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Serum Hepatitis B Surface Quantification is Predictive of A Sustained Response in Chronic Hepatitis B Patients after Cessation of Anti-Viral Therapy

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ABSTRACT

Background and Aims: In the current international guideline for management of chronic hepatitis B (CHB), there was no clear consensus when long-term oral nucleos(t)ide analogs (NUCs) therapy should be stopped. This was mainly because of the ideal endpoint with HBsAg seroclearance is rarely achieved by oral anti-viral therapy and also because of the high relapse rate after stopping treatment. We evaluated whether quantification of HBsAg could predict a sustained response and a seroclearance in chronic hepatitis B patients after stopping oral NUCs.

Methods: Seventy-seven CHB patients (35 HBeAg-positive and 42 HBeAg-negative patients) who had received long-term oral NUCs 61.86 ± 25.82 months and had maintained normal ALT and undetectable HBV DNA were enrolled for stopping oral NUCs.

Results: The cumulative incidence of HBsAg seroclearance and the sustained response in both HBeAg-positive and -negative patients at 12 months after stopping oral NUCs treatment were 13% and 62.3% respectively. The re-treatment rate in HBeAg-positive with HBeAg seroclearance was higher significant than in HBeAg-negative patients (p<0.001), but not significant in HBeAg-positive with HBeAg seroconversion patients (p=0.74). Lower serum HBsAg at the end of treatment (EOT) was associated with a higher rate of HBsAg seroclearance and a sustained response. The optimal cut-off value for predicting HBsAg seroclearance was 100 IU/mL (p=0.007) and for predicting a sustained response was and 345 IU/mL (p=0.048).

Conclusion: Serum HBsAg level at the end of treatment is a useful predictor for HBsAg seroclearance and a sustained response in both HBeAg-positive and -negative after stopping oral NUCs.

Key words : Hepatitis B surface quantificaiton, HBsAg, chronic hepatitis B

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INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem with an estimated two billion individuals who had been exposed to the virus and resulting in 600,000 deaths each year[1]. Of the 350 million with chronic hepatitis B (CHB) infection, 75 % reside in the Asia Pacific region[2]. Long-standing active viral replication and liver necroinflammation can potentially progress to cirrhosis, decompensated liver disease and hepatocellular carcinoma (HCC), all of which contribute to a major health and economic burden worldwide[3-5]. The goal of treatment of CHB is to suppress viral replication to the lowest possible level, thus halting disease progression and the onset of complications with an ultimate goal of clearing the surface antigen (HBsAg). There are two categories of drugs: a finite course of therapy with interferons (IFN), an immune modulator with antiviral capacities, including interferon-alfa or pegylated IFN, and specific nucleoside or nucleotide HBV inhibitors that target the reverse transcriptase function of the HBV DNA polymerase. Oral NUCs are widely used in the treatment of CHB patients, which are very effective for viral suppression and normalization of liver enzymes. However, oral NUCs cannot permanently eradicate a HBV infection ever after long-term therapy, as virological relapse is common after cessation of therapy[6-9].

In the recommendation by the current Asian Pacific Association for the Study of the Liver (APASL), discontinuation of antiviral drugs was recommended for HBeAg-positive patients when HBeAg seroconversion with HBV DNA loss has been noted for at least 12 months. However, approximately 25% to 50% of patients may still develop hepatitis relapse after stopping anti-viral therapy, even though these recommendations are followed[10-11]. Thus, the timing of cessation of oral anti-viral agents remains controversial. For HBeAg-negative patients, the optimal duration of NUCs treatment is unknown, the treatment goal being sustained suppression of HBV replication[12]. The ideal end-point is sustained loss of HBsAg expression and a satisfactory end-point is sustained virological and biochemical responses after therapy. However, the ideal end point is difficult to achieve in real-world clinical practice. A 5-year anti-viral therapy reportedly resulted in a reduced HBsAg expression level, usually lower than 5% in HBeAg-negative patients[13-14]. The patients who reached these end points for off therapy were eventually found to have episodes of either viral or clinical relapse, and HBsAg may be associated with relapse[15].

Serum HBsAg level reflects intrahepatic covalently closed circular DNA (cccDNA), and a reduction in HBsAg levels correlates well with that of cccDNA in HBeAg-positive patients[16-18]. Previous studies reported a correlation between the decline in intrahepatic cccDNA and serum HBsAg levels in HBeAg-positive patients[19-20]. Quantitation of HBsAg has been shown to be helpful in the management of CHB, the lower serum HBsAg level showed the higher chance of spontaneous HBsAg seroclearance. HBsAg level <200 IU/mL may predict HBsAg seroclearance in 3 years[21-22]. HBsAg level of <100 IU/mL is an appropriate cutoff for predicting HBsAg seroclearance over time. Serum HBsAg levels <100 IU/mL at the end of treatment are highly predictive of a sustained response after stopping oral anti-viral agents[23-24]. A recent study showed that serum HBsAg level at the end of treatment is a useful predictor to guide the timing for stopping lamivudine treatment in CHB patients. Different levels of HBsAg cut-offs at the end of treatment and further HBsAg reduction after stopping treatment were predictive of subsequent HBsAg seroclearance and the lowest risk of HBV relapse after cessation therapy in HBeAg-positive and -negative patients[25].

In this study, we aimed to investigate the role of serum hepatitis B surface quantification for predicting a sustained off-treatment response among CHB patients who had received long-term oral NUCs. We also wanted to analyze the factors associated with a sustained response at 12 months after stopping NUCs therapy. Based on the data, we defined the best timing and the best cut-off of HBsAg that could predict a sustained off-treatment response in our patients.

Patients and methods

The study was designed as an observational prospective cohort analysis. The observational study was performed by systematically reviewing medical records in 2007-2014, and the prospective study was carried out from 2014. The study protocol for clinical research was approved by Chiang Mai University Ethical Committee.

A total of 77 CHB patients were included (35 HBeAg-positive, 42 HBeAg-negative), all being HBsAg-positive for more than 6 months before treatment and treated with any one of standard oral anti-

viral agents (adefovir 10 mg/day, entecavir 0.5 mg/day, lamivudine 150 mg/day, telbivudine 600 mg/day, or tenofovir 300 mg/day). The choices of an initial anti-viral agent and any subsequent switching to or adding on another anti-viral agent were made according to the financial preference of the patients as well as the advice of the treating physicians. The exclusion criteria were as follow: the presence of antibody to human immunodeficiency virus (HIV), hepatitis C virus (HCV), history of malignancy, evidence of liver disease such as autoimmune hepatitis, alcoholic liver disease or decompensated cirrhosis. Patients were included if they fulfilled the following criteria: NUCs was/were stopped when ALT was normalized and HBV DNA was undetectable for more than 12 month in HBeAg-positive with HBeAg seroconversion patients, or more than 18 months in HBeAg-positive with HBeAg seroclearance patients, or in HBeAg-negative patients.

Serum quantitative HBsAg was tested at baseline and at the end of treatment. After discontinuation of anti-viral therapy, patients were followed up every month in the first three months, and then every three months for at least 12 months. Routine follow-up studies included clinical assessment, conventional liver biochemical tests, and serologic hepatitis B markers (serum quantitative HBsAg, anti-HBs, HBeAg and anti-HBe). Serum HBV DNA was assayed at baseline, after oral NUCs cessation and every 6 months thereafter. Re-treatment after discontinuation of NUCs therapy was considered when a sustained response had elevated ALT level > 2 times upper limit of normal and elevated serum quantitative HBsAg or an elevated serum HBV DNA level[26].

Laboratory methods

Serum ALT was measured using standard procedures. The upper limit of normal of serum ALT level was set by the laboratories at 41 U/L for male and 33 U/L for female patients. Serum virologic markers, including HBsAg, anti-HBs, HBeAg and anti-HBe were measured by the ARCHITECT i2000SR assay CMIA (Abbott laboratories, IL, USA). Hepatitis B virus DNA was measured by real-time polymerase chain reaction (PCR) using the Roche COBAS HBV Amplicor Monitor assay with a low limit of detection of 400 copies/mL and Abbott Real-time HBV assay with a low limit of detection of 34 copies/mL (Abbott Molecular Inc. Des plaines, IL, USA) as from 2011, respectively. Serum HBsAg was quantified using by the Architect CMIA (Abbott Laboratories, IL, USA) with the minimum detection limit of 0.05 IU/mL.

Statistical analysis

Data are presented as means ± standard deviation (SD), proportions, or median (range). To compare the values between the two groups, the chi-squared test or the Fisher's exact test was applied to analyse categorical variables, and the Student's *t* test or Mann-Whitney U test was used for continuous variables. Receiver operator characteristic (ROC) curve analysis was used to define the best cut-off point of HBsAg level or HBsAg decline for predicting a sustained response or HBsAg seroclearance. Cumulative incidences of sustained response or HBsAg seroclearance were analysed by Kaplan-Meier method with a log-rank test. Univariate and multivariate analyses were performed to identify factors associated with sustained response or HBsAg seroclearance using the Cox proportional hazards regression models. These statistical analyses were conducted by using the Statistical Package for the Social Sciences (SPSS) version 22.0 software (SPSS Inc., Chicago, IL, United States). All statistical tests were two-sided and a p- value < 0.05 was considered statistically significant.

Results

The baseline characteristics before oral NUCs cessation

The baseline characteristics of the 77 CHB patients who had discontinued oral NUCs are shown in Table 1. Most patients (70.1%) were male. The mean age was 52.74 ± 9.52 years. Thirty-five patients were HBeAg-positive (68.57% with HBeAg seroconversion, 31.43% with HBeAg seroclearance) and 42 were HBeAg-negative. Before discontinuation of oral NUCs, patients received any are of the five NUCs, most commonly lamivudine, telbivudine and tenofovir. The mean duration of treatment was 61.86 ± 25.82 (range 42.5-79.5) months. The mean duration of normalized serum ALT was 49.55 ± 25.02 (range 32-67) months. The mean duration of undetectable HBV DNA was 78.55 ± 230.46 (range 28-71) months, and the mean baseline serum HBsAg before NUCs cessation was 2.40 ± 1.16 $\log_{10}$IU/mL.

Table 1. Baseline characteristics of study patients.

	Total N=77	HBeAg-positive N=35	HBeAg-negative N=42	*p*-value
Male, n (%)	54 (70.1)	28 (51.9)	26 (48.1)	0.140
Age (mean±SD)	52.74±9.52	49.83±10.50	55.17±7.97	0.013*
NUCs, n (%)				
- Lamivudine	36 (46.8)	17 (47.2)	19 (52.8)	0.950
- Telbivudine	32 (41.6)	15 (46.9)	17 (53.1)	1.000
- Entecavir	16 (20.8)	9 (56.3)	7 (43.7)	0.489
- Adefovir	6 (7.8)	4 (66.7)	2 (33.3)	0.402
- Tenofovir	32 (41.6)	18 (56.3)	14 (43.7)	0.170
Mean treatment duration, months±SD	61.86±25.82	69.68±27.43	55.33±22.72	0.014*
HBV DNA before treatment, log_{10}copies/mL (range)	6.13 (5.22-7.44)	7.47 (2.67-10.19)	5.82 (2.10-7.60)	<0.001*
Undetectable HBV DNA, months (range)	48 (28-71)	44 (4-189)	50 (1-140)	0.351
ALT before treatment, IU/mL (range)	84.50 (52.00-157.25)	89 (28-572)	89 (25-1,085)	0.111
HBsAg levels before treatment, log_{10}IU/mL (range)	3.14 (2.41-3.46)	3.27 (2.20-6.50)	3.17 (0.37-4.36)	0.311
HBsAg levels at the end of treatment, log_{10}IU/mL (range)	2.69 (1.84-3.02)	2.64 (-1.70-4.05)	2.73 (-0.64-4.33)	0.487

Re-treatment and sustained response after discontinuation of NUCs

Of the 77 patients, 29 (37.7%) had to restart treatment during a regular follow-up period of up to 12 months, as shown in Table 2. Comparison of the factors between the re-treatment group and the sustained response group after stopping NUCs showed that the re-treatment rate in HBeAg-positive with HBeAg seroclearance patients was statistically significant compared with HBeAg-positive with HBeAg seroconversion patients (p=0.005) and with the HBeAg-negative patients (p<0.001), respectively. Re-treatment rates in HBeAg-positive with HBeAg seroconversion patients and in HBeAg-negative patients were not statistically different (p=0.74), as shown in Figure 1. The univariate and the stepwise multivariate analysis revealed that HBeAg-positive with HBeAg seroclearance were factors associated with high re-treatment rate (RR=4.139, 95% CI=1.748-9.801, p=0.001). Younger age and tenofovir as the chosen NUC was significant factors associated with sustained response. There were no statistical significances regarding gender ratio, treatment duration, duration of normalised ALT, duration of undetectable HBV DNA level before oral NUCs cessation, and baseline serum HBsAg level before treatment or before cessation.

HBsAg seroclearance after the end of treatment

Ten patients (13%) experienced HBsAg seroclearance after the end of treatment (EOT), 5 received lamivudine, 2 received telbivudine and 3 received entecavir during continuous follow-up for 12 months. All HBsAg seroclearance patients did not achieve HBsAg seroconversion. None experienced relapse during the follow-up period. The following variables were assessed as possible predictors of HBsAg seroclearance: age, sex, HBeAg status (pretreatment, EOT), choice of NUC, HBV DNA level and serum ALT before treatment, serum HBsAg level (pretreatment, EOT), duration with NUC treatment, time to normalization of ALT, and undetectable DNA at EOT (Table 3). Serum HBsAg quantification measured at the end of NUCs therapy in patients with a sustained response who finally cleared HBsAg was lower [median (range) = 3.96 (0.02-957.7)] IU/mL compared with that in patients without HBsAg seroclearance [median (range)

Table 2. Characteristics of CHB patients with or without a sustained response.

	Total N=77	No Sustained response N=29	Sustained response N=48	*p*-value
Male, n (%)	54 (70.1)	23 (42.6)	31 (57.4)	0.266
Age, year	52.74±9.52	55.69±9.59	50.96±9.12	0.034*
HBeAg status, n (%)				<0.001*
- Negative	42 (54.5)	11 (26.2)	31 (73.8)	
- Positive				
- with HBe seroclearance	11 (14.3)	10 (90.9)	1 (9.1)	
- with HBe seroconversion	24 (31.2)	8 (33.3)	16 (66.7)	
NUCs, n (%)				
- Lamivudine	36 (46.8)	9 (25.0)	27 (75.0)	0.056
- Telbivudine	32 (41.6)	17 (53.1)	15 (46.9)	0.034*
- Entecavir	16 (20.8)	3 (18.8)	13 (81.2)	0.143
- Adefovir	6 (7.8)	3 (50.0)	3 (50.0)	0.667
- Tenofovir	32 (41.6)	19 (59.4)	13 (40.6)	0.002*
Mean treatment duration, months±SD	61.86±25.82	65.45±23.16	59.69±27.31	0.346
HBV DNA before treatment, log_{10}copies/mL (range)	6.09±1.72	6.37±1.24	5.93±1.92	0.257
Undetectable HBV DNA, months (range)	48 (28-71)	41 (1-187)	64 (1-1,889)	0.166
ALT before treatment, IU/mL(range)	84.50 (52.00-157.25)	73 (32-1085)	89 (25-458)	0.969
HBsAg levels before treatment, log_{10}IU/mL (range)	3.14 (2.41-3.46)	3.18 (2.20-4.36)	3.18 (1.27-6.50)	0.256
HBsAg levels at the end of treatment, log_{10}IU/mL (range)	2.69 (1.84-3.02)	2.69 (1.01-4.33)	2.73 (-1.70-4.19)	0.168

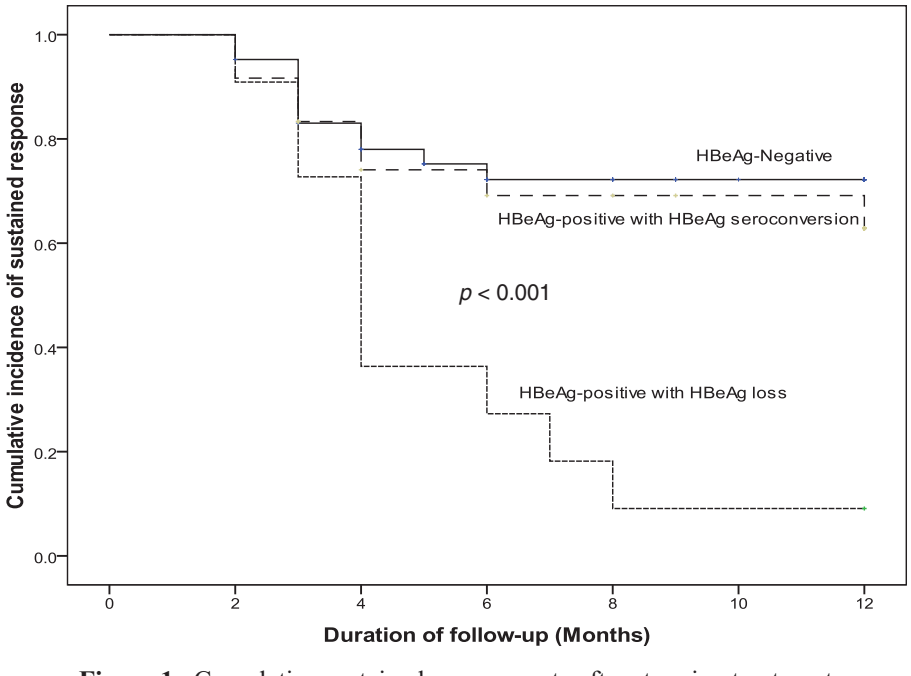

Figure 1. Cumulative sustained response rate after stopping treatment.

Table 3. Characteristics of CHB patients with or without HBsAg seroclearance.

	Total N=77	HBsAg seroclearance N=10	No HBsAg seroclearance N=67	*p*-value
Male, n (%)	54 (70.1)	6 (11.1)	48 (88.9)	0.474
Age, year	52.74±9.52	52.40±8.46	52.79±9.73	0.905
HBeAg status, n (%)				0.459
- Negative	42 (54.5)	7 (16.7)	35 (83.3)	
- Positive				
- with HBe seroclearance	11 (14.3)	0 (0.0)	11 (100)	
- with HBe seroconversion	24 (31.2)	3 (12.5)	21 (87.5)	
NUCs, n (%)				
- Lamivudine	36 (46.8)	5 (13.9)	31 (86.1)	1.000
- Telbivudine	32 (41.6)	2 (6.3)	30 (93.7)	0.180
- Entecavir	16 (20.8)	3 (18.8)	13 (81.2)	0.425
- Adefovir	6 (7.8)	0 (0.0)	6 (100)	1.000
- Tenofovir	32 (41.6)	0 (0.0)	32 (100)	0.004*
No sustained response	29 (37.7)	0 (0.0)	29 (100)	0.011*
Sustained response	48 (62.3)	10 (20.8)	38 (79.2)	
Mean treatment duration, months±SD	61.86±25.82	47.60±26.60	63.98±25.22	0.061
ALT before treatment, IU/mL (range)	84.50 (52.00-157.25)	125 (28-249)	84.5 (25-1085)	0.502
HBV DNA before treatment, log_{10}copies/mL (range)	6.13 (5.22-7.44)	5.09 (2.10-8.56)	6.29 (2.60-10.19)	0.533
Undetectable HBV DNA, months (range)	48 (28-71)	40 (1-71)	50 (1-1,889)	0.290
HBsAg levels before treatment, log_{10}IU/mL (range)	3.14 (2.41-3.46)	1.57 (-0.41-650)	3.15 (1.04-5.50)	0.168
HBsAg levels at the end of treatment, log_{10}IU/mL (range)	2.69 (1.84-3.02)	0.20 (-1.70-2.99)	2.73 (0.71-4.33)	<0.001*

= 519.27 (10.98-21,626) IU/mL; $p<0.001$].

Optimal HBsAg cut-off levels

We analyzed serum HBsAg level at the EOT and found that it correlated with a likelihood for HBsAg seroclearance (univariate analysis HR=1.60, 95% CI=1.25-2.05, $p<0.001$ multivariate analysis HR=1.57, 95% CI=1.21-2.03, $p=0.001$). We also tried to find the optimal cut-off values of HBsAg at the EOT to predict HBsAg seroclearance after stopping oral NUCs treatment (Figure 2). The cut-off value of 100 IU/mL could predict HBsAg seroclearance ($p=0.007$). We, therefore, used HBsAg level of 100 IU/mL as a marker to predict HBsAg seroclearance at the EOT. Nine of 22 patients (40.9%) with HBsAg <100 IU/mL, and 1 of 54 patients (1.8%) with HBsAg >100 IU/mL, experienced HBsAg seroclearance respectively after stopping oral NUCs treatment.

Overall, lower serum HBsAg at the EOT correlated with a high sustained response rate at 12 months. The area under ROC curve for serum HBsAg to predict re-treatment was 0.594 (95%CI 0.468-0.720, sensitivity 69%, specificity 50%). We tried to find the optimal HBsAg cut-off levels at the EOT to predict sustained response at 12 months after the EOT. Serum HBsAg levels up to 345 IU/mL significantly correlated with a sustained response ($p=0.048$) (Figure 3). Using this cut-off value, a sustained response was achieved in 24 of 33 patients (72.7%) in whom HBsAg, levels were <345 IU/mL. For those with HBsAg >345 IU/

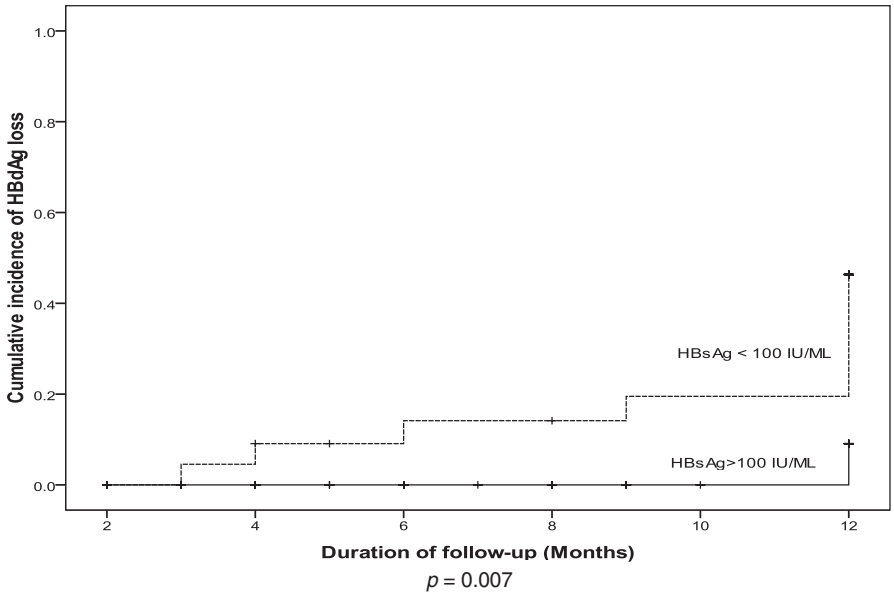

Figure 2. Cumulative incidence of HBsAg seroclearance according to HBsAg level at the end of treatment.

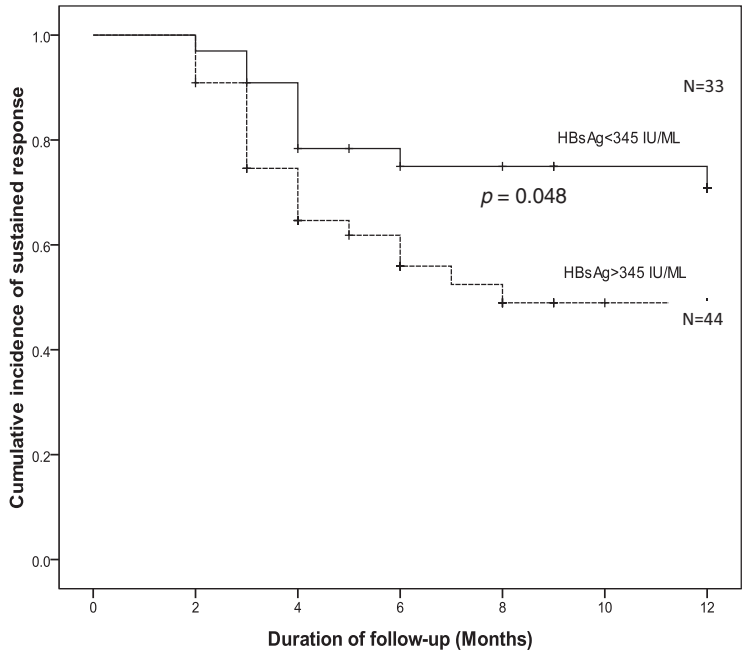

Figure 3. Cumulative incidence of a sustained response according to HBsAg level at the end of treatment.

mL, 24 of 44 cases (54.5%) achieved a sustained response at the last follow-up.

DISCUSSION

Nucleos(t)ide analogues such as LAM, ADV, LDT, ETV and TDF are standard treatment agents for CHB infection. Spontaneous HBeAg seroclearance and seroconversion predict long-lasting suppression of HBV, reduced infectivity, and improved clinical prognosis[27]. Thus, NUCs-induced HBeAg seroclearance and seroconversion have been considered potential endpoints for stopping antiviral treatment. In this cohort study of 77 patients, we found that long-term treatment (median 61 month) with oral NUCs in CHB patients with either HBeAg-positivity or HBeAg negativity, serum ALT became normalized and HBV DNA undetectable at about 48 months, and the cumulative

sustained response rate at 12 months after the EOT was 62.3%. The HBeAg-positive with HBeAg-seroconversion patients and the HBeAg-negative patients had comparable rates of sustained virological response (SVR). HBeAg-positive with HBeAg seroclearance patients had the highest chance of re-treatment at 12 months. The serum HBsAg level at the EOT could also serve as a predictor of HBsAg seroclearance and a sustained response.

Previous studies have suggested that a longer duration of consolidation therapy after HBeAg seroclearance or e-seroconversion was strongly associated with the probability of a sustained response, and both HBeAg seroclearance and e-seroconversion were appropriate parameters for considering cessation of lamivudine. An extended therapy for more than another 12 months after HBeAg seroclearance or seroconversion might be needed to achieve a sustained response in Asian patients[28]. That previous study showed a 1-year cumulative probability of virological relapse of 44%. Both HBeAg-positive and HBeAg-negative patients had a comparably high risk of virological relapse in 1 year[15]. Our results showed that after antiviral therapy discontinuation the cumulative sustained response at 12 months in HBeAg-positive with HBeAg-seroconversion patients was 66.6% (16/24), while HBeAg seroclearance was 9.1% (10/11). Such findings were in keeping with a with support previous study showing that HBeAg seroconversion was a stronger predictor than HBeAg seroclearance. Our results also suggested that HBeAg-positive with HBeAg seroclearance patients should continue taking oral antiviral agents until HBeAg seroconversion appeared.

In HBeAg-negative patients, a recent report from China showed the cumulative probability of virological relapse after stopping antiviral therapy of 43.82% (71/162) in 1 year, and the cumulative probability of virological relapse in the group with HBsAg ≥ 1500 IU/L was higher than that in the group with HBsAg ≤ 1500 IU/L[29]. In our study, the cumulative re-treatment was noted in 26.2%, which was lower than in a previous study, and our sustained response at 12 months was 73.8% (31/42), which was similar to that in patients with HBeAg seroconversion. In addition, the baseline serum HBsAg before oral NUCs cessation was 2.73 $\log_{10}$IU/mL (-0.64-4.33), which was lower than in the previous study. However, we suggest that in HBeAg-negative patients, oral NUCs therapy could be discontinued after a period of prolong treatment with permanent normal ALT and undetectable DNA and with a low HBsAg level. Thereafter, a close follow-up should be made to monitor on incidence of relapse.

Serum HBsAg level recently become a hot biomarker as it was shown to be associated with the amount of covalently closed circular (ccc) DNA in the liver cells[30-31]. Studies on the natural history of HBV suggested that lower serum HBsAg levels were associated with an improved immunity against the virus[32-33]. Serum HBsAg levels were lower in patients with an inactive disease compared with those with elevated HBV DNA and/or ALT[34]. A previous study of 17 HBeAg-positive patients on telbivudine treatment suggested that serum HBsAg levels <100 IU/mL at the end of treatment were highly predictive of a sustained response at 2 years off-treatment[23]. A study in HBeAg-negative patients similarly showed that serum HBsAg levels <100 IU/mL at the end of treatment were highly predictive of a sustained response after stopping NUCs[24]. A recent study showed that serum HBsAg <200 IU/mL at the end of treatment was the best prediction for a sustained response after stopping lamivudine treatment[25]. Our study also suggested that serum HBsAg <100 IU/mL at the end of treatment was associated with a higher likelihood for HBsAg clearance. Serum HBsAg cut-off <345 IU/mL was the higher value that showed a statistical significance for a sustained response (p=0.048) in ether HBeAg-positive or HBeAg-negative CHB patients on who long-term oral NUCs therapy who achieved ALT normalization and HBV DNA clearance. However, the number of subjects, the choice of oral antiviral agents, HBeAg status, duration of treatment, and the proportion of cases with undetectable HBV DNA were different between ours and the previous studies[23,25].

There were limitations in our study. First, the choice of anti-viral agents varied widely among cases and TDF was almost always used in non-naive CHB patients. Second, NUCs treatment was widely durations. Third, our post-treatment follow-up of 12-month was relatively short compared with other studies. However, based on previous reports in Chinese subjects, most of the virological relapse occurred in the first year post-treatment[10,11,35]. Forth, HBV genotype was not available in our study. A previous study indicated that HBV genotype A strongly influenced HBsAg clearance during NUCs therapy[36]. We do not know whether the studied HBsAg cut-offs could be applied in other HBV genotypes.

In conclusion, serum hepatitis B surface antigen quantification at the end of treatment was an important predictor for HBsAg seroclearance and a sustained response in both HBeAg-positive and HBeAg-negative patients after stopping oral NUCs. Different levels of HBsAg cut-offs at the end of treatment were seemingly predictive of a sustained response and a lower risk of HBV relapse after termination of oral NUCs therapy, in both HBeAg-positive and HBeAg-negative patients. However, HBeAg-positive patients with just HBeAg seroclearance should continue oral anti-viral agents while awaiting HBeAg seroconversion.

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