Role of Hepatitis B Virus Genotypes in Chronic Hepatitis B Exacerbation

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Hepatitis B virus (HBV) genotypes and precore and core promoter mutations were determined in 318 patients with HBV. Patients infected with HBV genotype B had a higher median alanine aminotransferase level and bilirubin level and a lower median albumin level during exacerbations of disease, compared with patients infected with HBV genotype C (all $P < .001$). By logistic regression analysis, HBV genotype B infection ($P = .014$) and low albumin levels ($P = .006$) were independently associated with a higher risk of hepatic decompensation during severe exacerbations of disease. Patients infected with genotype B had a significantly higher mortality due to hepatic decompensation than did patients with genotype C (70% vs. 27.8%; $P = .05$).

There is growing evidence that hepatitis B virus (HBV) genotypes may play some role in causing different disease profiles in chronic hepatitis B (CHB). Among Asians, who constitute ⩾75% of the worldwide population of individuals with CHB [1], genotypes B and C are the 2 most common HBV genotypes [2]. Though genotype B can be subdivided into genotype Bj, representing genotype B found among infected individuals from Japan, and genotype Ba, representing genotype B found among individuals from the rest of Asia [3], most infected non-Japanese Asians have genotype Ba only. In this article, references to genotype B refer to genotype Ba unless otherwise noted.

A study from Taiwan shows that young patients with hepatocellular carcinoma are more likely to be infected with HBV genotype B than genotype C, whereas patients with more-advanced liver disease are more likely to be infected with genotype C than genotype B [4]. Other studies demonstrate that, compared with patients with genotype C infection, patients with genotype B infection have more serious liver disease [5–7]. Recent studies show that patients with genotype B achieve hepatitis B e antigen (HBeAg) seroconversion a decade earlier than do patients with genotype C [8, 9]. Regarding responsiveness to treatment, there is some evidence that patients with genotype B respond better to IFN-α when compared with patients with genotype C [10, 11].

However, the effect of HBV genotypes on HBV disease exacerbations has not been studied. We aimed to investigate, in a cross-sectional study, the relationship of HBV genotypes to the probability and severity of HBV disease exacerbations among Chinese patients with CHB.

Patients and methods. During the period 2000–2001, 73 patients (group I) who were admitted to Queen Mary Hospital, The University of Hong Kong, Hong Kong, with severe exacerbations of hepatitis B disease and symptoms of hepatitis were recruited for our study. All 73 patients had tested positive for hepatitis B surface antigen for >6 months. "Severe exacerbation" of disease was defined as an increase of alanine aminotransferase (ALT) levels to >10 times the upper limit of normal (ULN). Patients with evidence of other hepatotrophic virus infection, checked by testing with antibodies to hepatitis A, C, D, and E, were excluded. Patients with a history and clinical features of drug-induced hepatitis, alcoholic hepatitis, and steatohepatitis were also excluded. Of the 73 patients with severe exacerbations of disease, 30 patients had hepatic decompensation, defined as an elevated bilirubin level >2 times ULN and prolonged prothrombin time (PT) of 5 s greater than the control value with or without development of ascites or hepatic encephalopathy. The liver biochemistry, PT, and HBV DNA levels (determined by Digene Hybrid Capture II assay [Digene; lower limit of detection, 140,000 copies/mL]) were measured at presentation. Patients were monitored for development of ascites and hepatic encephalopathy, and liver functions and PT were measured throughout the study period.

Patients first seen at the Hepatitis Clinic, Queen Mary Hospital, The University of Hong Kong, Hong Kong, during the same period of recruitment as the patients in the severe exacerbation group (group I) were recruited as control subjects. They were categorized into 3 different groups, as follows: 44 patients (group II) with moderate exacerbation of disease (defined as ALT levels 5–10× ULN), 80 patients (group III) with mild exacerbation of disease (ALT levels 2–5× ULN), and 121 patients (group IV) with no exacerbation of disease (ALT levels <2× ULN).

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Table 1. Demographic and clinical data of 4 groups of patients with hepatitis B virus (HBV) infection.

Variable	Disease exacerbation group			
	Severe (group I)	Moderate (group II)	Mild (group III)	None (group IV)
No. of patients	73	44	80	121
Sex, no. male/no. female	55/18	24/20	63/17	85/36
Age in years, median (range)	37.2 (17.8–67.9)	36.3 (18.5–65.2)	38.0 (21.2–68.3)	38.1 (22.7–80.5)
HBeAg/anti-HBe, n/n[a]	43/30	26/18	36/44	71/50
Laboratory values, median (range)				
ALT, U/L	719 (400–3840)	278 (162–498)	136 (75–249)	41 (7–104)
Albumin, g/L	38 (19 –51)	42 (31–53)	45 (29–55)	44 (32–53)
Bilirubin, μmol/L	56.5 (6–810)	13.5 (5–298)	12 (3–38.7)	11 (4–36)
HBV DNA, $\times 10^6$ copies/mL	0.93 (<0.14–1261.5)	3.8 (<0.14–986.1)	2.6 (<0.14–1380.2)	1.2 (<0.14–313.1)
US evidence of cirrhosis, *n/N* (%)[b]				
All genotypes	12/50 (24)	4/22 (18.2)	10/43 (23.3)	8/35 (22.9)
Genotype B	5/24 (20.8)	2/6 (33.3)	3/13 (23.1)	2/11 (18.2)
Genotype C	6/23 (26.1)	2/16 (12.5)	6/28 (21.4)	5/22 (22.7)

NOTE. "Severe exacerbation" was defined as an increase of alanine aminotransferase (ALT) levels to >10 times the upper limit of normal (ULN). "Moderate exacerbation" was defined as an increase of ALT levels to 5–10× ULN. "Mild exacerbation" was defined as an increase of ALT levels to 2–5× ULN. "No exacerbation" was defined as an increase of ALT levels to <2× ULN. The median ALT levels increased significantly from group I to group IV (all $P < .001$). Group I had a significantly lower median albumin level and higher bilirubin level compared with those of other groups (all $P < .001$). US, ultrasonographic.

[a] No. of patients testing positive for hepatitis B e antigen/no. of patients testing positive for antibody to hepatitis B e antigen.

[b] No. of patients with evidence of cirrhosis/no. for whom ultrasonography was performed (%).

The genotypes as well as the precore and core promoter mutations of the infecting HBV strains in all patients were determined with a line probe assay (INNO-LiPA HBV Genotyping and INNO-LiPA HBV Precore, developed by Innogenetics). The methodologies of these assays have been described in our previous studies [9, 12].

All statistical analyses were performed using the SPSS version 10.0 for Windows (SPSS). Continuous variables with skewed distribution were tested by Mann-Whitney *U* test. Categorical variables were tested by χ^2 test or Fisher's exact test. Logistic regression was applied to test independent association of various variables with outcome.

Results. The demographic and clinical data for the patient groups are listed in table 1. There were no differences in the median age, sex ratio, proportion of HBeAg to antibody to HBeAg positivity, proportion of patients with ultrasonographic evidence of cirrhosis, and median HBV DNA level between the 4 groups of patients. Group I patients (i.e., with severe exacerbations) had a significantly higher median ALT level, lower median albumin level, and higher median bilirubin level compared with the other 3 groups (all $P < .001$).

The number and percentages of patients with single genotype B or C infection and the prevalences of HBV precore and core promoter mutations in different groups are listed in table 2. There were no significant differences in the prevalence of genotype B and genotype C between the 4 groups (all $P =$ NS). In total, there were 102 patients with single genotype B infection and 183 patients with single genotype C infection. Infection with genotype B was associated with a higher prevalence of precore mutations (84 [82.4%] of 102 patients), compared with infection with genotype C (54 [29.5%] of 183; OR 11.1; 95% CI, 6.1–20.3; $P < .001$). In contrast, infection with genotype C was associated with

Table 2. Distribution of hepatitis B virus (HBV) genotypes and mutations in and among 4 groups of patients with HBV infection.

Infecting genotype, mutation location and class	No. (%) of patients, by disease exacerbation group			
	Severe ($n = 67$)	Moderate ($n = 41$)	Mild ($n = 67$)	None ($n = 110$)
Genotype B	31 (46.3)	13 (31.7)	19 (28.4)	39 (35.5)
Precore region				
Wild-type	4 (12.9)	3 (23.1)	1 (5.3)	10 (25.4)
Mutant	27 (87.1)	10 (76.9)	18 (94.7)	29 (74.6)
Core promoter				
Wild-type	18 (58.1)	9 (69.2)	11 (57.9)	29 (74.6)
Mutant	13 (41.9)	4 (30.8)	8 (42.1)	10 (25.4)
Genotype C	36 (53.7)	28 (68.3)	48 (71.6)	71 (64.5)
Precore region				
Wild-type	23 (63.9)	19 (67.9)	37 (77.1)	50 (70.4)
Mutant	13 (36.1)	9 (32.1)	11 (22.9)	21 (29.6)
Core promoter				
Wild-type	6 (16.7)	2 (7.1)	2 (4.2)	8 (11.3)
Mutant	30 (83.3)	26 (92.9)	46 (95.8)	63 (88.7)

a higher prevalence of core promoter mutations (165 [90.2%] of 183 patients), compared with infection with genotype B (35 [34.3%] of 102; OR 17.5; 95% CI, 9.3–33.1; $P < .001$).

All patients with exacerbations of disease (groups I, II, and III) were categorized according to whether they were infected with HBV genotypes B or C. The clinical and the liver biochemistry data obtained during periods of exacerbation are listed in table 3. Patients infected with genotype B had a higher median ALT level, higher median bilirubin level, and lower median albumin level during periods of exacerbation, compared with patients infected with genotype C. This means that patients infected with genotype B had more severe exacerbations compared with those had by patients infected with genotype C.

The prevalence of genotype B among and the liver biochemistry data for the 73 patients who had severe exacerbations with and without hepatic decompensation are reported in table 4. By logistic regression analysis, infection with genotype B and low albumin levels were independently associated with a higher risk of hepatic decompensation in patients with severe exacerbations ($P = .014$ and $P = .006$, respectively), though it is difficult to distinguish whether the low albumin levels were of causal significance or were only the outcome of the hepatic decompensation.

Of the 30 patients with hepatic decompensation, 13 were given lamivudine (1–20 days before admission, for 8 patients; on admission, for 4; and on day 19 after admission, for 1). Single infection with genotype B was found in 10 patients and single infection with genotype C was found in 18 patients. The remaining 2 patients had coinfection with genotypes A and C and genotypes A and B. Patients infected with genotype B had a higher mortality due to hepatic decompensation caused by severe exacerbations (7 [70%] of 10), compared with patients infected with genotype C (5 [27.8%] of 18; OR 6.1; 95% CI, 1.1–33.4; $P = .05$).

Discussion. Because nearly all patients we studied with HBV infection in the Chinese population became infected during the perinatal period or within the first 1–2 years of life, it is unlikely that there is any difference in the duration of infection for patients infected with genotypes B and C. In the present study, there was no difference in the probability or the severity of exacerbations of disease, graded according to the ALT levels at presentation, for patients infected with genotypes B and C; this was because the prevalence of genotypes B and C was similar in all 4 groups. Though group I patients had a higher prevalence of genotype B (46.3%), compared with the other 3 groups (range, 28.4–35.5%) (table 2), the difference was not statistically significant. However, when all the patients with exacerbations were grouped together (table 3), patients infected with genotype B had more severe exacerbations, compared with patients infected with genotype C, as reflected by the higher ALT, higher bilirubin, and lower albumin levels ($P = .047$, $P < .001$, and $P = .02$, respectively).

We also found that, when the exacerbations were severe, patients infected with genotype B had a higher risk of hepatic decompensation, compared with patients infected with genotype C. Furthermore, among patients who had hepatic decompensation caused by severe exacerbations, patients infected with genotype B had a higher mortality rate than did patients infected with genotype C . The higher rates of hepatic decompensation

Table 3. Differences in demographic and clinical data between patients infected with hepatitis B virus (HBV) genotype B and those infected with genotype C.

Variable	Patients infected with HBV		*P*
	Genotype B ($n = 63$)	Genotype C ($n = 112$)	
Median age (range), years	37.6 (17.8–68.28)	37.0 (18.5–67.93)	.19
Sex, no. male/no. female	53/10	75/37	.014
HBeAg/anti-HBe, n/n[a]	31/32	66/46	.21
Laboratory values, median (range)			
ALT, U/L	304 (71–3840)	229 (69–2420)	.047
Albumin, g/L	41 (19–51)	43 (24–55)	.02
Bilirubin, μmol/L	23 (5–801)	13 (3–810)	<.001
HBV DNA, copies $\times 10^6$/mL	1.6 (<0.14–1261.5)	3.7 (<0.14–1380.2)	.61

NOTE. ALT, alanine aminotransferase.

[a] No. of patients testing positive for hepatitis B e antigen/no. of patients testing positive for antibody to hepatitis B e antigen.

Table 4. Prevalence of genotype B among and liver biochemistry data for 73 patients with severe exacerbations of hepatitis B virus (HBV) infection with and without hepatic decompensation.

Variable	Patients with hepatic decompensation (n = 30)	Patients without hepatic decompensation (n = 43)	P
HBV genotype B, n/N (%)[a]	20/28 (71.4)	11/39 (28.2)	.0001[b]
Median albumin level (range), g/L	32.5 (19–49)	44 (29–51)	<.001
Median bilirubin level (range), μmol/L	224.5 (17–740)	16 (6–583)	<.001

NOTE. "Severe exacerbation" was defined as an increase of alanine aminotransferase (ALT) levels to >10 times the upper limit of normal.

[a] No. of patients with HBV genotype B infection/no. for whom HBV genotype information was known (%).

[b] OR, 6.4; 95% CI (2.2–18.7).

and mortality among patients infected with genotype B compared with patients infected with genotype C suggests that HBV genotype B may be more immunogenic and hence cause more severe immune-system–mediated damage. Studies have shown that patients infected with genotype B have earlier HBeAg seroconversion, compared with patients with genotype C [8, 9]. These studies suggest that the immune-system–mediated attack during the immunoclearance phase may be more pronounced and, hence, associated with a higher rate of HBeAg seroconversion in patients infected with genotype B. Immunological studies examining the cytotoxic T lymphocyte responses against the hepatitis B core antigen and HBeAg in patients infected with genotypes B and C are necessary to prove this hypothesis. With reference to clinical symptoms, a longitudinal study measuring the ALT and HBV DNA levels and monitoring the duration of exacerbations is also required.

Infection with genotype B was associated with precore mutations and infection with genotype C was associated with core promoter mutations. This finding is similar to the findings of our previous report, as well as the findings of studies by other groups [9, 13]. It would be interesting to examine whether the association between genotype B infection and precore mutations contributes to the adverse outcome for patients with severe exacerbations of disease. Unfortunately, among the 31 patients in the present study infected with genotype B infection who had severe exacerbations, only 4 patients (12.9%) had HBV with a wild-type precore region (table 2).

In conclusion, the present study suggests that patients with HBV genotype B infection had more severe exacerbations of disease and a higher risk of hepatic decompensation and mortality due to severe exacerbations, compared with patients with HBV genotype C infections. Previous longitudinal studies of acute exacerbations in patients with chronic HBV infection have demonstrated convincingly that acute exacerbations are usually not associated with infection with viral genotypes other than the original genotype [14, 15]. Further longitudinal studies should be designed to follow up a large population of patients with CHB and define the impact of the difference in exacerbations of disease among patients infected with genotypes B and C on the progression of the disease and the development of complications.

References

1. Lai CL. Chronic hepatitis B in Hong Kong: immunization strategies for the control of hepatitis B virus infection. In: Zuckerman A, ed. Hepatitis B in the asian-pacific region: screening, diagnosis, and control. Vol. 1. London: Royal College of Physicians of London, **1997**:79–87.
2. Tsubota A, Arase Y, Ren F, Tanaka H, Ikeda K, Kumada H. Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. J Med Virol **2001**; 65:257–65.
3. Sugauchi F, Orito E, Ichida T, et al. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J Virol **2002**; 76:5985–92.
4. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology **2000**; 118:554–9.
5. Ding X, Mizokami M, Ge X, et al. Different hepatitis B virus genotype distributions among asymptomatic carriers and patients with liver diseases in Nanning, southern China. Hepatol Res **2002**; 22:37–44.
6. Shina S, Fujino H, Uta Y, et al. Relationship of HBsAg subtypes with HBeAg/anti-HBe status and chronic liver disease Part I: analysis of 1744 HBsAg carriers. Am J Gastroenterol **1991**; 86:866–71.
7. Kobayashi M, Arase Y, Ikeda K, et al. Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. J Gastroenterol **2002**; 37:35–9.
8. 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**; 122:1756–62.
9. Yuen MF, Sablon E, Yuan HY, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirhosis-related complications, and hepatocellular carcinoma. Hepatology **2003**; 37:562–7.
10. 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.
11. Wai CY, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with a higher response rate to interferon therapy than genotype C. Hepatology **2002**; 36:1425–30.
12. Yuen MF, Sablon E, Yuan HJ, et al. The relationship between the

development of precore and core promoter mutations and HBeAg seroconversion in chronic hepatitis B. J Infect Dis **2002**; 186:1335–8.
13. Orito E, Mizokami M, Sakugawa H, et al. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotype B and C. Japan HBV Genotype Research Group. Hepatology **2001**; 33:218–23.
14. Kao JH, Chen PJ, Lai MY, Chen DS. Acute exacerbations of chronic hepatitis B are rarely associated with superinfection of hepatitis B virus. Hepatology **2001**; 34:817–23.
15. Liu CJ, Chen PJ, Lai MY, et al.. A prospective study characterizing full-length hepatitis B virus genomes during acute exacerbation. Gastroenterology **2003**; 124:80–90.

ERRATUM

An error appeared in a Brief Report published in the 15 August 2003 issue of the journal (Yuen MF, Sablon E, Wong DKH, Yuan HJ, Wong BCY, Chan AOO, Lai CL. Role of hepatitis B virus genotypes in chronic hepatitis B exacerbation. Clin Infect Dis 2003; 37:593–7). In the last paragraph of Results, after the first sentence, it should read, "Single infection with genotype B was found in 18 patients, and single infection with genotype C was found in 10 patients. The remaining 2 patients had coinfection with genotypes A and C and genotypes A and B. There was no significant difference in the mortality rate due to hepatic decompensation caused by severe exacerbation between patients with genotype B and C (7 [38.9%] of 18 vs. 5 [50%] of 10, respectively; $P = .87$)" (*not* "Single infection with genotype B was found in 10 patients and single infection with genotype C was found in 18 patients. The remaining 2 patients had coinfection with genotypes A and C and genotypes A and B. Patients infected with genotype B had a higher mortality due to hepatic decompensation caused by severe exacerbations (7 [70%] of 10), compared with patients infected with genotype C (5 [27.8%] of 18; OR 6.1; 95% CI, 1.1–33.4; $P = .05$"). The authors regret this error.

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