Prevalence of hepatitis B virus genotype B in Vietnamese patients with chronic hepatitis B

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Abstract

Purpose Hepatitis B virus (HBV) genotypes can affect treatment response to interferon-based therapy and disease outcomes in patients with chronic hepatitis B (CHB). Little data exist to characterize HBV genotypes in Vietnamese, one of the largest minority groups in the United States and also one with one of the highest CHB and liver cancer disease burdens. The goal of this study was to compare the distribution of HBV genotypes in Vietnamese and Chinese patients.

Methods We performed a cross-sectional study of 567 consecutive patients of Vietnamese ($n = 478$) or Chinese ($n = 89$) descent, with HBV genotype mutation analysis performed between 7/2,005 and 6/2,008 at a community gastroenterology clinic and a university-affiliated liver clinic in the United States.

Results There were no significant differences between the Vietnamese and Chinese groups in mean age (45 and 44 years), gender (58% and 61% male), HBeAg status (64% and 65% negative), median alanine aminotransferase (33 and 41 U/L), and $\log_{10}$ HBV DNA (4.9 and 5.0

$\log_{10}$ IU/ml), or the prevalence of precore/basic core promoter mutations (72% and 71%), respectively. Vietnamese patients had a much higher prevalence of HBV genotype B and a lower prevalence of genotype C than Chinese patients: 74% and 25% vs. 55% and 43% ($P = 0.001$).

Conclusions Chinese patients with CHB often carry either B or C genotype. Vietnamese patients with CHB mostly have HBV genotype B. Additional studies are needed to further characterize the clinical significance of HBV genotype in the natural history and treatment outcomes of CHB in Vietnamese patients.

Keywords Epidemiology · Asian · HBV

Introduction

Hepatitis B virus (HBV) infects more than 350 million people worldwide and 1.25 million individuals in the United States alone, and chronic hepatitis B (CHB) is one of the major causes of chronic liver diseases leading to cirrhosis [1, 2]. In addition, the number of those infected with HBV in the United States may be both underreported and on the rise. In recent years, the number of immigrants from the Asia/Pacific region, an area in which HBV is endemic, has increased dramatically to approximately 14 million individuals [3, 4].

Among the diverse subgroups of Asian Americans and Pacific Islanders (AAPI), Vietnamese men exhibit hepatocellular carcinoma (HCC) mortality that is almost two times higher than Chinese men (and almost seven times higher than their non-Hispanic white counterparts), whereas globally, Chinese individuals account for nearly 55% of all HCC cases.

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HBV genotypes A–H have distinct geographic distributions, and their clinical significance continues to unfold [5–20]. Recent literature has linked HBV genotypes to the amount of time needed to clear HBeAg, with genotypes C and F associated with delayed seroconversion, exposing patients to longer periods of active viral replication and liver inflammation, as indicated by high alanine aminotransferase (ALT) levels and a higher risk for cirrhosis and HCC [14, 17, 18]. Patients with HBV genotype B, in addition to achieving seroconversion earlier than their genotype C counterparts, do so at much higher rates [15].

Other studies have associated genotypes B, C, and D with a higher prevalence of mutations in the precore and basic core promoter (BCP) regions and a greater risk for hepatitis B e antigen (HBeAg) relapse postseroconversion, both of which prolong viral activity and increase the potential for permanent liver damage [12]. Genotype B has also been associated with a greater T-helper cell immune response than with genotype C [19]. In addition, multiple studies suggest that for interferon users, HBeAg loss is lower in genotype C than genotype B, further strengthening the link between HBV genotype and clinical outcomes [13, 16].

The aim of this study was to evaluate the HBV genotype distribution in a large population of Chinese and Vietnamese patients with CHB in northern California.

Methods

Study design and data collection

We queried and compiled a list of 2,071 patients with CHB seen at a community gastroenterology clinic and a university liver clinic from June 2005 to June 2008. A total of 567 patients completed the HBV genotyping and mutation analysis test during the specified study period. Patients were identified by their physicians or self as either Chinese or Vietnamese.

Both study sites serve a large Asian American patient population of the San Francisco Bay area, particularly Vietnamese Americans. The Western Institutional Review Board (Olympia, WA) and the Stanford Institutional Review Board (Stanford, CA) approved the study protocol.

The medical records of all study patients were reviewed in their entirety. A case report form was created and used for data abstraction. All data were entered into a Microsoft Access 2003 database (Redmond, WA) to be exported into statistical software.

Laboratory tests, including quantitative serum HBV DNA polymerase chain reaction (PCR) and HBV DNA genotyping with mutation analysis, were performed at local community clinical laboratories operated by either Quest

Diagnostics (San Juan Capistrano, CA) or Stanford Medical Center Laboratories (Palo Alto, CA).

HBV genotyping was performed with the Nichols Institute “Hepatitis B Virus Drug Resistance, Genotype, and BCP/Precore Mutations Test” (test code 10529x), a direct sequencing PCR test.

Liver biopsies were obtained via percutaneous techniques and histologic examinations were evaluated with the Batts-Ludwig scoring system at a single pathology laboratory.

Statistical analysis

Various patient clinical and viral characteristics were collected and analyzed including the prevalence of precore and BCP mutations by genotype and by ethnicity. Categorical variables were evaluated with χ^2 tests. The Student *t* test was applied to normally distributed noncategorical variables, whereas nonparametric statistics were applied to all others. Differences with a two-tailed $P \leq 0.05$ were considered significant. A Bonferroni correction was applied when multiple tests were conducted on a set of data to account for the increased possibility of chance results.

We determined odds ratios (OR) and their 95% confidence intervals (CI) relating HBV genotype to sex, age, ethnicity, HBeAg status, and HBV DNA level at genotyping using both uncontrolled univariate logistic regression and adjusted multivariate logistic regression.

All statistical analysis was performed with Stata 10.0 (StataCorp LP, College Station, TX).

Results

HBV genotypes for Chinese and Vietnamese patients

The study population’s HBV genotype distribution was overwhelmingly skewed toward genotypes B and C, accounting for 97% of the viral genotypes. Genotype A or D was found in only 5 patients (3%).

Vietnamese patients had a much higher prevalence of genotype B and a lower prevalence of genotype C than Chinese patients: 74% and 25% vs. 55% and 43% ($P = 0.001$).

When the small number of patients with genotype A or D was removed from study analysis, a Bonferroni correction was applied to ensure the power of statistical significance remained intact. Figure 1 shows the HBV genotype distribution by ethnicity, with the lowered $P \leq 0.025$ threshold.

Patient characteristics by ethnicity and HBV genotypes

A total of 567 consecutive Vietnamese or Chinese patients with CHB, with HBV genotyping performed between June

Fig. 1 Hepatitis B viral genotype distribution by ethnicity. * $P = 0.001$

2005 and June 2008, were included in the data analysis. From this group, 478 (85%) patients were Vietnamese and 89 (15%) were Chinese. This proportion of Vietnamese to Chinese patients is similar to what was seen in the 2,071 HBV-infected patients screened for study inclusion (Vietnamese: 81%, Chinese: 19%). All 567 were included in the study analysis. The patient clinical characteristics of the study population are shown in Table 1.

The Chinese and Vietnamese patient subgroups had very few statistically significant differences in their demographic characteristics (Table 1). Both patient populations had a similar proportion of males (58% for Vietnamese and 61% for Chinese, $P = 0.66$) and a similar mean age (45 ± 13 for the Vietnamese vs. 44 ± 14 years for the Chinese, $P = 0.62$).

Both the Vietnamese and Chinese groups had a comparable proportion of patients with HBeAg negative status (64% vs. 65%, $P = 0.86$), similar HBV DNA levels at genotyping (4.9 ± 1.8 IU/ml for the Vietnamese vs. 5.0 ± 1.7 log IU/ml for the Chinese, $P = 0.78$), and a comparable number of patients with precore and/or BCP HBV mutations (72% vs. 71%, $P = 0.82$). Vietnamese and Chinese patients also had similar median ALT levels at the time of genotyping (33 vs. 41 U/L, $P = 0.74$) and a statistically insignificant difference in the number of patients with advanced liver disease (stage 3–4 fibrosis; 8% vs. 15%, $P = 0.54$). No cases of HCC were found in the study participants at the time of mutation analysis.

Vietnamese patients reported fewer cases of family history of HBV, HCC, liver disease, and/or liver-related death than reported by Chinese patients, though this finding did not reach statistical significance (28% vs. 35%, $P = 0.20$).

When we compared the Chinese and Vietnamese subgroups with the same HBV genotypes, we found no

significant differences between Vietnamese patients with genotype B and Chinese patients with genotype B in the characteristics already detailed in Table 1, including gender distribution, mean age, HBeAg negativity, median ALT, mean HBV DNA, and the presence of either precore or BCP mutations. When patients with genotype C were stratified by Vietnamese and Chinese ethnicity, again there were no significant differences in patient characteristics other than the lower proportion of Vietnamese patients with genotype C having a lower rate of combined precore and BCP mutations (7% vs. 18%, $P = 0.03$).

Clinical and virologic characteristics by genotypes and severity of liver disease

When those who had completed the mutation analysis were stratified according to HBV genotypes B and C, several differences in patient characteristics emerge: those with genotype B were older at the time of testing (46 ± 14 vs. 43 ± 12 years of age, $P = 0.03$; Table 2), were much more likely to be HBeAg negative (71% vs. 47%, $P < 0.001$). In addition to having lower median HBV DNA PCR, genotype B patients were more likely to have precore mutations alone or precore plus BCP mutations (45% vs. 3%, $P < 0.001$ and 20% vs. 9%, $P = 0.003$, respectively), whereas genotype C patients were more likely to have BCP mutations alone (54% vs. 9%, $P < 0.001$).

In the 96 patients who had liver biopsies, various viral characteristics, including HBV genotype, HBV DNA, and the presence of precore and/or BCP mutations, were analyzed according to the severity of their liver disease with significant disease defined as stage 2 fibrosis or greater or stage 1 fibrosis plus grade 2 inflammation or greater. There was no exhibited difference in viral characteristics between those with significant disease and those without.

Prevalence of precore and BCP mutations by genotype

Of the patients with either precore or BCP mutations, those with HBV genotype B tended to have only precore mutations, as opposed to only BCP mutations (61% vs. 12%), and 27% of these genotype B patients had both precore and BCP mutations. HBV genotype C patients with confirmed precore or BCP mutations exhibited the opposite trend. Patients with genotype C were much more likely to have only BCP mutations than only precore mutations (82% vs. 4%), and 14% of the genotype C patients with confirmed precore or BCP mutations had both. The statistical significance threshold for this data set was again $P < 0.025$ due to the omission of CHB patients with genotype A or D. Figure 2 illustrates these relationships.

When we compared the two ethnic subgroups, no significant difference was found in the prevalence of specific

Table 1 Patient characteristics by ethnicity

	All patients (<i>n</i> = 567)	Vietnamese patients (<i>n</i> = 478)	Chinese patients (<i>n</i> = 89)	<i>P</i>
Male	332 (59%)	278 (58%)	54 (61%)	0.66
Age, mean $\pm$ SD	45 $\pm$ 13	45 $\pm$ 13	44 $\pm$ 14	0.62
HBeAg negative	364 (64%)	307 (64%)	58 (65%)	0.86
Median ALT (5%–95% range)	34 (13–165)	33 (12–164)	41 (15–231)	0.74
Mean HBV DNA (log ₁₀ IU/ml)	4.9 $\pm$ 1.8	4.9 $\pm$ 1.8	5.0 $\pm$ 1.7	0.78
HBV genotype				0.001
A	1 (1%)	1 (1%)	0 (0%)	
B	404 (70%)	355 (74%)	49 (55%)	
C	158 (28%)	120 (24%)	38 (43%)	
D	4 (1%)	2 (1%)	2 (2%)	
Any PreC/BCP mutations	407 (72%)	344 (72%)	63 (71%)	0.82
PreC only	186 (33%)	164 (34%)	22 (25%)	0.08
BCP only	124 (22%)	98 (21%)	26 (29%)	0.07
PreC and BCP	96 (17%)	82 (17%)	15 (17%)	0.95
No PreC or BCP	160 (28%)	134 (28%)	26 (29%)	0.82
Biopsy result (<i>n</i> = 96)				
Grade				0.40
1	67 (70%)	60 (72%)	7 (54%)	
2	24 (25%)	19 (23%)	5 (38%)	
3–4	5 (5%)	4 (5%)	1 (8%)	
Stage				0.54
1	64 (67%)	57 (69%)	7 (54%)	
2	23 (24%)	19 (23%)	4 (31%)	
3–4	9 (9%)	7 (8%)	2 (15%)	
Family history				
HBV	105 (19%)	81 (17%)	24 (27%)	0.03
HCC	55 (10%)	44 (9%)	11 (12%)	0.60
Liver disease	29% (5%)	24 (5%)	5 (6%)	0.82
Liver-related death	55 (10%)	45 (9%)	10 (11%)	0.60
Any of the above	165 (29%)	134 (28%)	31 (35%)	0.20

Abbreviations: SD standard deviation, HBeAg hepatitis B e antigen, ALT alanine aminotransferase, HBV DNA hepatitis B viral deoxyribonucleic acid, PreC/BCP precore/basic core promoter region, HCC hepatocellular cancer

base pair mutations in BCP regions. However, when stratified by genotype, genotype B tended to have fewer instances of the A1762 mutation with G1764 than genotype C (80% vs. 92%) and more instances of the A1762 substitution with no other BCP mutations (11% vs. 4%) ($P = 0.03$; Table 3). All patients with confirmed precore mutations had a substitution at the G1896A base pair.

Predictors of HBV genotype C

Unadjusted univariate and adjusted multivariate logistic regression analyses were performed for the genotype B or C subset to determine whether or not gender, age, ethnicity, HBeAg status, and/or HBV DNA level at genotyping were independent predictors of HBV genotype.

Chinese ethnicity and HBeAg negative status each independently predicted HBV genotype C after adjusting for gender, age, and HBV DNA level ($P \leq 0.001$ and $P \leq 0.001$, respectively; Table 4).

Discussion

We found that Vietnamese patients with CHB were nearly three times more likely to carry HBV genotype B than genotype C, whereas the Chinese were nearly as likely to have genotype C as were genotype B.

The nearly 3:1 genotype B to C distribution in our Vietnamese patients is comparable with what was found in prior smaller studies or studies of patients with primarily acute hepatitis [21, 22].

Table 2 Patient characteristics by HBV genotypes

Patient characteristics	All patients (<i>n</i> = 567)	HBV genotype B patients (<i>n</i> = 404)	HBV genotype C patients (<i>n</i> = 158)	<i>P</i>
Male	332 (59%)	242 (60%)	86 (54%)	0.24
Age, mean $\pm$ SD	45 $\pm$ 13	46 $\pm$ 14	43 $\pm$ 12	0.03
HBeAg negative	364 (64%)	286 (71%)	75 (47%)	<0.001
Median ALT (5%–95% range)	34 (13–165)	34 (13–156)	38 (11–197)	0.86
Mean HBV DNA (log ₁₀ IU/ml)	4.9 $\pm$ 1.8	5.0 $\pm$ 1.8	5.5 $\pm$ 1.9	0.006
Any PreC/BCP mutations	407 (72%)	299 (74%)	105 (66%)	0.07
PreC only	186 (33%)	181 (45%)	4 (3%)	<0.001
BCP only	124 (22%)	37 (9%)	86 (54%)	<0.001
PreC and BCP	96 (17%)	81 (20%)	15 (9%)	0.003
No PreC or BCP	160 (28%)	105 (26%)	53 (34%)	0.07
Biopsy result (<i>n</i> = 96)				
Grade				0.31
1	67 (70%)	45 (69%)	22 (71%)	
2	24 (25%)	18 (28%)	6 (19%)	
3–4	5 (5%)	2 (3%)	3 (10%)	
Stage				0.16
1	64 (67%)	47 (72%)	17 (55%)	
2	23 (24%)	14 (22%)	9 (29%)	
3–4	9 (9%)	4 (6%)	5 (16%)	
Family history				
HBV	105 (19%)	73 (18%)	32 (20%)	0.55
HCC	55 (10%)	36 (9%)	19 (12%)	0.27
Liver disease	29 (5%)	17 (4%)	12 (8%)	0.01
Liver-related death	55 (10%)	38 (9%)	17 (11%)	0.63
Any of the above	165 (29%)	115 (29%)	50 (32%)	0.44

Abbreviations: SD standard deviation, HBeAg hepatitis B e antigen, ALT alanine aminotransferase, HBV DNA hepatitis B viral deoxyribonucleic acid, PreC/BCP precore/basic core promoter region, HCC hepatocellular cancer

Fig. 2 Prevalence of precore and/or basic core promoter viral mutations by genotype. † PreC = precore region; ‡ BCP = basic core promoter region. * *P* = 0.001

Table 3 Basic core promoter point mutation distribution by ethnicity and genotype

Ethnicity or HBV genotype	A1762T/A/G	G1764A/G	A1762T/A/G + G1764A/G	<i>P</i>
Vietnamese	15 (8%)	12 (7%)	153 (85%)	0.62
Chinese	2 (5%)	4 (10%)	35 (85%)	
Genotype B	13 (11%)	11 (9%)	94 (80%)	0.03
Genotype C	4 (4%)	4 (4%)	93 (92%)	

Our study also showed a significant difference in the distribution of HBV viral mutations between genotypes: CHB patients with genotype B tended to have only precore mutations, whereas those with genotype C tended to have only BCP mutations. There was also a statistically significant difference in the specific base pair location of these BCP mutations between genotypes. This supports the findings of several other studies that claim a higher prevalence of precore mutations for patients with HBV

Table 4 Predictors of hepatitis B viral genotype C

Predictors	Unadjusted OR (95% CI)	<i>P</i>	Adjusted OR (95% CI) ^a	<i>P</i>
Gender		0.24		0.26
Female	1.0		1.0	
Male	0.8 (0.6–1.2)		0.8 (0.5–1.2)	
Age	1.0 (1.0–1.0)	0.04	1.0 (1.0–1.0)	0.43
Ethnicity		0.001		<0.001
Vietnamese	1.0		1.0	
Chinese	2.3 (1.4–3.7)		2.5 (1.5–4.2)	
HBeAg status		<0.001		<0.001
Positive	1.0		1.0	
Negative	0.4 (0.3–0.5)		0.3 (0.2–0.5)	
HBV DNA (log ₁₀ IU/ml)	1.2 (1.0–1.3)	0.007	1.0 (0.9–1.1)	0.97

Abbreviations: OR odds ratio, CI confidence interval, HBeAg hepatitis B e antigen, HBV DNA hepatitis B viral deoxyribonucleic acid

^a Adjusted for gender, age, ethnicity, HBeAg status, and HBV DNA level

genotype B than genotype C and a higher rate of BCP mutations in patients with HBV genotype C than genotype B [22, 23].

To our knowledge, this study is the largest comparing the distribution of HBV genotypes in Vietnamese and Chinese patients. In addition, the vast majority of our patients were neither cirrhotic nor did they have cancer at the time of study analysis, a sample that is likely more representative of the general CHB patient population as a whole. This is in contrast to other studies, which tend to include a higher proportion of patients with more advanced liver disease.

We believe this helps explain the dramatic discrepancy between our findings and those of Toan et al. [24]. Toan's group found a nearly 3:1 ratio of genotype C to genotype B among Vietnamese hepatitis B patients, whereas our study revealed a nearly 3:1 ratio of genotype B to genotype C. The study patients in the study of Toan had much more severe liver diseases and were unlikely representative of the average Vietnamese patient with CHB. Of the 375 Vietnamese patients included in this study, only 86 were asymptomatic and 289 were symptomatic with liver cancer, liver cirrhosis, acute hepatitis, and renal failure requiring hemodialysis whereas the vast majority of our patients were asymptomatic, with only four cases of known stage 3–4 fibrosis and no liver cancer. This selection bias could lead to the high prevalence of genotype C seen in their study that is unlikely to be generalizable to the general Vietnamese population with CHB. Our patient population is likely more representative of the general Vietnamese patient population with CHB. Our findings are similar to results published by Thuy Le et al. [25], who showed a 63% to 18% distribution between HBV genotypes B and C. In this smaller study, Thuy Le et al. characterized a total of 34 patients with CHB and only 4 with acute hepatitis B and 2 with HCC.

Our study has some limitations. The HBV mutation analysis assay performed by Quest Diagnostics did not

provide information on HBV subgenotypes. Also, although our study includes a large sample of patients, it is still clinic based and not population based. In addition, the Chinese CHB patients included in the study comprise only 15% of our sample population, with the remainder being Vietnamese. In addition, our study could not distinguish patients from Taiwan with those from mainland China. Although they may share similar ethnic origins, several studies have shown that their respective HBV genotype distributions are very different [9, 14–16, 26–28]. It is worth noting, however, that the distribution of HBV genotypes in our Chinese group is fairly similar to the findings of several other studies of mainland Chinese patients [27, 28].

With growing evidence suggesting that HBV viral genotype C can lead to a more hastened disease progression and worse clinical outcomes, especially when compared with genotype B, our findings may indicate that the much higher observed incidence of HCC in the Vietnamese population previously reported by the California Cancer Registry is likely due to factors other than HBV genotype. Further studies are needed to characterize CHB in Vietnamese patients.

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