Original Article

HBsAg Quantitation Cannot Predict Virological Flare in Chronic Inactive Hepatitis B Infection

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Background: Chronic hepatitis B, HBeAg negative infection with inactive HBV DNA can become active during follow-up. Previous studies have demonstrated that male, younger age and higher HBV virus at entry are among predictors of subsequent virological flare. There are studies which demonstrated that quantitative HBsAg (qHBsAg) correlated with cccDNA and might predict HBV DNA flare.

Objective: This study was aimed to determine whether qHBsAg and/or HBV DNA can predict HBV DNA flare.

Materials and Methods: This is a prospective study includes inactive chronic hepatitis B patients naïve to treatment with HBV DNA <2,000 IU/mL at hepatitis clinic, Siriraj Hospital from May 1, 2008 to December 31, 2009. The patients were followed every 6 months with ALT, HBV DNA viral load and qHBsAg to evaluate virological flare at 1 year of follow-up.

Results: There were 91 patients (49 male, 42 female), mean age of 50 years old with mean follow-up time of 308 days. Baseline mean HBV DNA, qHBsAg and ALT were 364 IU/ml (range 6 to 1,930), 2,831 IU/mL (range 0.15 to 23,794) and 28 U/L (range 8 to 78), respectively. At last follow-up, the mean values of HBV DNA, qHBsAg, and ALT were 1,310 IU/ml (range 6 to 51,500), 2,239 IU/mL (range 0.04 to 29,242) and 26 U/L (range 9 to 88), respectively. Twenty-one of 91 patients (23%) had virological flare at 1 year of follow-up. There is no correlation of qHBsAg and HBV DNA over the time (r = 0.25 (p<0.001). The only predictor of virological flare was the higher baseline DNA (p<0.001). Overall, qHBsAg level was not the predictor of virological flare, however, when we analyzed the patients with initial HBsAg level ≤250 IU/mL we found that this level can predict absent of virological flare with the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 76.2, 55.7, 34.0, and 88.6%, respectively, with AUC of 0. 66 (95% CI, 0.54 to 0.77, p = 0.03). qHBsAg combined with HBV DNA below detection limit even offered better predictive values with sensitivity, specificity, PPV and NPV of 100, 23, 28, and 100%, respectively (p = 0.018). Those with virological flare, the only predictor of sustained virological flare, were high baseline ALT.

Conclusion: Inactive chronic hepatitis B can have virological flare of 23% in 1 year. Our study has found poor correlation between qHBsAg and HBV DNA. However, low baseline HBV DNA together with low qHBsAg level (≤250 IU/mL) is useful to predict the absence of subsequent virological flare in inactive CHB patients. However, more data with more patients and longer follow-up period are needed to confirm this finding.

Keywords: qHBsAg, HBV DNA, Predicting factors of HBV flare

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Hepatitis B virus (HBV) is the major health problem worldwide. It is estimated by WHO that as many as 350 million people are chronically infected with HBV virus. Of these, about 15 to 40% will die prematurely due to existent of HBV, either from complication of cirrhosis or development of hepatocellular carcinoma (HCC)[1]. The infection usually starts during early childhood and remains nonpathogenic until reaching adulthood when liver inflammation occurs. After a period of inflammation or immune clearance phase, the immune can take control and become silence known as non-replicative phase or carrier state in the past[1]. This results in HBeAg seroconversion where 67 to 80% of non-replicative chronic hepatitis B (CHB) has low or undetectable HBV DNA and normal ALT levels with minimal or no necroinflammation on liver biopsy known as the "inactive carrier state"[2,3]. Approximately 4% to 20% of these inactive carriers can have one or more reversion which can be back to HBeAg positive, whereas 10% to 20% may have reactivation of HBV while HBeAg is negative and repeat exacerbations of hepatitis can occur after many years of quiescence[3]. Therefore, serial testing is necessary to determine if an HBsAg-positive, HBeAg-negative carrier is truly in the "inactive carrier state" and lifelong follow-up is required to confirm that the inactive state is maintained. Most guidelines recommend follow-up with ALT and HBV DNA every 3 to 6 months and treatment is recommended for those who have HBV DNA greater than 2,000 IU/mL with evidence of inflammation or significant fibrosis[1,8].

Recent development of more sensitive quantitative

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HBsAg such as Architect HBsAg QT has shown to be simple, sensitive, specific, reproducible, and inexpensive method of measuring HBsAg quantitatively. Previous studies suggested that quantitative hepatitis B surface antigen (qHBsAg) studied by this chemiluminescent microparticle immunoassay can be a surrogate marker and there was a significant correlation between the HBsAg concentration and HBV DNA level. In some study, serum HBsAg increased a few months before the increase of HBV DNA[4-7]. There were studies which demonstrated that qHBsAg correlated with DNA, which is precursor of HBV DNA production[11-13]. Some studies have demonstrated factors that was used to predict reactivation of CHB such as HBV genotype C, male sex, ALT level more than 5 times of upper limit of normal (ULN) during HBeAg-positive phase before HBeAg seroconversion, age at HBeAg seroconversion of more than 40 years, high virus at baseline, precore mutants[9,10,16].

This study is aimed to investigate whether qHBsAg and HBV DNA levels correlates with HBV virological flare as defined by HBV DNA >2,000 IU/mL at 1 year of follow-up in inactive CHB. The second aim is to identify factors that can predict subsequent virological flare in the patients with low HBV DNA, including qHBsAg level.

Materials and Methods

This prospective study included male or female who were inactive chronic hepatitis B defined as HBeAg negative, HBV DNA <2,000 IU/mL, with no evidence of advanced liver disease or cirrhosis and naive to any HBV treatment. The subjects were excluded if they were co-infected with HCV and/or HIV, required treatment with immunosuppressive, significant underlying diseases according to investigator decision. The study was conducted at hepatitis clinic, Siriraj Hospital from May 1, 2008 to December 31, 2009 and the study was approved by Siriraj IRB.

After consent, patient demographics, liver biochemistries, virological parameters were collected. Serum samples for HBV DNA, qHBsAg, ALT was obtained every 6 months period by the average of 3 points. HBV DNA was measured by COBAS® TaqMan® HBV Test (Roche, Mannheim, Germany) with the range of HBV DNA detection 6 (below detection limit) to 110,000,000 IU/mL. Sera were stored at -80°C until tested for qHBsAg using the Abbott Architect chemiluminescent microparticle immunoassay (Abbott, Sligo, Ireland, version April 2009) with dynamic range of detection from 0.05 to 250 IU/mL. Values greater than 250 IU/mL were diluted according to manufacturer's recommendation.

Statistical analysis

All analyses were performed using SPSS version 13.0. Continuous variables were expressed as mean (min, max) and percentage where appropriate. Univariate analysis of parameters was used to identify any predictor of virological flare. Spearman correlation coefficient was tested for correlation of HBV DNA levels and qHBsAg. Area under the receiver operating characteristic (ROC) curve was performed to assess the predictive values of qHBsAg for virological flare. Statistical significance was defined as a *p*-value of less than 0.05 and all statistical tests were 2-sided.

Results

There were 91 patients (49 male, 42 female) with mean age of 50-year-old and mean body weight of 65 Kg (range 43 to 115). The mean follow-up time was 308 days. Baseline mean HBV DNA, qHBsAg and ALT were 364 IU/ml (range 6 to 1,930), 2,831 IU/mL (range 0.15 to 23,794), and 28 U/L (range 8 to 78), respectively. At last follow-up, the mean values of HBV DNA, qHBsAg, and ALT were 1,310 IU/ml (range 6 to 51,500), 2,239 IU/mL (range 0.04 to 29,242), and 26 U/L (range 9 to 88), respectively as shown in Table 1.

There were 47 patients with initial HBsAg level greater than dynamic range of 250 IU/mL and dilution was required, 44 patients had level ≤ 250 IU/mL. Spearman's correlation coefficient between HBV DNA and HBsAg level over time was poor r = 0.25 (p<0.001). Twenty-one patients (23%) developed virological flare during follow-up and the only predictor of virological flare was the higher baseline DNA (p<0.001). Overall, qHBsAg level was not the predictor of virological flare, as shown in Table 2. One patient (1.1%) developed virological flare with biological flare (defined by ALT more than twice ULN). However, when we analyzed CHB patients with initial HBsAg level ≤250 IU/mL, we found that this level can predict absent of virological flare with the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 76.2, 55.7, 34.0, and 88.6%, respectively, as shown in Table 3 with the AUC of 0.66 (95% CI, 0.54 to 0.77, p = 0.03) as shown in Figure 1. When initial HBsAg level ≤250 IU/ mL and HBV DNA below detection limit were combined, we found that combination of both factors can predict absent of virological flare with sensitivity, specificity, PPV and NPV of 100, 23, 28, and 100%, respectively (p = 0.018) as shown in Table 3. Moreover, that combination of both factors can predict absence of both virological flare and biological flare with

Table 1. Demographic and characteristics of inactive CHB patients

Characteristic	Total (n = 91)
Men: women	49:42 (54%:46%)
Age (years)	50 (21, 72)
BW (kg)	65 (43, 115)
Baseline HBV DNA (IU/mL)	364 (6, 1,930)
Last HBV DNA (IU/mL)	1,310 (6, 51,500)
Initial HBsAg (IU/mL)	2,831 (0.15, 23,794)
Last qHBsAg (IU/mL)	2,239 (0.04, 29,242)
Initial ALT (IU/mL)	28 (8, 78)
Last ALT (IU/mL)	26 (9, 88)
Follow-up period, mean (days)	308

* Values are given mean (min, max)

Table 2. Predictor of virological flare

	Flare (n = 21) mean (min, max)	No flare (n = 70) mean (min, max)	*p*-value
Age (year)	48 (21, 72)	51 (28, 70)	0.47
Initial HBV DNA(IU/mL)	793 (6, 1,930)	235 (6, 1,780)	0.001
ALT at entry (IU/mL)	26 (11, 59)	29 (8, 78)	0.54
qHBsAg at entry (IU/mL)	3,422 (23, 21,472)	2,653 (0.15, 23,794)	0.547
Body weight (kg)	64 (43, 115)	65 (45, 94)	0.76

* Values are given mean (min, max)

Table 3. qHBsAg level and qHBsAg and/or HBV DNA for predicting HBV virological flare

	Flare (n = 21)	No flare (n = 70)
HBsAg level >250 IU/ml	16 (76.2%)	31 (44.3%)
HBsAg level ≤250 IU/ml	5 (23.8%)	39 (55.7%)
HBsAg ≤250 and HBV DNA <6 IU/mL	0	16 (23%)
HBsAg >250 and/or HBV DNA ≥6 IU/mL	21 (100%)	54 (77%)

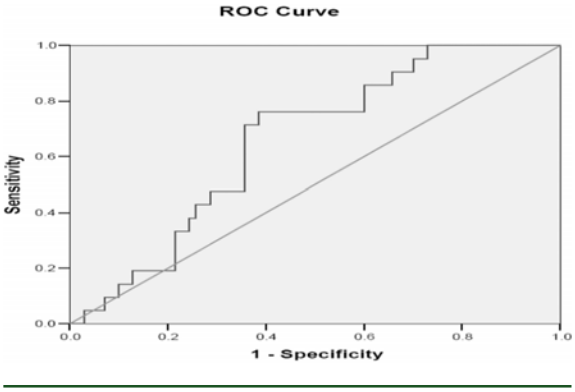

Figure 1. ROC of HBsAg level more than 250 IU/ml for predict virological flare.

sensitivity, specificity, PPV and NPV of 100, 17.8, 1.3, and 100%, respectively ($p = 1.00$) as shown in Table 4. Twenty-one patient developed virological flare. However, most of patients with HBV DNA flare were transient. Seventeen (81%) patient in this group had regular visit after virological flare and only 4 (19%) had persistently high HBV DNA and only predictor of sustained virological flare was the higher baseline ALT at baseline ($p = 0.02$), as shown in Table 5.

Discussion

HBV infection is a life-long disease that need follow-up continuously. Usually we define chronic non-replicative HBV as having HBV DNA less than 2,000 IU/ml all the time. However, the clinical course of chronic hepatitis B is very dynamic depending on the complex interaction between host immunity and the virus. Previous studies showed that qHBsAg correlated with DNA which is precursor of HBV DNA production also correlated with intrahepatic HBV DNA but did not correlate with serum HBV DNA. Low pretreatment qHBsAg can predict good response to treatment[11-13].

In this study, we found no correlation between HBV DNA and qHBsAg level in chronic inactive hepatitis even during virological flare. This may be due to serum HBsAg is produced by transcription and translation of the surface genes of the HBV. The production of HBsAg by the cccDNA is independent of the replication of the virus and HBsAg is produced many times excess of HBV DNA, some even can integrate to host gene. Previous studies showed factors that were used to predict reactivation of inactive CHB such as genotype C, male sex, ALT level greater than 5 times ULN during the HBeAg-positive phase before HBeAg seroconversion, age at HBeAg seroconversion more than 40 years, high virus at entry, precore mutants[9,10,16].

We found that 21 patients (23%) developed virological flare during 1 year follow-up and the only predictor of virological flare was the higher baseline HBV DNA. Overall, HBsAg level was not the predictor of virological flare. However, when we looked at the patients with initial qHBsAg level ≤250 IU/mL we found that this level can predict absence of virological flare with the sensitivity, specificity, PPV and NPV of 76.2, 55.7, 34.0, and 88.6%, respectively, with AUC of 0.66. When both qHBsAg ≤250 IU/mL and HBV DNA <6 IU/mL was combined, we found that combination of both parameters can predict absent of virological flare with the sensitivity, specificity, PPV and NPV of 100, 23, 28, and 100%, respectively ($p = 0.018$). This finding was similar to other studies in HBV genotype B or C which were similar to HBV patients in Thailand which found that combination of HBV DNA <2,000 IU/mL plus qHBsAg less than 1,000 IU/

Table 4. Subgroup analysis of diagnosis test of HBsAg and HBV DNA for predict both virological and biological flare

	Virological plus biological flare (n = 1)	No virological and/or no biological flare (n = 90)
HBsAg ≤250 and HBV DNA ≤IU/mL	0	16
HBsAg >250 and/or HBV DNA >IU/mL	1	74

Table 5. Predictor of sustained virological flare

	HBV DNA decreased (n = 13)	HBV DNA increased (n = 4)	*p*-value
Age (year)	46 (21, 72)	60 (51, 72)	0.61
Initial HBV DNA (IU/mL)	537 (6, 1,520)	970 (380, 1,800)	0.19
ALT ratio at entry (IU/mL)#	0.95 (0.41, 1.32)	1.4 (1.1, 1.95)	0.02
HBsAg at entry (IU/mL)	3,790 (23, 21,472)	2,147 (844, 4,391)	0.63
Body weight (kg)	63 (43, 115)	67 (60, 80)	0.68

* Values are given mean (min, max)
# ALT ratio is ratio between that level and upper limit of normal

mL had diagnostic accuracy of predicting inactive carrier 78% with PPV of 83%[17].

Low qHBsAg can be useful in predicting truly inactive CHB, especially when combined with low HBV DNA. This can be useful to guide monitoring of CHB patients who have low HBV DNA at baseline since HBV DNA measurement is more expensive and cannot be done everywhere. Moreover, those with qHBsAg below 100 IU/mL and HBV DNA of less than 2,000 had 98% specificity and 97% PPV for predicting inactive carrier but sensitivity only 35%[18]. In the patients who developed HBV DNA flare during follow-up, we found that the only predictor that may predict sustained virological flare required treatment was when HBV DNA flare occurred in the patients with high baseline ALT (p = 0.02). This result was also found in previous studies which found higher ALT level was predictor of reactivation of Hepatitis B virus in inactive chronic hepatitis B infection. The annual rates of spontaneous ALT flares or reactivation of hepatitis B (ALT ≥twice ULN) and HBV DNA more than 2,000 IU/mL) vary from 1.5% to 2.2% in studies involving asymptomatic patients[10,14-16]. Some studies from Southeast Asia have shown that reactivation of hepatitis B following HBeAg seroconversion correlates significantly with genotype C (compared with genotype B), male sex, ALT levels ≥5 ULN during the HBeAg positive phase, and age at HBeAg seroconversion[10,16].

The importance of this study is that to define "inactive carrier state" is very difficult and need many information. The patients must have no evidence of significant or advance fibrosis since presence of advanced fibrosis may require HBV treatment even HBV DNA is low. In the patients with low HBV DNA (<2,000 IU/mL) without significant liver fibrosis, long-term follow-up is required. In this study, we found that the 1-year likelihood of HBV DNA flare and HBV DNA flare plus ALT flare was 23% and 1.1%, respectively. In fact, even in the patient without ALT flare, significant fibrosis can be found[19] and treatment may be indicated.

Low baseline qHBsAg may be predictive for low likelihood of HBV DNA flare, however, large number of patients and longer time of follow-up as well as monitoring of liver fibrosis such as current non-invasive test likes transient elastography may be helpful in monitoring and classifying these patients.

Conclusion

There was poor correlation between HBV DNA and qHBsAg level in chronic inactive hepatitis B, even during virological flare. However, low HBsAg level at baseline is useful to predict absence of subsequent virological flare in chronic inactive hepatitis B patients especially when the level of HBsAg is less than 250 IU/mL (NPV of 88.6%). Moreover, low HBsAg (≤250 IU/mL) combined with HBV DNA below detection limit is useful to predict absence of subsequent virological flare in chronic inactive hepatitis B patients during 1 year of follow-up. In patients who had HBV DNA flare, the factor that can predict sustained virological flare was higher baseline ALT.

What is already known on this topic?

HBV DNA level and ALT are common markers used for follow-up CHB patients. HBV DNA can fluctuate overtime and it is costly, especially, in resource-limit countries. There were some studies showing that qHBsAg may reflect cccDNA which is reservoir of HBV in the liver. Level of qHBsAg can predict HBV DNA flare.

What this study adds?

Correlation between HBV DNA and qHBsAg is poor, even during HBV DNA virological flare, level of qHBsAg

is still low. Use of qHBsAg alone cannot replace HBV DNA monitoring in CHB with low HBV DNA, however, combine qHBsAg <250 IU/mL and HBV DNA below detection limit may be useful in predicting absence of subsequent virological flare.

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Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-2.
2. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis 2005;25 Suppl 1:3-8.
3. Hsu YS, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002;35:1522-7.
4. Deguchi M, Yamashita N, Kagita M, Asari S, Iwatani Y, Tsuchida T, et al. Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. J Virol Methods 2004;115:217-22.
5. Chen CH, Lee CM, Wang JH, Tung HD, Hung CH, Lu SN. Correlation of quantitative assay of hepatitis B surface antigen and HBV DNA levels in asymptomatic hepatitis B virus carriers. Eur J Gastroenterol Hepatol 2004;16:1213-8.
6. Kohmoto M, Enomoto M, Tamori A, Habu D, Takeda T, Kawada N, et al. Quantitative detection of hepatitis B surface antigen by chemiluminescent microparticle immunoassay during lamivudine treatment of chronic hepatitis B virus carriers. J Med Virol 2005;75:235-9.
7. Ozaras R, Tabak F, Tahan V, Ozturk R, Akin H, Mert A, et al. Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. Dig Dis Sci 2008;53:2995-8.
8. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1-98.
9. Kumar M, Chauhan R, Gupta N, Hissar S, Sakhuja P, Sarin SK. Spontaneous increases in alanine aminotransferase levels in asymptomatic chronic hepatitis B virus-infected patients. Gastroenterology 2009;136:1272-80.
10. Chu CM, Liaw YF. Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. Gastroenterology 2007;133:1458-65.
11. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004;126:1750-8.
12. Chan HL, Wong VW, Tse AM, Tse CH, Chim AM, Chan HY, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol 2007;5:1462-8.
13. Wong GL, Chan HL. Predictors of treatment response in chronic hepatitis B. Drugs 2009;69:2167-77.
14. Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med 2004;116:829-34.
15. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology 2007;45:1187-92.
16. Chu CM, Liaw YF. Spontaneous relapse of hepatitis in inactive HBsAg carriers. Hepatol Int 2007;1:311-5.
17. Liu J, Yang HI, Lee MH, Jen CL, Batrla-Utermann R, Lu SN, et al. Serum Levels of Hepatitis B Surface Antigen and DNA Can Predict Inactive Carriers With Low Risk of Disease Progression. Hepatology 2016;64:381-9.
18. Brouwer WP, Chan HL, Brunetto MR, Martinot-Peignoux M, Arends P, Cornberg M, et al. Repeated Measurements of Hepatitis B Surface Antigen Identify Carriers of Inactive HBV During Long-term Follow-up. Clin Gastroenterol Hepatol 2016;14:1481-9.
19. Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology 2008;134:1376-84.