Int J Mol Epidemiol Genet 2020;11(2):26-30
www.ijmeg.org /ISSN:1948-1756/IJMEG0115593

Original Article

Association of hepatitis B genotypes with clinical profile of patients with chronic hepatitis B

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Received June 4, 2020; Accepted October 12, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Hepatitis B virus (HBV) infection is a common cause of chronic liver disease and is responsible for HBV-related deaths due to cirrhosis and HCC. It is well recognized that viral genotypes play an important role on the outcome of HBV infection. Ten HBV genotypes have been identified and the prevalence varies geographically. A hospital-based cross-sectional study was conducted to determine the association of HBV genotypes with the clinical profile of CHB patients. PCR-RFLP was performed to identify HBV genotypes. In this study, majority (70%) of patients were males; with ages between 22 to 67 years with a mean of 42.5 years. The ALT ranged from 23 to 111 U/L (mean 72.5 U/L). HBV DNA levels varied from less than 6 to more than 110,000,000 IU/ml. Forty-seven percent of the patients had chronic active hepatitis at the time of diagnosis. Of these, 36% were HBeAg positive while 64% were HBeAg negative. Inactive HBsAg carrier was found in 53% of cases. No significant association was established between HBV genotypes and fibrosis. PCR-RFLP analysis showed that 57%, 10%, and 13% of the samples belonged to HBV/A, HBV/B, and HBV/C, respectively and the remaining 20% had non-detectable HBV genotype. HBV/D to HBV/J were not observed in this study. Taken together, the patient's clinical profile such as sex, ALT levels, HBeAg status, HBV DNA levels and liver histology were not found to be significantly associated with HBV genotypes. A large-scale longitudinal study examining multiple HBV strains are needed to determine significant correlation of clinical profile.

Keywords: Chronic active hepatitis, clinical profile, HBV genotypes, inactive HBsAg carrier

Introduction

Hepatitis B virus (HBV) infection remains to be a major health problem worldwide and is responsible for approximately 500,000 deaths annually due to HBV-related liver cirrhosis and hepatocellular carcinoma (HCC). In the Philippines, the HBsAg seroprevalence among adults was 16.7%, corresponding to approximately 7.3 million individuals. As of 2016, it has been reported that the factors underlying the high seroprevalence of chronic hepatitis B (CHB) include inadequate use of vaccination and the lack of treatment for many Filipinos [1, 2].

The incidence rates of developing HBV-related liver cirrhosis and HCC vary worldwide. Host and viral factors have been identified that could influence the rate of severe complications resulting from HBV infection which include but not limited to age, sex (with higher rates of HCC in men), ethnicity, alcohol consumption, HBV DNA levels, and viral genotypes [3-5].

From an epidemiological point of view, HBV genotyping is relevant to better understand the source of HBV infection in various countries including the Philippines. Here, we aimed to determine the association of HBV genotypes with the clinical, virological, and histopathological profile of CHB patients such as sex, ALT levels, HBeAg status, HBV DNA levels, Metavir score, Knodell Histology Activity Index, and stage of fibrosis.

Methods

Patients and samples

A total of 30 stored plasma obtained from patients clinically diagnosed and biopsy-pro-

Table 1. Restriction digestion patterns of HBV-DNA positive samples

Genotypes	Fragment sizes obtained (bp)			
	*Bsr*I	*Sty*I	*Hpa*II	*Eae*I
HBV/A	300 and 285	585	585	485 and 100
HBV/B	459 and 126	585	-	-
HBV/C	585	332 and 253	-	-

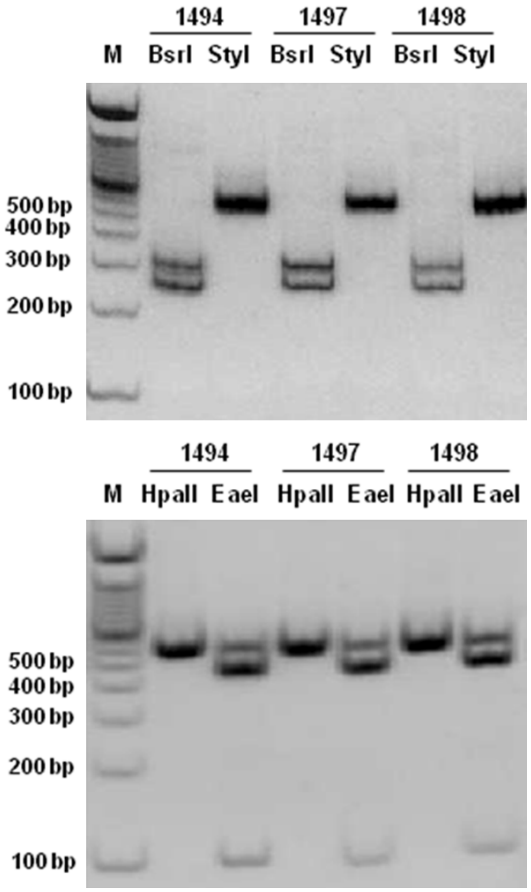

Figure 1. Ten percent polyacrylamide gels showing HBV/A genotype. M: molecular weight marker.

ven CHB at St. Luke's Medical Center-Quezon City were retrospectively analyzed. Chronic active hepatitis (CAH) was defined as HBsAg positive and HBV DNA levels of more than 20,000 IU/ml if HBeAg positive or HBV DNA is between 2,000 to 20,000 IU/ml if HBeAg negative. Inactive HBsAg carrier was defined as HBeAg negative, Anti-HBe positive with HBV DNA level of less than 2,000 IU/ml [6]. Patients who were co-infected with hepatitis C virus were excluded. The study was approved by the Institutional Ethics Committee of St. Luke's Medical Center (Ethics Review Board Reference No. 12-002). The study participants gave written informed consent prior to enrollment to this study.

HBV DNA extraction and genotyping by PCR-RFLP

Viral nucleic acid was extracted from stored plasma using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). HBV DNA amplification of the surface (S) gene was done as previously described [7]. Amplified products were digested with *Sty*I, *Dpn*I, *Hpa*II, and *Eae*I restriction enzymes (New England Biolabs). The restriction digestion patterns are shown in **Table 1**. The digested products were visualized under a UV transilluminator (**Figures 1** and **2**).

HBV DNA quantitation by real-time PCR and HBV serologic testing

Quantitation of HBV DNA levels and serological markers which include but not limited to HBsAg, HBeAg and AntiHBe were measured by standard laboratory procedures in the Institute of Pathology of St. Luke's Medical Center-Quezon City.

Liver histology

The Metavir scoring system and Knodell Histology Activity Index were used to assess the histopathological status of liver biopsies. The degree of fibrosis was graded as F0 to F4 by an experienced pathologist.

Statistical analysis

The distribution of the different HBV genotypes and the qualitative clinical, virological, and histopathological parameters was done using frequency and percentages. The determination of the association between HBV genotypes and the clinical, virological, and histopathological parameters was analyzed using chi-square test. Continuous variables were expressed as mean ± SD. Statistical analysis was conducted using Statistical Package for Social Sciences. Statistical significance was determined at $P < .05$.

Results

Our results showed that majority (70%) of the CHB patients were males. The age range of

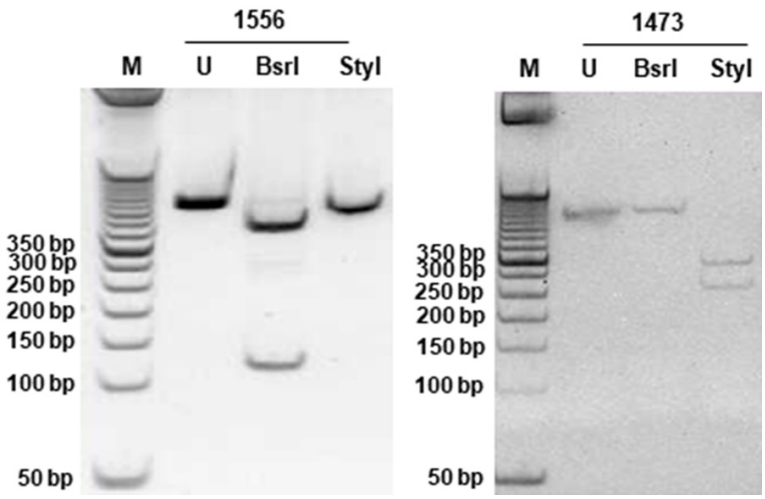

Figure 2. Ten percent polyacrylamide gels showing HBV/B and HBV/C genotypes. M: molecular weight marker; U: undigested PCR product.

Table 2. Clinical, virological, and histopathological profile of CHB patients

Parameter	HBV/A N (%)	HBV/B N (%)	HBV/C N (%)	Non-detectable genotype N (%)	*p* value
Sex					0.077
Male	12 (71)	3 (100)	4 (100)	2 (33)	
Female	5 (29)	0 (0)	0 (0)	4 (67)	
ALT					0.566
> 50 U/L	7 (41)	1 (33)	3 (75)	2 (33)	
≤ 50 U/L	10 (59)	2 (67)	1 (25)	4 (67)	
HBeAg					0.151
Positive	2 (12)	1 (33)	2 (50)	0 (0)	
Negative	15 (88)	2 (67)	2 (50)	6 (100)	
HBV DNA					0.330
> 20,000 IU/ml	5 (29)	1 (33)	2 (50)	0 (0)	
≤ 20,000 IU/ml	12 (71)	2 (67)	2 (50)	6 (100)	
CAH					0.080
Yes	10 (59)	2 (67)	2 (50)	0 (0)	
No	7 (41)	1 (33)	2 (50)	6 (100)	
Metavir score					0.819
A1	11 (73)	2 (100)	2 (67)	4 (67)	
A2-A3	4 (27)	0 (0)	1 (33)	2 (33)	
Knodell HAI					0.920
< 2	4 (27)	1 (50)	1 (33)	2 (33)	
> 2	11 (73)	1 (50)	2 (67)	4 (67)	
Fibrosis stage					0.651
F0	9 (60)	2 (100)	2 (67)	3 (50)	
F1-F2	6 (40)	0 (0)	1 (33)	3 (50)	

HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; CAH, chronic active hepatitis; HAI, histology activity index.

CHB patients was 22 to 67 years with a mean of 42.5. The ALT levels ranged from 23 to 111 U/L with a mean of 72.5 U/L. The HBV DNA levels varied from less than 6 to more than 110,000,000 IU/ml. Forty-seven percent of the patients had chronic active hepatitis at the time of diagnosis. Of these, 36% were HBeAg positive while 64% were HBeAg negative. Inactive HBsAg carrier was found in 53% of cases. Out of the 30 patients, 13% were tested positive for both HBsAg and HBeAg with a viral load of more than 110,000,000 IU/ml. One patient had history of interferon-α treatment, 1 patient had received lamivudine, and 1 patient had received lamivudine for 12 months then shifted to entecavir.

Distribution of HBV genotypes in patients with CHB

Several methods have been used for HBV genotyping including restriction fragment length polymorphism. PCR-RFLP analysis showed that 57%, 10%, and 13% of the samples belonged to HBV/A, HBV/B, and HBV/C genotypes, respectively and the remaining 20% had non-detectable HBV genotype. HBV/D to HBV/J genotypes were not observed in this study (**Figures 1** and **2**).

Association of HBV genotypes with histopathological profile of CHB patients

The Knodell Histology Activity Index ranged from 2 to 14 with degree of fibrosis from F0 to F2. No significant association was established between HBV genotypes and fibrosis (**Table 2**).

Discussion

Accumulating evidence suggests that HBV genotypes are associated with clinical outcomes in

patients with CHB [3, 8]. Particularly, it has been reported that HBV/C is associated with more severe liver disease, while HBV/B may be associated with the development of hepatocellular carcinoma (HCC) in young Taiwanese patients [9]. In another study, it has been shown that HBV/B and HBV/C genotypes did not differ in the risk for subsequent development of HCC [10]. Based on the results of this study, no statistically significant association was established between HBV genotypes and the clinical, virological, and histopathological profile of patients with CHB. Our findings were similar with previous studies, which showed no difference between HBV genotypes with the outcome of liver damage [5, 11-12]. It is widely accepted that biopsy remains to be the gold standard for assessing the degree of liver injury such as inflammatory activity and the stage of fibrosis [13, 14]. However, biopsy is invasive and based on our results, the findings on liver biopsy did not correlate with the viral genotypes. Moreover, Galizzi and colleagues reported that CHB infection can present different outcomes according to immune and host genetic factors [4]. In addition, viral factors such as an elevated HBV DNA levels, HBV genotypes (HBV/C and HBV/B), as well as precore and core promoter mutations (A1762T and G1764A) also influence the outcome of infection [15-17]. Thus, it is likely that a combination of both host and viral factors among various populations contribute to differences in outcomes.

The distribution of HBV genotypes among Filipino patients with CHB was likewise investigated. Consistent with a previous study, HBV/A (57%), HBV/C (13%), and HBV/B (10%) were the predominant circulating HBV genotypes in the country [16]. In 2006, Sakamoto and colleagues reported that the proportion of HBV/B and HBV/C genotypes were higher in patients with cirrhosis and HCC as compared to HBV/A. Thus, it is likely that certain HBV genotypes or subgenotypes (HBV/C5 and HBV/B5) may increase the risk of HCC development.

It is noteworthy to mention that these findings have major implications for epidemiologic investigations such as monitoring the distribution of different HBV strains, and identifying risk factors for transmission [18, 19]. Additionally, this information on circulating HBV strains may prove to be a useful tool in shaping new health policies on the prevention of HBV infection, as well as formulating new guidelines for the management of CHB patients.

Interestingly, the HBV genotype was non-detectable in 20% (6/30) of cases. One of the possible reasons for this may be explained by the low or undetectable HBV DNA levels of these patients. In this study, 33% (2/6) of CHB patients with undetectable genotype had HBV DNA levels of less than 6 IU/ml with mean ALT of 53.5 U/L. The present study corroborates with a previous finding showing that 24% (12/50) of CHB patients had non-detectable HBV genotype [6]. Thus, if clinically indicated, follow-up testing for HBV DNA detection and quantitation should be done in one to two months.

Our study comes with limitations. First, although comparable with previous reports, the sample size is relatively small which may limit the ability to detect novel HBV strains [16, 20]. Second, the subtype classification of the samples cannot be determined by traditional RFLP method. Thus, sequence and phylogenetic analysis is recommended in future studies to identify HBV subgenotypes. In addition, full-length genome sequencing is recommended in detecting recombinant viruses, as well as minor variants [21-23]. Third, the retrospective research design will not allow correlation with disease progression to be drawn.

Taken together, the patient's clinical profile such as sex, ALT levels, HBeAg status, HBV DNA levels, and liver histology were not found to be significantly associated with HBV genotypes. A large-scale longitudinal study examining multiple HBV strains are needed to determine significant correlation of clinical profile.

Acknowledgements

This work was supported by a research grant to St. Luke's Liver Diseases Study Group (Project No. 12-002) through the Research and Biotechnology Group. The authors would like to thank Myko Tapia and Doris Aceberos for data encoding.

Disclosure of conflict of interest

None.

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