Serum Toll-Like Receptor-2, Toll-Like Receptor-4 Levels in Patients with HBeAg-Negative Chronic Viral Hepatitis B

Erdem Akbal,¹ Erdem Koçak,¹ Seyfettin Köklü,² Bilal Ergül,³ Ömer Akyürek,⁴ and Fatma Meriç Yılmaz⁵

Abstract

Toll-like receptors (*TLRs*) may play an important role in hepatitis-B pathogenesis. However, serum *TLR-2* and *TLR-4* levels and their association with serum liver enzymes, hepatitis B virus (HBV) DNA, quantitative HBsAg levels, and liver biopsy findings, are unknown. A total of naive 40 HBeAg (–) chronic hepatitis B (CHB) patients and 20 healthy control subjects were recruited in this study. Liver tests, HBV DNA, serum *TLR-2* and *TLR-4*, and quantitative HBsAg levels were evaluated among all groups. The relationship among *TLR-2*, *TLR-4*, quantitative HBsAg levels and liver tests, and liver histological findings were investigated with correlation analysis. Serum *TLR-2* and *TLR-4* levels in HBeAg (–) CHB patients were higher than in the control group. There was a positive correlation between serum *TLR-2*, *TLR-4*, and HBV DNA and ALT levels. We have further demonstrated that serum *TLR-2* levels are correlated with AST and quantitative HBsAg levels. However, *TLRs* levels were not linked to the liver biopsy findings. *TLR* can have an important role in hepatitis B pathogenesis. Liver injury in CHB may cause elevated *TLR-2* and *TLR-4* levels.

Keywords: chronic hepatitis B, *TLR-2*, *TLR-4*, quantitative HBsAg

Introduction

HEPATITIS B VIRUS (HBV) is an important health problem, which can lead to cirrhosis and hepatocellular carcinoma (7). Numerous factors are responsible for the pathogenesis of chronic hepatitis B (CHB) infection. The immune system plays a major role of CHB pathogenesis. The degree of liver damage in CHB is determined by the interaction between hepatitis B-infected hepatocytes and the immune response of the host (3,4). Toll-like receptors (*TLRs*) have a significant role in the regulation of host defense. They play important roles in identification of pathogens, initiation, and maintenance of the host antimicrobial defense mechanism and natural immunity. They also play a crucial role in the regulation of the interaction between initial response and adaptive immunity (2). *TLRs* are demonstrated to be intensively expressed in several liver cells such as Kupffer cells, hepatocytes, and biliary epithelial cells (6,10,19). Therefore, some authors have claimed that *TLRs*, especially *TLR-2* and *TLR-4*, are linked to several liver diseases.

Recently, researchers have shown an increased interest to clarify whether *TLR-2* and *TLR-4* may play a role in the pathogenesis of CHB. Previous studies have commonly focused on

TLR expression in blood samples or tissues samples in CHB patients. However, there is still a discussion about the fact that *TLRs* are upregulated when the body becomes in CHB. There were some series of experimental studies reported on antigenic structure of HBV and *TLR* activation. In these studies, addition of HBeAg in cell cultures resulted in inhibition of *TLRs* (21). In HBeAg-positive patients, *TLR-2* activation is significantly reduced compared with HBeAg-negative patients. There was no difference in *TLR-4* expression. After HBeAg-positive patients receive treatment and become HBeAg negative and produce anti-HBe antibodies, a reincrease in *TLR* levels occurs (9). Previous study showed a positive correlation between HBV DNA and *TLR*, while another showed in a series of liver cirrhosis patients, although *TLR* expressions were elevated, they were negatively correlated with *TLR-2*, *TLR-4*, and HBV DNA levels (16,22). Expressions of *TLRs* are found to be increased in severe HBV infections and in cases of cirrhosis (5,12,13,16,30). It is a common finding in the literature that *TLR-2* expression significantly increases in HBeAg (–) CHB patients, meanwhile inconsistent results are reported regarding *TLR-4* expression (3,9,15,18,21,24, 29).

However, *TLRs* expression measuring methods are commonly difficult and complex analysis. In the same time,

¹Department of Gastroenterology, Florence Nightingale Hospital, Faculty of Medicine, İstanbul Bilim University, İstanbul, Turkey.

²Department of Gastroenterology, Faculty of Medicine, Hacettepe University, Ankara, Turkey.

³Department of Gastroenterology, Kırşehir State Hospital, Kırşehir, Turkey.

⁴Department of Internal Medicine, Medova Hospital, Konya, Turkey.

⁵Department of Medical Biochemistry, Numune Education and Research Hospital, Ankara, Turkey.

there is a lack of data in the relationship between *TLRs* expression levels and liver tests. Measuring of serum *TLRs* levels by enzyme-linked immunosorbent assay (ELISA) method is a simple method and provides quantitative values. However, *TLR 2–4* serum levels have not been investigated in CHB patients until now (3,5,9,12,13,15,16,18,21,24,29,30).

Recent studies show that quantitative HBsAg levels play significant roles in CHB pathogenesis. Several reports have demonstrated the relationship between HBsAg and immune cells and several cytokines. *TLRs* increased expression of viral cytokines such as IFN, and proinflammatory cytokines such as TNF- α is observed after *TLRs* upregulation (24,26). Therefore, *TLRs* may be related to severity of CHB. However, the relationship between HBsAg quantitative levels and serum *TLRs* has not been investigated.

Our aim in this study is to evaluate quantitative HBsAg levels together with serum *TLR-2* and *TLR-4* levels in HBeAg-negative CHB patients and to find out their exact roles in HBV pathogenesis, hepatic histological activity, and fibrosis.

Methods

Subjects

HBeAg-negative CHB group. HBeAg (–) CHB patients aged between 18 and 70 years and admitted to gastroenterology outpatient clinics of Ankara Training and Research Hospital were included in this study. The HBeAg (–) CHB group constituted of patients who were HBsAg (+) previously for at least 6 months and kept being HBsAg negative for the last 6 months plus HBV DNA levels more than 2,000 IU/mL plus persistent or intermittent ALT elevation plus no previous treatment for CHB.

Control group. Control group subjects were randomly sampled from healthy individuals who were found to be HBsAg, Anti-HCV, and Anti-HIV negative on their routine controls.

Exclusion criteria. Healthy subjects and HBeAg (–) CHB patients with chronic renal insufficiency, coronary artery disease, vasculitis, diabetes mellitus, hypertension, cirrhosis were excluded from this study. We also excluded patients who were not using alcohol, had mass lesions on hepatobiliary ultrasonography, and had hepatocellular carcinoma.

Biochemical measurements. Fasting venous blood samples were taken from all patients. Routine biochemical measurements were studied and remaining blood samples were centrifuged and stored at -80°C until the quantitative analyses of *TLR-2* and *TLR-4*, and quantitative HBsAg measurements were performed. All biochemical measurements were undertaken by Abbott Architect C 8000—Abbott system. Viral serological tests were performed by ELISA method for HBsAg, anti-HCV, Vitros ECI Immunodiagnostic, and anti-HBe, antidelata antibodies, while HBV DNA was detected by BIORAD-iCycler IQ Multicolor Real-Time PCR Detection System. Quantitative HBsAg measurement was undertaken by Elecsys (Indianapolis, IN) device of Roche Diagnostics. Serum *TLR-2* and *TLR-4* levels were measured by ELISA method (Cusabio Biotech, Wuhan, China).

Liver biopsy. Patients in the HBeAg (–) CHB group underwent ultrasound-guided liver biopsy upon internaliza-

tion into the gastroenterology inpatients clinics. All liver biopsies were evaluated by a single pathologist who did not know about the clinical status of the patients to whom the samples belong. All biopsies were scored using Knodell scoring system (11).

Statistical analyses

Data analyses were performed using SPSS 15.0 software. The normal distribution of the variables was analyzed with Kolmogorov–Smirnov test. Continuous variables are presented as mean $\pm$ standard deviation and categorical variables are presented as percentages (%). Parametric variables between the patients and control groups were compared using Student's *t* test. Nonparametric variables were analyzed with Mann–Whitney *U* test. The correlation between serum levels of *TLRs* and HBsAg was evaluated with Pearson correlation test. A *p*-value <0.05 was delineated as statistically significant.

Results

Study subjects demographical and biochemical results

A total of 60 patients aged between 18 and 70 were included in this study. The number of patients in HBeAg (–) CHB group and healthy control group were 40 and 20, respectively. There were no statistically significant differences between two groups in age, gender, and smoking status ($p=0.586$, $p=0.675$ and $p=0.458$, respectively). The liver enzyme levels and other routine biochemical test results were compared between two groups. ALT, AST, and LDH levels were higher in HBeAg (–) CHB group than in control group ($p=0.001$, $p<0.001$, and $p=0.008$, respectively). Other parameters (total bilirubin, direct bilirubin, urea, creatinine, glucose, GGT, and ALP) were comparably different between groups (all $p>0.05$). Thrombocyte count in HBeAg (–) CHB group was significantly lower compared with control group ($p<0.001$). Other parameters, erythrocyte sedimentation rate and C-reactive protein levels, were similar in two groups ($p=0.569$ and $p=0.302$) (Table 1). The HBV DNA level was measured to be 3,070,000 IU/mL (minimum=4,759, maximum= 10^9 IU/mL) in HBeAg (–) CHB group. The median quantitative HBsAg level is 7,875 IU/mL (minimum=413.8 IU/mL, maximum=50,090 IU/mL) in HBeAg (–) CHB group. When HBV DNA and quantitative HBsAg levels were correlated, a statistically significant strong positive correlation was observed between HBV DNA and HBsAg levels ($\rho=0.830$, $p<0.001$).

Liver biopsy findings

Liver biopsy was performed to all 40 patients in HBeAg-negative CHB group. The median histological stage index was 5 (2–10), and the median fibroblastic stage was 2 (0–4). When the findings of liver biopsy were correlated with HBV DNA and quantitative HBsAg levels, we observed a positive correlation of HBV DNA with HAI ($\rho=0.403$, $p=0.037$), but HBV DNA levels were not correlated with fibroblastic stage ($p=0.180$). Quantitative HBsAg levels did not show any correlation with HAI and fibroblastic stage ($p=0.375$, $p=0.570$).

Serum *TLRs* levels. *TLR-2* levels were 0.88 ± 0.82 ng/mL and 1.39 ± 1.19 ng/mL, respectively, in control group

TABLE 1. DEMOGRAPHIC AND LABORATORY PARAMETERS
IN HBeAg-NEGATIVE CHRONIC HEPATITIS B GROUP AND CONTROL GROUP

Variables	Control group	HBeAg-negative CHB group	p
Age, years	43.1 ± 11.2	46.3 ± 14.3	0.196
Sex, male, %	40	56	0.148
HBV DNA, IU/mL	—	457,508 (3,482–10 ⁹)	
AST, U/L	21.6 ± 5.6	48.7 ± 31.0	<0.001
ALT, U/L	20.8 ± 11.3	84.9 ± 80.6	0.001
ALP, U/L	81.4 ± 31.4	83.9 ± 23.5	0.726
GGT, U/L	24.5 ± 10.5	36.8 ± 31.3	0.129
Total bilirubin, mg/dL	0.7 ± 0.2	1.0 ± 1.1	0.422
Direct bilirubin, mg/dL	0.12 ± 0.04	0.21 ± 0.47	0.096
LDH, U/L	192.5 ± 38.7	184.1 ± 34.6	0.007
Glucose, mg/dL	92.4 ± 8.0	103.5 ± 43.1	0.926
BUN, mg/dL	28.0 ± 9.6	30.0 ± 10.0	0.235
Creatinine, mg/dL	0.9 ± 0.2	0.9 ± 0.2	0.122
Albumin, g/L	4.3 ± 0.2	4.0 ± 0.4	0.06
CRP, mg/L	0.4 ± 0.28	0.50 ± 0.49	0.124
Sedimentation, mm/h	12.4 ± 10.2	10.4 ± 7.0	0.569
Hemoglobin, g/dL	13.8 ± 1.3	14.4 ± 1.5	0.698
Leukocyte, mm ³	6,725 ± 1,679	7,029 ± 2,544	0.992
Thrombocyte, mm ³	256,659 ± 52,204	192,000 ± 104,000	<0.001

CHB, chronic hepatitis B; CRP, C reactive protein; HBV, hepatitis B virus.

and HBeAg (–) CHB group. There was statistically significant difference between HBeAg (–) CHB group and control group ($p=0.022$) (Fig. 1). *TLR-4* levels were significantly higher in HBeAg (–) CHB group compared with control group. *TLR-4* levels were 0.14 ± 0.06 ng/mL and 0.55 ± 1.38 ng/mL ($p < 0.001$), respectively, in control and HBeAg (–) CHB group (Fig. 2).

Correlation analysis

When the correlation of *TLR-2* and *TLR-4* levels with HAI, fibroblastic stage, quantitative HBsAg, HBV DNA levels, AST, and ALT were evaluated, *TLR-2* positive correlated with HBV DNA ($\rho=0.399$, $p=0.005$), ALT ($\rho=0.302$, $p=0.037$), AST ($\rho=0.285$, $p=0.047$), and quantitative HBsAg ($\rho=0.432$, $p=0.027$) was noted. *TLR-2* levels were not correlated with HAI and fibroblastic stage

($p=0.887$, $p=0.804$). We detected that a positive correlation among *TLR-4*, HBV DNA ($\rho=0.447$, $p=0.001$), and ALT ($\rho=0.386$, $p=0.006$), but there was apparently no correlation of *TLR-4* with other parameters (Table 2).

Discussion

To study for the first time, we investigated the serum *TLR-2* and *TLR-4* levels with ELISA method. We also evaluated serum *TLR-2* and *TLR-4* levels that associated with liver enzymes, HBV DNA, quantitative HBsAg levels, and liver histological findings. We detected that serum *TLR-2* and *TLR-4* levels increased in HBeAg (–) CHB patients, and they are also associated with ALT and HBV DNA levels. Indeed, serum *TLR-2* levels are associated with quantitative HBsAg levels and AST levels. However, serum

FIG. 1. Serum *TLR-2* levels show in control and HBeAg CHB patients. TLR, toll-like receptor; CHB, chronic hepatitis B.

FIG. 2. Serum *TLR-4* levels show in control and HBeAg CHB patients.

TABLE 2. CORRELATION ANALYSES OF TOLL-LIKE RECEPTOR-2 AND TOLL-LIKE RECEPTOR-4

	TLR-2		TLR-4	
	<i>Rho</i>	p	<i>Rho</i>	p
HAI	-0.029	0.887	0.114	0.572
Fibroblastic stage	0.040	0.844	0.152	0.449
HBV DNA	0.399	0.005	0.447	0.001
Quantitative HBsAg	0.432	0.027	-0.232	0.254
ALT	0.307	0.032	0.386	0.006
AST	0.285	0.0047	0.1249	0.084

TLR, toll-like receptor.

TLR-2 and *TLR-4* levels are not linked to liver histological findings.

In our study, serum levels of *TLR-2* were detected to be higher in HBeAg (–) CHB compared with the control group. Most probably, increased serum *TLR-2* levels are reflecting to HBV antigenic stimulus. HBV DNA and HBsAg are important antigenic stimulus for started immunopathological response in CHB patients. The pathogenesis and clinical manifestations of hepatitis B are due to the interaction of the virus and the host immune system. The *in vitro* and *in vivo* studies to date show that *TLRs* can play an important role in the pathogenesis of HBV (9,11,13–16,18,20,23,27–30). Increased expression of *TLR-2* in peripheral blood monocytes and liver biopsy specimens with CHB patients has been shown (14,18,21,24,26,31,32). Previous studies on HBV-infected cell cultures have shown that expression of *TLR-2* is increased (5,8,12,13). *TLR-2* plays an important role in the pathogenesis of HBV-related liver damage through increasing proinflammatory cytokines, IFN secretion, and activation of monocyte macrophage system. *TLR-2*-dependent induction of type I interferon occurred only in response to viral ligands and also in CHB induces the production of type I interferon (1).

There are few studies whether expression levels of *TLR-2* can be related with HBV DNA and HBsAg levels. These studies have shown different results as these studies used different methods of measuring *TLR-2* levels and at different stages of CHB. A study previously showed a positive correlation between HBV DNA levels and expression of *TLRs* (22). Another study showed expression of *TLR-2* and *TLR-4* negative correlated with HBV DNA levels in a series of liver cirrhosis patients (16). Our findings show that there is a significant positive correlation between serum *TLR-2* and HBV DNA levels. Our results indicated that HBV DNA levels are an important factor for elevation of serum *TLR-2* levels. Similar mechanisms can be said about *TLR-4*, because our study revealed that serum *TLR-4* levels were positively correlated with HBV DNA levels. On the contrary, we did not detect that serum *TLR-2* and *TLR-4* levels were not linked to liver biopsy findings, histological activity, or fibroblastic stage. Only we showed that HBV DNA levels were related to HAI. Thus increased serum *TLR-2* and *TLR-4* levels did not seem a good estimate of the severity of liver biopsy findings.

HBsAg is the other important antigenic stimuli for *TLRs* activation. However, there was no study evaluating the relationship between quantitative HBsAg and *TLR-2* or *TLR-4*.

Wang *et al.* showed that the HBsAg was related to decreased cytokine production induced by the *TLR-2* ligand from CHB (25). Our study results show that quantitative HBsAg are positively correlated with serum *TLR-2* levels. Therefore, we think that increased HBsAg levels are related to immunological response in CHB patients. Our study demonstrated that *TLR-4* levels were not associated with quantitative HBsAg levels. There might be several reasons for these results. First and more important cause, *TLR-4* expression role of CHB pathogenesis are not clear compared with *TLR-2* expressions. Previous studies showed that *TLR-4* activation related to the induce of IFN- β . However, the relationship between increased TNF- β and inhibition HBV replication in hepatocytes is not exactly known (31). Other reason is that this study included relatively a small number of patients. It would be interesting to confirm these results with larger number of patients. In addition, this study did not include HBeAg-positive patients. HBeAg is one of the most important antigenic stimuli for started immune defense. Therefore, serum *TLR-2* and *TLR-4* levels would be performed investigating different stage of CHB. Another important result is that serum *TLR-2* and *TLR-4* levels are positively correlated with ALT levels. A previous study showed that serum *TLR-2* levels were correlated with ALT levels in HBeAg (–) CHB patients (17). However, this study did not include control subjects. Our study serum *TLR-2* and *TLR-4* levels were associated with ALT levels in CHB patients; however, they were not linked in histopathological findings of liver biopsy.

In conclusion this study analyzed for the first time the serum *TLR-2* and *TLR-4* levels with ELISA method, and their relationship with HBV DNA, ALT, and quantitative HBsAg levels were analyzed in HBeAg (–) CHB patients. Serum *TLR-2* and *TLR-4* measuring with ELISA methods were similar to previous studies, which included *TLR-2* and *TLR-4* expression in CHB patients. ELISA is a useful method to reflect *TLR-2* and *TLR-4* activation. Indeed HBV DNA and HBsAg play an important role in *TLR-2* activation in HBeAg (–) CHB patients.

Acknowledgment

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

Author Disclosure Statement

The authors declare no competing financial interest.

References

1. Barbalat R, Lau L, Locksley RM, *et al.* Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol* 2009;10:1200–1207.

2. Belvin MP, and Anderson KV. A conserved signaling pathway: the *Drosophila* toll-dorsal pathway. *Annu Rev Cell Dev Biol* 1996;12:393–416.
3. Bertoletti A, and Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. *Postgrad Med J* 2013;89:294–304.
4. Bertoletti A, Maini MK, and Ferrari C. The host-pathogen interaction during HBV infection: immunological controversies. *Antivir Ther* 2010;15:15–24.
5. Broering R, Lu M, and Schlaak JF. Role of Toll-like receptors in liver health and disease. *Clin Sci* 2011;121:415–426.
6. Broering R, Wu J, Meng Z, *et al.* Toll-like receptor-stimulated non-parenchymal liver cells can regulate hepatitis C virus replication. *J Hepatol* 2008;48:914–922.
7. Custer B, Sullivan SD, Hazlet TK, *et al.* Global epidemiology of hepatitis B virus. *J Clin Gastroenterol* 2004;38:158–168.
8. Guo H, Jiang D, Ma D, *et al.* Activation of pattern recognition receptor-mediated innate immunity inhibits the replication of hepatitis B virus in human hepatocyte-derived cells. *J Virol* 2009;83:847–858.
9. Han YP, Li J, Wan YF, *et al.* The influence of hepatitis B e antigen on the expression of toll-like receptor 2 on peripheral monocytes. *Zhonghua Gan Zang Bing Za Zhi* 2008;16:739–742.
10. Hans M, and Hans VM. Toll-like receptors and their dual role in periodontitis: a review. *J Oral Sci* 2011;53:263–271.
11. Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696–699.
12. Isogawa M, Robek MD, Furuichi Y, *et al.* Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol* 2005;79:7269–7272.
13. Lai Y, Yi G, Chen A, *et al.* Viral double-strand RNA-binding proteins can enhance innate immune signaling by toll-like Receptor 3. *PLoS One* 2011;6:e25837.
14. Leber B, Mayrhofer U, Rybczynski M, *et al.* Innate immune dysfunction in acute and chronic liver disease. *Wien Klin Wochenschr* 2009;121:732–744.
15. Li N, Li Q, Qian Z, *et al.* Impaired TLR3/IFN-beta signaling in monocyte-derived dendritic cells from patients with acute-on-chronic hepatitis B liver failure: relevance to the severity of liver damage. *Biochem Biophys Res Commun* 2009;390: 630–635.
16. Lian JQ, Wang XQ, Zhang Y, *et al.* Correlation of circulating TLR2/4 expression with CD3+/4+/8+ T cells and Treg cells in HBV-related liver cirrhosis. *Viral Immunol* 2009;22:301–308.
17. Moradzadeh M, Tayebi S, Poustchi H, *et al.* The possible role of TLR2 in chronic hepatitis B patients with precore mutation. *Adv Virol* 2013;2013:780319.
18. Mozer-Lisewska I, Kowala-Piaskowska A, Mania A, *et al.* Expression of pattern recognition receptors in liver biopsy specimens of children chronically infected with HBV and HCV. *Folia Histochem Cytobiol* 2011;49:410–416.
19. Pradere JP, Troeger JS, Dapito DH, *et al.* Toll-like receptor 4 and hepatic fibrogenesis. *Semin Liver Dis* 2010;30:232–244.
20. Preiss S, Thompson A, Chen X, *et al.* Characterization of the innate immune signalling pathways in hepatocyte cell lines. *J Viral Hepat* 2008;15:888–900.
21. Riordan SM, Skinner N, Kurtovic J, *et al.* Reduced expression of toll-like receptor 2 on peripheral monocytes in patients with chronic hepatitis B. *Clin Vaccine Immunol* 2006;13:972–974.
22. Testro AG, Visvanathan K. Toll-like receptors and their role in gastrointestinal disease. *J Gastroenterol Hepatol* 2009;24:943–954.
23. Thompson AJ, Colledge D, Rodgers S, *et al.* Stimulation of the interleukin-1 receptor and Toll-like receptor 2 inhibits hepatitis B virus replication in hepatoma cell lines in vitro. *Antivir Ther* 2009;14:797–808.
24. Visvanathan K, Skinner NA, Thompson AJ, *et al.* Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007;45:102–110.
25. Wang S, Chen Z, Hu C, *et al.* Hepatitis B virus surface antigen selectively inhibits TLR2 ligand-induced IL-12 production in monocytes/macrophages by interfering with JNK activation. *J Immunol* 2013;190:5142–5151.
26. Wei XQ, Wen ZF, Zheng FP, *et al.* Changes of Toll-like receptor (TLR) 2 and TLR4 on the peripheral blood mononuclear cells in patients with chronic hepatitis B and chronic severe hepatitis B. *Zhonghua Gan Zang Bing Za Zhi* 2007;15:354–357.
27. Wu J, Lu M, Meng Z, *et al.* Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. *Hepatol* 2007;46:1769–1778.
28. Wu J, Meng Z, Jiang M, *et al.* Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. *Immunology* 2010;129:363–374.
29. Xia C, Lu M, Zhang Z, *et al.* TLRs antiviral effect on hepatitis B virus in HepG2 cells. *J Appl Microbiol* 2008;105:1720–1727.
30. Xing T, Li L, Cao H, *et al.* Altered immune function of monocytes in different stages of patients with acute on chronic liver failure. *Clin Exp Immunol* 2007;147:184–188.
31. Zhang F, Lu M. Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. *Med Microbiol Immunol* 2015;204:11–20.
32. Zhang Z, Zou ZS, Fu JL, *et al.* Severe dendritic cell perturbation is actively involved in the pathogenesis of acute-on-chronic hepatitis B liver failure. *J Hepatol* 2008;49:396–406.

Address correspondence to:

Dr. Erdem Akbal

Department of Gastroenterology

Florence Nightingale Hospital

Faculty of Medicine

İstanbul Bilim University

İstanbul 34387

Turkey

E-mail: drerdemakbal@gmail.com