Original Article

Predictors of Sustained Virologic Response after Discontinuation of Nucleos(t)ide Analog Treatment for Chronic Hepatitis B

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ABSTRACT

Background/Aims: The aim of this study was to identify the predictors for relapse after discontinuation of oral nucleos(t)ide analog treatment for chronic hepatitis B (CHB). **Patients and Methods:** We evaluated patients who were receiving long-term, regular antiviral therapy with nucleos(t)ide analogs, and subsequently achieved the discontinuation criteria from the Asia-Pacific guideline. After they voluntarily discontinued the drug therapy, data were prospectively collected to observe the potential virologic relapse, and the parameters that predicted recurrence were analyzed. **Results:** Sixty-five patients met the inclusion criteria, and were included in this study. Twenty-eight patients relapsed, and the accumulative recurrence rates at the 3-month, 6-month, and 1-year follow-ups were 13.85%, 32.31%, and 49.23%, respectively. There was no difference in the accumulative recurrence rate 12 months after discontinuation among patients who were positive or negative for the hepatitis B e antigen (HBeAg) before they received the medication. Logistic regression analysis revealed that the time to complete response, age at discontinuation, and HBsAg levels at discontinuation affected the rate of relapse. **Conclusions:** Among patients who received orally administrated nucleos(t)ide analogs, serum levels of HBsAg, age at discontinuation, and the time to complete response might be used as a guide to discontinue treatment. Among younger patients, those with low serum HBsAg levels, and those with an earlier complete response, the risk of relapse is lower and discontinuation is much safer.

Key Words: Chronic hepatitis B, complete response time, nucleos(t)ide analogs, surface antigen titer, virologic relapse

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Chronic hepatitis B (CHB) is a serious global challenge with major advances in the treatment and management of CHB. Approximately 2 billion people have been infected by the hepatitis B virus (HBV), and approximately 5% were chronically infected.[1] About 1 million people died of HBV-related complications such as liver failure, cirrhosis, and liver cancer,[2] and in China CHB is the most common cause of cirrhosis and liver cancer.[3,4]

Anti-HBV drugs are currently divided into two groups: Injectable interferon and oral nucleos (t) ide analogs (NUCs).

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NUCs could inhibit the activity of HBV polymerase, thereby inhibiting viral replication and reducing the virus load, which was the short-term goal. And then NUCs would reduce liver inflammation, and prevent the development of liver disease,[5,6] and improving the patients' survival rate and quality of life,[7,8] which was the long-term aim. Before giving NUCs, care-givers should consider the acceptance, compliance, economic burden, and long-term side effects experienced by patients receiving long-term antiviral therapy, as well as the effect on childbearing capacity among patients of childbearing age. Another major concern is long-term use of NUCs might lead to the evolution of resistant viral mutants, which might cause severe liver inflammation or even liver failure.[9,10] Although European and American liver disease guidelines recommended that discontinuation could be considered when HBsAg seroconversion occurs, the possibility of HBsAg seroconversion in NUCs therapy is very small.[11,12] In addition, the Asia-Pacific Liver Guidelines and the 2nd Edition of the Chinese chronic hepatitis B prevention

and treatment guidelines (2010) recommend that treatment for HBeAg-positive patients should last at least 12 months, and after the patient has reached a complete response (CR; when HBV DNA is undetectable, HBeAg seroconversion has occurred and alanine aminotransferase [ALT] levels have normalized), they should be consolidated for at least 12 months before discontinuation is considered. Similarly, treatment for HBeAg-negative patients should last at least 12 months, and after CR has been reached (HBV DNA is undetectable and ALT levels have normalized), the patient should be consolidated for at least 18 months before discontinuation is considered.

With these concerns and high relapse rate of HBV with long-term NUCs treatment, we carried out this study to identify predictors of a sustained response, as well as the optimal treatment endpoint for minimizing the risk of recurrence.

PATIENTS AND METHODS

Patients

This study included patients with CHB who received NUCs therapy at Nanfang Hospital, Southern Medical University, between January 2003 and July 2011. Patients voluntarily discontinued NUCs, and were prospectively followed-up between November 1, 2012, and May 31, 2014. The diagnosis of CHB met the Chinese "Chronic hepatitis B prevention and treatment" guidelines criteria. The inclusion criteria for this observational study were (1) Adult patients (>18 years old) who were willing to discontinue treatment, understood and signed the informed consent form, and complied with the study requirements. (2) Patients who were HBeAg positive before the treatment must have received continuous oral NUCs treatment until their HBV DNA levels were below the detection limit and their ALT levels had normalized. Their therapy was then consolidated for at least 12 months after the HBeAg seroconversion occurred. Patients who were HBeAg negative before the treatment underwent consolidation for at least 18 months after their HBV DNA levels were below the detection limit and their ALT levels had normalized. (3) Before discontinuation, the decrease of HBV DNA levels to below the detection limit (<20 IU/mL) was confirmed using the Cobas TaqMan assay (Roche Molecular Systems, Inc., Pleasanton, CA, USA). (4) Patients who had compensated liver disease before the treatment, with no evidence of cirrhosis. The exclusion criteria were (1) The patient exhibited clinical manifestations of decompensated liver disease during the previous treatment. (2) The liver biopsy (taken before discontinuation) exhibited significant bridging hepatic fibrosis (>S3), or the FibroScan (before discontinuation) indicated significant liver fibrosis or cirrhosis. (3) Co-infection with the hepatitis C virus, hepatitis D virus, or human immunodeficiency virus, which was associated with autoimmune liver disease or alcohol history. (4) The patient had any other serious or active disease, including uncontrolled cancer, kidney, heart, lung, metabolic (eg, diabetes, thyroid disease, and adrenal disease), or immunodeficiency disease. (5) The patient had received, or was planning to receive, a liver transplant. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Southern Medical University. Written informed consent was obtained from all the participants.

Methods

Among patients who met the criteria for discontinuation, the various data were collected from the initiation of NUCs therapy to discontinuation, including pretreatment ALT levels, HBeAg status, HBV DNA levels, time to CR, and consolidation treatment (CT) time. A prospective postdiscontinuation follow-up was also included in this observational study. Patient's demographic data, ALT levels, HBV DNA levels, and the serum hepatitis B surface antigen (HBsAg) titers at discontinuation were also collected. In this study, patients were followed-up every month for the first 3 months, then once every 3 months. Additional biweekly or weekly appointments were arranged if their ALT levels increased by > 2 times the upper normal limit. Each follow-up included testing for HBV DNA, ALT, HBsAg, HBeAg, and antibodies to the hepatitis B e antigen (HBeAb). Abdominal ultrasound and liver FibroScan examinations were also performed once per year. The basic data for the patients after discontinuation are listed in [Table 1]. The endpoint for follow-up was set as clinical relapse or the last follow-up after discontinuation. The standards for a sustained virologic response (SVR) were HBV DNA < 2000 IU/mL, HBeAg-negative, and normal ALT levels. Virologic relapse (VR) was defined as HBV DNA > 2000 IU/mL, or serum HBeAg reversion in two consecutive weekly measurements. Clinical relapse was defined as VR with ALT levels > 2 times the upper normal limit. Clinical relapse patients were re-started on NUCs or interferon for antiviral treatment, and their follow-up was terminated. Follow-up was continued for patients with only VR and who did not experience clinical relapse.

Laboratory testing

During the follow-up, specimens from every patient were collected in the Center Laboratory of Nanfang Hospital, Southern Medical University, for further testing. The pretreatment HBV DNA quantitation were detected by using the Daan test (Daan Gene Co, Ltd of Sun Yat-sen University, Guangdong, China) with a lower limit quantification at 1000 copies/mL. The postdiscontinuation baseline points, as well as the HBV DNA quantitation for each follow-up point, (linear range 20–1.7 × 10^8 IU/mL). HBsAg titers were quantified by using the Architect HBsAg system (Abbott Ireland Diagnostics

Table 1: Baseline characteristics of patients and the comparison between SVR and non-SVR

Characteristics	All patients (*n*=65)	SVR (*n*=37)	Non-SVR (*n*=28)	*P*
Age at EOT (yr)	36.08±8.14	33.78±8.56	39.11±6.52	0.006
Male: Female	56:9	30:7	26:2	0.280
Pretreatment HBeAg (+:−)	44:21	26:11	18:10	0.808
Pretreatment HBV DNA (Logcps/mL)	6.46±1.24	6.56±1.10	6.30±1.41	0.409
Pretreatment ALT (U/L)	245 (41-2244)	222 (41-2244)	263 (45-944)	0.907
Total treatment course (months)	56.12±25.19	54.41±23.11	58.39±27.97	0.532
Course for CR (months)	19.35±18.09	14.19±11.75	26.18±22.52	0.014
Course for CT (months)	36.71±22.02	40.16±23.81	32.14±18.85	0.147
Level of HBsAg at EOT (LogIU/mL)	2.50±1.09	2.18±1.25	2.91±0.64	0.003
FibroScan value at EOT (Kpa)	5.63±1.52	5.56±1.61	5.72±1.42	0.669
Serum ALT at EOT (U/L)	20 (6-61)	20 (6-61)	21.5 (12-52)	0.891

SVR: Sustained virological response, EOT: End of the treatment, HBeAg: Hepatits B e antigen, HBV: Hepatitis B virus, DNA: Deoxyribonucleic acid, HBsAg: Hepatitis B surface antigen, ALT: Alanine aminotransferase, CR: Complete response, CT: Consolidation treatment. There were only 3 factors between the patients who got SVR or not, the older ones and longer course for CR and lower serum HBsAg level at EOT had relation with non-SVR

Division, Sligo, Ireland) at Nanfang Hospital, according to the manufacturer's instructions. The linear range of the Architect assay is 0.05–250 IU/mL, therefore samples with HBsAg titers > 250 IU/mL were retested after dilution (1:500–1000) to bring the reading within the linear range.

Statistical analyses

Statistical analyses were performed using SPSS (version 19.0; SPSS, Inc., Chicago, IL, USA). The count data were expressed as mean ± standard deviation or median and range, whereas the measurement data were expressed as percentages. The categorical variables were evaluated using the Chi-square test, and the continuous variables were evaluated using the *t*-test or Mann–Whitney *U* test, depending on whether the data was normally distributed. The logistic regression model (Forward LR) was used for the multivariate analysis to identify the independent predictors of a sustained response. The Kaplan–Meier method was used to calculate the accumulative recurrence rate, and the log-rank was used to test the difference. Serum HBsAg levels were logarithmically transformed for analysis. The area under the receiver operating characteristics (AUROC) curve was analyzed to define the optimal cutoff points for clinical parameters that predicted VR. Statistical significance was defined as a $P < 0.05$, and all statistical tests were two sided.

RESULTS

Patient characteristics

The prospective follow-ups for NUCs discontinuation began in November 2012, and the last follow-up was in May 2014. During this time, data were collected from 79 patients with CHB who discontinued NUCs treatment. After excluding 1 patient who was lost to follow-up, and 13 patients who were followed-up for < 6 months with no relapse, 65 patients were considered eligible for this study. Among these 65 patients, 25 received entecavir (ETV) monotherapy, 13 received telbivudine (LdT) monotherapy, 1 received lamvudine (LAM) monotherapy, 21 received adefovir (ADV) monotherapy, and 5 received combination therapy (LAM and ADV). Fifty-five patients were treatment-naive, whereas 10 had a history of NUCs treatment and were re-treated due to recurrence. The data for these 65 patients are listed in Table 1.

Among the 65 patients, 28 developed VR and 18 experienced clinical relapse. Among the patients who experienced clinical relapse, 11 were HBeAg positive before the NUCs treatment and 7 were HBeAg negative. All 11 patients who were HBeAg positive experienced HBeAg seroconversion before discontinuation, although 5 patients experienced HBeAg reversion after discontinuation (1 case in the 3rd month after discontinuation, 3 cases in the 6th month, and 1 case in the 9th month). Patients who experienced clinical relapse were re-treated with NUCs or interferon, and were subsequently excluded from this study.

The Kaplan–Meier method was used to calculate the accumulative VR rate after discontinuation [Figure 1], and the rates at months 3, 6, and 12 were 13.85%, 32.31%, and 49.23%, respectively.

Impacts of different NUCs on VR

Among the 65 patients, 25 received ETV and 13 received LdT (these are considered more powerful antiviral drugs), whereas the remaining 27 received less powerful antiviral drugs. When patients were divided into the potent antivirus group (ETV or LdT, Group A) or the non-potent antivirus group (Group B), 16 of the 38 (42.11%) patients in Group A relapsed, whereas 15 of the 27 (55.56%) patients in Group B relapsed. The intergroup comparison revealed no significant difference ($\chi^2 = 0.035$, $P = 0.851$) between these two groups. When patients were divided into a potent antiviral drug/low resistance group (ETV) and a non-potent antiviral drug/high resistance group (non-ETV), 14 patients in the ETV

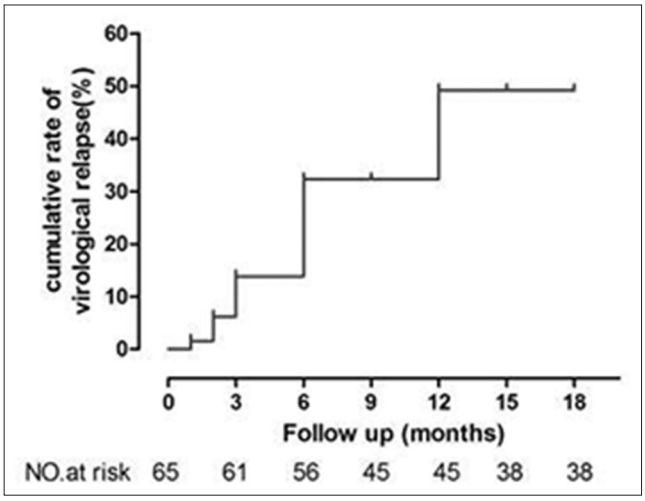

Figure 1: Cumulative virologic relapse rates among the 65 patients who stopped anti-viral therapy

group relapsed (44%), whereas 17 patients in the non-ETV group relapsed (42.5%). The intergroup comparison revealed no significant difference (χ^2 = 0.014, P = 0.905) between these two groups. Among the relapse patients, 3 were NUCs experienced who formed a small minority of the overall group.

The impact of HBeAg status on VR

Among the 44 patients who were HBeAg positive, the accumulative VR rate within the 12 months after discontinuation was 48.08%, whereas the rate among the 21 patients who were HBeAg negative was 51.85%; this difference was not statistically significant (P = 0.567).

The 44 patients who were HBeAg positive received antiviral treatment for a total of 55.39 ± 25.48 months (range, 16–124 months), whereas their CT time (from HBeAg seroconversion to discontinuation) was 33.27 ± 21.48 (range, 12–111) months. Eighteen patients experienced VR after an average of 6 (range, 2–12) months of follow up. The median follow-up period for the remaining 26 patients with SVR was 12 (range, 6–18) months. The 3-, 6- and 12-month accumulative VR rates were 11.36%, 29.55%, and 48.09%, respectively. The CR (from treatment initiation to HBeAg seroconversion) and CT times in the VR and SVR groups were compared. The average CR time in the VR group was 31.50 ± 24.75 (range, 3–73) months, which was significantly higher than that in the SVR group (15.46 ± 11.33 months; range, 1–45 months; P = 0.017). However, the average CT time in the VR group was 28.78 ± 17.33 (range, 12–66) months, whereas the CT time in the SVR group was 36.38 ± 23.76 (range, 12–111) months (P = 0.253).

The 21 patients who were HBeAg negative received antiviral treatment for a total of 57.67 ± 25.10 (range, 17–104) months, and their CT time (from the time HBV DNA below the detection limit to discontinuation) was 43.90 ± 21.89 (range, 14–77) months. Among these patients, 10 experienced VR, with a median VR time of 6 (range, 1–12) months. The 3-, 6-, and 12-month accumulative VR rates were 19.05%, 38.10%, and 51.85%, respectively. The median follow-up time for the 11 patients with SVR was 12 (range, 6–18) months. We also compared the CT and CR times in the VR and SVR groups, and found that the CR time in the VR group was 16.60 ± 14.33 (range, 1–43) months, which was similar to that in the SVR group (11.18 ± 12.71 months; range, 2–3 months; P = 0.370). The CT time in the VR group was 38.20 ± 20.85 (range, 17–73) months, which was also similar to that in the SVR group (49.09 ± 22.47 months; range, 14–77 months; P = 0.265).

Comparison of VR and SVR

We compared the HBV DNA levels, HBeAg status, ALT levels at initial treatment, total treatment time, CR time, CT time, age at discontinuation, gender, FibroScan values, and HBsAg levels at the initiation of NUCs treatment between the VR and SVR groups, and found that there was no significant difference between the two groups for most indicators [Table 1]. However, the CR time before discontinuation in the VR group was 26.18 ± 22.52 months, which was significantly longer than that in the SVR group (14.19 ± 11.75 months) (P = 0.014). In addition, the average age in the VR group was 39.11 ± 6.52 years, which was significantly higher than that in the SVR group (33.78 ± 8.56 years) (P = 0.006). The average HBsAg levels in the VR group (2.91 ± 0.64 logIU/mL) was also significantly higher than that in the SVR group (2.18 ± 1.25 logIU/mL) (P = 0.003).

Factors associated with VR

The logistic regression model was carried out to assess the predictors that were associated with VR, including pretreatment HBV DNA levels, HBeAg status, ALT levels, total treatment time, CT time, age at baseline screening, gender, FibroScan values, and HBsAg levels. We observed that the serum HBsAg levels and age at discontinuation, as well as CR time before discontinuation, were significant predictors of VR [Table 2]. Patients who experienced VR after discontinuation of NUC antiviral therapy were older, had higher HBsAg levels, and longer CR times before discontinuation, compared with patients with SVR.

Therefore, we constructed receiver operating characteristic (ROC) curve for HBsAg levels and age at discontinuation, as well as CR time before discontinuation [Figure 2]. At discontinuation, the AUROC curve for serum HBsAg was 0.690 (95% CI: 0.560–0.819). When the cutoff value for serum HBsAg was set at 2.715 logIU/mL, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for off-treatment VR were 75.0%, 62.16%, 60.0%, and 76.67%, respectively. Higher serum HBsAg at discontinuation

Table 2: Factors associated with virologic relapse by logistics regression

Characteristics	Univariate analysis			Multivariate analysis		
	OR	OR (95% C.I)	*P*	OR	OR (95% C.I)	*P*
Gender	0.330	(0.063,1.728)	0.189			
Age at EOT	1.095	(1.020,1.176)	0.012	1.093	(1.011,1.183)	0.026
FibroScan at EOT	1.074	(0.775,1.486)	0.669			
Pretreatment HBeAg status	0.762	(0.268,2.168)	0.610			
Pretreatment HBVDNA	0.842	(0.563,1.260)	0.403			
Pretreatment ALT	1.000	(0.999,1.001)	0.905			
Total treatment course	1.006	(0.987,1.026)	0.525			
Course for CT	0.982	(0.958,1.007)	0.150			
Course for CR	1.041	(1.009,1.074)	0.012	1.038	(1.002,1.076)	0.039
ALT level at EOT	0.997	(0.960,1.036)	0.889			
Level of HBsAg at EOT	2.199	(1.180,4.097)	0.013	2.734	(1.272,5.877)	0.010

EOT: End of the treatment, HBeAg: Hepatits B e antigen, HBV: Hepatitis B virus, DNA: Deoxyribonucleic acid, HBsAg: Hepatitis B surface antigen, ALT: Alanine aminotransferase, CT: Consolidation treatment, CR: Complete response, OR: Odds ratio, C.I: Confidence interval. The logistics regression showed that old age and long course for CR as well as the high HBsAg load at EOT were the three risk factors for non-SVR

was associated with an increased risk of VR. Once we had identified the optimal cutoff value as 2.715 logIU/mL, we had measured the difference in the cumulative VR rates between patients with HBsAg > 2.715 logIU/mL and patients with HBsAg < 2.715 logIU/mL [Figure 3]. The 12-month cumulative VR rate in patients with HBsAg < 2.715 logIU/mL (26.15%) was significantly lower than that in patients with HBsAg > 2.715 logIU/mL (65.71%) ($P = 0.0079$). Among the 35 patients with HBsAg > 2.715 logIU/mL at discontinuation, 21 experienced VR by the end of the follow-up. The 3-, 6-, and 12-month cumulative VR rates in these patients were 20.0%, 42.86%, and 65.71%, respectively. Among the 30 patients with HBsAg < 2.715 logIU/mL at discontinuation, only 7 experienced VR by the end of the follow-up, and their 3-, 6-, and 12-month cumulative VR rates were 6.67%, 20.0%, and 26.15%, respectively.

The AUROC for age at discontinuation was 0.679 (95% CI: 0.560–0.819). The optimal cutoff value was found as 38.5 years, and the sensitivity, specificity, PPV, and NPV for predicting off-treatment VR were 67.86%, 64.86%, 59.38%, and 72.73%, respectively. Elderly patients had a higher risk of VR. Among the 32 patients who were >38.5 years old at discontinuation, 19 (59.38%) had experienced VR by the end of the follow-up. Their 3-, 6-, and 12-month cumulative VR rates were 18.75%, 43.75%, and 65.39%, respectively. Among the 33 patients who were < 38.5 years old at discontinuation, only 9 (27.27%) experienced VR by the end of the follow-up, and their 3-, 6-, and 12-month cumulative VR rates were 9.09%, 21.21%, and 31.72%, respectively.

The AUROC for CR time was 0.647 (95% CI: 0.505–0.788). The optimal cutoff value was 32.5 months, and the sensitivity, specificity, PPV, and NPV for predicting off-treatment VR were 39.29%, 91.89%, 78.57%, and 66.67%, respectively. Among the 14 patients with a CR time of > 32.5 months, 11 (78.57%) experienced VR by the end of the follow-up. Their 3-, 6-, and 12-month cumulative VR rates were 21.43%, 64.29%, and 78.57%, respectively. Among the 51 patients with a CR time of < 32.5 months, 17 (33.33%) experienced VR by the end of the follow-up, and their 3-, 6-, and 12-month cumulative VR rates were 11.77%, 23.53%, and 40.15%, respectively.

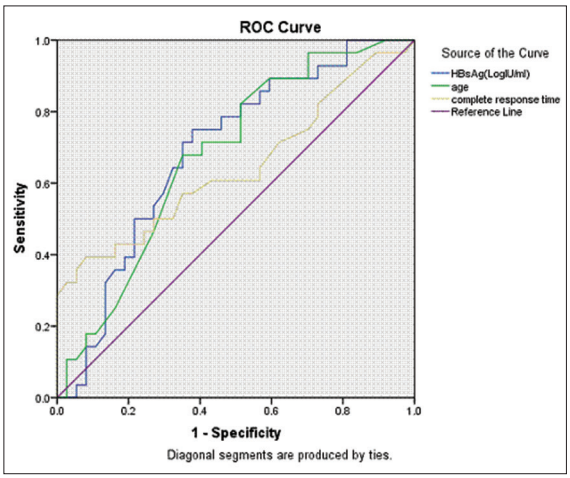

Figure 2: Receiver operating characteristic curves of postdiscontinuation HBsAg, age and complete response time in predicting virological relapse

Viral load and serum HBsAg levels in SVR groups

Among the 42 patients who were followed-up for > 1 year after discontinuation, 21 exhibited VR and 21 exhibited SVR. We further analyzed the virologic changes among the 21 SVR cases [Table 3]. The HBV DNA levels of 5 patients were below the detection limit throughout the 1-year follow-up, and these included 2 patients who were negative for HBsAg after discontinuation. HBV DNA levels above the detection limit were observed in 16 cases at least once during the 1-year follow-up [Table 3], which included 5 cases with positive HBV DNA levels in the 3–6 months

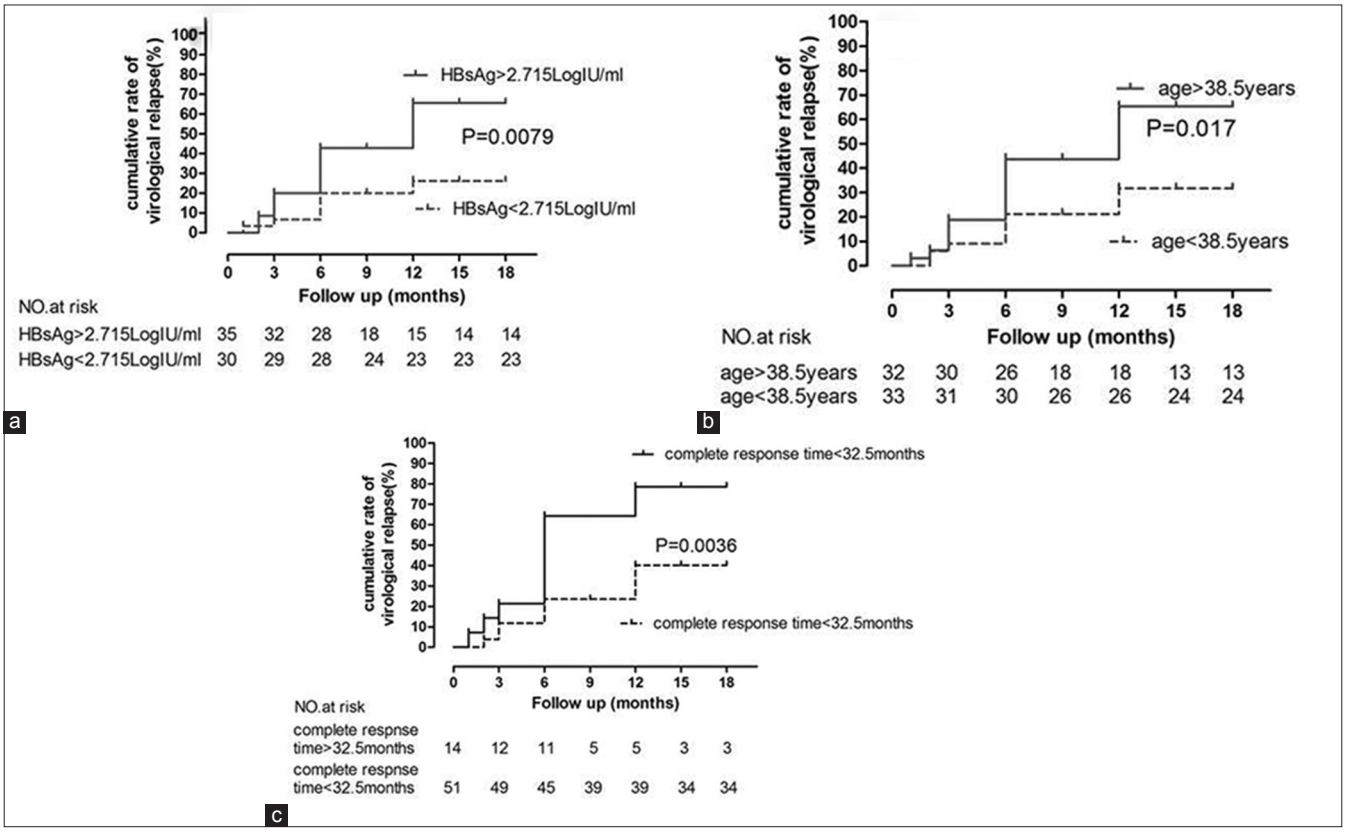

Figure 3: (a) Cumulative virologic relapse rates in patients with HBsAg <2.715 LogIU/ml and HBsAg >2.715 LogIU/ml at the end of treatment, log-rank test *P* = 0.0079; (b) Cumulative virologic relapse rates in patients with age <38.5 years and age >38.5 years at the end of treatment, log-rank test *P* = 0.017; (c) Cumulative virologic relapse rates in patients with complete response time < 32.5months and complete response time >32.5 months, log-rank test *P* = 0.0036; HBsAg,hepatitis B surface antigen

after discontinuation. These levels subsequently decreased to below the detection limit (Patients 9, 15, 20, 32, and 34). Five additional cases exhibited positive levels of HBV DNA 6 months after discontinuation, although they were maintained at lower levels and subsequently declined by > 1 logIU/mL (Patients 3, 5, 36, 39, and 40). Six cases exhibited positive levels of HBV DNA in the 3–6 months after discontinuation, which were maintained at lower levels and changed by <1 logIU/mL: (Patients 4, 14, 16, 24, 35, and 41).

We analyzed the HBsAg curve for the 21 patients with SVR who had discontinuation within 1 year [Figure 4]. The HBsAg levels did not significantly change during the postdiscontinuation follow-up. One patient (Patient 22) was HBsAg negative throughout the entire year, whereas another (Patient 33) experienced HBsAg clearance during that year. Both cases exhibited HBV DNA levels below the detection limit throughout the entire year.

DISCUSSION

In recent decades, NUCs have been widely used to treat CHB, thereby preventing or delaying the development of liver disease. However, many clinical studies have reported a high relapse rate after discontinuation of NUCs treatment.[13] The Asia-Pacific Association for the Study of the Liver (APASL) Guidelines recommend that treatment for patients who are HBeAg positive should be continued for at least 12 months, and after reaching CR, their treatment should be consolidated for at least 12 months before discontinuation is considered. Treatment for patients who are HBeAg negative should be continued for at least 12 months, and after reaching CR, their treatment should be consolidated for at least 18 months before discontinuation is considered.[14] However, the APASL recommendations lack sufficient evidence-based support, as there are currently only a small number of factors that are known to be related to recurrence after discontinuation. One study of 467 South Korean patients with CHB, who were HBeAg positive and received LAM antiviral therapy, reported that patients younger than 40 years and with a CT time >12 months after HBeAg seroconversion exhibited a 20% relapse rate. In contrast, patients younger than 40 years, or with a CT time > 12 months after HBeAg seroconversion, exhibited a 50% relapse rate, whereas patients older than 40 years and with a CT time < 12 months after HBeAg seroconversion, exhibited a relapse rate of up to 90%. Thus,

Table 3: Changes of HBV DNA level of the sustained virological response patients in the first year after the discontinuation

Patient no.	Subject (LogIU/mL)	Baseline	3 months	6 months	12 months
13	HBV DNA	0	0	0	0
22	HBV DNA	0	0	0	0
30	HBV DNA	0	0	0	0
33	HBV DNA	0	0	0	0
43	HBV DNA	0	0	0	0
9	HBV DNA	0	0	3.36	0
15	HBV DNA	0	2.25	0	0
20	HBV DNA	0	3.31	0	0
32	HBV DNA	0	2.21	3.37	0
34	HBV DNA	0	2.4	2.94	0
3	HBV DNA	0	0	3.21	1.77
5	HBV DNA	0	0	5.03	1.82
36	HBV DNA	0	1.42	5	2.94
39	HBV DNA	0	2.09	2.87	1.71
40	HBV DNA	0	3.66	3.86	2.33
4	HBV DNA	0	1.55	1.63	2.51
14	HBV DNA	0	2.01	1.75	1.95
16	HBV DNA	0	1.79	2.27	1.98
24	HBV DNA	0	0	2.31	1.87
35	HBV DNA	0	0	2.04	1.95
41	HBV DNA	0	1.97	1.38	1.14

0 represents that HBV DNA was under the detection line (Cobas TaqMan < 20 IU/mL). HBV: Hepatitis B virus, DNA: Deoxyribonucleic acid

the relapse rate is directly related to the patient's age and the duration of CT.[15] Another study of 17 patients from Shanghai who had CHB and were HBeAg positive revealed that HBsAg < 2 $\log_{10}$IU/mL at the 104th week of treatment accurately predicted postdiscontinuation SVR after 2 years of LdT antiretroviral therapy and 2 years of follow-up. In addition, the decreasing levels of HBsAg at the 24th and 52nd weeks of treatment could more accurately predict postdiscontinuation SVR compared with HBV DNA levels (in the same period).[16]

The purpose of this study was to identify the predictors of a sustained response after NUCs discontinuation. All patients enrolled into this study were strictly screened according to the APASL guidelines for discontinuation, and we then analyzed the postdiscontinuation VR rates. Using logistic regression analysis, we observed that HBsAg levels and age at discontinuation, as well as CR time, were predictors of postdiscontinuation VR. Low HBsAg levels at discontinuation indicated a low risk, with younger patients less prone to VR compared with elderly patients. In addition, shorter CR time during the treatment predicted a lower risk of postdiscontinuation VR. However, we did not observe any other significant predictors of relapse among the various parameters examined. Likewise, in a study of

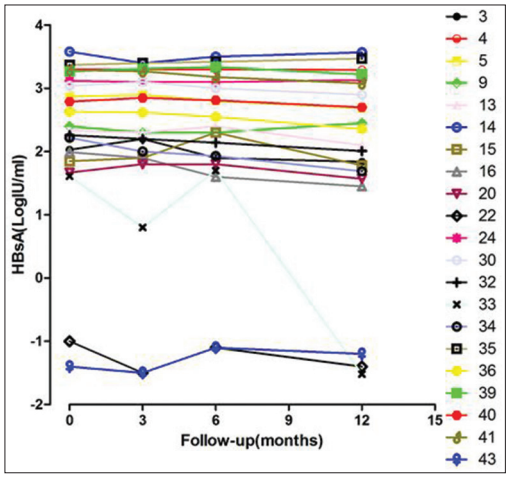

Figure 4: Serum HBsAg level profiles of the 21 patients with sustained virologic response during the first year of follow-up in patients who had discontinued treatment at least one year

NUCs use in 48 South Korean patients with CHB (HBeAg positive) reported that patient age (>40 years) and the duration of CT (≥15 months) were significant predictive factors for off-treatment durability [P = 0.049; relative risk (RR): 0.31; 95% CI: 0.096–0.998 and P = 0.005; RR: 11.29; 95% CI: 2.054–65.12, respectively]. However, younger patients (≤40 years) with extended CT (≥15 months) had significantly improved durability (P = 0.014). These results suggest that treatment for >15 months (after HBeAg seroconversion) in patients who are ≤40 years old may provide a sustained virologic response.[17] Another study from Guangzhou, China, reported that the age at discontinuation, baseline ALT levels, and prolonged CT were associated with VR after discontinuation.[18] The results of these clinical studies indicate that during NUCs treatment, CT time, age at discontinuation, and ALT levels before treatment could be used to guide discontinuation of NUCs treatment. However, we did not observe a significant difference in the CT time and baseline ALT levels among VR and SVR patients. Feng *et al.*, has reported from data collected after the discontinuation of LAM treatment in 61 patients who were HBeAg negative. Their total treatment time was ≥24 months, the CT time was ≥18 months, and relapse was defined as HBV DNA > 10^4 copies/mL. Cox regression analysis revealed that age was the only predictor in that study, with younger patients exhibiting a lower relapse rate.[19] Another study investigated ADV antiretroviral therapy in 145 patients with CHB (HBeAg negative) from Shanghai, and reported that the total treatment time for all patients was ≥ 24 months and the CT time was ≥ 18 months. During the postdiscontinuation follow-up period, 95 cases relapsed (relapse was defined as HBV DNA > 10^4 copies/mL), with 93% of these patients experiencing relapse within 12 months of discontinuation.[20] In their Cox correlation analysis, age was the only factor that was significantly associated with relapse. The results of

these studies indicated that age is an important predictor for postdiscontinuation relapse, which is similar to our results; therefore, younger patients do not appear to be prone to relapse after discontinuation.

In this study, follow-up revealed that only 5 of the 44 cases of HBeAg-positive CHB experienced HBeAg reversion, indicating that NUC-induced HBeAg seroconversion was durable. This result is consistent with the results of a 2-year drug discontinuation study, which reported that 80% patients maintained HBeAg seroconversion 2 years after discontinuation.[21]

The loss of HBsAg and the development of anti-HBs antibodies (HBsAg seroconversion) are the ultimate goals of anti-HBV therapy, and therefore the HBsAg levels might be a useful prognostic indicator. In recent years, serum HBsAg quantitation has become a popular field for research. In addition, it has been reported that HBsAg levels can reflect the levels of HBV DNA or covalently closed circular DNA inside liver cells, as this DNA acts as a transcription template for viral RNA. Therefore, HBsAg levels are recommended as an alternative indicator for HBV infection of liver cells.[22] In the natural history of hepatitis B, it was discovered that low serum HBsAg levels were related with disease improvement and virus removal.[22] In studies regarding interferon therapy, it has been reported that low HBsAg levels during treatment could predict a sustained response to the therapy.[23,24] Another study reported that LdT treatment induced a significant decline in HBsAg levels during treatment, and that HBsAg clearance was related to the sustained remission of disease.[25] In the present study, we observed that the serum HBsAg levels in the VR group were higher than those in the SVR group. Interestingly, 60% of patients with HBsAg > 2.715 IU/mL experienced VR after discontinuation, whereas only 23.33% of patients with HBsAg < 2.715 IU/mL experienced VR. These findings agree with recent findings from Guangxi, China, where HBsAg < 2 logIU/mL could predict sustained remission among 84 patients with hepatitis B, who had patients who had discontinued NUCs treatment.[26]

In the present study, we also observed that the risk of postdiscontinuation VR was lower among patients who achieved CR earlier compared with those who achieved CR later. Among the 51 patients who achieved CR within 32.5 months, only 17 cases (33.33%) experienced VR by the end of the follow-up, whereas 11 of 14 patients (78.57%) who achieved CR in >32.5 months experienced VR by the end of the follow-up. In addition, we concluded that serum HBsAg, age at discontinuation, and CR time were predictors of postdiscontinuation relapse. These factors could be used to guide clinical discontinuation of treatment, as younger patients, those with lower serum HBsAg levels, and those with earlier CR would have a lower risk of postdiscontinuation relapse.

This study also had several limitations. The first one is the small sample size, which may lead to bias from the real world treatment. As our data from the beginning of NUCs therapy to discontinuation was retrospectively obtained, and we had no serum samples from the time the patients started treatment and during the treatment period. Therefore, we were unable to evaluate any possible quantitative changes in HBsAg levels during treatment, and could not analyze the impacts of these changes on the incidence of relapse. With the limitation of retrospective information such as this, we may be able to plan prospective studies in future. In addition, the quantitation of HBV DNA before discontinuation was performed using a Chinese PCR-assay (detection limit: 1000 copies/mL), which is not as accurate as the Cobas TaqMan method used in the follow up (detection limit: 20 IU/mL).[27]

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