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Kinetics and Clearance of Hepatitis B Surface Antigen in Patients with HIV-HBV Co-infection Receiving Long Term Tenofovir-Containing Antiretroviral Therapy

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ABSTRACT

Background and Aims: Hepatitis B surface antigen (HBsAg) clearance is an important endpoint in the management of chronic hepatitis B (CHB). The aim of this retrospective longitudinal study was to evaluate HBsAg kinetics and HBsAg clearance rates in HIV-HBV co-infected patients receiving tenofovir (TDF)-containing antiretroviral therapy (ART).

Methods: A total of 102 Thai patients with HIV-HBV co-infection were recruited. Rates of HBsAg clearance and longitudinal changes in HBsAg levels were performed.

Results: At baseline, 55 (54%) and 47 (46.1%) patients were classified as HBeAg-positive and HBeAg-negative CHB, respectively. Over a median follow-up of 97 months, HBsAg declined steadily in >75% of patients receiving ART and 11 (10.8%) patients achieved HBsAg clearance. HBsAg clearance was significantly associated with HBeAg seroconversion, rapid HBsAg decline and HBsAg kinetic pattern, but was not related to baseline levels of ALT, HBV DNA, HBsAg and CD_4 counts.

Conclusions: Long-term TDF therapy could lead to a significant decline in HBsAg levels in patients with HIV-HBV co-infection, particularly those with HBsAg clearance. Monitoring HBsAg levels may help identify patients who might have a high probability in clearing HBsAg on long-term ART therapy.

Key words : HIV-HBV coinfection, tenofovir, HBsAg

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INTRODUCTION

Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection is common because both viruses share similar routes of transmission through blood and sexual contact. Currently, there are approximately 40 and 400 million people worldwide chronically infected with HIV and HBV, respectively[1,2]. In addition, an estimated 10% of HIV-infected individuals have chronic HBV infection, with a higher prevalence found in HBV endemic areas including countries in Africa and Asia. It is generally known that HIV can accelerate the natural course of HBV-associated liver disease, such as the progression of cirrhosis and hepatocellular carcinoma[3]. In addition, liver-related complications have emerged as an important cause of morbidity and mortality in HIV-HBV co-infected patients receiving antiretroviral therapy (ART)[1].

Antiviral therapy using tenofovir (TDF)-containing ART regimens have allowed the combined treatment of HIV-HBV co-infection by suppression of viral replication. TDF appears to be one of the most potent anti-HBV agents so far with a very low rate of drug resistance[4]. It has been demonstrated that long-term TDF therapy could reduce the risk of cirrhosis in HIV-HBV co-infected patients[5]. Although suppression of HBV DNA to undetectable levels is achievable in most HIV-HBV co-infected individuals, hepatitis B s antigen (HBsAg) clearance, which represents the ultimate goal of HBV treatment, remains uncommon after long-term TDF therapy. A recent study in HIV-HBV co-infected patients showed that HBsAg clearance rate was approximately 2.5% per year[6]. Recent studies have showed that serum HBsAg level is a non-invasive surrogate marker for the intrahepatic covalently closed circular DNA (cccDNA), which is the template of viral replication. Thus, the quantification of HBsAg may be useful for the prediction of subsequent HBsAg clearance and sustained virological response[7]. Currently, data on the predictive value of quantitative HBsAg during long-term potent viral suppression in HIV-HBV co-infected patients are limited. The aim of this retrospective study was to evaluate on-treatment HBsAg kinetics and HBsAg clearance in HIV-HBV co-infected patients receiving long-term TDF-containing ART regimens.

PATIENTS AND METHODS

HIV-HBV co-infected patients were enrolled from the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) of Thai Red Cross Society (HIV-NAT 006 long-term cohort). The criteria for patient selection were as the following: (1) patients' age more than 18 years, (2) HIV infection (anti-HIV and HIV-RNA positivity), (3) chronic HBV infection (detectable serum HBsAg at least 6 months), (4) hepatitis B e antigen (HBeAg) positive or negative, and (5) patients who received TDF-containing regimens. Patients were excluded if co-infected with hepatitis C virus (HCV). Prior treatment with pegylated interferon (PEG-IFN), lamivudine (LAM) and other HBV antiviral drugs before starting TDF was not excluded.

Routine laboratory and patient care were conducted. Serum samples collected during follow-up of every 6 months were stored at -20˚C until used for the quantitative determination of HBsAg and HBV DNA. Informed consent was given for subjects in the study. The study was approved by Research Ethics Committees/Institutional review boards of Faculty of Medicine, Chulalongkorn University, Bangkok.

Serological and virological assays

Qualitative HBsAg, anti-HBs, HBeAg and anti-HBe were measured by commercially available enzyme-linked immunosorbent assay kits (Abbott Laboratories, Chicago, IL). HBsAg levels were quantified by the Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, Indianapolis, IN). HBV DNA levels were quantified by the Abbott RealTime HBV assay (Abbott Laboratories, Chicago, IL). The lower limit of detection of serum HBV DNA is 10 IU/mL.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD), and percentages as appropriate. Comparisons between groups were analyzed by the ≥2 or Fisher' s exact test for categorical variables and by the Mann-Whitney *U*-test or Student's *t*-test for quantitative variables. *P*-values < 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS software for windows 21.0 (SPSS Inc., Chicago, IL).

Results

Baseline patient characteristics

A total of 102 patients with HIV-HBV co-infection were enrolled (Table 1). The majority of the patients were men (66.7%) at a mean age of 37.1±8.9 years. In most patients, the combination of TDF and LAM was used (n=98; 96.1%), while the remaining 4 (3.9%) patients were treated with TDF alone. Mean baseline ALT level was 29.3±51.2 U/L, while mean HBV DNA and HBsAg levels were 6.3±1.7 and 4.1±0.7 IU/mL, respectively.

Regarding HBeAg status at baseline, 55 (53.9%) and 47 (46.1%) patients were positive and negative for HBeAg, respectively. Patients with HBeAg-positive chronic hepatitis B (CHB), compared to those with HBeAg-negative CHB, were younger but had significantly higher mean of HBV DNA levels. Patients with HBeAg-positive CHB tended to have lower CD4 T-cell counts than those with HBeAg-negative CHB, although did not reach statistical significance (*P*=0.053). There was no significantly difference between groups regarding gender distribution, mean ALT and HBsAg, as well as HBV treatment regimens.

Table 1. Baseline characteristics of the patients.

Characteristic	All (n=102)	HBeAg positive CHB (n=55)	HBeAg negative CHB (n=47)	*p*-value
Age, years	37.1±8.6	34.6±7.8	39.2±8.0	0.007*
Sex (%)				0.905
Male	68 (66.7)	35 (63.6)	24 (64.9)	
Female	34 (33.3)	20 (36.4)	13 (35.1)	
ALT level (U/mL)	52.2±35.4	51.9±36.1	53.3±37.5	0.852
HBV DNA level (log_{10} IU/mL)	5.1±2.5	6.2±2.2	3.8±2.0	0.001*
HBsAg level (log_{10} IU/mL)	3.4±1.5	3.4±1.5	3.8±0.6	0.176
CD_4 cell count (cells/μL)	343.0±319.4	273.5±258.3	404.3±378.3	0.053
Treatment regimen (%)				0.415
TDF	4 (3.9)	1 (1.8)	0 (0)	
TDF+LAM	98 (96.1)	54 (98.2)	37 (100)	
Naïve for ART	57 (55.9)	27 (49.1)	23 (62.2)	0.222

Data presented as mean ± SD or n (%)

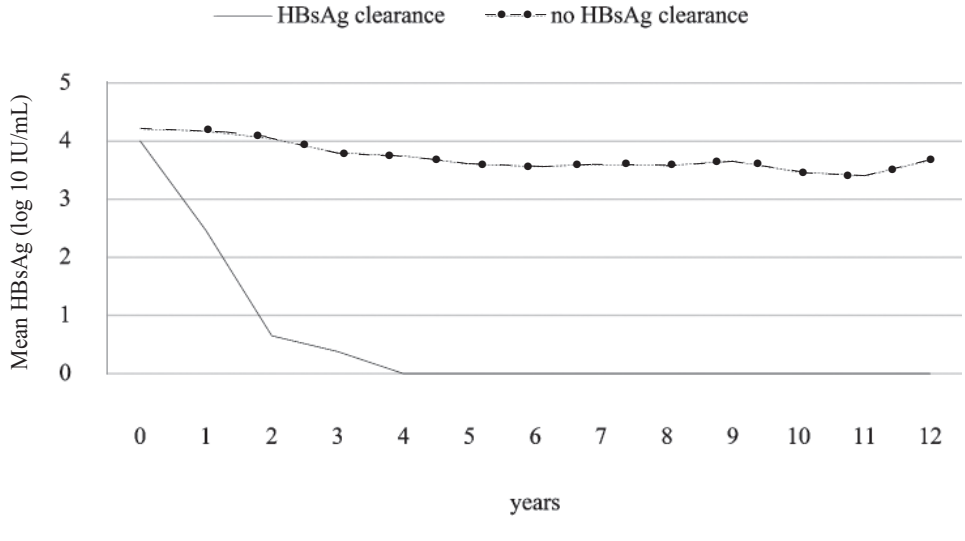

Figure 1. Kinetics of serum HBsAg levels with respect to HBsAg clearance.

Pattern of HBsAg change and HBsAg clearance during follow-up

Patients were followed for a median of 97 months (range, 9-146 months) after ART initiation. During follow-up, three different patterns of HBsAg kinetics were revealed. The increase HBsAg pattern was defined as ≥0.5 log10 IU/mL increase of HBsAg titer from baseline. Decrease HBsAg pattern was defined as ≥0.5 log10 IU/mL decrease of HBsAg titer from baseline. If the difference between baseline and last follow-up was less than 0.5 log10 IU/mL, it was categorized as steady pattern. In this study, 11 (10.8%) and 12 (11.8%) patients were categorized as having the increase and steady patterns, respectively, while 79 (77.4%) patients had the decreased pattern.

A total of 11 (10.8%) patients had HBsAg clear-

Table 2. Characteristics of patients who achieved HBsAg clearance.

No.	Age (year)	Sex	ALT (U/mL)	Baseline HBV DNA (IU/mL)	Baseline HBsAg (IU/mL)	Baseline CD_4 (cells/μL)	Last follow-up CD_4 (cells/μL)	HBsAg clearance (months)	HBeAg status
1	35	F	60	12	4152.4	702	639	12	Positive (negative)
2	49	M	144	40	No data	349	487	20	Negative
3	32	M	34	40	No data	311	537	12	Positive (negative)
4	23	M	67	20,000,000	52,000	59	142	12	Positive (negative)
5	32	F	66	6,658,137	1,035	31	83	57	Positive (negative)
6	30	F	42	20,000,000	52,000	19	120	12	Positive (negative)
7	24	M	65	1,100,000	No data	242	373	43	Positive (negative)
8	33	M	49	44	No data	245	372	49	Positive (negative)
9	43	M	30	11	No data	746	976	12	Negative
10	63	M	33	<10	2,233.6	1015	967	25	negative
11	41	M	52	1,388,640	3,571	51	128	12	Positive (negative)

Table 3. Characteristics of the patients with respect to HBsAg clearance.

Characteristics	HBsAg clearance (n=11)	No HBsAg clearance (n=91)	*p*-value
Age (years)	36.8±11.6	37.2±8.2	0.9
Sex			0.656
Male	8 (72.73)	60 (65.9)	
Female	3 (27.27)	31 (34.1)	
Baseline ALT level (U/mL)	58.4±31.5	51.5±35.9	0.512
Baseline HBV DNA level (log_{10} IU/mL)	3.8±2.9	5.2±2.4	0.068
Baseline HBsAg level (log_{10} IU/mL)	2.6±2.5	3.5±1.3	0.304
Baseline CD_4 count (cells/μL)			
Before	342.7±336.5	302.5±273.3	0.659
After	438.6±322.7	388.1±249.9	0.549
ΔCD_4 cell count (cells/μL)	95.8±93.4	85.6±101.2	0.753
HBeAg seroconversion	8 (72.7)	21 (23.1)	0.001*
HBsAg decline (log_{10} IU/mL)	3.5±0.9	2.5±1.2	0.049*
HBsAg kinetic pattern			0.035*
Increase or steady	0 (0)	23 (25.3)	
Decrease	11 (100)	68 (74.7)	

Data presented as mean ± SD or n (%)

ance during follow-up, all of which occurred within 5 years after initiation of therapy (Figure 1). Of these patients, 8 (72.7%) were HBeAg-positive CHB, who achieved HBeAg clearance with the presence of anti-HBe. All patients were categorized as having the decreased pattern of HBsAg kinetics. Table 2 summarizes the characteristics of patients who had HBsAg clearance.

Factors associated with HBsAg clearance

Table 3 compares the characteristics of patients with respect to HBsAg clearance. There was no significance between patients with or without HBsAg clearance in terms of age, gender distribution, baseline ALT, HBV DNA, HBsAg levels and baseline CD_4 cell counts. Patients with HBsAg clearance had higher frequency of HBeAg seroconversion, higher mean HBsAg decline and different pattern of HBsAg kinetics compared to those without HBsAg clearance.

DISCUSSION

HBsAg clearance is considered to be an important serological endpoint in the management of chronic HBV infection, indicating immunological control of the disease. Patients who achieved HBsAg clearance have considerably decreased liver-related complications, including cirrhosis and HCC[4]. This retrospective longitudinal study provided data of HBsAg clearance and kinetics in patients with HIV-HBV co-infection under TDF-based ART regimens. The overall rate of HBsAg clearance in this study (approximately 11%) was to some extent higher than that observed in most studies conducted in HBV mono-infected patients, which reported the clearance rates under nucleos(t)ide analogue (NA) therapy between 0-2%[8,9]. In general, the clearance rates of HBsAg remain uncommon in patients with HBV mono-infection treated with potent NA, such as entecavir (ETV) or TDF. In the real world setting, HBsAg clearance rate among 355 HBV-mono-infected patients treated with ETV or TDF was approximately 1% after 4 years of therapy[10]. In a recent large scale study of Asian patients with HBV mono-infection treated with TDF, the rate of HBsAg clearance was 0% through 5 years of treatment[11].

Compared with other cohorts of HIV-HBV co-infected patients, the rates of HBsAg clearance seemed to be diverse but higher than those found in HBV mono-infected patients. For instance, there was no HBsAg clearance in 65 cases receiving TDF-based ART regimens from the TECOVIR study after 12 months of therapy[12]. Such a low rate of HBsAg clearance might reflect a relatively short duration of follow-up. In the France cohort, HBsAg clearance occurred in 4 (2.7%) patients after median of 2.5 years of follow-up[13]. Another recent prospective study evaluating the efficacy of TDF-based ART regimens in co-infected patients demonstrated that 17 of 290 (5.9%) patients had HBsAg clearance after a median 4.6 years of therapy[14]. In a multicenter European cohort of 51 co-infected patients retrospectively followed for a mean of 43 months, 4 (8%) cases of HBsAg clearance were observed[15]. Interestingly, in two recently published multicenter studies conducted in Dutch and Austrian cohorts, HBsAg clearance in HIV-HBV co-infection appeared to be relatively high with approximately 12% and 17%, respectively[16, 17]. This discrepancy among reports is probably related to the heterogeneity of studies in terms of populations, HBV genotypes, sample size, treatment regimen and length of follow-up.

Recent studies have demonstrated the clinical application of HBsAg quantification in monitoring HBV during PEG-IFN therapy[18]. In particular, on-treatment kinetics of serum HBsAg levels can predict post-treatment response to PEG-IFN therapy in patients with HBeAg-positive and HBeAg-negative CHB[18]. Unlike in patients treated with PEG-IFN, the clinical utility of quantitative HBsAg under NA therapy remains largely unclear. Among patients with HBV mono-infection treated with potent NA, the decline in HBsAg is generally slow despite a marked suppressive effect on HBV DNA levels[7]. For instance, a recent study based on long-term follow-up of patients with HBV mono-infection treated with potent NA, it was showed that HBsAg clearance was rare and the average HBsAg decline was only 0.1 $\log_{10}$ IU/mL/year[19]. However, higher chance of HBsAg clearance might be observed particularly in those who had rapid HBsAg decline of more than 1 log10 IU/mL within 12 months of starting NA treatment[20].

In this study, we demonstrated that serum HBsAg declined significantly in those with HBsAg clearance. However, the decline in HBsAg was also observed in those without HBsAg clearance, which might reflect an ongoing decrease in intrahepatic cccDNA. The decline of HBsAg more than 1-2 $\log_{10}$ IU/mL in patients without HBsAg clearance in this report was comparable to that previously reported in the French study

conducted in HIV-HBV co-infected individuals[13]. In fact, lower HBsAg levels seem to reflect improved immune status with less active liver disease[19]. In addition, patients showing rapid on-treatment HBsAg declines are more likely to achieve virological end-points including sustained viral suppression and serological end-points including HBsAg clearance[7]. These findings may anticipate the high probability of future HBsAg clearance among co-infected individuals who have continuous reduction in HBsAg levels over time.

The mechanisms underlying higher HBsAg decline and clearance in patients with HIV-HBV co-infection when compared to those with HBV mono-infection are unclear but might be associated with several factors. First, unlike PEG-IFN therapy, TDF mainly affects HBV replication but lacks direct immunomodulatory effects. However, initiation of anti-HBV agents in co-infected patients not only influences viral replication but also leads to the restoration of immune activity that could enhance stronger HBsAg declines, and may eventually lead to HBsAg clearance[15]. As demonstrated in this report, the HBsAg decline was observed particularly in patients with an increase CD_4 cell counts at the last follow-up. Thus, it could be speculated that HBsAg clearance in co-infected patients was mainly a secondary effect of ART in increasing CD_4 cell counts, instead of a direct effect on viral suppression of TDF[21]. These results also support the recommendations to initiate TDF-containing ART regimens early in co-infected patients as immune restoration in an earlier phase of HBV infection may be essential for achieving higher rates of HBsAg decline and clearance. Second, our data showed that HBsAg clearance was mostly confined to patients with HBeAg-positive CHB achieving HBeAg seroconversion. This observation was in line with previous reports demonstrating that the magnitude of HBsAg decline and the probability of HBsAg clearance tended to be higher among HBV mono- and co-infected patients with HBeAg seroconversion[20,21]. Finally, host genetic variations such as human leukocyte antigen (HLA)-DP polymorphisms might influence HBV clearance, as demonstrated in HBV mono-infected patients[22]. Additional investigations are needed to identify host and virus-related factors, as well as the accurate mechanisms of HBsAg clearance in response to TDF-containing ART regimens.

The study might have limitations due to the relatively sample size and incomplete data of virological and serological markers in some patients. In addition, HBV genotypes and mutations, which might have influenced on HBsAg kinetics, were completely unknown. However, current data on HBsAg kinetics in Asian patients with HIV-HBV co-infection are still limited. Moreover, our cohort was based on long-term follow-up with a median of approximately 8 years. Thus, these data might reflect patients seen in real-life clinical practice in a resource-limited setting, where the prevalence of HIV-HBV co-infection is high.

In conclusion, our data demonstrated that long-term TDF therapy could lead to a significant decline in HBsAg levels in patients with HIV-HBV co-infection, particularly those with HBsAg clearance. Monitoring HBsAg levels may help identify some patients who might have a high probability in clearing HBsAg on long-term ART therapy.

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REFERENCES

1. Thio CL. Hepatitis B and human immunodeficiency virus coinfection. Hepatology 2009;49:S138-45.
2. McGovern BH. The epidemiology, natural history and prevention of hepatitis B: implications of HIV coinfection. Antivir Ther 2007;12 Suppl 3:H3-13.
3. Price JC, Thio CL. Liver disease in the HIV-infected individual. Clin Gastroenterol Hepatol 2010;8:1002-12.
4. Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384:2053-63.
5. Tuma P, Medrano J, Resino S, *et al.* Incidence of liver cirrhosis in HIV-infected patients with chronic hepatitis B or C in the era of highly active antiretroviral therapy. Antivir Ther 2010;15:881-6.
6. Martin-Carbonero L, Teixeira T, Poveda E, *et al.* Clinical and virological outcomes in HIV-infected patients with chronic hepatitis B on long-term nucleos(t)ide analogues. AIDS 2011;25:73-9.
7. Martinot-Peignoux M, Asselah T, Marcellin P. HBsAg quantification to optimize treatment monitoring in chronic hepatitis B patients. Liver Int 2015;35 Suppl 1:82-90.
8. Chang TT, Gish RG, de Man R, *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. NEJM 2006;354:1001-10.
9. Marcellin P, Chang TT, Lim SG, *et al.* Adefovir dipivoxil for

the treatment of hepatitis B e antigen-positive chronic hepatitis B. NEJM 2003;348:808-16.

10. Idilman R, Gunsar F, Koruk M, *et al.* Long-term entecavir or tenofovir disoproxil fumarate therapy in treatment-naive chronic hepatitis B patients in the real-world setting. J Viral Hepatol 2015;22:504-10.
11. Tsai NC, Marcellin P, Buti M, *et al.* Viral suppression and cirrhosis regression with tenofovir disoproxil fumarate in Asians with chronic hepatitis B. Dig Dis Sci 2015;60:260-8.
12. Benhamou Y, Fleury H, Trimoulet P, *et al.* Anti-hepatitis B virus efficacy of tenofovir disoproxil fumarate in HIV-infected patients. Hepatology 2006;43:548-55.
13. Maylin S, Boyd A, Lavocat F, *et al.* Kinetics of hepatitis B surface and envelope antigen and prediction of treatment response to tenofovir in antiretroviral-experienced HIV-hepatitis B virus-infected patients. AIDS 2012;26:939-49.
14. Boyd A, Gozlan J, Miailhes P, *et al.* Rates and determinants of hepatitis B "e' antigen and hepatitis B surface antigen seroclearance during long-term follow-up of patients coinfected with HIV and hepatitis B virus. AIDS 2015;4.
15. Arendt E, Jaroszewicz J, Rockstroh J, *et al.* Improved immune status corresponds with long-term decline of quantitative serum hepatitis B surface antigen in HBV/HIV co-infected patients. Viral immunology 2012;25:442-7.
16. de Vries-Sluijs TE, Reijnders JG, Hansen BE, *et al.* Long-term therapy with tenofovir is effective for patients co-infected with human immunodeficiency virus and hepatitis B virus. Gastroenterology 2010;139:1934-41.
17. Kosi L, Reiberger T, Payer BA, *et al.* Five-year on-treatment efficacy of lamivudine-, tenofovir- and tenofovir + emtricitabine-based HAART in HBV-HIV-coinfected patients. J Viral Hepat 2012;19:801-10.
18. Hadziyannis E, Hadziyannis SJ. Hepatitis B surface antigen quantification in chronic hepatitis B and its clinical utility. Expert Rev Gastroenterol Hepatol 2014;8:185-95.
19. Chevaliez S, Hezode C, Bahrami S, *et al*. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol 2013;58:676-83.
20. Wursthorn K, Jung M, Riva A, *et al.* Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. Hepatology 2010;52:1611-20.
21. Zoutendijk R, Zaaijer HL, de Vries-Sluijs TE, *et al.* Hepatitis B surface antigen declines and clearance during long-term tenofovir therapy in patients coinfected with HBV and HIV. J Infect Dis 2012; 206:974-80.
22. Nishida N, Sawai H, Matsuura K, *et al.* Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. PloS one 2012; 7:e39175.