

Original Article

Hepatitis B surface antigen levels at 6 months after treatment can predict the efficacy of lamivudine-adeфовir combination therapy in patients with lamivudine-resistant chronic hepatitis B

Jeong Han Kim¹, Hee Won Moon², Soon Young Ko¹, Won Hyeok Choe¹, and So Young Kwon¹

¹Department of Internal Medicine, Konkuk University School of Medicine, Seoul, Korea; ²Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea

Background/Aims: Quantitation of hepatitis B surface antigen (HBsAg) is an increasingly popular method to determine the treatment response in chronic hepatitis B (CHB) patients. The clinical value of HBsAg level measurement during rescue therapy for lamivudine (LMV)-resistant CHB patients have not been evaluated to date. Therefore, this study investigated the correlation between HBsAg level and treatment response in LMV-resistant CHB patients treated with adefovir (ADV) add-on therapy.

Methods: LMV-resistant CHB patients treated with LMV-ADV combination therapy for over 2 years were included. HBsAg levels were measured at 6 month intervals until 1 year, and annually thereafter. Treatment response was assessed by determining the virological response (VR, undetectable HBV DNA levels) during treatment.

Results: Fifty patients were included, of which 40 showed a VR. HBsAg levels were not different significantly at baseline (4.0 vs. 3.6 Log₁₀ IU/mL, $P=0.072$). However, the HBsAg level decreased after 6 months of treatment in patients with a VR and became different significantly between the groups thereafter (3.9 vs. 3.3 at 6 months, $P=0.002$; 3.8 vs. 3.2 at 1 year, $P=0.004$; 3.9 vs. 3.2 at 2 years, $P=0.008$; 3.7 vs. 3.1 at 3 years, $P=0.020$).

Conclusions: The HBsAg level at 6 months after treatment can help predict treatment response. (*Clin Mol Hepatol* 2014;20:274-282)

Keywords: Hepatitis B, Chronic; Hepatitis B surface antigen; Lamivudine; Adefovir; Resistance

INTRODUCTION

Quantitation of hepatitis B surface antigen (HBsAg) is an increasingly popular method to determine the treatment response in chronic hepatitis B (CHB) patients. Measurement of serum HBsAg levels during treatment may help identify sustained responders to

pegylated interferon therapy more reliably than measurement of serum hepatitis B virus (HBV) DNA¹. HBsAg kinetics is different during pegylated interferon and nucleoside analog therapy². Although HBsAg levels remain unchanged during lamivudine (LMV) treatment, recent studies report an association between a reduction of serum HBsAg level and viral suppression after entecavir

Abbreviations:

HBsAg, hepatitis B surface antigen; CHB, chronic hepatitis B; LMV, lamivudine; ADV, adefovir; VR, virological response; ETV, entecavir; PCR, polymerase chain reaction; ALT, alanine aminotransferase, HBeAg, hepatitis B 'e' antigen; RFMP, restriction fragment mass polymorphism

Corresponding author : Jeong Han Kim

Digestive Disease Center, Department of Internal Medicine, Konkuk University School of Medicine, Konkuk University Medical Center, 120-1 Neungdong-ro, Gwangjin-gu, Seoul 143-729, Korea
Tel: +82-2-2030-7764, Fax: +82-2-2030-5029
E-mail: 93haan@hanmail.net

Received : Jul. 28, 2014 / Revised : Aug. 28, 2014 / Accepted : Sep. 11, 2014

(ETV) treatment.³⁻⁵ However, these studies involved treatment-naïve patients with CHB. Moreover, the clinical value of HBsAg level measurement during rescue therapy for lamivudine (LMV)-resistant CHB patients have not been evaluated.

Therefore, this study investigated the correlation between the HBsAg level and treatment response in LMV-resistant CHB patients treated with adefovir (ADV) add-on therapy.

METHODS

Patients

LMV-resistant CHB patients who were treated with LMV-ADV combination therapy for over 2 years at Konkuk University Medical Center were included in this study. Their medical records were reviewed, and their HBsAg levels were measured at 6 month intervals until 1 year, and annually thereafter. Data were censored if patients had a change in treatment, died due to liver-related or other causes, were lost to follow up, or underwent liver transplantation. The final follow up visit was in August 2012. The clinical diagnosis of liver cirrhosis was performed based on imaging findings, using modalities such as abdominal ultrasonography, computed tomography or magnetic resonance imaging, together with clinical findings consistent with liver cirrhosis such as esophageal or gastric varices, ascites, hepatic encephalopathy and thrombocytopenia.

All patients provided written informed consent for CHB treatment and the storage of remnant serum samples. This study was approved by the Institutional Review Board of Konkuk University Medical Center.

HBsAg levels measurement and evaluation of treatment response

Serum samples were serially collected from each patient when rescue treatment with LMV-ADV combination was initiated and every 3 months thereafter; all samples were frozen and stored at -80°C.

HBsAg levels in the stored samples were measured at baseline, 6 months, and annually from 1 year to 5 years after treatment, using a chemiluminescent microparticle immunoassay (Architect HBsAg QT, Abbott Diagnostics, Chicago, IL, USA; Measurable range 0.05 - 250 IU/mL). HBsAg was quantified at a 1:500 dilution according to the manufacturer's instruction. To bring HBsAg levels

within the measurable range, samples with above and below this range required a lower and higher dilution, respectively.

HBV DNA levels were also measured by using real-time polymerase chain reaction (PCR) (Cobas Amplicor PCR, Roche Molecular Systems, Inc., Branchburg, NJ, USA; lower detection limit, 20 IU/mL). Alanine aminotransferase (ALT), bilirubin, hepatitis B 'e' antigen (HBeAg) and antibody to HBeAg (anti-HBeAg) were measured.

Virological response (VR) was defined as undetectable HBV DNA by real-time PCR, and LMV-resistance was detected using restriction fragment mass polymorphism (RFMP) method, as described previously.⁶

Statistical analysis

Continuous and categorical variables are expressed as means \pm standard deviations and numbers (percentiles) respectively. The correlation between HBsAg and HBV DNA titers at baseline was analyzed by calculating Pearson's correlation coefficient. Groups were divided according to VR and compared using the chi-square test or Fisher's exact test for categorical variables and the Student *t*-test for continuous variables. The area under the receiver operating characteristic (AUROC) curve of the HBsAg level at 6 months, 1 year, 2 years and 3 years was calculated for predicting VR; AUROC were compared using DeLong's test.⁷ The Youden index was calculated to choose the optimal cutoff value for VR. Kaplan-Meier analysis with the log-rank test was used to validate this cutoff value. The level of significance was set at $P < 0.05$. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and MedCalc 12.7.0 (Medcalc Software, Mariakerke, Belgium) were used for statistical analysis.

RESULTS

Baseline characteristics

Fifty patients were included, of which 40 achieved virological response. All patients who did not achieve a VR were HBeAg positive ($P = 0.003$) and had high HBV DNA levels at baseline (7.7 vs. 6.7 Log₁₀ copies/mL, $P = 0.027$) (Table 1).

The correlation between HBsAg and HBV DNA levels at baseline

HBsAg and HBV DNA levels were well correlated ($r = 0.531$, $P < 0.001$), both in HBeAg positive patients ($r = 0.454$, $P = 0.013$)

Table 1. Baseline characteristics

Variables	Virological response (+) (n=40)	Virological response (-) (n=10)	P-value
Male (n, %)	25 (62.5%)	3 (30.0%)	0.084
Age (years)*	44.4±13.6	48.5±12.9	0.396
Cirrhosis (n, %)	11 (27.5%)	4 (40.0%)	0.462
Duration (month)*			
LMV	28.8±20.0	35.7±34.6	0.559
LMV+ADV	45.8±10.1	42.6±7.7	0.355
HBeAg positive (n, %)	19 (47.5%)	10 (100%)	0.003
HBV DNA (log ₁₀ IU/mL)*	6.7±1.2	7.7±1.1	0.027
HBsAg (log ₁₀ IU/mL)*	3.6±0.6	4.0±0.7	0.072
ALT (IU/mL)*	263.9±349.7	75.8±112.8	0.102
Albumin (mg/dL)*	4.3±0.3	4.4±0.2	0.251
Total bilirubin (IU/mL)*	1.0±0.5	0.8±0.3	0.179
Prothrombin time (INR)*	1.1±0.1	1.1±0.1	0.400
Child-Pugh score*	5.1±0.4	5.0±0.0	0.058
Resistance mutation (n, %)			0.198
M204I	15 (37.5%)	2 (20.0%)	
M204V	1 (2.5%)	0 (0%)	
L180M+M204I	13 (32.5%)	3 (30.0%)	
L180M+M204V	9 (22.5%)	2 (20.0%)	
L180M+M204IV	2 (5.0%)	3 (30.0%)	

*Mean±standard deviation.

LMV, lamivudine; ADV, adefovir; HBV, hepatitis B virus; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; INR, international normalized ratio.

Table 2. Comparison of HBsAg levels (log₁₀ IU/mL) and HBV DNA levels (log₁₀ IU/mL) between groups

Time	HBsAg levels			Reduction in HBsAg levels from baseline		
	Virological response (+)	Virological response (-)	P-value	Virological response (+)	Virological response (-)	P-value
0 month*	3.6±0.6	4.0±0.7	0.072			
6 months*	3.3±0.5	3.9±0.5	0.002	0.3±0.6	0.2±0.4	0.384
1 year*	3.2±0.5	3.8±0.5	0.004	0.4±0.6	0.3±0.5	0.479
2 years*	3.2±0.7	3.9±0.6	0.008	0.5±0.6	0.2±0.5	0.247
3 years*	3.1±0.8	3.7±0.5	0.020	0.6±0.6	0.4±0.6	0.556
Time	HBV DNA levels			Reduction in HBV DNA levels from baseline		
	Virological response (+)	Virological response (-)	P-value	Virological response (+)	Virological response (-)	P-value
0 month*	6.7±1.2	7.7±1.1	0.026			
6 months*	2.6±1.0	4.9±0.7	<0.001	4.1±1.3	2.8±0.9	0.001
1 year*	2.4±1.0	4.5±0.4	<0.001	4.3±1.2	3.3±0.9	0.013
2 years*	1.3±1.2	4.0±0.4	<0.001	5.5±1.6	3.8±1.1	0.004
3 years*	1.0±1.2	3.5±0.6	<0.001	5.9±1.7	4.2±1.4	0.006

*Mean±standard deviation

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

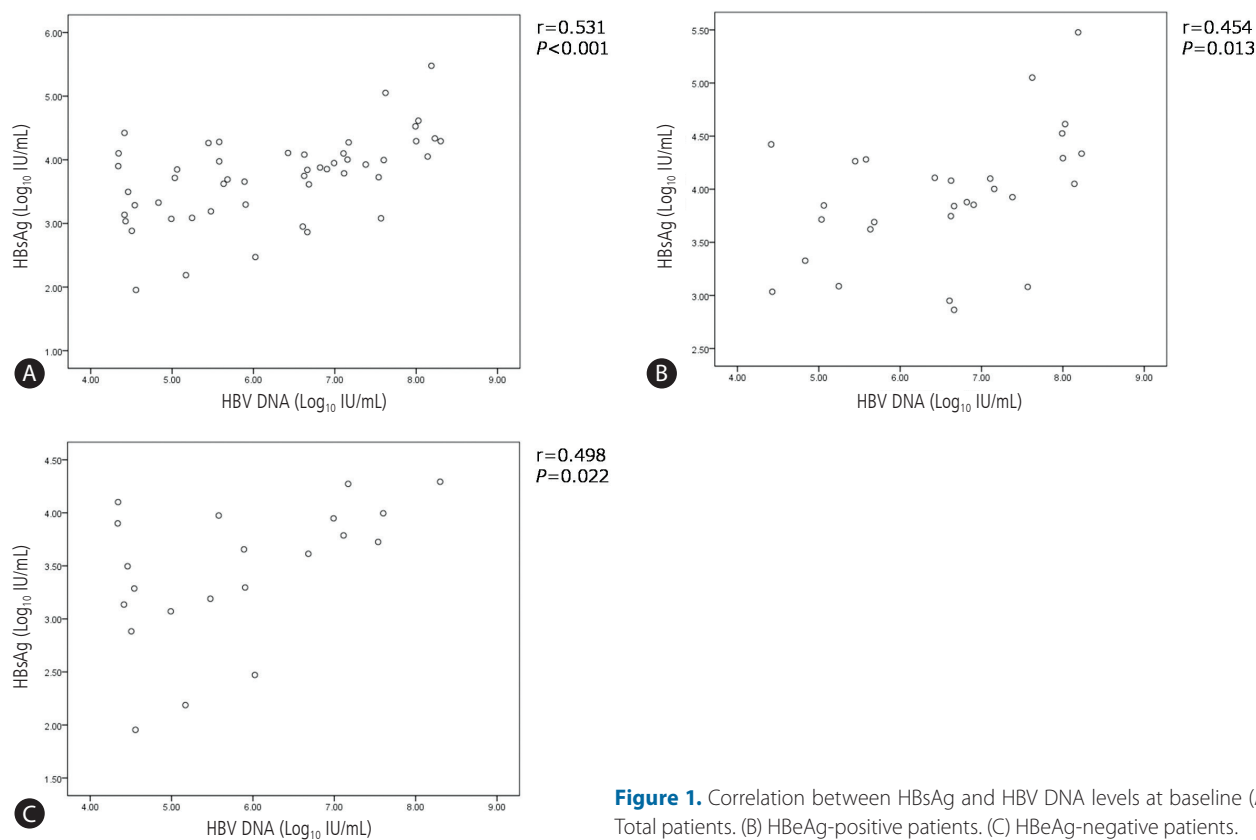


Figure 1. Correlation between HBsAg and HBV DNA levels at baseline (A) Total patients. (B) HBeAg-positive patients. (C) HBeAg-negative patients.

and HBeAg negative patients ($r=0.498$, $P=0.022$) (Fig. 1).

On treatment HBsAg and HBV DNA levels

Baseline HBsAg levels were not different significantly between patients without a VR and those with a VR (4.0 vs. 3.6 Log₁₀ IU/mL, $P=0.072$). However, HBsAg levels decreased after 6 months of treatment in patients who showed a VR and differed significantly between groups thereafter (3.9 vs. 3.3 at 6 months, $P=0.002$; 3.8 vs. 3.2 at 1 year, $P=0.004$; 3.9 vs. 3.2 at 2 years, $P=0.008$; and 3.7 vs. 3.1 at 3 years, $P=0.020$). The mean reduction in HBsAg levels from baseline was not different significantly between the two groups. The mean HBV DNA levels and reduction in HBV DNA levels from baseline were significantly different through entire time point (Table 2, Fig. 2).

Analysis according to VR within 1 year and 2 year, and HBeAg status

Thirty nine patients were HBeAg positive and 21 were negative. Twenty nine patients achieved VR within 1 year (eleven of HBeAg

positive patients and eighteen patients of HBeAg negative patients). HBsAg level at baseline was not significantly different according to VR (3.6 vs. 3.9 Log₁₀ copies/mL, $P=0.106$), but became lower in patients with VR at 6 months (3.3 vs. 3.6 Log₁₀ copies/mL, $P=0.044$) and 1 year (3.2 vs. 3.6 Log₁₀ copies/mL, $P=0.006$). HBV DNA level also showed significant differences at 6 months (2.3 vs. 4.2 Log₁₀ IU/mL, $P<0.001$) and 1 year (2.1 vs. 3.9 Log₁₀ IU/mL, $P<0.001$), but not at baseline (6.6 vs. 7.3 Log₁₀ IU/mL, $P=0.058$). On subanalysis according to HBeAg status, HBsAg levels at baseline, 6 months and 1 year were not different according to VR or not, in both HBeAg positive and negative patients. HBV DNA levels have become significantly lower in patients with VR at 6 months (2.4 vs. 4.3 Log₁₀ IU/mL, $P<0.001$) and at 1 year (2.2 vs. 3.9 Log₁₀ IU/mL, $P<0.001$) in HBeAg positive, and at baseline (6.2 vs. 8.0 Log₁₀ IU/mL, $P=0.025$) and at 1 year in HBeAg negative group (2.0 vs. 3.7 Log₁₀ IU/mL, $P=0.007$).

Thirty six patients achieved VR within 2 years (fifteen of HBeAg positive patients and all of HBeAg negative patients). HBsAg level was significantly lower in patients with VR at 6 months (3.3 vs. 3.8 Log₁₀ IU/mL, $P=0.001$), 1 year (3.2 vs. 3.7 Log₁₀ IU/mL, $P=0.005$) and 2 years (3.2 vs. 3.8 Log₁₀ IU/mL, $P=0.008$). HBV DNA level

Table 3. Comparison of HBsAg levels (\log_{10} IU/mL) and HBV DNA levels (\log_{10} IU/mL) according to virological response within 1 year and 2 year

Virological response within 1 year	Total			HBeAg positive			HBeAg negative		
	Positive (n=29)	Negative (n=21)	P-value	Positive (n=11)	Negative (n=18)	P-value	Positive (n=18)	Negative (n=3)	P-value
0 month									
HBsAg*	3.6±0.7	3.9±0.6	0.106	4.0±0.6	3.9±0.6	0.724	3.4±0.7	3.9±0.3	0.049
HBV DNA*	6.6±1.3	7.3±1.1	0.058	7.3±1.3	7.2±1.2	0.883	6.2±1.2	8.0±0.2	0.025
6 months									
HBsAg*	3.3±0.5	3.6±0.6	0.044	3.4±0.5	3.7±0.6	0.129	3.2±0.5	3.1±0.8	0.721
HBV DNA*	2.3±0.9	4.2±1.0	<0.001	2.4±0.9	4.3±1.0	<0.001	2.2±0.9	3.3±0.8	0.059
1 year									
HBsAg*	3.2±0.6	3.6±0.4	0.006	3.3±0.5	3.7±0.4	0.052	3.1±0.6	3.1±0.1	0.921
HBV DNA*	2.1±0.9	3.9±0.8	<0.001	2.2±0.7	3.9±0.8	<0.001	2.0±0.9	3.7±0.8	0.007
Virological response within 2 years	Total			HBeAg positive			HBeAg negative		
	Positive (n=36)	Negative (n=14)	P-value	Positive (n=15)	Negative (n=14)	P-value	Positive (n=21)	Negative (n=0)	P-value
0 month									
HBsAg*	3.6±0.7	4.0±0.6	0.102	4.0±0.6	4.0±0.6	0.834	3.4±0.7		N.A
HBV DNA*	6.8±1.3	7.4±1.2	0.172	7.3±1.1	7.4±1.2	0.906	6.5±1.3		
6 months									
HBsAg*	3.3±0.5	3.8±0.5	0.001	3.4±0.5	3.8±0.5	0.026	3.2±0.6		
HBV DNA*	2.6±0.9	4.7±0.8	<0.001	3.0±0.7	4.7±0.8	<0.001	2.4±1.0		
1 year									
HBsAg*	3.2±0.6	3.7±0.4	0.005	3.4±0.6	3.7±0.4	0.127	3.1±0.5		
HBV DNA*	2.4±0.9	4.3±0.5	<0.001	2.6±0.3	4.3±0.5	<0.001	2.2±1.1		
2 years									
HBsAg*	3.2±0.7	3.8±0.6	0.008	3.5±0.6	3.8±0.6	0.164	3.0±0.7		
HBV DNA*	1.2±1.1	3.7±1.1	<0.001	1.5±1.1	3.7±1.1	<0.001	1.0±1.1		

*Mean±standard deviation.

HBV, hepatitis B virus; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen.

also significantly lower in patients with VR at 6 months (2.6 vs. 4.7 \log_{10} IU/mL, $P<0.001$), 1 year (2.4 vs. 4.3 \log_{10} IU/mL, $P<0.001$) and 2 year (1.2 vs. 3.7 \log_{10} IU/mL, $P<0.001$). Because all of HBeAg negative patients achieved VR, only subanalysis for HBeAg positive patients were performed. HBsAg level was significantly lower in HBeAg positive VR group at 6 months (3.4 vs. 3.8 \log_{10} IU/mL, $P=0.026$). HBV DNA levels were lower in HBeAg positive VR group at 6 months (3.0 vs. 4.7 \log_{10} IU/mL, $P<0.001$), 1 year (2.6 vs. 4.3 \log_{10} IU/mL, $P<0.001$) and 2 years (1.5 vs. 3.7 \log_{10} IU/mL, $P<0.001$). (Table 3)

AUROC of HBsAg levels

The AUROCs of HBsAg levels at 6 months, 1 year and 2 years were 0.770, 0.781, and 0.768, respectively (Fig. 3). These values did not significantly differ (Table 4). With a cutoff value of 3.21 \log_{10} IU/mL for HBsAg at 6 months, the sensitivity and specificity for predicting a VR were estimated to be 45.0% and 100%, respectively, and the Youden index was 0.45. Kaplan-Meier analysis with the log-rank test showed a significance of difference in the VR rate according to this cutoff value ($P<0.001$, Fig. 4).

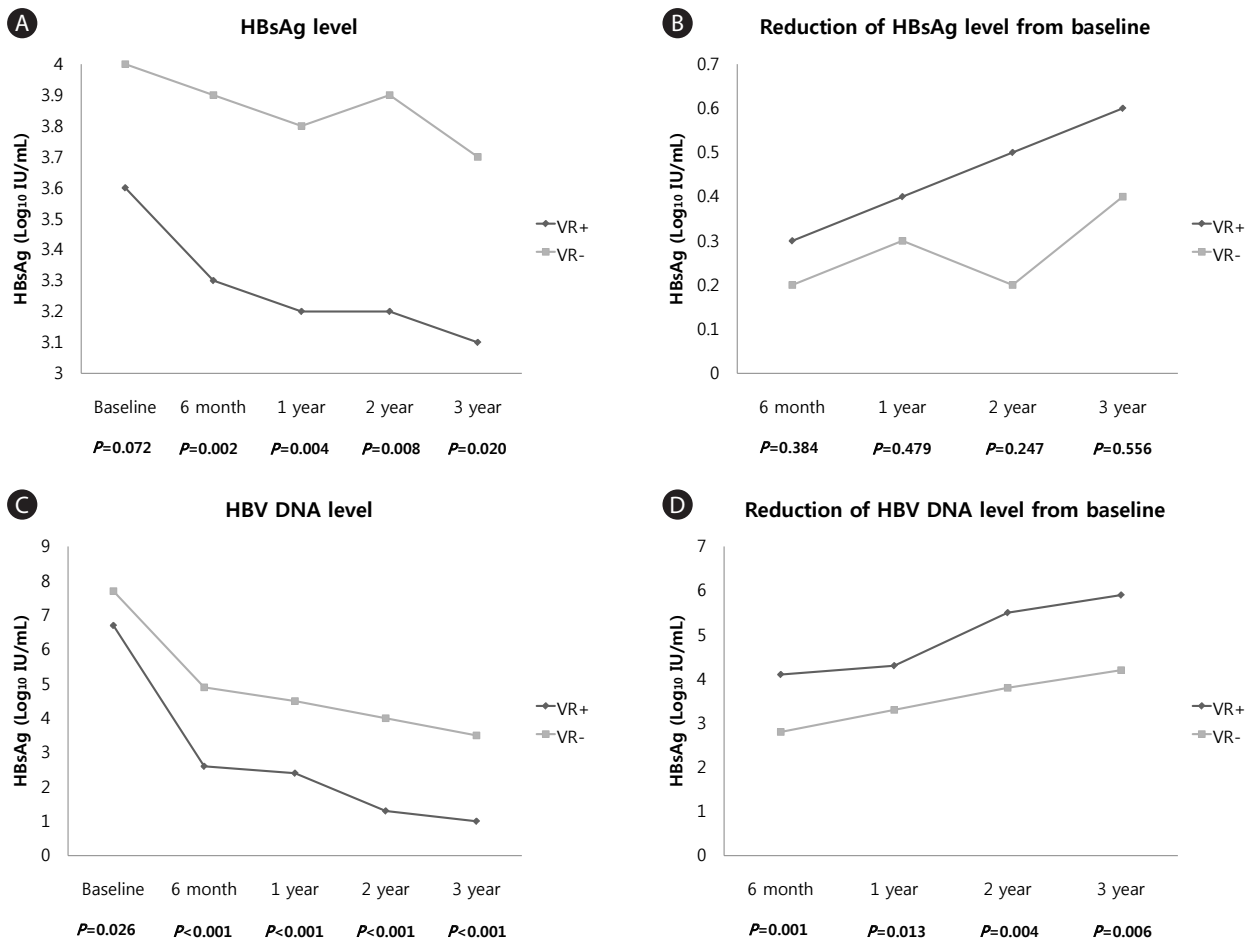


Figure 2. On treatment HBsAg and HBV DNA change. (A) HBsAg level, (B) Reduction of HBsAg from baseline, (C) HBV DNA level, (D) Reduction of HBV DNA level from baseline.

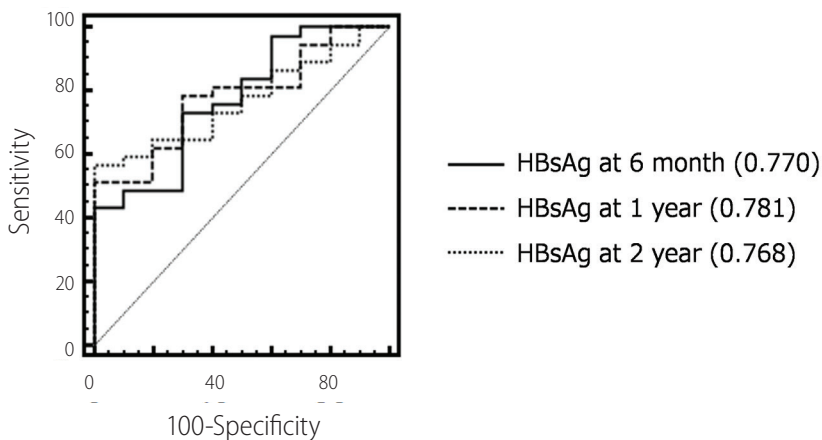


Figure 3. Areas under the receiver operating characteristics curve (AUROCs) of HBsAg levels at each time point for virological response.

DISCUSSION

CHB is an important cause of mortality and morbidity world-

wide, accounting for up to 1 million deaths annually.⁸ HBV infection is also the one of the most common etiology of chronic liver disease in Korea.⁹ Although the prevalence of chronic HBV infec-

Table 4. Pairwise comparison of AUROCs (HBsAg level for virological response)^a

	Difference between areas	Standard error	95% Confidence interval	P-value
6 month vs. 1 year	0.0108	0.0527	-0.0925 to 0.114	0.8375
6 month vs. 2 year	0.0027	0.0511	-0.0974 to 0.103	0.9578
1 year vs. 2 year	0.0135	0.0463	-0.0772 to 0.104	0.7703

HBsAg, hepatitis B surface antigen.

^aDeLong's test.

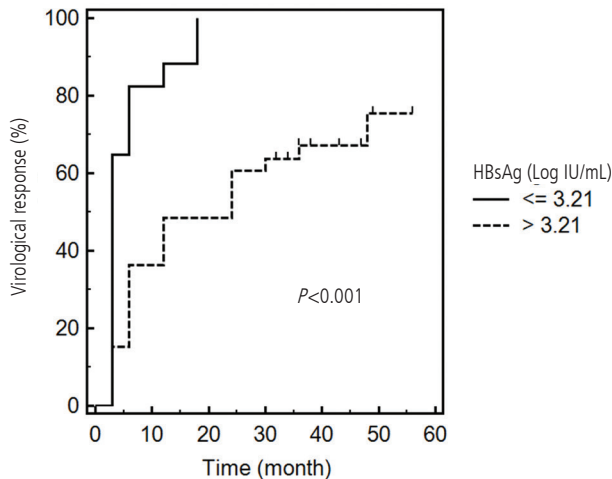


Figure 4. Kaplan-Meier curves of virological response with respect to HBsAg level 6 months.

tion is decreasing, this plays a major role in the etiology of liver cirrhosis and hepatocellular carcinoma in Korea.¹⁰

HBsAg has been the hallmark of HBV infection since the 1960s.¹¹ Several studies have shown a clear correlation between serum HBsAg level and covalently closed circular DNA (cccDNA)¹²⁻¹⁴ Studies using newly available automated quantitative assays have shown that serum levels of HBsAg are inversely proportional to the immune response against HBV, i.e., greater the immune response, lower the HBsAg level.¹⁵ Furthermore, several studies have shown that the decrease in serum HBsAg levels during pegylated interferon treatment mimics that in intrahepatic cccDNA, suggesting that a decrease in the serum HBsAg level is associated with the induction of an effective immune response.¹⁶⁻¹⁸ In contrast, treatment with nucleoside analogs may induce a marked decrease in HBV DNA levels; however, their effect on the serum level of HBsAg is limited, and the decrease in the HBsAg level during treatment is slower than the decrease in HBV DNA.^{3,19-21} Therefore, the clinical use of monitoring HBsAg levels in nucleoside analogs treatment for CHB patients is limited.¹⁵ Several studies show a correlation between the HBsAg level and treatment response during nucleoside analog treatment; however, these studies are limited

by their small sample size.³⁻⁵ To our knowledge, the present study is the first to evaluate the possibility of a correlation between HBsAg levels and treatment response during rescue therapy for LMV-resistant CHB patients. In the present study, levels of HBsAg were significantly lower in patients with a VR and AUROCs were not different at the different time points. Therefore, the HBsAg level at 6 months after treatment was sufficient to predict a VR. The results of Kaplan-Meier analysis corroborate this finding. Lee et al. showed baseline HBsAg level is useful in predicting VR.⁵ Although baseline HBsAg level was not significant value in our study, there was the tendency of lower in patients with VR (3.6 vs. 4.0, $P=0.072$). This became more prominent at 6 months. Both studies are limited by a small number of cases, and response pattern in treatment experienced patients may differ with naïve patients. Further study is warranted for this point of view.

In the present study, HBeAg negative patients were all achieved VR within 2 years. Therefore, the value of HBsAg level is higher for HBeAg positive patients. In HBeAg positive patients, HBsAg level was not significantly different according to VR within 1 year, but HBsAg level at 6 months became significant in the analysis for VR within 2 years. We can assume that HBsAg level is more profit for prediction of long term treatment response in comparison with HBV DNA level.

Current treatment guidelines suggest ETV or tenofovir (TDF) as the first-line antiviral agent for the CHB treatment.²²⁻²⁴ ETV and TDF have been available in Korea since 2007 and 2011, respectively. Therefore, majority of patients who started treatment before this period received LMV as first-line treatment and subsequently developed resistance. The proportions of patients with observed resistance who were treated with LMV for >1 and >4 years are reported to be 24% and 70% respectively.²⁵ If LMV resistance develops, current guidelines recommend a combination of nucleoside and nucleotide analogs as rescue therapy.²²⁻²⁴ LMV-ADV combination therapy is the current most commonly used strategy and is superior to both ETV monotherapy and ADV monotherapy.²⁶⁻³² However, some patients fail to achieve a VR with this combination and require more potent treatment. HBsAg quantitation can be helpful in determining whether a drug change is war-

ranted in such cases.

Many studies report a good correlation between HBsAg and HBV DNA levels in treatment-naïve CHB patients³³⁻³⁶ However, this correlation has not been evaluated in LMV-resistant patients. The HBV polymerase gene mutation can alter the amino acid codon sequences of the surface region and vice versa, because the polymerase gene has overlapping reading frames with the surface genes. Therefore, selection of a drug-resistant HBV polymerase gene mutation may have important implications^{37,38} The P120A mutation in the surface region results in a failure to detect HBsAg in LMV-treated patients.³⁹ The rtV173L (sE164D), one of LMV-resistant polymerase mutation, is reported to reduce the antigenicity of HBsAg.⁴⁰ The results of the present study demonstrate a good correlation between HBsAg and HBV DNA levels. Therefore, it can be assumed HBsAg kinetics is similar to that in treatment-naïve cases and that the clinical implications of LMV-resistant polymerase mutations on HBsAg levels are limited. However, HBsAg quantitation studies on other types of resistance mutations remain limited. The overlap of surface region mutations, such as sW172stop in patients carrying rtA181T, and sL173F in patients carrying rtA181V, has been reported in CHB patients treated with ADV. However, the clinical consequences of these mutations have not been determined.^{37,41} One study reported no significant difference in the levels of HBsAg in such patients, but the study had a small sample size; therefore, this point requires further evaluation.⁴²

In conclusion, HBsAg levels at 6 months of LMV-ADV combination therapy can help predict treatment response. More potent treatments should be considered for cases positive for HBeAg, with high baseline HBV DNA and high HBsAg levels after 6 months treatment.

Conflicts of Interest

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

REFERENCES

1. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* (Baltimore, Md.) 2009;49:1151-1157.
2. Perrillo R. Editorial: Measurement of HBsAg concentration during antiviral therapy of hepatitis B: who, when, and why. *Am J Gastroenterol* 2011;106:1774-1776.
3. Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *Hepatology* 2009;49:1141-1150.
4. Jung YK, Kim JH, Lee YS, Lee HJ, Yoon E, Jung ES, et al. Change in serum hepatitis B surface antigen level and its clinical significance in treatment-naïve, hepatitis B e antigen-positive patients receiving entecavir. *J Clin Gastroenterol* 2010;44:653-657.
5. Lee JM, Ahn SH, Kim HS, Park H, Chang HY, Kim do Y, et al. Quantitative hepatitis B surface antigen and hepatitis B e antigen titers in prediction of treatment response to entecavir. *Hepatology* 2011;53:1486-1493.
6. Yeon JE, Yoo W, Hong SP, Chang YJ, Yu SK, Kim JH, et al. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. *Gut* 2006;55:1488-1495.
7. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;44:837-845.
8. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004;11:97-107.
9. Lee SS, Byoun YS, Jeong SH, Kim YM, Gil H, Min BY, et al. Type and cause of liver disease in Korea: single-center experience, 2005-2010. *Clin Mol Hepatol* 2012;18:309-315.
10. Chae HB, Kim JH, Kim JK, Yim HJ. Current status of liver diseases in Korea: hepatitis B. *The Korean J Hepatol* 2009;15 Suppl 6:S13-24.
11. Rotman Y, Brown TA, Hoofnagle JH. Evaluation of the patient with hepatitis B. *Hepatology* 2009;49:S22-27.
12. Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. *J Hepatol* 2010;52:508-513.
13. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. *J Hepatol* 2010;52:514-522.
14. Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 2010;51:1933-1944.
15. Janssen HL, Sonneveld MJ, Brunetto MR. Quantification of serum hepatitis B surface antigen: is it useful for the management of chronic hepatitis B? *Gut* 2012;61:641-645.
16. Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology* 2006;44:675-684.
17. Chan HL, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, et al. Se-

- rum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. *Clin Gastroenterol Hepatol* 2007;5:1462-8.
18. Janssen HL, Kerhof-Los CJ, Heijtk RA, Schalm SW. Measurement of HBsAg to monitor hepatitis B viral replication in patients on alpha-interferon therapy. *Antiviral Res* 1994;23:251-257.
 19. Reijnders JG, Rijckborst V, Sonneveld MJ, Scherbeijn SM, Boucher CA, Hansen BE, et al. Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir. *J Hepatol* 2011;54:449-454.
 20. Borgniet O1, Parvaz P, Bouix C, Chevallier P, Trépo C, André P, et al. Clearance of serum HBsAg and anti-HBs seroconversion following antiviral therapy for chronic hepatitis B. *J Med Virol* 2009;81:1336-1342.
 21. Kim JH, Choi YJ, Moon HW, Ko SY, Choe WH, Kwon SY. HBsAg level and clinical course in patients with chronic hepatitis B treated with nucleoside analogue: five years of follow-up data. *Clin Mol Hepatol* 2013;19:409-416.
 22. Korean Association for the Study of the Liver. KASL Clinical Practice Guidelines: Management of chronic hepatitis B. *Clin Mol Hepatol* 2012;18:109-162.
 23. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012;57:167-185.
 24. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009;50:661-662.
 25. Zoulim F, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009;137:1593-608.e1-2.
 26. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006;44:283-290.
 27. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* 2007;45:307-313.
 28. Ijaz S, Arnold C, Dervisevic S, Mechurova J, Tatman N, Tedder RS, et al. Dynamics of lamivudine-resistant hepatitis B virus during adefovir monotherapy versus lamivudine plus adefovir combination therapy. *J Med Virol* 2008;80:1160-1170.
 29. Vassiliadis TG, Gioulema O, Koumerkeridis G, Koumaras H, Tziomalos K, Patsiaoura K, et al. Adefovir plus lamivudine are more effective than adefovir alone in lamivudine-resistant HBeAg- chronic hepatitis B patients: a 4-year study. *J Gastroenterol Hepatol* 2010;25:54-60.
 30. Ryu HJ, Lee JM, Ahn SH, Kim do Y, Lee MH, Han KH, et al. Efficacy of adefovir add-on lamivudine rescue therapy compared with switching to entecavir monotherapy in patients with lamivudine-resistant chronic hepatitis B. *J Med Virol* 2010;82:1835-1842.
 31. Kim HJ, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, et al. Rescue therapy for lamivudine-resistant chronic hepatitis B: comparison between entecavir 1.0 mg monotherapy, adefovir monotherapy and adefovir add-on lamivudine combination therapy. *J Gastroenterol Hepatol* 2010;25:1374-1380.
 32. Chung GE, Kim W, Lee KL, Hwang SY, Lee JH, Kim HY, et al. Add-on adefovir is superior to a switch to entecavir as rescue therapy for Lamivudine-resistant chronic hepatitis B. *Dig Dis Sci* 2011;56:2130-2136.
 33. Lee HJ, Kim SY, Lee SM, Heo J, Kim HH, Chang CL, et al. Elecsys hepatitis B surface antigen quantitative assay: performance evaluation and correlation with hepatitis B virus DNA during 96 weeks of follow-up in chronic hepatitis B patients. *Ann Lab Med* 2012;32:420-425.
 34. Lee JH, Kim SJ, Ahn SH, Lee J, Park Y, Kim HS. Correlation between quantitative serum HBsAg and HBV DNA test in Korean patients who showed high level of HBsAg. *J Clin Pathol* 2010;63:1027-1031.
 35. Su TH, Hsu CS, Chen CL, Liu CH, Huang YW, Tseng TC, et al. Serum hepatitis B surface antigen concentration correlates with HBV DNA level in patients with chronic hepatitis B. *Antivir Ther* 2010;15:1133-1139.
 36. Ozaras R, Tabak F, Tahan V, Ozturk R, Akin H, Mert A, et al. Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. *Dig Dis Sci* 2008;53:2995-2998.
 37. Bartholomeusz A, Locarnini S. Hepatitis B virus mutations associated with antiviral therapy. *J Med Virol* 2006;78 Suppl 1:552-555.
 38. Torresi J. The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. *J Clin Virol* 2002;25:97-106.
 39. Hsu CW, Yeh CT, Chang ML, Liaw YF. Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology* 2007;132:543-550.
 40. Torresi J, Earnest-Silveira L, Deliyannis G, Edgton K, Zhuang H, Locarnini SA, et al. Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. *Virology* 2002;293:305-313.
 41. Locarnini S, Mason WS. Cellular and virological mechanisms of HBV drug resistance. *J Hepatol* 2006;44:422-431.
 42. Kim JH, Jung YK, Joo MK, Yim HJ, Park JJ, Kim JS, et al. Hepatitis B viral surface mutations in patients with adefovir resistant chronic hepatitis B with A181T/V polymerase mutations. *J Korean Med Sci* 2010;25:257-264.