Short Communication

Detection of hepatitis B virus subgenotypes D1, D3 and A1 in blood donors in NCT of Delhi, India

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Abstract
Phylogenetically hepatitis B virus (HBV) has been classified into eight major genotypes. The dominant genotype in New Delhi is genotype D. HBV genotype D and A have been well documented from different parts of India. In this study, the sequences of HBV isolates from 12 asymptomatic blood donors were amplified; the resulting amplicons were sequenced. Analysis showed that 6 isolates belonged to HBV genotype D1, 3 belonged to D3 and rest 3 belonged to A1.

Keywords: Hepatitis; Hepatitis B virus; HBV

1. Introduction
On the basis of more than 8% genomic variability HBV has been classified mainly in eight genotypes namely A, B, C, D, E, F, G and H. In addition to this, based on 4 to 8% genetic variability within a particular genotype, subgenotypes to these HBV genotypes have been designated. HBV is one of the principal causes of mortality among infectious diseases in developing and underdeveloped countries. The Indian subcontinent has been tagged to have intermediate HBV endemicity due to 2-7% HBsAg carrier rate. HBsAg has been used universally for the diagnosis of HBV, but the advancement of molecular techniques has divided developed and developing countries in terms of diagnostic standards. The developed countries have started nucleic acid testing (NAT) technology in blood banks for screening HBV, HCV and HIV in late 90s. The developing countries including India are still in the process of implementing this technology. In year 2013, Delhi government has taken steps to implement this technology for its blood banks.

The objective of this study was to find out sub-genotypes of Hepatitis B virus in HBV infected blood donors in national capital territory of Delhi.

2. Material and Methods
Plasma samples from seventy six blood bags found positive for HBsAg, during the screening at blood banks located in NCT of Delhi were selected. HBV DNA was extracted using QIAmpMinElute virus spin kit (QIAGEN, Germany). The genomic region encoding for precore/core and partial pol genes was amplified using Taq PCR kit (New England Biolabs) with 20 pmoles of each forward primer (5'-GAAGGATGGGGAGGAGATA-3', ntd. 1734-1755) and reverse primer (5'-AGGCGCTACGTGTTTCTC-3', ntd. 2785-2805). The thermal cycling was performed in GeneAmp PCR system 9700 (Applied Biosystems) with: denaturation at 94 °C for 5 min followed by 40 cycles consisting of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1.5 min and final extension at 72 °C for 7 min. The HBV DNA amplicons were purified using QIAquick PCR purification kit (QIAGEN, Germany). The nucleotide sequences of the twelve amplified products were determined in both directions by using BigDye Terminator v3.1 Cycle Sequencing kit and Genetic Analyzer 3130xl (Applied Biosystems). The sequences were analyzed using BLAST and clustal W alignment tools. The sequences were submitted to EMBL/GenBank under accession numbers LK995384 to LK995395.

3. Results
Hepatitis B virus DNA was detected in 56 of 76 samples taken from blood donor units found HBsAg positive at various blood banks in NCT of Delhi. Twelve samples were selected randomly for determining nucleotide sequences. The viral loads were similar for all samples. Out of these 12 samples, the nucleotide sequences of 6 samples showed 96-99% homology to D1 subgenotype; nucleotide sequences of 3 samples showed 95-98% homology to D3 subgenotype and the nucleotide sequences of 3 samples showed 95-98% homology to A1 subgenotype of HBV. Therefore, of the 12 isolates, 6 were classified as subgenotypes D1, 3 as subgenotype D3 and remaining 3 as subgenotype A1. The same has been supported by phylogenetic analysis and clustering of these isolates with the respective subgenotype sequences extracted from Genbank (Fig 1).
Fig 1: Phylogenetic relationship of 12 HBV isolates from New Delhi (in rectangles) to corresponding sequences of other HBV isolates obtained from GenBank established using neighbour-joining tree. Each sequence is designated by its accession number and subgenotype.

D1 isolates of this study showed 87-98% nucleotide homology among each other, D3 isolates showed 74-96% nucleotide homology to each other and A1 isolate showed 98% nucleotide identity to each other.

4. Discussion

Interest in decoding HBV genotypes has increased since it has been revealed that they affect different infection outcomes and treatment responses, but the factors explaining such influences are still unknown. In context to the subgenotypes present in human population, so far three subgenotypes of genotype A and five of genotype D have been identified. In this study we report D1, D3 and A1 subgenotypes from blood donors in New Delhi. Transfusion is associated as the most important risk factor of HBV transmission in all. Genotypes A and D have already been reported from New Delhi. There have been studies about revealing of HBV genotypes from patient samples but no study about revealing of subgenotypes from blood donors from New Delhi. The genotype D has been demonstrated as prevalent and our study inspite of less (12) samples also indicates towards it. Genotype D predominates in India and ninety five percent of the genotype A isolates are represented by subgenotype A1 in India and as well as in the world. We have also detected A1 subgenotype in all HBV A genotype samples. HBV genotype A is more often associated with severe liver disease in northern India than is genotype D. Determining the subgenotype could be helpful for predicting the outcome of antiviral therapy in patients with chronic hepatitis B.

References