P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(12):2695-2697
World Journal of Gastroenterology
 ISSN 1007-9327

• VIRAL HEPATITIS •

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

Henry Lik-Yuen Chan, May-Ling Wong, Alex Yui Hui, Angel Mei-Ling Chim, Ada Mei-Ling Tse, Lawrence Cheung-Tsui Hung, Francis Ka-Leung Chan, Joseph Jao-Yiu Sung

Henry Lik-Yuen Chan, May-Ling Wong, Alex Yui Hui, Angel Mei-Ling Chim, Ada Mei-Ling Tse, Lawrence Cheung-Tsui Hung, Francis Ka-Leung Chan, Joseph Jao-Yiu Sung, Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong
Correspondence to: Dr. Henry Lik-Yuen Chan, Department of Medicine and Therapeutics, 9/F Prince of Wales Hospital, Shatin, Hong Kong. hlychan@cuhk.edu.hk
Telephone: +852-26323594 **Fax:** +852-26373852
Received: 2003-07-04 **Accepted:** 2003-10-08

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.

Chan HLY, Wong ML, Hui AY, Chim AML, Tse AML, Hung LCT, Chan FKL, Sung JJY. Hepatitis B virus genotype has no impact on hepatitis B e antigen seroconversion after lamivudine treatment. *World J Gastroenterol* 2003; 9(12): 2695-2697
http://www.wjgnet.com/1007-9327/9/2695.asp

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[3-6]. There are increasing evidences showing that genotype C HBV is associated with a more aggressive disease as compared with genotype B HBV[3,7-10]. 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[3,17]. 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' -GTGGTGGACTTCTCTCAATTTTC 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 (19-49)	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, %)	28 (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 ratio, 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, %)			0.40
Nil	29 (67 %)	67 (76 %)	
Lamivudine	14 (33 %)	21 (24 %)	

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

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

P value 1: unadjusted comparison of genotype B versus genotype C; P value 2: comparison of genotype B versus genotype C after adjustment for age, gender, initial ALT levels and follow-up duration.

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[14]. 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

patients who have consented for liver biopsy. The consecutive patient recruitment and prospective follow-up in our series has minimized the bias of sampling. Although the age of spontaneous HBeAg seroconversion did not differ between the 2 HBV genotypes, our previous studies agreed with others that genotype C HBV is associated with a more aggressive disease course than genotype B HBV[3,7].

In this study, the rate of HBeAg seroconversion was comparable between the treated and untreated patients. The rate of HBeAg seroconversion after 1 year of lamivudine treatment was comparable to that reported in the previous multicenter Asian lamivudine trial[19]. The rate of spontaneous HBeAg seroconversion (25 % in 3 years) was also comparable to previous reported Asian series after taken into consideration the young age and relatively lower ALT level of the control patients in this study[20,21]. As patients in the control group were followed up for longer duration and some of these patients might have milder disease as compared with the treated patients, the effect of treatment could not be concluded by the results of this study. This study was also not designed to assess the predictive factor of treatment response to the nucleoside analogues.

In conclusion, the results of this study did not support a 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.

REFERENCES

1 **Norder H,** Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 1994; **198**: 489-503

2 **Stuyver L,** De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; **81**: 67-74

3 **Chan HLY,** Wong ML, Hui AY, Hung LCT, Chan FKL, Sung JJY. Hepatitis B virus genotype C takes a more aggressive disease course than hepatitis B virus genotype B in hepatitis B e antigen-positive patients. *J Clin Microbiol* 2003; **41**: 1277-1279

4 **Sung JJY,** Chan HLY, Wong ML, Tse CH, Yuen SCH, Tam JSL, Leung NWY. Relationship of clinical and virological factors with hepatitis activity in hepatitis B e antigen-negative chronic hepatitis B virus-infected patients. *J Viral Hepat* 2002; **9**: 229-223

5 **Orito E,** Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, Okita K, Okanoue T, Iino S, Tanada E, Suzuki K, Watanabe H, Huge S, Mizokami M. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 2001; **34**: 590-594

6 **Kao JH,** Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcome in patients with chronic hepatitis B. *Gastroenterology* 2000; **118**: 554-559

7 **Chan HLY,** Tsang SWC, Liew CT, Tse CH, Wong ML, Ching JYL, Leung NWY, Tam JSL, Sung JJY. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. *Am J Gastroenterol* 2002; **97**: 406-412

8 **Kao JH,** Chen PJ, Lai MY, Chen DS. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. *J Clin Microbiol* 2002; **40**: 1207-1209

9 **Chu CJ,** Hussain M, Lok ASF. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; **122**: 1756-1762

10 **Sumi H,** Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, Kanda T, Fukai K, Kato M, Saisho H. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37**: 19-26

11 **Akuta N,** Suzuki F, Kobayashi M, Tsubota A, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. *J Hepatol* 2003; **38**: 315-321

12 **Kao JH,** Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; **33**: 998-1002

13 **Wai CT,** Chu CJ, Hussain M, Lok ASF. HBV genotype B is associated with better response in interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002; **36**: 1425-1430

14 **Kao JH,** Liu CJ, Chen DS. Hepatitis B viral genotypes and lamivudine resistance. *J Hepatol* 2002; **36**: 303-305

15 **Westland C,** Delaney W, Yang HL, Fry J, Brosgart C, Gibbs C, Miller M, Xiong S. Distribution and clinical response of HBV genotypes in phase III studies of adefovir dipivoxil. *J Hepatol* 2002; **36**(Supp1): 105

16 **Chan HLY,** Leung NWY, Lau TCM, Wong ML, Sung JJY. Comparison of three different sensitive assays for hepatitis B virus DNA in monitoring of responses to anti-viral therapy. *J Clin Microbiol* 2000; **38**: 3205-3208

17 **Chan HLY,** Leung NWY, Hussain M, Wong ML, Lok ASF. Hepatitis B e antigen negative chronic hepatitis B in Hong Kong. *Hepatology* 2000; **31**: 763-768

18 **Lindh M,** Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographical origin of hepatitis B virus – large-scaled analysis using a new genotyping method. *J Infect Dis* 1997; **175**: 1285-1293

19 **Lai CL,** Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998; **339**: 61-68

20 **Lok ASF,** Lai CL. Acute exacerbation in Chinese patients with chronic hepatitis B virus (HBV) infection. *J Hepatol* 1990; **10**: 29-34

21 **Yuen MF,** Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, Wong BC, Lai CL. 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

Edited by Ma JY