Is it necessary to delay antiviral therapy for 3–6 months to anticipate HBeAg seroconversion in patients with HBeAg-positive chronic hepatitis B in endemic areas of HBV genotype C?

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Background/Aims: Spontaneous HBeAg seroconversion occurs frequently in the immune reactive phase in HBeAg-positive chronic hepatitis B (CHB). Therefore, observation for 3–6 months before commencing antiviral therapy is recommended in patients with alanine aminotransferase (ALT) levels that exceed twice the upper limit of normal (ULN). However, HBeAg seroconversion occurs infrequently in patients infected with hepatitis B virus (HBV) genotype C. The aim of the present study was to determine whether the waiting policy is necessary in endemic areas of HBV genotype C infection.

Methods: Ninety patients with HBeAg-positive CHB were followed prospectively without administering antiviral therapy for 6 months. Antiviral therapy was initiated promptly at any time if there was any evidence of biochemical (i.e., acute exacerbation of HBV infection or aggravation of jaundice) or symptomatic deterioration. After 6 months of observation, antiviral therapy was initiated according to the patient’s ALT and HBV DNA levels.

Results: Only one patient (1.1%) achieved spontaneous HBeAg seroconversion. Biochemical and symptomatic deterioration occurred before 6 months in 17 patients (18.9%) and 5 patients, respectively. High ALT and HBV DNA levels were both independent risk factors for biochemical deterioration. Of 15 patients with HBV DNA ≥5.1 × 10^7 IU/mL and ALT ≥5×ULN, biochemical deterioration occurred in 7 (46.7%), including 1 patient receiving liver transplantation due to liver failure.

Conclusions: Spontaneous HBeAg seroconversion in patients with HBeAg-positive CHB is rare within 6 months. Biochemical deterioration was common and may lead to liver failure. Immediate antiviral therapy should be considered, especially in patients with high ALT and HBV DNA levels in endemic areas of genotype C infection. (Clin Mol Hepatol 2014;20:355-360)

Keywords: Hepatitis B virus, chronic hepatitis B; acute exacerbation of hepatitis B, HBV genotype C

INTRODUCTION

Hepatitis B virus (HBV) is the most common cause of chronic liver disease including liver cirrhosis and hepatocellular carcinoma. Recently, potent oral nucleos(t)ide analogues, including entecavir and tenofovir, have been widely used in the treatment of chronic hepatitis B (CHB). The potent antiviral treatment in patients with CHB can suppress the viral replication and prevent progression to liver cirrhosis and hepatocellular carcinoma.
During the natural course of CHB infection, spontaneous HBeAg seroconversion, defined as loss of HBeAg and detection of anti-HBe in a person who was HBeAg positive and anti-HBe negative, occurred frequently in the immune reactive phase in patients with HBeAg-positive CHB ranging from 2-17% per year depending on serum alanine aminotransferase (ALT) levels. Spontaneous HBeAg seroconversion is usually followed by normalization of serum aminotransferase levels and sustained suppression of HBV DNA (<2,000 IU/ml) and is associated with favorable long-term outcomes in patients with CHB. Therefore, in HBeAg-positive CHB patients with elevated serum ALT, most guidelines recommend observation for 3-6 months before antiviral therapy to anticipate spontaneous HBeAg seroconversion.

However, spontaneous HBeAg seroconversion occurred infrequently in patients with genotype C compared to those with other genotypes. Therefore, the strategy for initiation of antiviral therapy would be different according to the distribution of HBV genotype. The purpose of this study was to investigate the necessity of the waiting strategy before antiviral therapy in HBeAg-positive CHB patients in endemic areas of HBV genotype C infection.

PATIENTS AND METHODS

We prospectively enrolled HBeAg-positive CHB patients with HBV DNA levels of more than 20,000 IU/mL and ALT levels of more than two times the upper limit of normal (ULN) between June 2009 and September 2013 in Jeju National University Hospital. Patients were excluded if they had concurrent hepatitis C or other liver disease (alcoholism, autoimmune liver disease, toxic hepatitis, liver cirrhosis and hepatocellular carcinoma). Pregnant women and patients who refused this study were also excluded.

The patients were followed without antiviral treatment to anticipate spontaneous HBeAg seroconversion during 6 months. They were followed at 1-3 month intervals or more frequently if clinically needed. At every visit, serial liver function tests, including ALT and bilirubin, and HBV DNA were measured. HBeAg and anti-HBe were analyzed by third-generation microparticle enzyme immunoassays using commercial enzyme immunoassay kits (Abbott, North Chicago, IL, USA). Serum HBV DNA levels were measured by the Cobas Amplicor HBV Monitor (detection limit, 60 IU/mL) (Roche Molecular Diagnostics, Pleasanton, CA, USA) before December 2009 or the Cobas AmpliPrep/Cobas TaqMan® HBV Test, v2.0 (detection limit, 20 IU/mL) (Roche Molecular Diagnostics) after December 2009.

Antiviral therapy was initiated promptly at any time if there was any evidence of biochemical (acute exacerbation of hepatitis B or aggravation of jaundice, defined by bilirubin ≥2 mg/dL) or symptomatic deterioration which disturbed the patients’ daily life, and the patients wanted to receive antiviral therapy. Acute exacerbation of hepatitis B was defined as elevations of serum ALT levels to more than 10 times the ULN and more than twice the baseline value. In patients with biochemical deterioration, other causes of acute hepatitis were excluded.

Data analysis was performed using SPSS version 12.0 for Windows (SPSS Corp, Chicago, IL, USA). The data were analyzed by the Student’s t-test for continuous variables and the chi-square test for categorizing variables. A logistic regression analysis was used to identify the independent predictive factors for biochemical or symptomatic deterioration. P values less than 0.05 were considered significant.

The study protocol was approved by the ethics committees of our institution and written informed consents were obtained from the patients.

RESULTS

Patient characteristics

During the study period, 119 consecutive patients fulfilled the inclusion criteria. Among them, 29 patients were excluded because of exclusion criteria (n=19) and were lost to follow-up after the first visit (n=10). Therefore, 90 patients with HBeAg-positive CHB were analyzed. Baseline characteristics of the 90 patients are shown in Table 1. The mean age of the patients was 37.9 years and 60 patients (67.0%) were male. Fifty-four patients (60%) were vertically infected.

During follow-up, only one (1.1%) of 90 HBeAg-positive CHB patients achieved spontaneous HBeAg seroconversion at 2 months. In another patient, HBeAg loss, but not anti-HBe positive occurred at 3 months. However, in this patient, persistent elevation of HBV DNA above 2,000 IU/mL and elevation of ALT levels was observed over 6 months.

Biochemical deterioration was observed in 17 patients (18.9%); acute exacerbations in 8, aggravation of jaundice in 2, and both in 7 patients) and symptomatic deteriorations without biochemical deterioration occurred in 5 patients before 6 months.

Biochemical deterioration occurred more often in patients with
high ALT, high bilirubin and high HBV DNA levels in univariate analysis (Table 1). Among these, high serum ALT and HBV DNA levels were two independent risk factors for biochemical deterioration (Table 2). Serum HBV DNA levels were dichotomized at the levels of $5.1 \times 10^7$ IU/mL (median value). We categorized the patients into the following 4 groups: Group 1 (n=24), low HBV DNA (<$5.1 \times 10^7$ IU/mL) and low ALT (<5×ULN); group 2 (n=15), low HBV DNA and high ALT ($\geq$5×ULN); group 3 (n=36), high HBV DNA ($\geq$5.1×$10^7$ IU/mL) and low ALT; and group 4 (n=15), high HBV DNA and high ALT. The percentages of biochemical or symptomatic deteriorations in each group are demonstrated in Fig. 1.

In 17 patients with biochemical deterioration and 5 patients with symptomatic deterioration (n=22), immediate antiviral therapy was recommended before 6 months of follow up. Twenty patients received antiviral therapy (entecavir or tenofovir) promptly (mean 3 months, range 1-5 months). However, in 2 patients with acute exacerbation of hepatitis B during follow-up, antiviral therapy was initiated at 8 and 9 months as the patients wanted to delay the treatment for several months anticipating spontaneous HBeAg seroconversion. Among 17 patients with biochemical deterioration during follow-up, a patient received living-related liver transplantation because of acute-on-chronic liver failure even introducing entecavir therapy (Fig. 2).

In remaining 68 patients without biochemical or symptomatic deterioration within 6 months, 56 patients received antiviral therapy at or after 6 months of follow-up (mean 12.9 months, range 6-48 months), 6 patients did not satisfy the treatment indication until the last follow-up, and 6 patients were lost to follow-up after 6 months. As a total, 78 of 84 patients (93%) who were followed, received antiviral therapy.

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Antiviral therapy was initiated in 17, 14, 31 and 16 patients during follow-up in group 1, 2, 3 and 4, respectively. In each group, antiviral therapy was initiated at a mean of 8.9 months (3-32 months), 18.2 months (1-48 months), 9.8 months (2-36 months) and 6.1 months (1-18 months) in group 1, 2, 3 and 4, respectively.

### Table 1. Baseline characteristics of the study patients

| Variables                        | Total (n=90) | No biochemical deterioration (n=73) | Biochemical deterioration (n=17) | P-value |
|----------------------------------|-------------|-------------------------------------|---------------------------------|--------|
| Age (years)                      | 37.9±10.7   | 37.2±10.7                           | 40.9±10.5                       | 0.2    |
| Sex (M/F)                        | 60/30       | 51/22                               | 9/8                             | 0.18   |
| ALT (IU/mL)                      |             |                                     |                                 | 0.03   |
| <5×ULN                           | 58          | 51 (87.9)                           | 7 (12.1)                        |        |
| ≥5×ULN                           | 32          | 22 (68.8)                           | 10 (31.3)                       |        |
| Bilirubin (mg/dL)                | 0.9±0.6     | 0.8±0.4                             | 1.2±1.1                         | 0.03   |
| HBV DNA (log_{10} IU/mL)         | 7.4±1.2     | 7.3±1.3                             | 7.9±1.1                         | 0.07   |
| Median (range, IU/mL)            | $5.1 \times 10^7$ (2×$10^4$-3.4×$10^9$) |                         |                                 |        |

### Table 2. Independent risk factors for biochemical deterioration in patients with HBeAg-positive CHB

| Variables                        | Odds ratio (95% CI) | P-value |
|----------------------------------|---------------------|--------|
| Serum ALT ≥5×ULN                 | 4.1 (1.3-13.1)      | 0.016  |
| HBV DNA (Log_{10})               | 1.8 (1.1-3.0)       | 0.04   |

ALT, alanine aminotransferase; CI, confidence interval.

Figure 1. Biochemical or symptomatic deterioration in patients with HBeAg-positive CHB according to serum HBV DNA and ALT levels. The patients were categorized into the following four groups: group 1 (n=24), low HBV DNA (<$5.1 \times 10^7$ IU/mL) and low ALT (<5×ULN); group 2 (n=15), low HBV DNA and high ALT (≥5×ULN); group 3 (n=36); group 4 (n=15), high HBV DNA (≥$5.1 \times 10^7$ IU/mL) and high ALT. The percentages of biochemical or symptomatic deteriorations in each group are demonstrated in Fig. 1.
Clinical course of a 62-year-old female patient with HBeAg-positive CHB who received liver transplantation because of acute-on-chronic liver failure. This patient was followed at weekly intervals over a 6-month period without any antiviral therapy before week 0, at which point her serum ALT, bilirubin, and HBV DNA levels were 35 IU/mL, 0.6 mg/dL, and \(3.1 \times 10^6\) IU/mL, respectively; at 12 weeks these levels had increased to 282 IU/mL, 0.8 mg/dL, and \(2.8 \times 10^7\) IU/mL. It was requested that the patient be followed without antiviral therapy even though her ALT levels were elevated to more than twice the ULN. At 14 weeks her serum ALT and bilirubin levels were 314 IU/mL and 0.7 mg/dL, respectively. At 16 weeks, the patient visited the emergency room because of severe anorexia and nausea. Her serum ALT, bilirubin, and HBV DNA levels at that point were 2,039 IU/mL, 19 mg/dL, and \(3.85 \times 10^8\) IU/mL, respectively. Entecavir was introduced immediately. It did not occur within 6 months, even in patients with ALT levels more than 4-5 times the ULN in patients with genotype C. In addition, the difference in the rate of HBeAg seroconversion was predominantly noted in patients who had elevated ALT levels on presentation. In patients with genotype B, HBeAg seroconversion was dependent on serum ALT levels on presentation. However, in patients with HBV genotype C, HBeAg seroconversion was not dependent on serum ALT levels.

In general, high serum ALT levels are considered a surrogate marker for vigorous host immune response caused by cytotoxic T-cell–mediated immune hepatolysis and eventual immune clearance of HBV. However, HBeAg seroconversion occurred only in 1.1% within 6 months of follow-up in this study. Previous studies and this data suggests that some abortive immune clearance mechanism might exist in patients infected with HBV genotype C.

A study in Korea reported that HBeAg seroconversion occurred in 35.5% within 6 months in patients with ALT levels more than 5 times the ULN. We could not explain the exact mechanism of the discrepancy between the previous studies and our data. Previous study showed that non-vertical infection of HBV was one of the independent predictive factors for HBeAg seroconversion. In their study, 31% of the subjects were vertically infected. In our study, 60% of the subjects were vertically infected. The difference of the proportion of vertical infection in subject might partially explain the difference in HBeAg seroconversion rate within 6 months. Yoon et. al also reported that HBeAg seroconversion within 6 months in patients with elevated ALT (mean 179 IU/mL) levels was less than 3%.
Acute exacerbation of hepatitis B may lead to hepatic decompensation and death from liver failure. In this study, acute exacerbation of hepatitis B or aggravation of jaundice developed in 17 patients (18.9%) and one patient received liver transplantation even after introducing antiviral because of acute-on-chronic liver failure following acute exacerbation of hepatitis B. Jeng et al. reported that hepatic decompensation occurred in 5.1%, especially in patients with serum HBV DNA >1.55×10^8 IU/mL (around 3×10^5 copies/mL) in CHB patients with abrupt increase in ALT more than 5 times the ULN. In this study, in patients with high ALT (≥5 ULN) and high viral load (HBV DNA ≥ 5.1×10^7 IU/mL), acute exacerbation of hepatitis B or aggravation of jaundice developed in 46.7% of the patients. Lok et al. also reported that acute exacerbation of hepatitis B occurred frequently in patients with ALT levels more than 5 times the ULN. In addition, Kim et al. reported that patients with serum HBV DNA levels more than 2×10^8 IU/mL had little possibility of spontaneous HBeAg seroconversion. After spontaneous HBeAg seroconversion, those with genotype C were more likely to revert to the HBeAg-positive state and reactivation of hepatitis B occurred in 50-80% of patients during long-term follow-up. In addition, reactivation of hepatitis B occurred frequently in patients with genotype C compared with those with genotype A and serum ALT levels more than 5 times the ULN.

Considering previous results and the results of our study, serum ALT and HBV DNA cut-off levels for immediate antiviral therapy might be 5 times the ULN and 5.1×10^7 IU/mL, respectively.

In conclusion, spontaneous HBeAg seroconversion in patients with HBeAg-positive CHB in the endemic area of genotype C infection was rare within 6 months. Biochemical deterioration was common. Therefore, immediate antiviral therapy should be considered, especially in patients with high ALT and HBV DNA levels.

Acknowledgements

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Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Korean Association for the Study of the Liver. KASL Clinical Practice Guidelines: Management of chronic hepatitis B. Clin Mol Hepatol 2012;18:109-162.
3. European Association for the Study of the Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-185.
4. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
5. Liaw YF, Kao JH, Piratvisuth T, Chan HLY, Chin R, Liu C, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531-561.
6. Schiff ER, Lee SS, Chao YC, Kew Yoon S, Bessone F, Wu SS, et al. Long-term treatment with entecavir induces reversal of advanced fibrosis or cirrhosis in patients with chronic hepatitis B. Clin Gastroenterol Hepatol 2011;9:274-276.
7. Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, et al. Long-term entecavir treatment results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886-893.
8. Marcellin P, Gane E, Buth M, Alfdal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468-475.
9. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology 2013;58:98-107.
10. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuan H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.
11. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology 2013;58:98-107.
12. Wong GL, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, et al. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. Hepatology 2013;58:1537-1547.
13. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, et al. Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology 1986;6:167-172.
14. Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology 1987;92:1839-1843.
15. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated...
with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology 2002;122:1756-1762.

16. Chu CM, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol 2005;43:411-417.

17. Yuen MF, Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, et al. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. Gut 2003;52:416-419.

18. Kim HS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, et al. Predictive factors for early HBeAg seroconversion in acute exacerbation of patients with HBeAg-positive chronic hepatitis B. Gastroenterology 2009;136:505-512.

19. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. J Med Virol 2004;72:363-369.

20. Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology 1983;84:216-225.

21. Lim YS, Han S, Heo NY, Shim JH, Lee HC, Suh DJ. Mortality, liver transplantation, and hepatocellular carcinoma among patients with chronic hepatitis B treated with entecavir vs lamivudine. Gastroenterology 2014;147:152-161.

22. Hsu YS, Chien RN, Yeh CT, Sheen IS, Chou HY, Chu CM, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology 2002;35:1522-1527.

23. Hui CK, Leung N, Shek TW, Yao H, Lee WK, Lai JY, et al. Sustained disease remission after spontaneous HBeAg seroconversion is associated with reduction in fibrosis progression in chronic hepatitis B Chinese patients. Hepatology 2007;46:690-698.

24. Livingston SE, Simonetti JP, Bullkow LR, Homan CE, Snowball MM, Cagle HH, et al. Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F. Gastroenterology 2007;133:1452-1457.

25. Sarin SK, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). Hepatol Int 2009;3:269-282.

26. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med 2001;135:759-768.

27. Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. Intervirology 2003;46:329-338.

28. Song BC, Cui XJ, Kim H. Hepatitis B virus genotypes in Korea: an endemic area of hepatitis B virus infection. Intervirology 2005;48:133-137.

29. Bae SH, Yoon SK, Jang JW, Kim CW, Nam SW, Choi JY, et al. Hepatitis B virus genotype C prevails among chronic carriers of the virus in Korea. J Korean Med Sci 2005;20:816-820.

30. Chu CM, Liaw YF. Intrahepatic distribution of hepatitis B surface and core antigens in chronic hepatitis B virus infection. Hepatocyte with cytoplasmic/membranous hepatitis B core antigen as a possible target for immune hepatocytolysis. Gastroenterology 1987;92:220-225.

31. Tsai SL, Chen PJ, Lai MY, Yang PM, Sung JL, Huang JH, et al. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J Clin Invest 1992;89:87-96.

32. Yoon JH, Rhee PL, Lee HS, Kim CY. Spontaneous HBeAg clearance rate and its affecting factors inpatients with chronic hepatitis B in Korea. Korean J Gastroenterol 1992;24:1313-1319.

33. Sheen IS, Liaw YF, Tai DI, Chu CM. Hepatic decompensation associated with hepatitis B e antigen clearance in chronic type B hepatitis. Gastroenterology 1985;93:732-735.

34. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. Gastroenterology 1984;86:230-235.

35. Lok AS, Lai CL. Acute exacerbations in Chinese patients with chronic hepatitis B virus (HBV) infection. Incidence, predisposing factors and etiology. J Hepatol 1990;10:29-34.

36. Jeng WJ, Sheen IS, Liaw YF. Hepatitis B virus DNA level predicts hepatic decompensation in patients with acute exacerbation of chronic hepatitis B. J Gastroenterol Hepatol 2010;25:541-545.