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ORIGINAL ARTICLE

Baseline hepatitis B surface antigen quantitation can predict virologic response in entecavir-treated chronic hepatitis B patients

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quantitative hepatitis B surface antigen

Background/Purpose: Several anti-viral drugs are approved for the treatment of hepatitis B virus (HBV) infection. However, whether quantitative hepatitis B surface antigen (qHBsAg) can predict the therapeutic response during long-term entecavir treatment remains unclear.
Methods: Fifty-five chronic hepatitis B (CHB) patients who received entecavir for more than 2 years were enrolled. The serum qHBsAg level was measured by HBsAg II quant immunoassay. A significant decline in the qHBsAg level was defined as $>$ 1 log reduction from baseline to 6 months of entecavir treatment.
Results: Of the 55 patients (41 males and 14 females with a mean age of 48.3 $\pm$ 11.4 years), 23 patients were positive for hepatitis B e antigen (HBeAg). The median treatment period was 34 months, and ranged from 26 months to 43 months. A total of 288 serum samples were used to determine the qHBsAg levels. At year 3 of entecavir therapy, one (1.8%) patient had HBsAg seroclearance. A high qHBsAg level was defined as greater than 10,000 IU/mL. Patients with a high baseline qHBsAg level had a lower rate of virologic response at year 1 (37.5% vs. 89.7%, $p < 0.001$) and year 2 (56.2% vs. 94.9%, $p = 0.001$). In this study population, 14.5% had a significant decline of the qHBsAg level. A significant decline could not predict HBeAg loss in HBeAg-positive or virologic response in all patients.

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Conclusion: The baseline serum qHBsAg level can predict virologic response in entecavir-treated CHB patients. However, a significant decline in the qHBsAg level cannot predict serologic or virologic response of entecavir treatment.

Introduction

Hepatitis B virus (HBV) infection affects about 350 million people and is the tenth leading causes of death in the world.[1] Its complications include cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC), leading to more than 600,000 premature deaths annually.[2] Although new HBV infection can be effectively prevented by hepatitis B vaccination, the existing chronic hepatitis B (CHB) patients continue to be a health burden worldwide. Interferon-based or antiviral therapies have recently been proven to decrease hepatic inflammation and reduce the development of hepatic complications in patients with chronic HBV infection.[3]

Entecavir (ETV) is a nucleoside analogue used for the treatment of CHB. Previous studies have proven its efficacy in HBeAg-positive and HBeAg-negative CHB patients.[4–6] Better histological improvement, higher rate of undetectable serum HBV DNA, and normalization of serum alanine aminotransferase (ALT) were noted at 48 weeks of therapy with ETV in comparison to lamivudine (LMV) therapy. However, the hepatitis B e antigen (HBeAg) seroconversion rate was similar between ETV and LMV in HBeAg-positive CHB patients. Because of the minimal risk of developing resistance to ETV, several guidelines have recommended the drug as a first-line agent for the treatment of CHB.[7,8]

In 1965, hepatitis B surface antigen (HBsAg) was initially identified and named "Australia antigen".[9] This finding subsequently contributed to the identification of HBV. The antigenic protein on the outer surface of HBV virion could be used as the target for the development of serologic test and effective vaccines. In the past decades, the HBsAg test was only qualitative. In clinical practice, a positive result indicates ongoing HBV infection. The HBsAg level has recently been quantitatively measured by newly developed techniques. The serum quantitative HBsAg (qHBsAg) level is reportedly correlated with intrahepatic HBV DNA and with covalently closed circular DNA (cccDNA) levels.[10] The on-treatment level or dynamic change of HBsAg is also an indicator of treatment response in pegylated interferon (PEG INF)-treated CHB patients.[11,12] However, whether the kinetics of qHBsAg during entecavir treatment can predict the treatment response remains unclear and deserves further investigation.

Materials and methods

In 2003, the Bureau of National Health Insurance in Taipei, Taiwan began a treatment program for CHB. To date, oral agents such as lamivudine, telbivudine, or entecavir can be reimbursed for 3 years in HBeAg-positive or HBeAg-negative CHB patients, and reimbursed indefinitely in cirrhotic patients. In this prospective study, patients were recruited from the Buddhist Tzu Chi General Hospital between October 2007 and March 2009. The inclusion criteria of eligible patients were the presence of HBsAg for at least 6 months. The patients should meet one of the following criteria prior to receiving ETV at a dose of 0.5 mg daily: (1) a serum alanine aminotransferase (ALT) level greater than five times the upper limit of normal (ULN) in HBeAg-positive patients; (2) a serum ALT level greater than 2 times the ULN and a serum HBV DNA level greater than 20,000 IU/mL in HBeAg-positive patients; (3) a serum ALT level greater than two times the ULN, obtained twice in a period of at least 3 months, and a serum HBV DNA level greater than 2,000 IU/mL in HBeAg-negative patients; (4) hepatic decompensation with documentation of a total bilirubin level greater than 2 mg/dL or a prolonged prothrombin time greater than 3 seconds, compared to a normal control; or (5) cirrhosis with evidence of portal hypertension such as splenomegaly or varices. All participants received regular follow-up at 3-month intervals during entecavir treatment and hepatic ultrasound examination every 6 months.

The exclusion criteria consisted of coinfection with hepatitis C virus or the presence of other known liver diseases such as alcoholism or autoimmune or metabolic liver disease. Each participant provided written, informed consent. All patients agreed to have serum samples stored before treatment, and at 3 months, 6 months, 1 year, 2 years, and 3 years after treatment. The sera were stored at -80°C until tested. The HBV genotype, the status of serum HBeAg, and the pattern of the precore or core promoter region were determined at study entry. The serologic response was defined as HBeAg loss and/or seroconversion in HBeAg-positive CHB patients. The virologic response was defined as an undetectable level of serum HBV DNA in HBeAg-positive or HBeAg-negative CHB patients. Because most patients continued ETV treatment at the end of this study, the on-treatment response was adopted in the current study. At the time of data analyses, 123 patients had given informed consent. Of these, 67 patients received ETV treatment for at least 2 years. Twelve (17.9%) patients were excluded because of the lack of available data of baseline qHBsAg levels. Fifty-five patients were recruited for statistic analyses.

Laboratory assays

The serum ALT level was measured by using the automated method (Roche Analytics; Roche Professional Diagnostics, Penzberg, Germany) with the UNL of 40 IU/mL. The serum HBsAg, HBeAg, and anti-HBe were tested by commercial enzyme-linked immunosorbent assays.

The serum HBV DNA level was measured with a real-time polymerase chain reaction Cobas TaqMan HBV assay (Roche Molecular Systems, Branchburg, NJ, USA) with the lower detection limit at 60 IU/mL and the upper limit at 1.1×10^8 IU/mL. The genotypes of HBV were determined by using a real-time polymerase chain reaction (PCR)-based single-tube assay, as previously described.[13] For HBV genomic analysis, precore and core promoter genes were amplified by PCR.[14] Nucleotide sequences of the amplified products were directly determined by using fluorescence-labeled primers with a 3100 Automatic Sequencer (Applied Biosystems, Foster City, CA, USA).

The Elecsys HBsAg II quant immunoassay (Roche Analytics; Roche Professional Diagnostics, Branchburg, New Jersey, USA) was used to quantify the serum HBsAg level, according to the manufacturer's instructions. This is a one-step sandwich assay: the HBsAg in the samples is bound to an antibody to HBsAg (anti-HBs) and streptavidin-coated microparticle. The chemiluminescence reaction was initiated by applying a voltage to the sample solution to detect the reaction complex. The analyzer automatically performed the on-board dilution. The range of measurement was 0.05—52,000 IU/mL.

Endpoints

The primary endpoint of this study was to understand the kinetics of the qHBsAg level in patients receiving ETV treatment. The secondary endpoint was to elucidate the factors affecting the qHBsAg level and the significant decline of qHBsAg.

Ethical considerations

The study was performed in accordance with the principles of the 1975 Declaration of Helsinki and approved by the Ethical Committee of Buddhist Tzu-Chi General Hospital (98-IRB-018-X). Each participant provided written, informed consent.

Statistical analysis

Continuous variables were expressed as the mean and the standard deviation or as the median and the range. They were analyzed with a *t* test. Categorical variables were included by using the frequency table and the Chi-square test. A p value <0.05 was considered statistically significant.

Results

Baseline demographics and background characteristics

Table 1 summarizes the baseline clinical and virologic characteristics of 55 CHB patients [41 males (74.6%) and 14 females (25.4%)]. The mean age at enrolment was

Table 1 Baseline characteristics of the chronic hepatitis B cohort.

	HBeAg(−) (n = 32)	HBeAg(+) (n = 23)	p
Age (y; age range)	52.0 ± 10.0 (31.9—80.8)	43.1 ± 11.4 (25.8—69.0)	0.004[a]
Sex			
Female	8 (25.0)	6 (26.1)	
Male	24 (75.0)	17 (73.9)	> 0.99
Cirrhosis			
No	15 (46.9)	18 (78.3)	
Yes	17 (53.1)	5 (21.7)	0.026[a]
Baseline HBV DNA level (log_{10} IU/mL)	5.2 ± 1.5	7.3 ± 1.5	<0.001[a]
Baseline ALT level (U/L)	103.8 ± 83.1	144.8 ± 339.8	0.004[a]
Baseline qHBsAg level (log_{10} IU/mL), median (range)	3.3 (1.9—5.3)	4.2 (1.7—5.8)	0.002[a]
HBV genotype			
B	22 (68.8)	14 (63.6)	
C	1 (31.2)	8 (36.4)	0.773
Precore			
Wild	3 (9.4)	15 (68.2)	
Mutant	27 (84.4)	7 (31.8)	
Undetermined	2 (6.3)	0 (0.0)	< 0.001[a]
Basal core promoter			
Wild	15 (46.9)	12 (54.6)	
Mutant	17 (53.1)	9 (40.9)	
Undetermined	0 (0.0)	1 (4.5)	0.3957

Data are presented as n (%) or mean ± SD.
ALT = alanine aminotransferase; DNA = deoxyribonucleic acid; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; qHBsAg = quantitative hepatitis B surface antigen; SD = standard deviation.
[a] Indicates a significant difference ($p < 0.05$).

48.3 ± 11.4 years. In this study, seven (12.7%) patients were receiving treatment for hepatocellular carcinoma and seven patients had used LMV. The median treatment period was 34 months (range, 26—43 months). Of the 55 patients, 23 patients were HBeAg-positive and 32 were HBeAg-negative. For qHBsAg levels, 288 samples were analyzed. In one (1.8%) patient, HBsAg seroclearance was noted at year 3 of ETV therapy.

Comparison of baseline characteristics between HBeAg-positive and HBeAg-negative CHB patients

HBeAg-positive patients were younger and had higher serum ALT levels and higher baseline HBV DNA level, compared to HBeAg-negative patients. The qHBsAg level was higher in HBeAg-positive than in HBeAg-negative patients. The cirrhosis prevalence rate and precore mutation of the HBV genome were higher in HBeAg-negative patients, compared to HBeAg-positive patients. There was no difference between the two groups in sex, prevalence of HBV genotypes, and basal core promoter mutant (Table 1).

Treatment outcomes in HBeAg-positive and HBeAg-negative CHB patients

In HBeAg-positive patients, the rates of HBeAg loss at year 1 and year 2 were 21.7% and 47.8%, respectively. In HBeAg-negative patients, the rates of virologic response (HBV DNA <60 IU/mL) were 84.4% at year 1 and 93.7% at year 2. The rate of virologic response at year 2 was significantly higher in HBeAg-negative patients than in HBeAg-positive patients (Fig. 1). One (1.8%) patient had HBsAg seroclearance at year 3 of entecavir therapy. This was a 35-year-old female patient who was HBeAg-positive at baseline. The kinetics of qHBsAg was 19,404 IU/mL at baseline; it decreased to 2.1 IU/mL after 6 months of ETV treatment and became undetectable after 3 years of ETV treatment.

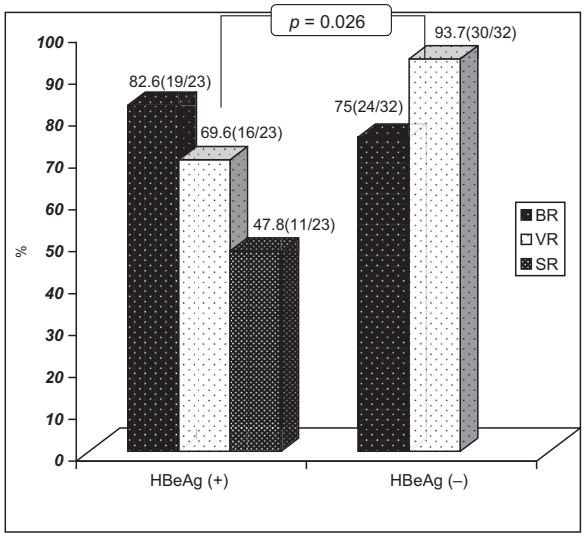

Figure 1 The 2-year response of entecavir treatment in HBeAg-positive and HBeAg-negative chronic hepatitis B patients. BR = biochemical response (defined as normalized ALT level after 2 years of entecavir treatment); HBeAg = hepatitis B e antigen; SR = serologic response (defined as HBeAg loss and/or seroconversion after 2 years of entecavir treatment); VR = virologic response (defined as undetectable HBV DNA levels after 2 years of entecavir treatment).

The dynamics of qHBsAg levels during entecavir treatment

The baseline qHBsAg levels were higher in HBeAg-positive patients than in HBeAg-negative patients (median 4.2 $\log_{10}$ IU/mL vs. 3.3 $\log_{10}$ IU/mL, respectively) ($p = 0.002$). In HBeAg-negative patients, the qHBsAg levels remained stationary after entecavir treatment for up to 24 months. By contrast, the qHBsAg levels declined rapidly after 3 months of entecavir treatment in HBeAg-positive patients, but remained stationary thereafter for up to 24 months (Fig. 2).

Baseline characteristics associated with a high qHBsAg level and the impact of a high qHBsAg level on treatment outcomes

A high qHBsAg level was defined as a level greater than 10,000 IU/mL. The patients with a high qHBsAg level were younger and had a higher frequency of positive HBeAg. High qHBsAg level was also correlated with high serum ALT and HBV DNA levels. The patients with a high qHBsAg level tended to have a lower frequency of cirrhosis, compared to qHBsAg-negative patients ($p = 0.068$). There was no difference in sex or distribution of HBV genotypes between the two groups. After adjusting for age, sex, cirrhosis, HBeAg status, serum ALT, and log HBV DNA levels, multivariate analysis showed that HBV DNA was the only independent factor associated with higher qHBsAg levels (odds ratio 4.08, 95% confidence interval 1.63—10.23; data not shown). In regard to the ability of qHBsAg to predict treatment responses, at year 1 patients with a high baseline qHBsAg level had a worse virologic response, compared to qHBsAg-negative patients (37.5% vs. 89.7%, respectively; $p < 0.001$) and at year 2 (56.2% vs. 94.9%; $p = 0.001$).

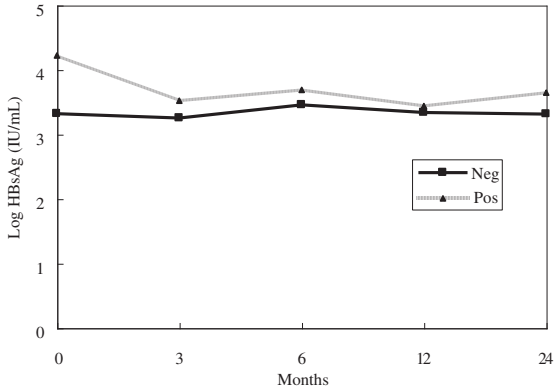

Figure 2 The dynamics of quantitative HBsAg levels during entecavir treatment. Neg = HBeAg-negative patients; Pos = HBeAg-positive patients.

Table 2 Clinical factors and outcome of treatment, according to baseline serum HBsAg levels ≥ 10,000 IU/mL.

	Baseline qHBsAg ≥ 10,000 IU/mL (n = 16)	Baseline qHBsAg <10,000 IU/mL (n = 39)	p
Age (y; age range)	42.5 ± 10.9 (27.1–59.0)	50.6 ± 10.9 (25.8–80.8)	0.019[a]
Sex			
Female	3 (18.8)	11 (28.2)	
Male	13 (81.2)	28 (71.8)	0.734
HBeAg			
Negative	3 (18.8)	29 (74.4)	
Positive	13 (81.2)	10 (25.6)	< 0.001[a]
ALT (U/L)	303.0 ± 396.8	132.8 ± 118.8	0.018[a]
HBV DNA ($\log_{10}$ IU/mL)	7.9 ± 1.5296	5.3 ± 1.3276	< 0.001[a]
Cirrhosis			
No	13 (81.3)	20 (51.3)	
Yes	3 (18.7)	19 (48.7)	0.068
HBV genotype			
B	10 (66.7)	26 (66.7)	
C	5 (33.3)	13 (33.3)	> 0.99
Undetectable serum HBV DNA at 1 y			
No	10 (62.5)	4 (10.3)	
Yes	6 (37.5)	35 (89.7)	< 0.001[a]
Undetectable serum HBV DNA at 2 y			
No	7 (43.8)	2 (5.1)	
Yes	9 (56.2)	37 (94.9)	0.001[a]
HBeAg loss at 1 y in HBeAg(+) patients			
No	11 (84.6)	7 (70.0)	
Yes	2 (15.4)	3 (30.0)	0.618
HBeAg loss at 2 y in HBeAg(+) patients			
No	8 (61.5)	4 (40.0)	
Yes	5 (38.5)	6 (60.0)	0.414

Data are presented as n (%) or mean ± SD.
ALT = alanine aminotransferase; DNA = deoxyribonucleic acid; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; qHBsAg = quantitative hepatitis B surface antigen; SD = standard deviation.
[a] Indicates a significant difference ($p < 0.05$).

However, the 1-year and 2-year HBeAg loss rate were comparable between patients with a high qHBsAg level and qHBsAg-negative patients (Table 2).

Baseline characteristics associated with significant decline of qHBsAg at 6 months of therapy and its impact on treatment outcomes

A significant decline of the qHBsAg level at 6 months was defined as a greater than 1 $\log_{10}$ IU/mL reduction from the baseline to 6 months of treatment. Eight patients achieved a significant decline of qHBsAg at 6 months. Of these patients, 6 patients were HBeAg-positive at baseline. Two patients had HBeAg seroconversion after 2 years of entecavir treatment. One patient subsequently achieved HBsAg loss at year 3. The baseline HBeAg status was negative in two patients. Their HBV DNA levels were undetectable in both patients after 2 years of entecavir treatment. Between patients with a significant decline in the HBV DNA level and those without a decline, there was no difference in age, sex, cirrhosis, or HBV DNA levels. A significant decline of the qHBsAg level 6 months after beginning entecavir treatment was associated with high baseline levels of ALT and qHBsAg. A significant decline of the qHBsAg level at 6 months could not predict 1-year or 2-year HBeAg loss in HBeAg-positive patients or the virologic response in CHB patients receiving ETV treatment (Table 3).

Discussion

In this study, 55 CHB patients received ETV for at least 2 years. After 6 months of treatment, 14.5% of the patients had a decrease of the qHBsAg level that was greater than 1 $\log_{10}$ IU/mL. A baseline qHBsAg level greater than 10,000 IU/mL was associated with a worse virologic response. If the baseline qHBsAg level was less than 10,000 IU/mL, then virologic response could be achieved in 89.7% of patients at year 1 and 94.9% of patients at year 2. A significant decline of the serum qHBsAg level cannot predict virologic response or HBeAg loss. These findings suggested that baseline qHBsAg level may serve as a predictor of virologic response in CHB patients receiving ETV treatment. However, a significant decline of the qHBsAg level could not predict both virologic and serologic responses in this study population.

Table 3 Clinical factors and therapeutic outcome associated with a significant decline in the serum HBsAg level (>1 log) at 6 months of entecavir treatment.

	Significant decline ($n = 8$)	No significant decline ($n = 47$)	p
Age (y; age range)	44.8 ± 13.6 (27.1–65.3)	48.8 ± 11.0 (25.8–80.8)	0.389
Sex			
Female	1 (12.5)	13 (27.7)	
Male	7 (87.5)	34 (72.3)	0.664
HBeAg			
Negative	2 (25.0)	30 (63.8)	
Positive	6 (75.0)	17 (36.2)	0.057
ALT (U/L)	444.3 ± 519.7	137.8 ± 122.9	<0.001[a]
qHBsAg ($\log_{10}$ IU/mL)	4.1 ± 0.8222	3.5 ± 0.6922	< 0.001[a]
HBV DNA ($\log_{10}$ IU/mL)	5.1 ± 2.3082	5.9 ± 1.6857	0.112
Cirrhosis			
No	6 (75.0)	27 (57.5)	
Yes	2 (25.0)	20 (42.6)	0.454
Undetectable HBV DNA at 1 y			
No	4 (50.0)	10 (21.3)	
Yes	4 (50.0)	37 (78.7)	0.181
Undetectable HBV DNA at 2 y			
No	2 (25.0)	7 (14.9)	
Yes	6 (75.0)	40 (85.1)	0.604
HBeAg loss at 1 y in HBeAg(+) patients ($n = 23$)			
No	5 (83.3)	13 (76.5)	
Yes	1 (16.7)	4 (23.5)	> 0.99
HBeAg loss at 2 y in HBeAg(+) patients ($n = 23$)			
No	4 (66.7)	8 (47.1)	
Yes	2 (33.3)	9 (52.9)	0.640

Data are presented as n (%) or mean ± SD.
ALT = alanine aminotransferase; DNA = deoxyribonucleic acid; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; qHBsAg = quantitative hepatitis B surface antigen; SD = standard deviation.
[a] Indicates a significant difference ($p < 0.05$).

In HBeAg-positive patients receiving PEG-IFN therapy, the therapeutic response was better in patients with a baseline qHBsAg level less than 10,000 U/mL.[15] Ninety-five CHB patients were recently treated with ETV for 2 years; 60% of these patients were HBeAg-positive. The baseline qHBsAg levels were useful in predicting the virologic response to entecavir treatment in 57 HBeAg-positive CHB patients. Using the area under the curve analysis, researchers have found that a baseline cutoff qHBsAg level of 9550 IU/mL had the highest predictive value.[16] Our study showed similar findings in that a baseline qHBsAg level less than 10,000 U/mL could predict a better virologic response in CHB patients receiving ETV treatment. In addition, this finding could be extrapolated to all CHB patients, regardless of the HBeAg status. Based on these lines of evidence, the qHBsAg level should be routinely assayed in CHB patients prior to starting ETV treatment. If the serum qHBsAg level was less than 10,000 IU/mL, the virologic response could be approximately 90% at year 1 and thereafter. In clinical practice, patients are advised to receive HBV DNA quantification once yearly in treatment-naïve CHB patients during the follow-up period, if the economic conditions allow this. For patients on anti-HBV therapy, measurement of the viral load is recommended at 3 months in case of primary treatment failure, at 6 months to obtain the status of the initial virologic response, and every 6 months thereafter. From our data, if the baseline qHBsAg level was less than 10,000 U/mL, the viral load could be measured at year 1 and once yearly thereafter because of the very high rate of virologic response in these CHB patients. Thus, our findings are clinically valuable. Because the measurement of serum HBV DNA level is expensive, the cost of HBV DNA monitoring could be reduced in this special population.

An earlier study of 28 HBeAg-positive CHB patients receiving ETV therapy found that a reduction in the serum qHBsAg levels greater than 1 $\log_{10}$ IU/mL from the baseline level after 12 months of treatment was associated with a significantly higher 1-year HBeAg loss rate.[17] This was the first study to seek the utility of the qHBsAg level in predicting HBeAg loss in CHB patients with ETV therapy. However, the sample size was relatively small and 1-year of entecavir treatment was suboptimal in clinical practice. A study with larger sample size subsequently showed inconsistent results. They found that the early decline of qHBsAg levels at 12 weeks and 24 weeks were not associated with HBV DNA suppression or HBeAg seroconversion in ETV-treated CHB patients.[18] Therefore, further studies are needed to clarify this important and interesting issue. Our data showed that a significant decline of qHBsAg level cannot predict virologic response in overall patients and

HBeAg loss in HBeAg-positive patients. Thus, these results could improve our understanding about the relationship between the decline in the qHBsAg level and the response to ETV treatment.

Serum qHBsAg levels are reportedly associated with intrahepatic HBV DNA and cccDNA levels.[10,19] It is also correlated with serum HBV DNA levels.[20] Previous studies indicate that qHBsAg levels varied during different phases of the natural history of HBV infection. For example, the qHBsAg level is highest in the immune tolerance phase, and lower during the immune clearance phase, and decreases progressively after HBeAg seroconversion.[21] The qHBsAg levels are higher in HBeAg-positive patients than in HBeAg-negative patients.[22] Our study's findings were consistent with previous studies. After adjusting for age, sex, cirrhosis, HBeAg status, serum ALT, and log HBV DNA levels, multivariate analysis showed that HBV DNA was the only independent factor associated with high qHBsAg levels (odds ratio 4.08; 95% confidence interval, 1.63—10.23). Therefore, the qHBsAg level was independently associated with the HBV DNA level, after adjusting for possible confounding factors. Thus, the dynamic change in the qHBsAg level in the natural history of HBV is similar to the change in the HBV DNA level. These findings provide additional evidence to improve our understanding about the change of qHBsAg in the natural history of HBV infection.

Interferon-based therapy can result in a greater qHBsAg decline, compared to treatment with nucleoside analogues (NAs).[23] Furthermore, the decline in the qHBsAg level is reportedly limited to ETV-treated patients with a baseline ALT level more than 2 times the ULN. In this study population, 14.5% of patients had a decrease in the qHBsAg level that was greater than 1 $\log_{10}$ IU/mL after 6 months of treatment. Patients who had a significant decline in the qHBsAg level also had a significantly higher baseline ALT level, indicating the serum ALT levels may reflect the intensity of host immunity to reduce the qHBsAg level.

The on-treatment measurement of the qHBsAg levels was useful as stopping rules to identify the patients receiving IFN-based therapy who had a low probability of sustained response. Unnecessary economic burden and side effects could thus be avoided.[24] HBsAg seroclearance is the ideal end-point, although it is rare in clinical practice, especially in Asian patients undergoing NAs treatment. Therefore, optimized end-points of NAs treatment, which could ensure sustained response with a low risk of relapse, are needed to prevent the likelihood of long term or even life-long treatment, especially in HBeAg-negative CHB patients. However, whether the cut-off value of qHBsAg can be identified as an optimized end-point of treatment needs further investigation.

This study has several unique features. First, the qHBsAg level was measured by the Elecsys method (Roche Professional Diagnostics), rather than by the Architect method that has been used in previous studies. Automatic dilution in the Elecsys method could avoid errors created by custom-made dilution. Second, all sera samples were stored within 1 hour in a refrigerator at −80 °C to ensure a good quality of serum proteins. Third, serial data of the qHBsAg level from baseline up to 3 years were obtained. Therefore, we could find the kinetics of qHBsAg during ETV treatment. Fourth, our paper is the first Taiwanese paper describing the predictive value of qHBsAg levels in the virological response of patients receiving entecavir therapy, and provides further evidence to strengthen the utility of the qHBsAg level during entecavir treatment.

However, a few limitations should be acknowledged. First, the patient number was not large and the study population included people at various stages of HBV infection; however, this real-world data could truly reflect the utility of qHBsAg in clinical practice. Furthermore, because patients with various stages of HBV infection were recruited in this study, we had the advantage of understanding the whole picture of qHBsAg change at different stages of HBV infection. Second, a German study found that patients with a significant decline in the HBsAg level at 6 months of treatment had a higher rate of HBsAg seroclearance at 3 years with telbivudine treatment than patients without a significant decline in the HBsAg level.[25] However, the impact of the qHBsAg level on HBsAg seroclearance during ETV treatment could not be determined in this study because there was only 1 patient with HBsAg seroclearance at year 3.

In conclusion, this real-world data provides further evidence to confirm the association between the qHBsAg levels and the HBV DNA levels. A baseline qHBsAg level greater than 10,000 IU/mL is also associated with a suboptimal virologic response to ETV therapy. However, a significant decline in the serum qHBsAg level cannot predict the virologic response or HBeAg loss in patients receiving ETV treatment. Therefore, a baseline serum qHBsAg level should be measured prior to beginning ETV treatment and can be used to predict virologic response in CHB patients.

Acknowledgments

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