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Long Term Quantitative HBsAg Kinetics in Chronic Hepatitis B Patients Treated with Entecavir

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의 학 과

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만성 B형 간염 환자에서 엔테카비어 치료 시
HBsAg 정량 검사의 역학

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연구 배경: 만성 B형 간염 환자에서의 HBsAg 소실은 B형 간염 바이러스 복제가 견고히 억제되며, 면역학적인 조절이 이루어졌음을 시사한다. HBsAg 소실이 된 만성 B형 간염 환자에서 간경변증으로의 진행, 간세포암의 발생은 유의하게 감소하며 장기적인 예후도 우수한 것으로 알려져 있다. HBsAg 소실에는 HBV DNA의 전사(transcription) 등의 주형인 covalently closed circular DNA (cccDNA)에 대한 정보가 중요하다. 혈청 HBsAg 정량값은 간세포 내의 cccDNA 의 전사를 반영한다고 알려져 있다. 본 연구의 목적은 장기간 엔테카비어로 치료한 만성 B형 간염 환자에서 HBsAg 정량 검사 결과에 대한 역학 (kinetics)을 확인하여 HBsAg 소실을 이루는데 필요한 기간을 확인하고자 한다.

방법: 만성 B형 간염 환자에서 엔테카비어를 초치료로 시작한

1,009명 환자의 코호트에서 치료 기간 중 HBsAg 정량 검사를 시행한 420 명을 선정하였다. 이들 환자군의 75.1% (321명)에서 2개 이상의 HBsAg 정량 검사 결과가 있었으며, HBsAg 정량 값 감소 역학 분석에 1,426개의 검체 결과를 이용하였다. 선형 혼합 모델(Linear mixed model)을 적용하기 위한 정규성 검정, 잔차 및 등분산성 확인에서 이상치를 제외한 410명에서 엔테카비어 치료 중 HBsAg이 소실되는 기간을 확인하였다.

결과: 271명의 환자(66.1%)가 남성이었으며, 중위 연령은 48세였다. 약 53.5개월의 중위 추적 기간이 확인되었다. 213명의 환자(52.0%)가 HBeAg-양성이였다. 평균 기저 HBV DNA 값은 6.24 ± 1.40 log_{10}IU/mL이였으며, 11.7%의 환자가 기저 간경변증이 확인되었다. 환자들의 추적 관찰 중 누적 바이러스 반응률은 1, 3, 5년째 각각 83.3%, 95.0%, 그리고 99.3%를 이루었다. HBeAg-음성, HBV DNA < 6 log_{10}IU/mL, 기저 간경변을 갖는 환자에서 유의하게 높은 누적 바이러스 반응률이 확인되었다.

엔테카비어 치료 중 HBsAg 정량 검사 결과의 변화는 HBeAg-양성, HBeAg-음성 환자에서 각각 3.4773-0.0039 x Months, 3.1853-0.0036 x Months 소실되는 것을 확인할 수 있었으며, 이 두 기울기의 차이는 통계적 유의성이 없었다. HBeAg-양성 환자에서 HBeAg 소실의 유무에 따른 HBsAg 정량값의 변화는 3.3395-0.0057 x Months, 3.5455-0.0037 x Months 으로 확인되었다. HBeAg 소실 유무에 따른 HBsAg 정량값 감소의 기울기의 차이는 통계적으로 유의하지 않았다. 기저 간경변증의 유무에 따른

HBsAg 정량값의 변화는 3.0312-0.0048 x Months과 3.3766-0.0036 x Months으로 확인되었으며, 두 군간의 HBsAg 감소 속도의 차이는 유의하지 않았다.

결론: 장기간 엔테카비어를 투여받은 만성 B형 간염 환자에서 HBsAg 정량 값의 변화에 대하여 선형 혼합 모델을 이용하여 분석 결과 HBsAg 소실을 위해서는 HBeAg-양성 유무, HBeAg 소실 유무, 기저 간경변증 유무와 무관하게 평생 복용을 해야 한다.

핵심 단어: 만성 B형 간염, HBsAg 정량 검사, 엔테카비어

INTRODUCTION

Patients with chronic hepatitis B virus (HBV) infection are at an increased risk for liver-related complications, including cirrhosis and hepatocellular carcinoma, and greater mortality.[1] The goals of therapy for chronic HBV infected patients is to prevent disease progression, improve survival and quality of life.[2-4] Over the past few decades, this goal has been achievable using highly potent oral nucleos(t)ide analogues (NUC), such as entecavir and tenofovir, inhibiting the HBV DNA reverse transcriptase with a high barrier to resistance. The continued suppression of HBV DNA levels leads to reduced hepatic inflammation, inhibition of liver fibrosis and decreased incidence of hepatocellular carcinoma. The loss of hepatitis B surface antigen (HBsAg), termed as functional cure, further lowers the rates of hepatic decompensation and the development of hepatocellular carcinoma.[5] It is currently the treatment goal recommended in current guidelines.[3,4,6]

In chronic HBV infection, HBsAg is produced as a result of translation of the messenger RNAs generated from transcriptionally active covalently closed circular DNA (cccDNA) or HBV DNA sequences integrated in the host genome.[7] Recent studies indicate that the decline in serum quantitative HBsAg (qHBsAg) levels during pegylated interferon (pegIFN) treatment is associated with a decrease in intrahepatic cccDNA and HBsAg.[8-11] Early on-treatment serum qHBsAg levels predict the sustained off-treatment response to therapy

and the subsequent HBsAg clearance in patients with both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative chronic hepatitis B treated with a finite duration of pegIFN.[8-10,12] Therefore, in patients treated with pegIFN, qHBsAg levels is associated with the induction of an effective anti-HBV immune response and a valuable tool for the monitoring of patients.[13,14]

Studies regarding HBsAg kinetics during treatment with nucleoside/nucleotide analogues is limited.[15-28] Compared to PegIFN treatment that has both direct antiviral and immune mediated effects, qHBsAg decline during nucleos(t)ide analogue therapy is much slower and less pronounced. Also, nucleos(t)ide analogues inhibiting only the reverse transcription of the pregenomic RNA (pgRNA) but not the cccDNA directly has minimal effect on the pathway for HBsAg secretion.[7] Still, reports of rapid decline in HBsAg titer early after initiation of treatment with nucleos(t)ide analogues have been associated with prediction of HBsAg loss.[20]

The aim of the present study was to assess the long-term quantitative HBsAg kinetics in order to achieve HBsAg loss and search for factors that predict HBsAg seroclearance. The long-term kinetics of quantitative HBsAg levels in patients with chronic hepatitis B treated with entecavir and the time for patients to achieve HBsAg seroclearance was assessed.

METHODS

Patients

From a cohort of 1,009 consecutive chronic hepatitis B treatment-naïve patients who were started on entecavir 0.5mg at Samsung Medical Center between 2007 and 2012, 420 patients with a quantitative HBsAg value whilst on treatment were selected. All patients were HBsAg-positive for at least 6 months prior to treatment. Among the patients, 321 patients (75.1%) had more than 2 serial samples of quantitative HBsAg. The kinetics of quantitative HBsAg decline was assessed using 1,426 samples collected during entecavir treatment. Patients who had less than 6 months of entecavir treatment, coinfection with hepatitis C virus or human immunodeficiency virus, prior treatment history with nucleos(t)ide analagoues or interferon, prior diagnosis of hepatocellular carcinoma, being younger than 18 years of age and inadequate medical record were excluded. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and principles of Good Clinical Practice.

Laboratory measurements

The complete blood counts, liver function tests, quantitative HBsAg, HBsAg, anti-HBs antibody, HBeAg, anti-HBe antibody, and HBV DNA titer were examined. HBsAg, anti-HBs antibody, HBeAg, and anti-HBe antibody levels were examined by enzyme immunoassay. Quantitative HBsAg levels were measured using an automated chemiluminescent microparticle immunoassay (Architect HBsAg, Abbott, IL, USA). The lower limit of detection was 0.05 IU/mL. HBV DNA levels were tested using the COBAS TaqMan HBV quantitative test (Roche Molecular Systems Inc., Branchburg, NJ, USA), with a lower detection limit of < 9 IU/mL. Virologic response was defined as a reduction in HBV DNA titers to < 60 IU/mL. Liver cirrhosis was determined through liver biopsy or an imaging modality combined with two positive laboratory findings; platelet < 100,000/μL, albumin < 3.5 g/dL, or prothrombin time [PT, international normalized ratio] > 1.3.

Statistical analysis

Continuous variables are presented as mean±standard deviation and a median value with range according to parametric and non-parametric distributions, respectively. The variables are compared parametrically using the Student t test or non-parametrically using the Mann-Whitney *U* test. Categorical

variables are presented as frequencies with percentages and compared by chi-square or Fisher's exact test. Cumulative rates of virologic responses were analyzed using the Kaplan-Meier method and the log-rank test was used to test for statistical difference. Multivariate analysis was performed using stepwise Cox proportional hazards regression with forward conditions to determine the factors independently associated with virologic response. Linear mixed model analyses were performed to assess the changes in quantitative HBsAg levels over various time points using subject effect as the random effect and time effect as the fixed effect. Outliers affecting the normality assumption and the residuals of the fits were excluded from the analysis. The interaction between 1) HBeAg and time; 2) Presence of liver cirrhosis and time for quantitative HBsAg were also assessed using linear mixed model. A two-sided $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 19.0 (IBM Co., Armonk, NY, USA), SAS version 9.3 (SAS Institute, Cary, NC) and R 2.13.2 (Vienna, Austria; http://www.R-project.org/).

RESULTS

Characteristics of the study patients

The baseline characteristics of all the patients (n=420) meeting the inclusion and exclusion criteria are listed in Table 1 as "All patients". The majority of the patients were male (66.0%) and the median patient age was 48 years old (range, 18 to 80). The patients were followed up for a median of 53.2 months (range, 6.8 to 73.3). Two hundred and nineteen (52.1%) patients were HBeAg-positive. The mean baseline HBV DNA titer was 6.25 ± 1.40 $\log_{10}$ IU/mL and 11.7% of the patients presented with liver cirrhosis at the time when entecavir treatment was initiated.

"Analyzed patients" in Table 1 represents patients included in all the subsequent analysis (n=410) after patients with quantitative HBsAg levels affecting the homoscedasticity and the normality assumption were excluded. Two hundred and seventy one patients were male (66.1%) and the median patient age was 48 years old (range, 18 to 80). The patients were followed up for a median of 53.5 months (range, 6.8 to 73.3). Two hundred and thirteen (52.0%) patients were HBeAg-positive. The mean baseline HBV DNA titer was 6.24 ± 1.40 $\log_{10}$ IU/mL and 11.7% of the patients presented with liver

Table 1. Baseline characteristics of chronic hepatitis B patients on entecavir

	All patients	Analyzed patients	P-value
N	420	410	
Age (years)	48.0±10.3	47.9±10.3	0.933
Male (n, %)	277 (66)	271 (66.1)	1.000
WBC ($x10^3$/uL)	5.02±1.64	5.02±1.65	0.985
Hgb (g/dL)	14.4±1.72	14.4±1.71	0.950
Platelet ($x10^3$/uL)	149.4±62.3	149.1±61.7	0.945
AST (U/L)	95.6±136.6	95.5±137.6	0.996
ALT (U/L)	125.8±211.5	126.1±213.5	0.985
Total bilirubin (mg/dL)	1.28±1.49	1.29±1.51	0.940
Albumin (g/dL)	3.96±0.45	3.95±0.45	0.937
Prothrombin Time (INR)	1.14±0.16	1.14±0.16	0.983

HBeAg (+) (n, %)	219 (52.1)	213 (52.0)	0.506
HBV-DNA (log IU/mL)	6.25±1.40	6.24±1.40	0.934
Treatment period (months)	53.2 (6.8-73.3)	53.5 (6.8-73.3)	0.714
Virological response (n, %)	404 (96.2)	394 (96.1)	1.000
HBeAg seroclearance	68 (16.2)	66 (16.1)	0.989
HBsAg seroclearance	6 (1.4)	6 (1.5)	0.999
Liver cirrhosis (n, %)	49 (11.7)	48 (11.7)	1.000
Child-Pugh A (n, %)	380 (90.5)	371 (90.5)	
Child-Pugh B (n, %)	35 (8.3)	34 (8.3)	
Child-Pugh C (n, %)	5 (1.2)	5 (1.2)	

cirrhosis when entecavir treatment was initiated. There were no statistically significant differences in the age, gender, baseline virological status, laboratory findings, presence of liver cirrhosis, and the virological response between all patients and the analyzed patients.

Virological response on entecavir treatment

The cumulative virologic response rates in the entecavir-treated patients were 83.3%, 95.0%, and 99.3% at years 1, 3, and 5, respectively (Fig 1A). Sixteen patients (3.9%) failed to achieve a virologic response during follow-up. The cumulative virologic response rate in HBeAg-negative patients was 96.8% at the first year and all the patients achieved virologic response by the third year. In HBeAg-positive patients, the virologic response rate at the first, third and fifth year was 71.9%, 91.4%, and 98.7%, respectively (Fig 1B). The difference in the cumulative virologic response between HBeAg-positive and –negative patients was statistically significant ($p < 0.001$). Compared to patients with HBV DNA titer < 6 $\log_{10}$ IU/mL, patients with HBV DNA ≥ 6 $\log_{10}$ IU/mL had a significantly lower virologic response ($p < 0.001$, Fig 1C). In patients with baseline liver cirrhosis, the virologic response at the first year was 93.7%. The virologic response rate at the first, third, and fifth year was 81.9%, 94.4%, and 99.2% in patients without baseline liver cirrhosis (Fig1D). The difference

Figure 1. Virologic response of patients on continuous entecavir treatment.

The cumulative virologic response in all patients during entecavir treatment was 83.3%, 95.0%, and 99.3% in years 1, 3, and 5, respectively (A). The HBeAg-negative patients had a significantly greater virologic reseponse compared to HBeAg-positive patients (B).

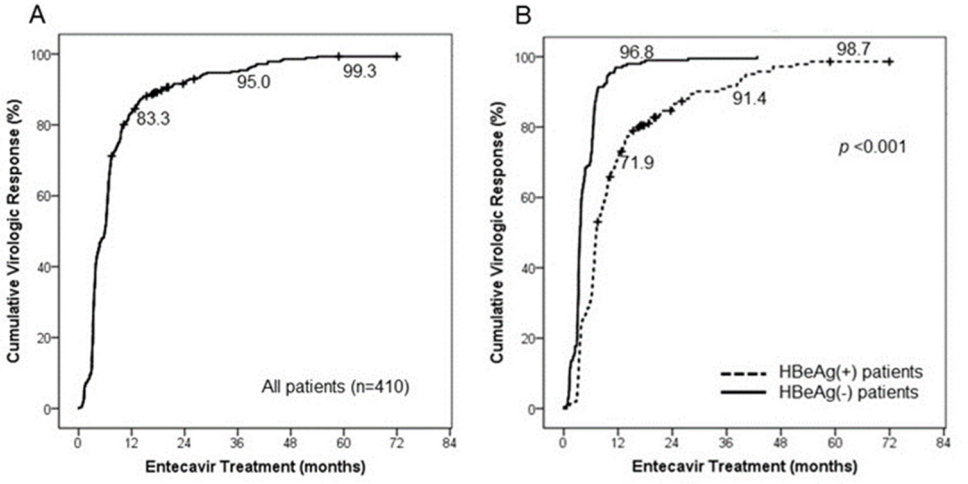
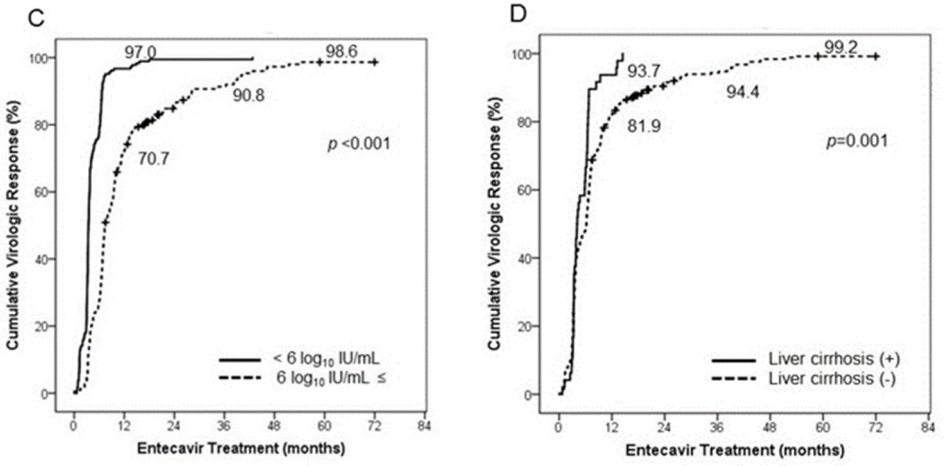


Figure 1. Virologic response of patients on continuous entecavir treatment.

The patients with HBV DNA < 6 $\log_{10}$ IU/mL had a significantly greater virologic response compared to patients with HBV DNA ≥ 6 $\log_{10}$ IU/mL (C). Patients with baseline liver cirrhosis had a significantly greater virologic response compared to patients without baseline liver cirrhosis (D).



in the cumulative virologic response was statistically significant (p=0.001).

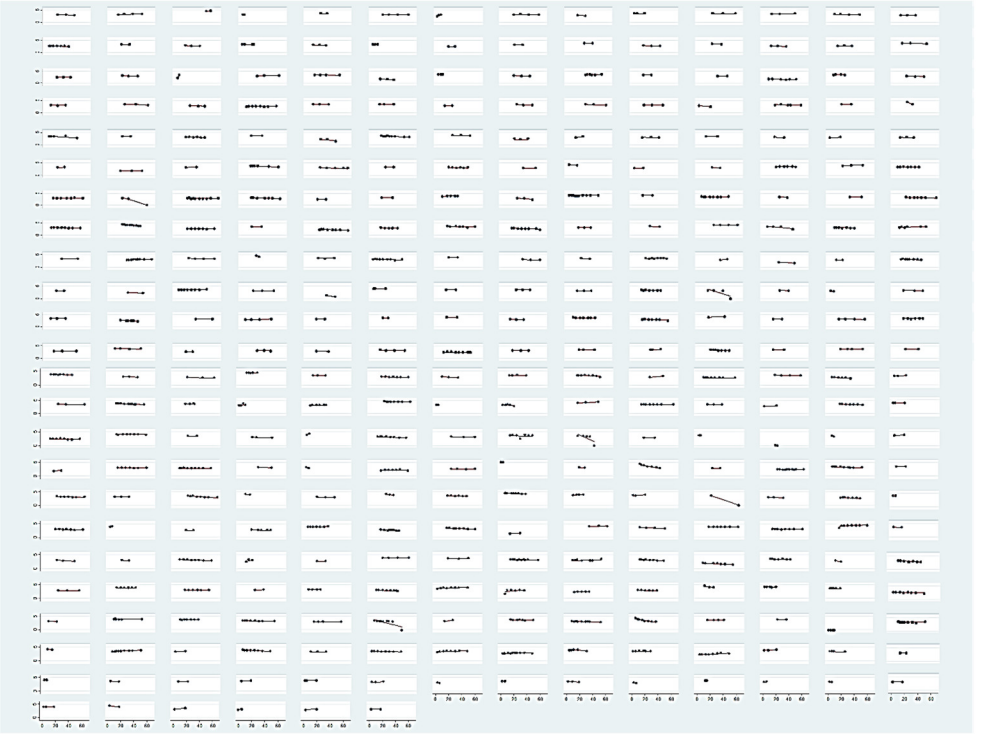
Univariate analysis revealed that older age, presence of liver cirrhosis, lower HBV DNA titer, HBeAg negativity, lower platelet count, and prolonged prothrombin time were statistically significant factors associated with the virologic response. In multivariate analysis, HBeAg-negativity, lower HBV DNA titer, and liver cirrhosis were independently associated with greater virologic response (Table 2).

The individual kinetics of log transformed quantitative HBsAg was plotted in function of time to determine the distribution and evaluate whether the application of linear mixed model was acceptable (Fig 2). A linear relationship with variation in the number of repeated measures was noted between the quantitative HBsAg and time in each patient. Therefore, linear mixed model analyses were done to evaluate the change in the quantitative HBsAg titers as a function of time in samples that fit the normality assumption and complied to homoscedasticity.

Table 2. Univariate and multivariate analysis of baseline factors associated with virologic response in chronic hepatitis B patients on entecavir (n = 410)

	Univariate		**Multivariate**	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Age (years)	1.022 (1.012-1.032)	<0.001		
Male	1.077 (0.873-1.328)	0.489		
WBC (x 10^3/μL)	0.979 (0.920-1.041)	0.497		
Hgb (g/dL)	0.970 (0.918-1.024)	0.269		
Platelet (x 10^3/μL)	0.997 (0.995-0.998)	<0.001		
AST (U/L)	1.000 (0.999-1.001)	0.981		
ALT (U/L)	1.000 (0.999-1.000)	0.740		
Total bilirubin (mg/dL)	1.062 (0.995-1.133)	0.070		
Albumin (g/dL)	0.957 (0.771-1.189)	0.694		
PT (INR)	3.281 (1.907-5.644)	<0.001		
HBeAg (+)	0.338 (0.274-0.418)	<0.001	0.545 (0.436-0.682)	<0.001
HBV DNA ($\log_{10}$ IU/mL)	0.597 (0.556-0.641)	<0.001	0.636 (0.586-0.689)	<0.001
Liver cirrhosis	1.696 (1.247-2.307)	0.001	1.507 (1.105-2.055)	0.009

Figure 2. Individual kinetics of quantitative HBsAg titer in function of time.



Long-term quantitative HBsAg kinetics based on HBeAg positivity

The baseline characteristics of patients based on HBeAg positivity are presented in Table 3. Compared to HBeAg-positive patients, HBeAg-negative patients are significantly older ($p < 0.001$), have a lower HBV-DNA titer ($p < 0.001$) and a greater virological response ($p < 0.001$). The expected log qHBsAg levels as a function of time during entecavir treatment in HBeAg(+) and HBeAg(-) patients was 3.4773-0.0039xMonths and 3.1853-0.0036xMonths, respectively (Fig 3A, B). The HBeAg-positive group had a mean slope of -0.0036 ± 0.0003 log_{10}IU/month and the HBeAg-negative group had a mean slope of -0.0037 ± 0.0004 log_{10}IU/month. The average annual decline of qHBsAg titer was 0.043 log_{10}IU/mL and 0.044 log_{10}IU/mL for HBeAg-positive and HBeAg-negative patients, respectively. The expected time to achieve HBsAg seroclearance while on entecavir treatment was 74.1 years in HBeAg(+) patients and 73.5 years in HBeAg(-) patients. The difference in the kinetics of qHBsAg according to time was not statistically significant based on HBeAg positivity (p=0.48). The predicted qHBsAg level using the linear mixed model conformed to the actual qHBsAg levels (Fig 3C, D).

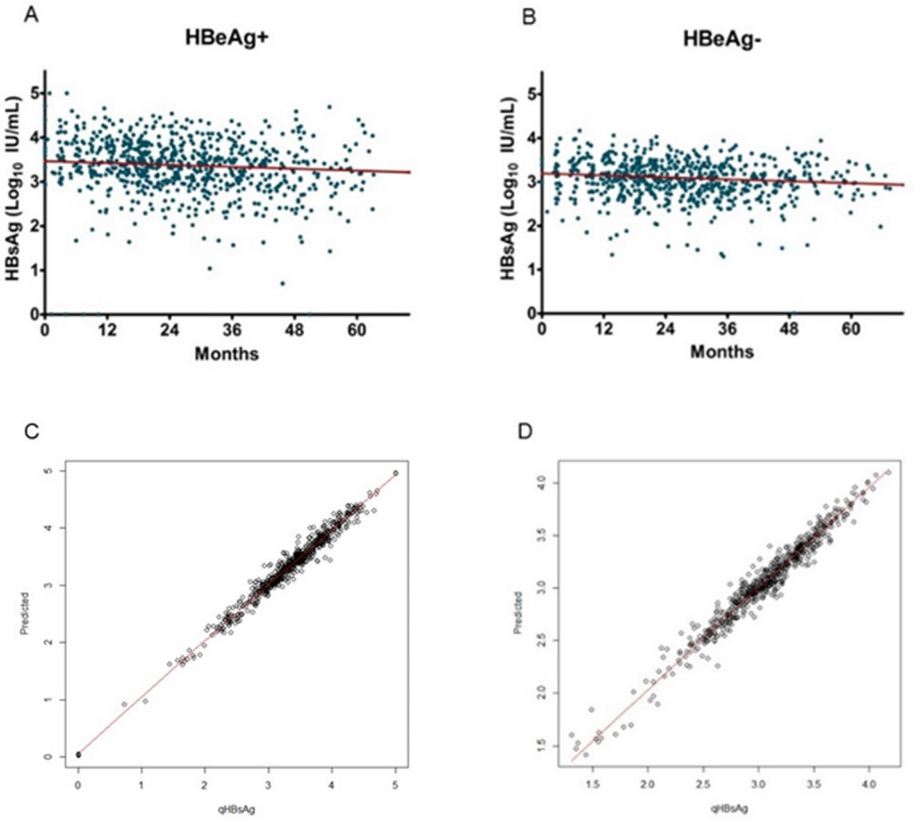
Table 3. Baseline characteristics of chronic hepatitis B patients on entecavir based on HBeAg positivity.

	HBeAg(+)	HBeAg(-)	P-value
N	213	197	
Age (years)	45.2±10.6	50.9±9.1	< 0.001
Male (n, %)	147 (69.0)	124 (62.9)	0.211
WBC ($x10^3$/uL)	5.02±1.83	5.02±1.43	0.988
Hgb (g/dL)	14.5±1.80	14.3±1.61	0.264
Platelet ($x10^3$/uL)	158.3±67.9	139.2±52.7	0.002
AST (U/L)	103.6±174.2	86.8±80.9	0.217
ALT (U/L)	139.0±268.6	112.1±129.3	0.202
Total bilirubin (mg/dL)	1.36±1.74	1.21±1.21	0.336
Albumin (g/dL)	3.92±0.46	3.99±0.45	0.122
Prothrombin Time (INR)	1.13±0.17	1.14±0.16	0.456

HBV-DNA (log IU/mL)	6.88±1.24	5.54±1.23	< 0.001
Treatment period (months)	53.1 (6.8-73.3)	53.9 (6.8-70.6)	0.718
Virological response (n, %)	197 (92.5)	197 (100)	< 0.001
HBeAg seroclearance (n, %)	68 (31.9)	.	.
Liver cirrhosis (n, %)	23 (10.8)	25 (12.7)	0.645

Figure 3. Quantitative HBsAg kinetics based on HBeAg positivity.

The expected log qHBsAg levels as a function of time during entecavir treatment in HBeAg(+) and HBeAg(-) patients was 3.4773-0.0039xMonths and 3.1853-0.0036xMonths, respectively (Fig 3A, B). The difference in the kinetics of qHBsAg according to time was not statistically significant based on HBeAg positivity (p=0.48). The predicted qHBsAg level using the linear mixed model conformed to the actual qHBsAg levels (Fig 3C, D).

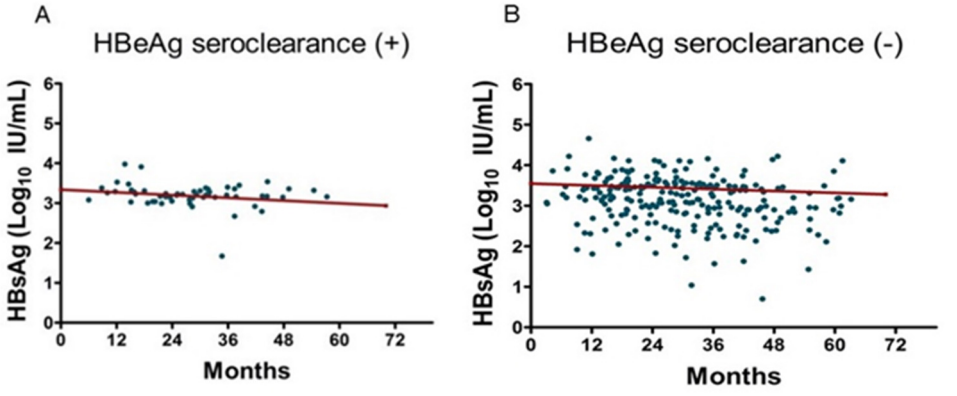


Long-term quantitative HBsAg kinetics based on achievement of HBeAg seroclearance in HBeAg-positive patients

During the follow up period, sixty-eight (31.9%) patients among the 213 HBeAg-positive patients achieved HBeAg seroclearance. The expected log qHBsAg levels as a function of time during entecavir treatment in patients who did not achieve HBeAg seroclearance was 3.5455-0.0037xMonths while patients who achieved HBeAg seroclearance was 3.3395-0.0057xMonths (Fig 4A, B). The average annual decline of qHBsAg titer was 0.068 log_{10}IU/mL and 0.044 log_{10}IU/mL for patients with and without HBeAg seroclearance, respectively. Although there was a trend for a greater decline in quantitative HBsAg titers in the group that achieved HBeAg seroclearance, the difference in the kinetics of qHBsAg according to time was not statistically significant (p=0.30). The estimated time to achieve HBsAg seroclearance in HBeAg(+) patients who achieved HBeAg seroclearance was 48.6 years while the expected treatment time in patients who did not achieve HBeAg seroclearance was 79.6 years.

Figure 4. Quantitative HBsAg kinetics based on achievement of HBeAg seroclearance in HBeAg-positive patients.

The expected log qHBsAg levels as a function of time during entecavir treatment in patients who achieved HBeAg seroclearance was 3.3395-0.0057xMonths and in patients who did not achieve HBeAg seroclearance was 3.5455-0.0037xMonths, respectively (Fig 4A, B). Although there was a trend for a greater decline in quantitative HBsAg titers in the group that achieved HBeAg seroclearance, the difference in the kinetics of qHBsAg according to time was not statistically significant (p=0.30).



Long-term quantitative HBsAg kinetics based on presence of baseline liver cirrhosis

The baseline characteristics of patients based on the presence of liver cirrhosis are presented in Table 4. The patients with baseline liver cirrhosis were significantly older ($p < 0.001$). Significant laboratory differences including lower Hgb, platelet and albumin, greater total bilirubin and prolonged prothrombin time were noted in the cirrhotic patients ($p < 0.001$).

The expected log qHBsAg levels as a function of time during entecavir treatment in patients with or without baseline liver cirrhosis was 3.0312-0.0048xMonths and 3.3766-0.0036xMonths, respectively (Fig 5A, B). The patients with baseline liver cirrhosis had a mean slope of -0.0048 ± 0.0006 $\log_{10}$IU/month and the patients without baseline liver cirrhosis had a mean slope of -0.0036 ± 0.0002 $\log_{10}$IU/month. The average annual decline of qHBsAg titer was 0.057 $\log_{10}$IU/mL and 0.043 $\log_{10}$IU/mL for liver cirrhosis patients and non-liver cirrhosis patients, respectively. The estimated time to achieve HBsAg seroclearance while on entecavir treatment in patients without liver cirrhosis was 77.9 years while patients with liver cirrhosis was 52.5 years. The difference in the kinetics of qHBsAg according to time was not statistically significant based on the severity of baseline liver disease (p=0.10).

The predicted qHBsAg level using the linear mixed model conformed to the actual qHBsAg levels (Fig 5C, D).

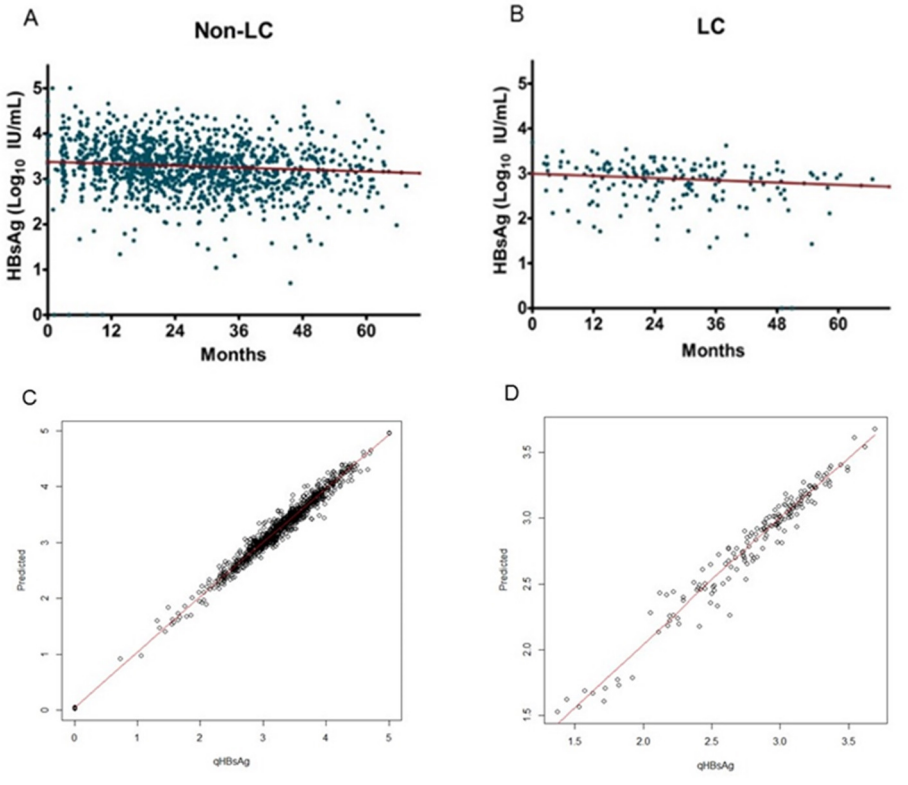
Table 4. Baseline characteristics of chronic hepatitis B patients on entecavir based on presence of liver cirrhosis.

	Non-Liver cirrhosis	Liver cirrhosis	P-value
N	362	48	
Age (years)	47.3±10.2	53.1±9.6	<0.001
Male (n, %)	246 (68.0)	25 (52.1)	0.035
WBC ($x10^3$/uL)	5.14±1.55	4.16±2.12	0.003
Hgb (g/dL)	14.6±1.58	12.6±1.64	<0.001
Platelet ($x10^3$/uL)	159.8±57.0	68.0±25.4	<0.001
AST (U/L)	93.6±136.3	110.3±148.0	0.461
ALT (U/L)	160.9±222.7	89.1±118.9	0.047
Total bilirubin (mg/dL)	1.13±1.30	2.47±2.29	<0.001
Albumin (g/dL)	4.05±0.37	3.22±0.38	<0.001
Prothrombin Time (INR)	1.10±0.10	1.42±0.23	<0.001

HBeAg (+) (n, %)	190 (52.5)	23 (47.9)	0.645
HBV-DNA (log IU/mL)	6.29±1.42	5.88±1.15	0.055
Treatment period (months)	53.2 (6.8-72.0)	50.2 (7.3-73.3)	0.149
Virological response (n, %)	346 (95.6)	48 (100)	0.235

Figure 5. Quantitative HBsAg kinetics based on disease severity.

The expected log qHBsAg levels as a function of time during entecavir treatment in patients with or without baseline liver cirrhosis was 3.0312-0.0048xMonths and 3.3766-0.0036xMonths, respectively (Fig 5A, B). The difference in the kinetics of qHBsAg according to time was not statistically significant based on the severity of baseline liver disease (p=0.10). The predicted qHBsAg level using the linear mixed model conformed to the actual qHBsAg levels (Fig 5C, D).



DISCUSSION

In the present study, the long-term quantitative HBsAg kinetics in treatment-naïve chronic hepatitis B patients on entecavir and the duration of treatment in order to achieve undetectable HBsAg levels was determined through mathematical modeling. To the best of the known literature, this is the largest study using on-treatment quantitative HBsAg at multiple time points up to 7 years to predict the trajectory of quantitative HBsAg decline.

Current clinical practice guidelines regard HBsAg seroclearance as the state of "functional cure".[4,6] Although nucleos(t)ide analogue treatment effectively suppress the HBV DNA level through inhibition of HBV DNA reverse transcriptase, the quantitative HBsAg decline is much slower and HBsAg seroclearance is a rarely observed event in NUC-treated patients.[16,18,19] Zoutendijk et al reported that the predicted median time to HBsAg loss was 36 years for HBeAg-positive and 39 years for HBeAg-negative patients.[28] Chevaliez et al reported 52.2 years of NUC treatment was needed to clear HBsAg.[17] The estimated time to clearance of quantitative HBsAg in this study was 74.3 years in HBeAg-positive patients and 73.7 years in HBeAg-negative patients. The prolonged period calculated is not surprising since the decrease in quantitative HBsAg reflects a decline in translation of mRNAs produced

from cccDNA and integrated HBV sequences and current NUCs do not actively target these sites.[29]

In HBV genotype D patients, a greater decline in HBsAg was observed in patients treated with more potent NUCs, such as entecavir or tenofovir, compared to telbivudine, with an estimated time to HBsAg loss of 17 years.[15] Although, the difference was not significant, a shortened period for HBsAg loss in entecavir treated patients (24.9 years) compared to adefovir treated patients (31.1 years) have been reported.[30] Compared to these results, the estimated time to HBsAg clearance in this study which exclusively included entecavir treated patients was more prolonged. One explanation to this may be associated with the differences in HBsAg kinetics based on HBV genotype, where genotype A was associated with the greatest decline.[27] Although genotyping of the patients was not done, as genotype C predominates in 95.6% to 100.0% of HBV infected patients in Korea,[31-33] the slower decline of quantitative HBsAg in this study may be representative of the decline in genotype C patients.

In this study, the difference in the quantitative HBsAg kinetics between HBeAg-positive and HBeAg-negative patients was not significant (p=0.48). On the contrary, several groups have reported a difference in the HBsAg

kinetic patterns between HBeAg-positive and –negative patients.[25] Serum HBsAg titers are reported to have a positive correlation with the serum HBV DNA and the liver cccDNA in treatment-naïve HBeAg-positive patients.[34] Nucleos(t)ide analogue treatment in HBeAg-positive patients resulted in a slow decrease in HBsAg levels.[11,27,35] In HBeAg-negative patients, there is a lack of correlation between HBsAg levels in the serum and the intrahepatic cccDNA levels.[34,36,37] In a study of the factors affecting the decline of quantitative HBsAg titers, a negative correlation with advancing fibrosis in HBeAg-positive patients and increased necroinflammation with a decrease of HBsAg titers in HBeAg-negative patients have been reported.[38] Also, the fact that serum quantitative HBsAg is representative of not only the transcriptional activity of the cccDNA, but also the integrated HBV DNA sequences must be considered.[13] The contradicting results of the kinetics based on the HBeAg-positivity warrants further studies.

HBeAg seroclearance is a predisposing factor to achieving HBsAg seroclerance.[5] Previous studies report that more pronounced decrease of HBsAg titers was noted in HBeAg-positive patients who achieved HBeAg seroclearance.[21,28] However in this study, although there was a trend for a faster decline in quantitative HBsAg titers in HBeAg-positive patients who

achieved HBeAg seroclearance, the difference in the decrease of HBsAg titers compared to HBeAg-positive patients without HBeAg seroclearance was not significant (p=0.300). The contradicting results may be from the difference of the baseline characteristics of the included patients. During a median follow-up of 53 months, 68 patients (31.9%) achieved HBeAg seroclearance in this study, while 71% (115/162) achieved HBeAg seroclearance by the third year of telbivudine treatment in a study by Wursthorn et al.[27] The estimated time to HBsAg seroclearance in HBeAg-positive patients who achieved HBeAg seroclearance still required a life-long antiviral therapy of 48.6 years.

The annual decline of quantitative HBsAg was greater in patients with baseline liver cirrhosis (0.057 $\log_{10}$IU/mL) compared to patients without liver cirrhosis (0.043 $\log_{10}$IU/mL), but without statistical significance. In biopsy proven hepatitis B patients, a decrease in quantitative HBsAg was noticed as the grade of fibrosis increased.[38,39] Although the baseline quantitative HBsAg was not evaluated in all the patients included in the study, the estimated baseline qHBsAg was 3.0312$\log_{10}$IU and 3.3766$\log_{10}$IU in patients with or without liver cirrhosis, respectively. Therefore, the shortened estimated time of HBsAg seroclearance while on entecavir treatment in cirrhotic patients (52.5 years) compared to non-cirrhotic patients (77.9 years) is also the result

of decreased baseline qHBsAg as the grade of fibrosis increased in chronic hepatitis B patients.

The mechanisms underlying HBsAg clearance remain unknown. The use of quantitative HBsAg in predicting the treatment response and eventual functional cure would be of great assistance to clinicians. This study aimed to determine the duration needed to achieve functional cure in entecavir treated patients and of predisposing factors that could predict an earlier HBsAg seroclearance. The HBeAg-positivity, achievement of HBeAg seroclearance in HBeAg-positive patients or the presence of baseline liver cirrhosis were not factors significantly associated with a greater decline of quantitative HBsAg.

The observation of this study supports several assumptions regarding HBV and gives new insight regarding HBV treatment. The interest in quantitative HBsAg started from the fact that serum HBsAg correlates with the transcription activity of the cccDNA. The nucleos(t)ide analogue targeting the HBV reverse transcriptase has minimal effect on the cccDNA or the DNA integrated in the host cell genome. Patients with suppressed HBV DNA through nucleos(t)ide analogues continue to produce large amounts of quantitative HBsAg from transcription-active cccDNA and coding sequences integrated in the host cell genome. The estimated time to HBsAg seroclearance

in entecavir treated patients using a mathematical model is life-long. The very low rate of HBsAg seroclearance reported in multiple studies from Korea support this finding. In effect, a new class of drug to actively eliminate the cccDNA, achieve HBsAg seroclearance is in order. Also, a novel biomarker that can determine the patients that do achieve HBsAg seroclearance within a relatively short period of treatment is warranted.

Compared to previous studies which generally assessed HBsAg kinetics for patients on 2-3 years of treatment, the patients included in this study had a substantially long-term follow up of 7 years.[8,15-28] An innovative approach using mathematical modeling incorporating quantitative HBsAg from multiple time points of enteavir treatment was developed and proved that life-long treatment is necessary with current nucelos(t)ide analogues. However, there are several limitations to the study. First, although meticulous reasoning was done before the exclusion of several outliers to achieve the assumptions needed to carry out the linear mixed regression, this could affect the model developed. Secondly, an external validating step would have further concreted the findings of this study.

In conclusion, the mathematical modeling from a long-term follow up of chronic hepatitis B patients on entecavir shows that HBsAg clearance requires

decades of treatment. Thus, life-long therapy is inevitable in patients to achieve functional cure. Further research on new classes of antiviral drugs for complete cure of HBV and novel biomarkers to actively detect the possibility of functional cure in patients with current nucleos(t)ide analogues is necessary.

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