First Report of Genotype E of Hepatitis B Virus in an Indian Population

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Key Words

Hepatitis B virus • Precore mutations • North Indian population

Abstract

Twenty-one hepatitis B virus (HBV) isolates from the state of Haryana (North India) were studied for genotype, subgenotype, serotype distribution and precore mutations. Assays of alanine aminotransferase (ALT) and HBeAg were performed on all samples. Genotypes, subgenotypes and serotypes were determined by amplification of pre-S1/S2 regions followed by RFLP and also by phylogenetic analysis of amplified products. Mutations were studied by amplification and sequencing of the precore region. Twenty-four percent of the samples had high ALT levels and 90% were HBeAg negative. It was observed that 90% of the samples were HBV D genotype, (subgenotype D1, serotype *ayw2*), 5% HBV A genotype (subgenotype A1, serotype *adw2*), and the remaining 5% were HBV E genotype (serotype *ayw4*). The subgenotype A1 was quite similar to the South African isolates. Phylogenetic analysis of the HBV isolates, based on the pre-S1/S2 gene sequences, confirmed genotype E. Amplification and sequencing of the precore region showed 1762^{A→T} and 1764^{G→A} mutations in 38 and 15% of the samples, respectively. 1809^T was observed in 5% of the cases under study. This

is the first report of the genotype E of hepatitis B virus in the Indian population. Efforts are underway to amplify and sequence the full length of this genotype E isolate.

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Hepatitis B virus (HBV) is a noncytopathic, hepatotropic DNA virus which belongs to the family *Hepadnaviridae*. It remains an important cause of morbidity and mortality worldwide, especially in developing countries. HBV infection leads to a wide spectrum of liver conditions including acute self-limited infection, inactive carriers, fulminant hepatitis and chronic hepatitis, with eventual progression to cirrhosis and hepatocellular carcinoma [1]. It is estimated that over 400 million people worldwide are infected chronically with HBV. The virus has a small-sized genome (approximately 3.2 kb long) arranged in a circular, partially double-stranded DNA molecule. The viral genome contains 4 open reading frames encoding the envelope, pre-C/C, polymerase and X proteins.

There are at least 8 genotypes of HBV, designated A–H, based on a divergence of >8% over the entire genomic sequence. There are significant differences in the worldwide distribution of these different genotypes [2–4]. India falls into the intermediate endemic zone with regards to the prevalence of HBV infection. The reported HBsAg

carrier rate in India ranges from 1 to 13%, with a national average of 4.7%. Vertical and horizontal transmission in the perinatal period and early childhood, respectively, are the major means of transmission of this infection in India [5]. There is increasing evidence that the clinical outcome, response to antiviral treatment and long-term prognosis may differ depending on the genotype of the patient [6]. An attempt has been made in this communication to study different genotype, subgenotype and serotype distribution, precore mutations and to carry out phylogenetic analysis of HBV in Haryana (North India), as there is a lack of information from this area.

The sera were obtained from 21 HBsAg-positive patients from Haryana who had not taken antiviral agents such as lamivudine or interferon. All patients were negative for HIV and the hepatitis C virus infection. The institute's ethics committee approved the study. Alanine aminotransferase (ALT) and HBeAg were determined in all the serum samples using an auto-analyzer and commercially available kits (Abbotts Diagnostics), respectively. The extraction of DNA from serum was performed using a QIAmp extraction kit (Qiagen, Germany). DNA extracted was eluted in 100 µl best quality water.

PCR for the amplification of the pre-S1/S2 region was done using nested PCR utilizing 230F (5'-TC-ACAATACCGCAGAGTCT-3') and 800R (5'-AACAGC-GGTATAAAGGGACT-3') primers for the first round. PCR was performed for 40 cycles involving denaturation at 94° for 1 min, annealing at 53° for 50 s and extension at 72° for 50 s. The initial denaturation was at 94° for 3 min, and final extension was performed at 72° for 10 min. Second-round PCR was performed using P7 (5'-GTGGTG-GACTTCTCTCAATTTTC-3') and P8 (5'-CGGTATA-AAGGGACTCAAGAT-3') primers, as per the method of Lindh et al. [7]. Genotyping was done using the restriction enzymes *Hinf*I and *Tsp*509I, again as per the method of Lindh et al. [7].

The precore region was amplified using nested PCR involving primers 1732F (5'-CTGGGAGGAGTTGGGG-GA-3') and 2045R (5'-CAATGCTCAGGAGACTCTA-ACGG-3') for the first round. PCR was performed for 40 cycles involving denaturation at 94° for 30 s, annealing at 62° for 50 s and extension at 72° for 50 s. Initial denaturation was at 94° for 5 min and final extension was performed at 72° for 10 min. Second round PCR was performed using primers 1765F (5'-GGTCTTTGTAC-TAGGAGGCTG-3') and 1968R (5'-GTCAGAAGGCA-AAAACGAGAG-3'). PCR was performed for 40 cycles involving denaturation at 94° for 30 s, annealing at 58° for 50 s and extension at 72° for 50 s. Initial denaturation

was at 94° for 5 min, and final extension was performed at 72° for 10 min. The results were analyzed on a 2% agarose gel.

Nucleotide sequences of samples were compared with the available wild-type sequences using BioEdit sequence comparison software (USA). A phylogenetic analysis was carried out as described previously [8].

Assays of ALT and HBeAg showed that 24% of the samples had elevated ALT levels and 90% of the samples were negative for HBeAg. The banding pattern of nested PCR (using 230F and 800R primers in the first round and P7 and P8 primers in the second round, followed by restriction analysis using *Hinf*I and *Tsp*509I for genotyping) was compared with the published results of Lindh et al. [7]. Our results showed that 15/17 (90%) of the samples were of the HBV D genotype (subgenotype D1, serotype *ayw*2), 1 sample (5%) belonged to subgenotype A1 (serotype *adw*2) and the remaining 1 (5%) to HBV E genotype (serotype *ayw*4). Genotype A was found to have subgenotype A1 quite similar to the South African isolates. Results for genotyping were confirmed using phylogenetic analysis and are shown in figure 1. This is the first report of the presence of the HBV E genotype in India, as this genotype is characteristic of West Africa [8]. Our results do not support the previous finding of Thakur et al. [9] in which genotypes D and A were detected in 48 and 46% of the individuals, respectively, in the Indian subcontinent. Similar to our findings, Thippavazzula et al. [10] reported that genotype D (subgenotype D1, serotype *ayw*) and genotype A (subgenotype A1, serotype *ayw*) were prevalent in a South Indian population. Banerjee et al. [11] had reported genotype D having subgenotypes D1, D3 and D5 as well as serotypes *ayw*2 and *ayw*3 in eastern India.

Amplification and sequencing of the precore region shows 1762^{A-T} and 1764^{G-A} mutations. A 1762^{A-T} mutation was observed in 38% of the samples; 5% had elevated ALT levels, and 85% of the samples were negative for HBeAg. A 1764^{G-A} mutation was observed in 15% of the samples, and 10% had elevated ALT and were negative for HBeAg. 1762^{A-T} and 1764^{G-A} mutations were higher in negative HBeAg and equally common in different genotypes, but with high ALT as observed by Chauhan et al. [12]. 1762^{A-T} and 1764^{G-A} mutations suppress HBeAg synthesis and may contribute to hepatocarcinogenesis [13, 14]. An 1809^T mutation was observed in 5% of Indian patients under study, all of whom had normal ALT and negative HBeAg. This is a missense mutation, which is found in 80% of African blacks [14] and represents the wild-type.

Therefore, this communication reports the first instance of HBV genotype E in an Indian population. The

Fig. 1. Phylogenetic analysis of S region position 375–790 from the *Eco*RI site on AY945307. Names of isolates sequenced in the present study are surrounded by boxes.

majority of the samples have HBV D genotypes, subgenotype D1 and serotype *ayw*2. 1762^{A-T} and 1764^{G-A} as well as 1809^T mutations have also been observed. Efforts to amplify and sequence the full length genotype E are ongoing.

Acknowledgement

Jasbir Singh is thankful to the Union of International Cancer Congress for providing financial assistance to carry out this work.

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