G1896A Mutation in Genotype D of Hepatitis B Virus Stabilizes the RNA Stem Loop Structure

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Hepatitis B is one of the most common infectious diseases in the world that causes chronic HBV infection in 400 million people worldwide. HBV is an enveloped, 3.2 kb double stranded DNA virus that can be classified into eight major genotypes (A-H).The HBV genome is organized into four overlapping open reading frames, the longest of which encodes the viral polymerase (P- ORF). The envelope ORF (consisting of Pre-S1, Pre-S2 and S genes) is completely overlapped with that of the polymerase, whereas the Core (C) and the X ORFs overlap partially with it. The C-ORF consists of two regions namely the core which encodes the viral nucleocapsid (HBcAg) of 183 amino acids and the precore encoding 29 amino acids. After being processed at its N-terminus and its C-terminus, the secretory or ripe HBeAg (precore protein) generally contains the aa20–29 of the precore sequence and aa1-149 of the core sequence. Formation of covalently closed circular (ccc) DNA is an essential and strategic step in the viral life cycle. The cccDNA is responsible for the production of four major RNA species 3.5, 2.4, 2.1 and 0.7kb. During the life cycle of HBV, there is formation of pregenomic RNA (pgRNA) of 3.5kb from the cccDNA. The stem loop structure in the pgRNA spanning from downstream to the nucleotide 1855 up to the nt. 1899 is extremely important in the process of viral life cycle since this is the region from where reverse transcription is initiated for the progress of viral replication. In order to investigate the association of G1896A mutation with the prevailing HBV genotypes in e negative CHB in India, we extracted the viral DNA from sera samples of 14 HBeAg negative chronic hepatitis B patients. PCR amplification was carried out to amplify the region of viral genome containing the stem loop structure. Purified PCR products were bidirectionally sequenced with respective primers in an automated DNA sequencer. Obtained DNA sequences were aligned with that of the known genotypes of HBV with the help of multalin alignment bioinformatics tool. Out of 14 patients, 9 were of genotype D (64.28%), 1 of genotype C (7.14%) and 4 of genotype A (28.57%). In agreement with the information available in the literatures, we too found considerably higher number of patients with genotype D having the G1896A mutation of viral stem loop. This finding can be explained in a way that this 'G' to 'A' substitution at nt.1896 in genotype D, stabilizes the hairpin structure by creating a U-A pair in the RNA, which might be advantageous for viral replication and breakthrough.