Original Research Article

The patterns of Hepatitis B virus genotypes and variants

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Received: 20-06-2021 / Revised: 18-07-2021 / Accepted: 12-08-2021

Abstract

Introduction: HBsAg, the most widely used marker for detecting current hepatitis B infection, is detected by kits using an antibody to HBsAg. HBsAg is the first serologic marker to appear, although HBV DNA may be detected slightly earlier. HBsAg usually appears 1 to 2 months after infection and before the onset of clinical illness and is the last protein marker to disappear. HBV DNA replication is slower than that of HCV; doubling time averages 2 to 3 days.HBV replication persists throughout the whole course of chronic HBV infection. **Material and methods:** In the present study, a total of 78 cases were included. Among all 53 cases were positive for the HBV screening test. This study was carried out at the Microbiology section of Central Laboratory (a NABH 2012 accredited laboratory), Shri Mahant Indiresh Hospital and Central Molecular Research Laboratory (CMRL) [a BSL-III laboratory], Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIMHS), Patel Nagar, Dehradun, Uttarakhand state. The duration of the study was over a period of two years.**Result:** The result of this study revealed that 53.48% cases were found with genotype D as compare to genotype A(23.33%),B(11.62%),C(4.65%),D(53.48%),E(4.65%) & F(2.33%).**Conclusion:** This study concludes that HBV genotype D is the most prevalent one, followed by genotype A in northern India, and a very rare genotype F is also found.

Keywords: Genotypes, HBV, Antibody.

Introduction

The Hepatitis B virus (HBV) is the property of the genus Orthohepadnavirus of the Hepadnaviridae family, and it is maybe severe liver infection can cause both acute and chronic inflammatory disease. Globally, an estimated 257 million public are existing with hepatitis B virus (HBV) infectious disease. In 2015, hepatitis B infection disease resulted in 887,000 mortality, mostly from complications that include cirrhosis and hepatocellular carcinoma [1].

Although carriers may clear HBsAg and develop antibodies to HBsAg, there appears to be a risk of severe liver complications later in life. HBV e antigen (HBeAg) is generally used as a secondary marker to indicate active HBV replication associated with progressive liver disease. Failure to clear HBeAg appears to increase the risk of end-stage liver disease. HBV is grouped into numerous genotypes based on the genome sequence, of which nine genotypes are well established. Some HBV genotypes are put forward classified as sub-genotypes. The HBV outcome is characterized by having more than 8% nucleotide differences for genotypes and more than 4–8% nucleotide differences for subgenotypes. Presently over 30 related sub-genotypes belonging to HBV genotypes have been establishing [2, 3].

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According to the molecular evolutionary analysis of genomic DNA sequence, HBV strains isolated in various countries are classified into ten genotypes, designated A–J, and arbitrarily defined by an inter-group sequence divergence of more than 8% based on complete genomes. The classification system separated the HBsAg into four major serological subtypes such as viz., adw, adr, ayw, and ayr, which, in turn, are correlated to HBV genotypes. In general, HBV genotypes of A, B, F, G, or H have the HBsAg subtype adw, whereas HBV genotype C have adr, and HBV genotype D and HBV genotype E have ayw [4, 5].

Hepatitis B virus is transmitted through body fluids, primarily by parenteral or sexual contact; it can be transmitted from mother to child, usually at or after delivery (termed vertical transmission). In parts of the world with high rates of chronic infection, much of the transmission is vertical. The residual risk from transfusion is estimated to be 1:600,000 [6].

Variant strains of HBV can either originate HBeAg that is not discoverable in serum, or the strain can lose the capability to make HBeAg even when an active infection is seen. Hence, using this marker to monitor disease progression may be of limited utility. The ability to detect HBV DNA in serum has been described to have prognostic value for the result of acute and chronic HBV infections. The methodology can allow the detection of HBV DNA after HBsAg clearance or detection of HBV lacking serologic markers. The efficacy of antiviral therapy to treat patients with HBV can also be assessed by serologic markers or by measurement of liver enzyme function. However, the most direct and reliable measurement of viral

replication is thought to be the Quantitation of HBV viral DNA in serum or plasma. A rapid and sustained drop in HBV DNA levels in patients receiving treatment with α-Interferon, Lamivudine, or Ganciclovir has been demonstrated to be a predictive factor for a beneficial outcome. Monitoring of HBV DNA levels can estimate the development of resistance to Lamivudine also. In consequence, a quantitative test for the measurement of HBV DNA is a considerable tool that can be applied in conjunction with other serological markers to manage HBV infection. Quantitation of Hepatitis B virus DNA (genotypes A to G) can be done over the range 6-110,000,000 IU/mL (Conversion factor: 5.82 copies= 1 IU). This is calculated for use in conjunction with clinical presentation and other laboratory markers as an aid in assessing viral response to antiviral therapy as measured by the change in HBV DNA levels [7,8] Persistence of HBsAg for longer than six months beyond the onset of acute hepatitis indicates chronic infection. Although carriers may clear HBsAg and develop antibodies to HBsAg, there appears to be a risk of severe liver complications later in life. HBV e antigen (HBeAg) is generally used as a secondary marker to indicate active HBV replication associated with progressive liver disease. Failure to clear HBeAg appears to increase the risk of end-stage liver disease.

Material & Methods

Ethical clearance was taken from the institutional ethical committee before beginning the present study. Two years cross-sectional study from December 2013 to November 2015 was conducted in the Microbiology section of Central Laboratory (NABH 2012 accredited laboratory), Shri Mahant Indiresh Hospital and Central Molecular ResearchLaboratory (BSL-III laboratory), and Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIMHS), Patel Nagar, Dehradun regions of Uttarakhand State, India. In the present study, 78 cases were included; among all, 53 cases were positive for the HBV screening test, the rest 25 were HBsAg negative (taken as control). The researchers clarified the purpose and procedure of the study, and informed consent was obtained from each subject before 5 ml venous blood samples were drawn in a serum separation tube (SST). Serum was separated, and tests were performed according to WHO guidelines and the manufacturer's instructions. Various procedures and assays involved in the study were carried out with the serum, which will include, Serological studies at Microbiology Laboratory of Central Laboratory, SMIH, and Molecular characterization to detect HBV Genotypes and quantitative estimation at Central Molecular Research Laboratory, Biochemistry Department, SGRRIM & HS. Patients from all age groups and gender were included in this study. A patient diagnosed with alcoholic hepatitis was excluded from this study. Data were analyzed by using Microsoft Excel. All statistical analyses were analyzed using the Statistical Program for Social Science Software (SPSS) 23.0 for Windows (SPSS Inc., Chicago IL., USA).

Results

In this study, we have included a total of 78 cases; out of 78 cases, 53 patients were HBsAg positive & the rest were negative.

Table: 1 Distribution of cases according to screening test (n=78).

Screening test	No. of cases (n=78)	Percentage
HBsAg positive	53	67.9%
HBsAg negative	25	32.1%
Total	78	100%

In the screening test of 78 patients, 53 (67.9%) were positive, and 25 (32.1%) were negative [Table 1].

Table:2 Distribution of cases according to virus detected in RT-PCR test (n=78).

RT-PCR	No. of sample	Percentage
Virus Detected	43	81.1%
Virus not detected	10	18.9%
Total	53	100%

We observed by RT-PCR in the study, 43 cases (81.1%) were virus detected positive, and 10 cases (18.9%) were negative for HBV [Table 2].

Table: 3 Age wise Prevalence of HBV Genotype

S. N.	Age group (in years)	No. of cases	HBV genotypes	HBV genotypes detected						Total
				A	B	C	D	E	F	
1.	0-20	03	Type D & E	00	00	00	02	01	00	03
2.	21-40	16	Type A, B, D, E & F	05	01	00	08	01	01	16
3.	41-60	15	Type A, B, C & D	02	03	02	08	00	00	15
4.	60 & Above	09	Type A, B & D,	03	01	00	05	00	00	09
TOTAL = 43				**10**	**05**	**02**	**23**	**02**	**01**	**43**
				23.33 %	**11.62%**	**4.65%**	**53.48%**	**4.65%**	**2.33%**	

We observed that 53.48% cases were found with genotype D as compare to genotype A (23.33%), B (11.62%), C(4.65%), D (53.48%), E (4.65%) & F(2.33%) [Table 3].

Table: 4 Titre wise HBV Viral Load and Genotypes detected

S. No.	HBV DNA titer (IU/ml)	No. of cases	HBV genotypes	HBV genotypes detected						Total
				A	B	C	D	E	F	
1.	1.00 x10^1 to 1.00 x10^3	15	Type A, B, C, D & E	05	04	02	03	01	00	15 (34.88%)
2.	1.01 x10^3 to 1.00 x10^6	18	Type A, B, D, E & F	02	01	00	13	01	01	18 (41.86%)
3.	More than 1.00 x10^6	10	Type A & D	03	00	00	07	00	00	10 (23.26%)
TOTAL = 43				**10**	**05**	**02**	**23**	**02**	**01**	**43**
				23.33%	**11.62%**	**4.65%**	**53.48%**	**4.65%**	**2.33%**	

It was observed that in cases having a viral load more than 1.1 x 106, only genotype A and D were present with the prevalence of genotype D, while other genotypes were absent. It was also seen that those maximum HBV infections were between viral loads1.00 x103 -1.00 x106, and all genotypes were present except genotype C. Out of 43 patients, 18 (41.86%) had a viral load in the range between 1.01 x103 to1.00 x106, next major group was of 15 (34.88%) patients, and their Viral load was between 1.0x101to1.00 x103 and rest 10 (23.26%) patients had a very high viral load (more than 106) [Table 4].

Discussion

In our study, when the association between HBV genotype and HBV DNA load was observed as a whole, no significant correlation was observed (p = 0.121), but when only two genotypes having greater prevalence, i.e., genotype D and genotype A, were considered, a significant relationship was found (p = 0.012). This was contrary to one study done earlier in northern India [9] and two previous studies done in Saudi Arabia [10, 11]. A rare genotypes B, C, and E were also found in our study. The finding of these genotypes are consistent with one study done in northern India [12], and genotype C was found in one study in northern India [13]. One reason for these findings may be because of Dehradun and adjoining areas like Mussoorie is tourist destinations for people of all over the world visit these places. Another possible reason may be the migration of people from Bihar and Uttar Pradesh to Dehradun and Adjoining areas like Hardwar and Mussoorie in search of a job. In an epoch of frequent international travel and human migration, the introduction of a new HBV genotype to a community might have far-reaching effects, including recombination between genotypes or replacement of one genotype by another. Genotype F is divided into four subgenotypes: F1–F4. Subgenotypes F1 and F2 have been further divided into F1a, F1b, F2a, and F2b.InVenezuelasubtypesF1, F2, and F3 are found in East and West Amerindians. Among South Amerindians, only F3 was found. Subtypes Ia, III, and IV exhibit are restricted geographic distribution (Central America, the North and the South of South America, resp.) while clades Ib and II are found in all the Americas except in the Northern South America and North America, respectively [14].A rare genotype F was also found in our study. One reason for this not expected finding may be because of Dehradun and adjoining areas like Mussoorie being tourist destinations and people from all over the world visit these places. In an era of frequent multinational travel and human migration, the introduction of a new HBV genotype to a community might have far-reaching effects, including recombination between genotypes or replacement of one genotype by another. However, there is an urgent need to explore other possible reasons forth is the unusual prevalence of genotype F. This suggests that genotype F may be indigenous to certain pockets of North India, clustering in and around western UP and Haryana. This unusual finding apparently contradicts the conventional knowledge that HBV genotype closely mirrors ethnic and geographical migration.

Conclusion

This study concludes that HBV genotype D is the most prevalent one, followed by genotype A in northern India, and a very rare genotype F is also found. Although there is no significant association between the various HBV genotypes and HBV DNA load, a significant association is found between HBV genotype A and D and HBV DNA load. The emergence of genotype F in India needs further study regarding its severity, clinical implications, and treatment modalities.

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Conflict of Interest: Nil
Source of support: Nil