Hepatitis B virus genotype has no impact on hepatitis B e antigen seroconversion after lamivudine treatment

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Abstract

AIM: To investigate the association of hepatitis B virus (HBV) genotype and HBeAg seroconversion after nucleotide analogue treatment.

METHODS: Chronic hepatitis B patients receiving lamivudine followed up for at least 6 months post-treatment were studied. Consecutive treatment-naïve patients who were prospectively followed up in the clinic for at least 18 months were studied as controls. HBeAg seroconversion was defined as loss of HBeAg, appearance of anti-HBe and normalization of alanine aminotransferase for at least 6 months.

RESULTS: Thirty-five patients on lamivudine and 96 control patients followed up for 39 (18-49) months were studied. Lamivudine was given for 12 (10-18) months, and patients were followed up for 15 (6-34) months after drug cessation. Genotype B and C HBV were found in 43 and 88 patients and HBeAg seroconversion occurred in 12 (28 %) and 16 (18 %) patients, respectively (P = 0.30). There was no difference in HBeAg seroconversion between patients infected by genotype B and C HBV in the control (35 % vs 21 %, P = 0.25) and lamivudine-treated (14 % vs 10 %, P = 1.00) groups.

CONCLUSION: HBeAg seroconversion after treatment by lamivudine was not influenced by the HBV genotype.

INTRODUCTION

Hepatitis B virus (HBV) has 7 different genotypes (A-G) according to the homogeneity of the viral sequence[1,2]. In Southeast Asia including Hong Kong, genotype B and C HBV are the predominant species[3,4]. There are increasing evidences showing that genotype C HBV is associated with a more aggressive disease as compared with genotype B HBV[5-10]. A recent Japanese study suggests that HBV genotype is not associated with the development of lamivudine resistance after a median of 1.8 years of treatment[11]. However, data on the association of HBV genotype and HBeAg seroconversion response to anti-viral treatment is scanty. This information is potentially important in the selection of patients for anti-viral treatment and development of future therapeutic regimens as in the case of chronic hepatitis C.

Previous studies suggest that genotype B HBV is associated with a higher rate of HBeAg seroconversion versus genotype C HBV among patients receiving interferon treatment[12,13]. This finding, however, cannot be extrapolated to nucleos(t)ide analogues which have completely different mechanisms of action as compared with interferon. A study including 31 Taiwanese HBeAg-positive chronic hepatitis B patients treated by lamivudine does not show any difference in HBeAg seroconversion between patients infected by genotype B and C HBV[14]. This study is limited by the small number of patients and the absence of control group. Phase III, multi-centered studies on adefovir dipivoxil reveal no difference in viral load reduction among patients infected by different HBV genotypes, but both HBeAg-positive and HBeAg-negative patients are included and HBeAg seroconversion has not been studied[15]. In this study, we aimed to investigate the association of HBV genotype and HBeAg seroconversion after treatment by lamivudine, and a control group of untreated HBeAg-positive chronic hepatitis B patients was included for comparison.

MATERIALS AND METHODS

Patients on anti-viral treatment

Clinical data and stored serum samples of patients on lamivudine treated in Prince of Wales Hospital from 1998 to 2000 were included for analysis. These patients had positive hepatitis B surface antigen (HBsAg) for at least 6 months and positive hepatitis B e antigen (HBeAg). HBV DNA >1 000 000 copies/ml by DNA cross-linking assay (NAXCOR XLnt™, NAXCOR, Menlo Park, CA) or branched DNA assay (bDNA, Chiron Diagnostic, Emeryville, CA) before treatment[16]. None of these patients had evidence of liver cirrhosis complications, hepatocellular carcinoma (HCC) or co-infection by hepatitis C or human immunodeficiency viruses. All patients received lamivudine 100 mg daily. HBeAg seroconversion was defined as loss of HBeAg, appearance of antibodies to HBeAg (anti-HBe) and normalization of ALT at the end of anti-viral treatment and the response sustained for at least 6 months after cessation of treatment till the last follow-up visit.

Control patients

Consecutive chronic hepatitis B patients with positive HBeAg at the initial clinic visit recruited since 1997 in our hospital were studied as controls[17-19]. Patients who had previously received interferon or anti-viral treatment, liver cirrhosis complications and HCC were excluded from the study. To match the follow-up duration of patients treated by nucleoside analogues, patients who were followed up for less than 18 months were also excluded. All patients were followed up every 6 months, or more frequently as clinically indicated, with monitoring of HBeAg status and liver biochemistry. HBeAg
seroconversion was defined as sustained loss of HBeAg, appearance of anti-HBe and normalization of ALT during the follow-up period lasting for at least 6 months till the last visit.

**HBV genotyping**

The baseline samples of patients in clinical trial before starting anti-viral treatment and the serum samples at the first clinic visits of control patients were studied for HBV genotyping by restriction fragment length polymorphism as described previously[7,18]. In short, extracted HBV DNA was amplified by polymerase chain reaction (PCR) using primers flanking the HBV genome between nucleotide 256 to 796 (sense primer 5’-GTGGTGGAACCTCTCATTAATTTT and anti-sense primer 5’-CGGTA(A/T)AAAGGGACTCA(A/C)GAT). PCR product was then mixed and incubated with restriction enzymes Tsp 5091 (New England Biolabs, Beverly, MA) and HinfI (Boehringer Mannheim, Mannheim, Germany) respectively. The samples were run on agarose gel and DNA was visualized by ethidium bromide staining. The restriction pattern was read accordingly.

**Statistical analysis**

Results were expressed as median (range). Data were analyzed using SPSS version 11.0 software package. Categorical variables were compared by Chi-square test and continuous variables were compared by Mann-Whitney U test. Proportion of patients developing HBeAg seroconversion in different HBV genotypes were also compared after adjustment of potential confounding variables with P value <0.1 by logistic regression analysis. All tests were two-tailed. Statistical significance was taken as P<0.05.

**RESULTS**

Thirty-five patients on lamivudine and 96 control patients were identified. The clinical data and viral genotype of the studied patients of two groups of patients are shown in Table 1. Lamivudine was given for 12 (10-18) months and the treated patients were followed up for 15 (6-34) months after the cessation of lamivudine. Only 1 of 35 lamivudine-treated patients had been treated for 10 months whereas the remaining patients had treatment for at least 12 months. All patients who did not achieve HBeAg seroconversion after lamivudine treatment developed biochemical relapse with ALT elevation after cessation of lamivudine. The 24 control patients who achieved sustained HBeAg seroconversion were followed up for 24.5 (8-40) months after the development of HBeAg seroconversion. Patients on lamivudine were significantly older with higher initial ALT levels and shorter follow-up duration as compared with the controls. There was no difference in the distribution of HBV genotypes and the percentage of HBeAg seroconversion between the treated and control patients.

**Table 1** Clinical characteristics of patients on anti-viral treatments and controls

|          | Overall (n=131) | Controls (n=96) | Lamivudine (n=35) | P value |
|----------|----------------|----------------|-------------------|---------|
| Age      | 30 (12-68)     | 29 (12-68)     | 38 (22-47)        | 0.007   |
| Male gender (n, %) | 75 (57 %) | 50 (52 %) | 25 (71 %) | 0.074 |
| Initial ALT (IU/l) | 73 (17-1122) | 57.5 (17-753) | 135 (36-1122) | <0.001 |
| Follow-up (months) | 39 (18-49) | 39 (18-49) | 27 (18-46) | 0.009 |
| Genotype |               |               |                   | 0.40    |
| B (n, %) | 43 (33 %)     | 29 (30 %)     | 14 (40 %)         |         |
| C (n, %) | 88 (67 %)     | 67 (70 %)     | 21 (60 %)         |         |
| HBeAg seroconversion (n, %) | 29 (21 %) | 24 (25 %) | 4 (11 %) | 0.15 |

Forty-three (33 %) and 88 (67 %) patients were infected by genotype B and C respectively. The clinical characteristics of patients infected by genotype B and C HBV are shown in Table 2. There was no difference in the age, gender ratio, initial ALT levels, follow-up duration and drug treatment between patients infected by the two HBV genotypes. There was no difference in HBeAg seroconversion among patients infected by the 2 HBV genotypes, and the overall trend was confirmed after adjusted for age, gender, initial ALT level and the follow-up duration (Table 3).

**Table 2 Clinical characteristics of patients infected by genotype B and C HBV**

|          | Genotype B (n=43) | Genotype C (n=88) | P value |
|----------|-------------------|-------------------|---------|
| Age      | 34 (13-67)        | 29 (12-68)        | 0.14    |
| Male gender (n, %) | 28 (65 %) | 47 (53 %) | 0.28    |
| Initial ALT (IU/l) | 70 (17-1094) | 77 (18-1122) | 0.60    |
| Follow-up (months) | 38 (18-49) | 39 (18-49) | 0.49    |
| Treatment (n, %) | 29 (67 %) | 67 (76 %) | 0.40    |

**Table 3 Comparison of HBeAg seroconversion among patients infected by genotype B and C HBV**

|          | Genotype B | Genotype C | P value 1 | P value 2 |
|----------|------------|------------|-----------|-----------|
| HBeAg seroconversion |            |            | 0.25 | 0.41 |
| Control | 10/29 (35 %) | 14/67 (21 %) |          |         |
| Lamivudine | 2/14 (14 %) | 2/21 (10 %) | 1.00 | 0.51 |

**DISCUSSION**

The results of this study concurred with previous data that HBV genotype could not predict HBeAg seroconversion after 1 year treatment of lamivudine. The percentages of patients infected by genotype B and C HBV undergoing HBeAg seroconversion were similar among the treatment and control groups.

In the previous study in Taiwan, the proportion of patients achieving HBeAg seroconversion after lamivudine treatment was similar between genotype B and C HBV. However, genotype B HBV was reported to associate with earlier spontaneous HBeAg seroconversion by several series[9,10]. It was therefore uncertain whether there was really no difference in lamivudine response between the 2 HBV genotypes or the improved response of genotype C HBV has closed up the existing difference. In this study, a control group of untreated patients was included in the analysis. As the control group was not recruited together with the treated patients in a randomized manner, it was not surprising that untreated patients were generally younger and had lower ALT levels than the treated ones. In this study, the absence of association between HBV genotypes and HBeAg seroconversion among patients treated by nucleotide analogues was confirmed as there was no difference in HBeAg seroconversion between the 2 HBV genotypes in both the treated groups and the control group after adjustment for all the differences between the two groups.

The earlier age of spontaneous HBeAg seroconversion associated with genotype B HBV reported by recent longitudinal studies was not shown in this study[9,10]. Patient recruitment in the previous studies may be biased as one of them was a retrospective study and the other included only
role of HBV genotype in response to lamivudine among HBeAg-positive patients. Further studies are required to probe into the association of HBV genotypes and treatment response in other geographical locations where other HBV genotypes prevail.

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