Chapter

Hepatitis B Genotyping and Clinical Implication

Damodar Paudel and Sushma Suvedi

Abstract

Hepatitis B is one of the killer diseases and distributed globally. Nepal sandwiched between India and China. China is the country with high prevalence of hepatitis B surface antigen (HBsAg) account 30% of the world's HBsAg carriers, and India which has intermediate HBsAg prevalence accounts 10% of the world's carriers. Nepal has a low prevalence (around 1%) of hepatitis B virus (HBV) infection in general population. A and D genotypes are more prevalent in India, while band C is in China. The survey done in 2012 elaborated the common genes that are A and D and recombinant C/D in Nepal, but the clinical consequences are unclear. The prevalence of hepatitis B is low in Nepal, but it is widely common in intravenous drug users, PLHA, and HCV positive. The implication of HBV genotyping has clinical implication for the treatment. Basically, response of peginterféron, and antiviral drugs (adefovir, lamivudine, telbivudine) in hepatitis B.

Keywords: hepatitis B, genotyping, distribution, clinical implication, management

1. Introduction

About 257 million people were infected chronically with hepatitis B (HBsAg positive), and 887,000 deaths were recorded in 2015 due to hepatitis B virus (HBV)-related liver diseases, mostly from complication with cirrhosis and hepatocellular carcinoma (HCC) indicating an urgent necessity for better ways to prevent such complication of HBV [1]. HBV infection is the tenth leading cause of death and main contributor of HCC, which is ranked the fifth leading cause of cancer in man [2, 3]. HBV can be differentiated from other hepatitis viruses by genotype and subgenotype. HBV sequence differs by >8% form other genotypes and 4–8% nucleotide differences for subgenotypes. Including newly identified genotypes I and J, there are 10 genotypes A–J. Some HBV genotypes are further classified into subgenotypes.

About 30 subgenotypes are available till date. Many studies have reported that different genotypes and subgenotypes show different geographical distribution and are related to disease progression, clinical progression, response to antiviral treatment, and prognosis [4].

As shown in **Figure 1**, genotype A is widespread in sub-Saharan Africa, Northern Europe, and Western Africa; genotypes B and C are common in Asia; genotype C is primarily observed in Southeast Asia and China; genotype D is dominant in Africa, Europe, Mediterranean countries, and India including Nepal. Genotype E is dominant in South Africa. As a minor genotype, genotype G is reported in France, Germany, and the United States; and genotype H is commonly encountered in

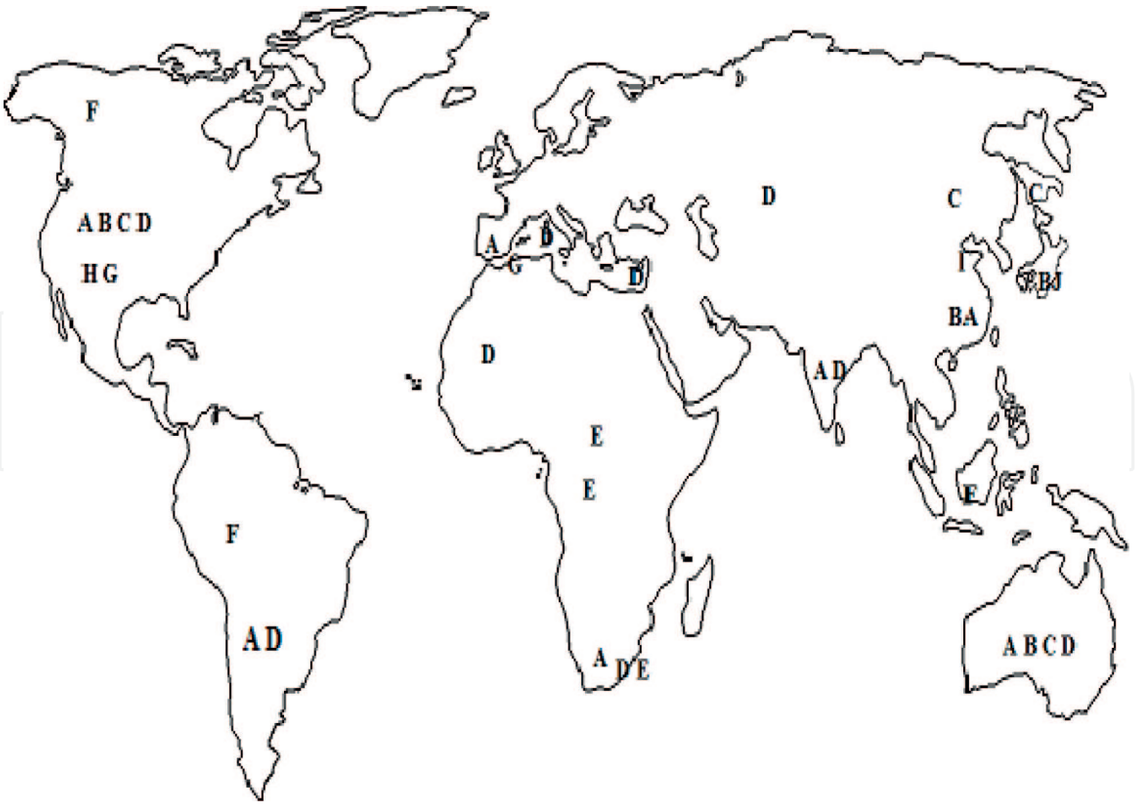

Figure 1.
Global distribution of HBV genotype.

Central and South America and genotype I in Vietnam and Laos. The newest HBV genotype, genotype J, has been identified in the Ryukyu Islands in Japan [5].

However, geographical distribution is still incomplete as data was based on very small numbers of patients and from only few countries.

Nepal is sandwiched between India and China, two countries with high prevalence of hepatitis B surface antigen (HBsAg) positive cases; 30% of the world's HBsAg carriers are in China and 10% in India. Nevertheless, Nepal has a low prevalence (around 1%) of hepatitis B virus (HBV) infection in general population [6]. Hepatitis virus A and D genotypes are dominant in India, while Band C is dominant in China. The prevalence of HBV infection is low in Nepal, but still it is widely common among intravenous drug users, PLHA, and HCV-positive populations. Although our previous study revealed only A, D, and C/D recombinant genotype [8], in the locality of Nepal, all four common genotypes (A, B, C, and D) were reported in the previous study [7].

Phylogenetic tree (**Figure 2**) was obtained from the aligned sequences combining with all human hepatitis B subgenotypes. The Nepal 10 strain was a C/D recombinant genotype, and it is differed from the genotypes A and D or other Nepalese strains. Genotype A1 in Nepal was more similar to that of Bangladesh and the Philippines, while A2 was more similar to the genotype A2 of Japan, Germany, Canada, and Russia. Genotype A1 (Nepal 31, 41, 24, 64, 50, 6, 9, 7, 47 strains) is similar to the A1 genotype of the Philippines, while strain 36, 16, and 18 are more similar to Bangladesh A1 strain. Almost all A1 strains of Nepal showed 88% similarity with Malawi A1 strain. Basically, HBV E genotype of Ghana and Sweden is nearby the genotype D of Nepal. Even some sequences from Germany and Italy are similar to the genotype D of Nepal.

Among genotype D, Nepal 12 and 55 isolates are similar with German D genotype isolates, while Nepal 60 and 57 isolates are more similar with D isolates of Italy and France and Russian isolates of D genotype. These isolates are 93% similar with Nepal 21 f isolates from where the strain Nepal 28 was evolved. Nepal 28 isolates of D genotype is evolved to more similar isolates 22, 32, and 14. Nepal isolates 40, 38

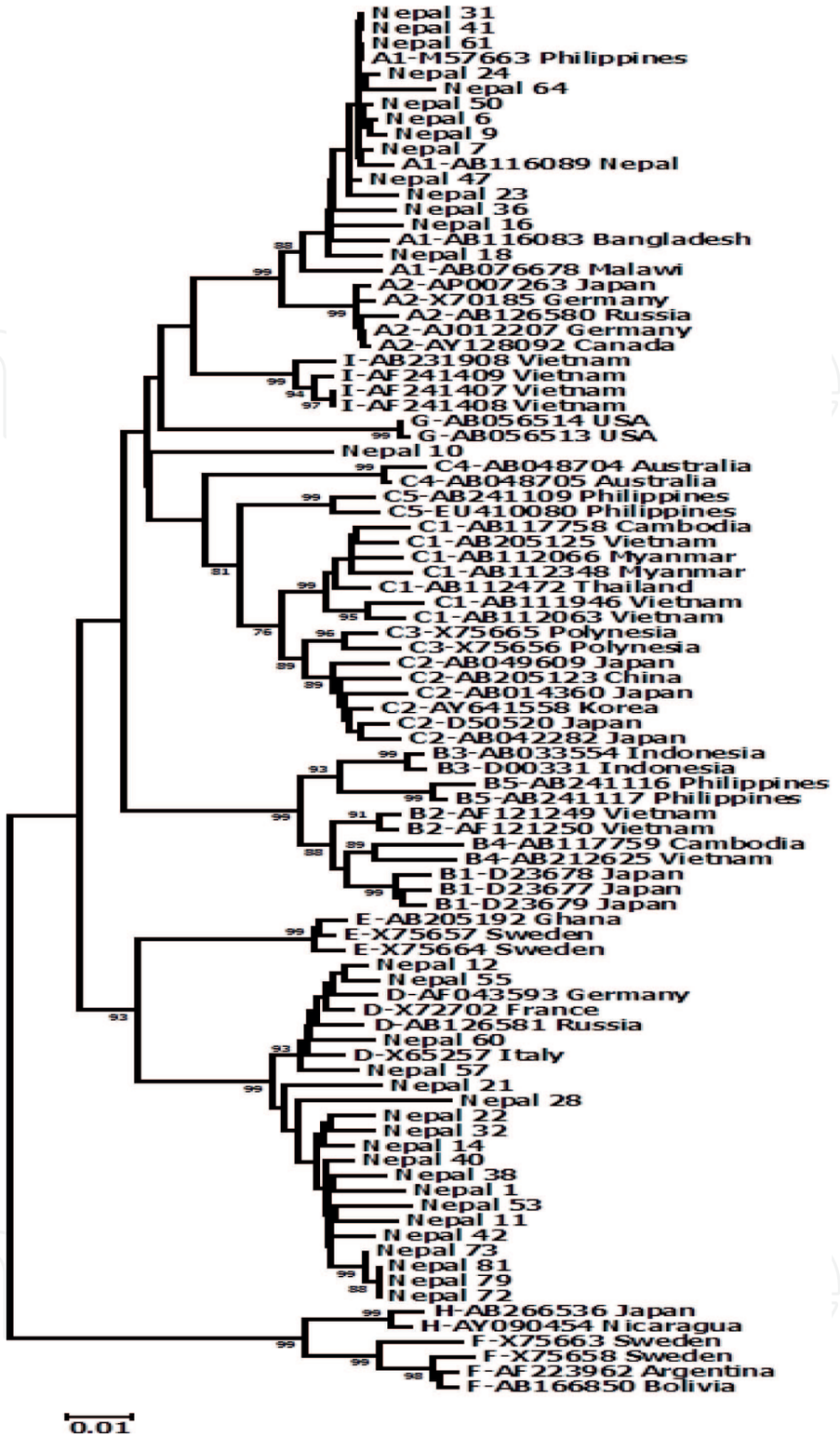

Figure 2.
Phylogenetic tree of Nepal study 2012.

1, 53, 11, 42, and 73 are more similar. Nepal 73 is more similar with 81, 79, and 72, while Nepal 81 and 79 is 88% similar with Nepal 72 isolates [8].

2. Clinical importance of HBV genotypes

As different regions have different serotypes and different clinical spectrum and different molecular epidemiological patterns, different HBV genotypes, but

not different rashes, may influence clinical outcome, HBeAg seroconversion rate, mutational patterns in the precore and core promoter regions, and response to interferon therapy [9].

Determination of genotypes: detection of the sequence differences in pre-S or S gene can be done by several methods, e.g., direct sequencing, restriction fragment length polymorphism, line probe assay, genotype-specific PCR, and mass spectrometry.

3. Treatment: noncirrhotic patients

Cirrhosis is defined as distortion of the hepatic architecture and the formation of regenerative nodules. It is generally irreversible in its late stage. It can progress even to hepatocellular carcinoma (HCC). The recommendation for the treatment initiation for cirrhotic patients are available as three different guidelines (EASL 2017, Asia pacific 2015, AASLD 2018) shown in **Table 1** [4, 10, 11].

All patients with compensated cirrhosis HBeAg positive and HBV DNA level >20,000 U/ml with increased ALT twice higher than the normal limit are recommended to be treated by all three regional guidelines. However, AASLD guideline recommends different upper limit of normal (ULN) of ALT in male and female, i.e., 35 and 25, respectively, although EASL and APSL guidelines recommend 40 as ULN of ALT. HBeAg-negative patients with cirrhosis and HBV DNA > 2000 U/ml and the ALT twice higher than normal or the family history of cirrhosis and HCC are also recommended for treatment. EASL recommended to treat patients if fibrosis is present [10–12]. Different guideline recommendations are shown in **Table 1**.

Guideline	HBeAg positive		HBeAg negative	ALT		Family history
	HBV DNA copies/ml	ALT		HBV DNA copies/ml		Cirrhosis, HCC
EASL2017	>20,000	Double (40)	30 years	>2000	Double + mod. fibrosis	Cirrhosis, HCC
Asia Pacific 2015	>2000	Double (40)		>2000	Double	
AASLD 2018	>2000	Double (m/F 35/25)	40 years	>2000	Double	Cirrhosis, HCC

Table 1.
Comparison of the recommendation of different guideline treatments of noncirrhotic HBV patients.

4. Treatment: cirrhotic patients

Patients with compensated cirrhosis and HBV DNA level >2000 U/ml are treated per recommendations for immune-active chronic hepatitis B (CHB). All three guidelines recommend to treat patients with decompensated cirrhosis and detectable viral load. In cases of compensated cirrhosis, there is discrepancy among the guidelines. In cases of HBV DNA <2000 copy/ml, AASLD and EASL both recommend to treat irrespective to ALT, whereas APSL recommended to such cases to treat only when ALT is above normal [10–12]. Treatment guideline is shown in **Table 2**.

Guideline	Compensated		Decompensated ALT	
	HBV DNA copies/ml	**ALT**	**HBV DNA copies/ml**	
EASL 2017	If >2000	Irrespective	Detectable	Irrespective
Asia Pacific 2015	>2000, if <2000		Detectable	Irrespective
ASLD 2018	2000	Irrespective	Detectable	Irrespective

Table 2.
Comparison of the recommendation of treatment of different guideline of cirrhotic HBV patients.

5. Clinical characteristics of different genotype are important

Genotype B is a common genotype in Asia. Some characteristics of genotype B are as follows [13–16]:

- Is commonly seen in young age less than 35 years as it is generally transmitted by perinatal or vertical route.
- The conversion from acute to chronic phase is less in this genotype.
- HBeAg seroconversion occurs earlier than genotype C.
- HBsAg seroclearance is faster than genotype C.
- Less patients develop cirrhosis.
- HCC development is less.
- Hepatic decompensation is less.
- HBV DNA is high in number.
- Less chance of getting fulminant hepatitis.

Genotype C is also a common genotype in Southeast Asia and China. Characteristics of genotype care are as follows [13–16]:

- Is commonly seen in young age less than 35 years generally transmitted by perinatal or vertical route.
- The conversion from acute to chronic phase is higher in this genotype.
- HBeAg seroconversion is less percentage and takes time than genotype B.
- HBsAg seroclearance is less than genotype B.
- High chance of getting cirrhosis.
- HCC development is more likely.

- Hepatic decompensation is higher in this genotype.
- HBV DNA is less in copy number.
- Less chance of getting fulminant hepatitis.

Genotype A is distributed worldwide. Characteristics of genotype A are as follows [13–16]:

- It is generally transmitted by horizontal route.
- The conversion from acute to chronic phase is frequent in this genotype.
- HBeAg seroconversion is earlier than genotype D.
- HBsAg seroclearance is also more frequent than genotype D.
- Less patients get cirrhosis.
- HCC development is less.
- Hepatic decompensation is higher.
- HBV DNA is high in copy number.
- Less chance of getting fulminant hepatitis.

Genotype clinical characteristics	**B**	**C**	**A**	**D**	**E–J**
Age	Common <35	>35	—	—	
Modes of transmission	Perinatal/vertical	Perinatal/ vertical	Horizontal	Horizontal	Horizontal
Chronicity	Lower	Higher	Higher	Lower	—
HBeAg seroconversion	Earlier	Later	Earlier	Later	Earlier
HBeAg seroclearance			Earlier	Later	
HBsAg seroclearance	More	Less	More	Less	_
Cirrhosis	Less active	Active			
HCC	Better	Worse	Better	Worse	Worse in genotype F
HBV DNA level	Higher	Lower	Higher	Lower	_
Hepatic decompensation	Lower	Higher	Higher	Higher	_
Fulminant hepatitis	Less	Less	Less	Higher	

Table 3.
Clinical characteristics of different genotypes of HBV.

Genotype D also has worldwide distribution. Characteristics of genotype D are as follows [13–16].

- Transmitted by horizontal route.
- The conversion from acute to chronic phase is less frequent in this genotype.
- HBeAg seroconversion takes longer time than genotype A.
- HBsAg seroclearance is less than genotype A.
- HCC development is most frequent.
- Hepatic decompensation is higher.
- HBV DNA is low in copy number.
- Higher chance of getting fulminant hepatitis.

Different HBV genotype clinical characteristics are compared in **Table 3**.

6. Antiviral treatment

The goal of therapy

- Decrease ALT—decrease necrotic inflammation in the liver
- Decrease viral load to the undetectable level
- HBeAg seroconversion to generate anti-HBeAg—to minimize the replication of virus
- To reduce HBsAg and seroconversion and anti-HBS
- Prevent development of new esophageal varices

Long-term goal is to reduce the risk of HCC and chronic liver diseases.

Peg-IFN alfa-2a or alfa-2b is recommended before starting peg-IFN therapy to HBeAg-positive patients; good responders should be identified; i.e., low viral load, HBV genotypes A and B, high serum ALT levels (above 2–5 times ULN) and high activity scores on liver biopsy are the predictors for better responding patients.

As the baseline predictor of response for the therapy to HBeAg-negative patient, HBV genotype, HBV DNA, ALT, HBsAg levels, and age are the crucial factors [17].

When to terminate Peg-IFN therapy: if low probability of response for HBeAg positive.

Or

No decline of HBsAg level for HBV genotype A and D at week 12 of treatment.

6.1 HBeAg loss

HBeAg loss with HBV DNA <2000 IU/ml for 6 months after treatment. Genotype D HBeAg-negative patient decreasing <2log decline in HBV DNA at week 12 of peg-IFN therapy [18].

Either entecavir or tenefovir is recommended for HIV

6.2 No response

HBeAg-positive patient with HBsAg level >20,000 IU/ml predicted no response after treatment. HIV positive, TNF + entecavir (lamivudine) + EFV is recommended. Other drugs are recommended as the second-line therapy if the first-line drug treatment is failed (if HBV DNA > log10 IU/ml in 3-month period [19].

6.3 Response to lamivudine

Genotype B has sustain responsiveness to lamivudine followed by genotype C. Genotype A is more resistant than genotype D so that virological responses to the drug are better in genotype D than genotype A. Pediatric patients have good tolerance to lamivudine. The younger the age at diagnosis, the longer consolidation treatment period [20].

6.4 Response to adefovirdipivoxil

Patients infected with genotypes A and D equally respond to 48-week treatment of adefovirdipivoxil, but there could be the adefovir resistance in patients having genotype D.

6.5 Response to entecavir or telbivudine

The relationship of HBV genotypes on drug resistance to entecavir was evaluated in lamivudine-refractory patients. Two-year therapy of telbivudine showed that HBeAg seroconversion, ALT normalization, and HBV negativity were comparable among different genotypes. Entecavir treatment has a chance of HBsAg seroloss in genotypes A and D, and the efficacy is better in Caucasian than in Asian population [20].

6.6 Tenefovir (TDF)

HBsAg loss in genotype A patient followed by genotype D (20 and 10%, respectively) was reported after completion of 144 weeks of treatment of TDF. The therapeutic effect was less in genotypes B and C [20].

Nucleotide analogue	Dose	Adverse	EASL	ASL	APSL
Lamivudine	100 mg/day		Undetectable DNA For 3 years	1–3 years after HBeAg-negative patient	Undetectable DNA For 2 years
Adefovir	10 mg/day				
Entecavir	0.5 mg/day				
Telbivudine	600 mg/day				
Tenofovir	300 mg/day	Bone and renal impairment			
Emcitrabine	200 mg/day				
Tenofovir alafenamide	25 mg/day				

Table 4.
Antiviral treatment doses and period of recommendation by different guidelines.

6.7 Management of incomplete responder

Incomplete responder should be checked for the drug adherence.

Patients who have previous exposure to lamivudine should be considered for entecavir resistance.

EASL recommend to switch to NA or combination therapy if detectable HBV DNA in 12 weeks of therapy. Combination of TDF + ETV has resulted in undetectable DNA even after 4 years, which is better than TDF alone [21]. Most of the study and the guideline do not recommend peg-IFN + nucleot(s)ide therapy, but recommend to use peg-IFN after NA therapy. Antiviral drugs and their continuation recommended by different guideline are illustrated in **Table 4**.

7. Conclusion

HBV genotyping is important for the proper management of chronic HBV patients. Clinical characteristics may be helpful to estimate the genotype and initiate antiviral therapy.

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