hepatitis, the stage and progression of fibrosis is as important aspect. Therefore, the sensitivity of the marker in detecting the fibrosis stage is important in terms of its convenience in clinical practice. In our literature survey, we found a few number of studies which evaluated the change in serum HMGB1 levels with respect to the stage of fibrosis. As examples to the studies on the subject, Albayrak et al,[3] Liu et al,[21] and Li et al[26] have reported in their studies that HMGB1 can be an effective biomarker in predicting advanced fibrosis. In addition, a recent meta-analysis reviewing 16 relevant published studies showed that HMGB1 levels were elevated in acute exacerbations of CHB and acute liver failure.[27] Our study group included only 35 patients (18%) with histologically proven severe liver injury. Although we have certain number of cases with severe injury, ALT levels of our cohort did not increase 5 times the upper limit of normal, which is generally accepted as the definition of hepatitis flare. Our cohort differed from the previous cohorts reviewed by Hu et al[27] in terms of severe flare-up cases.

Similar to previous studies, we divided 189 CHB patients into 2 groups as early and advanced fibrosis according to their fibrosis stage. HMGB1 levels of patients with advanced-stage fibrosis (stage 4 and 5) were detected to be higher than those of patients with early-stage fibrosis (stage 1–3). However, this difference was not statistically significant ($P > .05$). In addition, we classified the patients according to Knodell histological activity index. However, serum HMGB1 levels did not differ between different

Table 3

Serum ALT, HBV DNA, AFP, platelet, and albumin levels at different fibrosis stages.

	Fibrosis 1, n = 40	Fibrosis 2, n = 77	Fibrosis 3, n = 37	Fibrosis 4, n = 23	Fibrosis 5, n = 12	*P*
ALT, U/L	54 (32–144)	56 (32–124)	82 (32–164)	69 (43–145)	87 (54–144)	.001*
HBV DNA, copy/mL	6.7×10^4 (1.2×10^4–1.1×10^8)	9.7×10^4 (1.2×10^4–1.1×10^8)	1.1×10^9 (1.2×10^4–9.8×10^{10})	6×10^7 (4.5×10^5–1.2×10^{10})	1.1×10^9 (2.3×10^6–3.2×10^9)	.001**
AFP, ng/mL	5 (3–8)	5 (3–8)	6 (3–8)	6 (3–13)	6 (3–34)	.075***
Platelet, mm^3	2.77×10^5 (0.24×10^5–4.3×10^5)	2.78×10^5 (1.89×10^5–4.3×10^5)	2.89×10^5 (1.89×10^5–3.56×10^5)	2.74×10^5 (1.12×10^5–4.3×10^5)	1.83×10^5 (1.1×10^5–2.8×10^5)	.001****
Albumin, g/dL	4.0 (3.9–4.5)	4.0 (3.9–4.6)	4.0 (3.8–4.6)	3.9 (3.2–4.0)	3.5 (3.0–4.1)	.001*****

AFP = alpha fetoprotein, ALT = alanine aminotransferase, HBV DNA = hepatitis B virus DNA.

* Fibrosis 5 and 4 versus fibrosis 1 and 2.

** Fibrosis 5 and 4 versus fibrosis 1 and 2.

*** Fibrosis 5 and 4 versus fibrosis 1 and 2.

**** Fibrosis 5 versus fibrosis 1 to 3.

***** Fibrosis 5 versus fibrosis 1 to 3.

Table 4

Results of the regression analysis: factors associated with fibrosis.

	Standardized coefficients	T	*P*	Confidence interval (95.0%)	
Albumin	−0.427	−6.21	.001	−2.75	−1.42
ALT	0.238	3.4	.001	0.003	0.012

ALT = alanine aminotransferase.

histological activity scores. However, our data mirrors the real-life outpatient setting and suggests that HMGB1 levels are elevated in the very early stages of HBV infection related liver injury and cannot discriminate between different stages of liver injury. This finding might also be associated with the low number of patients with advanced-stage fibrosis.

HBV DNA, AST, ALT, AFP, thrombocyte, and albumin levels are still useful markers for follow-up of CHB. However, it is not possible to determine the level of liver fibrosis by assessing these markers alone. In our study, the relationship between fibrosis stage and HBV DNA, ALT, albumin, and platelet levels was found to be statistically significant (Table 3). In addition, according to the regression analysis results, ALT levels were correlated with fibrosis levels and inversely correlated with albumin levels (Table 4).

To our knowledge this is the most extensive study performed in the literature with well-defined patient and control group. In Turkey, liver biopsy is an obligatory test to determine the liver injury which provided us unique opportunity to demonstrate the liver damage histologically. The main limitation of this study is that it included small number of patients with advanced-stage fibrosis.

In conclusion, HMGB1 is elevated in serum of CHB patients. This elevation is not correlated with the degree of liver fibrosis. Based on our findings, it can be speculated that elevated HMGB1 level is reflecting hepatocyte injury rather than fibrotic process.

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