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Molecular Epidemiological Study of Hepatitis B Virus in the United Arab Emirates Based on the Analysis of Pre-S Gene

Mubarak S. Alfaresi

Department of Pathology and Laboratory Medicine, Zayed Military Hospital, Abu Dhabi, UAE

Abstract

This study was undertaken to investigate the prevalence of the hepatitis B virus (HBV) variant with the pre-S mutant circulating in the United Arab Emirates (UAE). The sequences of the pre-S and S regions were determined in serum samples of 90 HBV DNA-positive subjects who had been enrolled in the study. The results showed that genotypes D and A accounted for 77.8% and 17.8% respectively. The distribution of the HBV antigen subtypes was: ayw2 (78.9%), adw2 (14.4%), and adw (2.2%). Sequencing analysis showed that pre-S mutations were present in 4 samples (4.4%), with pre-S2 deletion as the most common mutant (50%). The pre-S mutations were associated with older age and a higher mean HBV DNA level. The study demonstrated that HBV genotypes D and A were the predominant strains circulating in the UAE and that the HBV pre-S mutant is very rare in this area, appearing only in genotype C.

Keywords: Hepatitis B virus; Genotype; Pre-S mutation; UAE

Introduction

Hepatitis B virus (HBV) is a small, enveloped 3.2 kb DNA virus with four Open Reading Frames (ORFs). HBV envelope proteins are encoded by three overlapping envelope genes contained within a single ORF: pre-S1, pre-S2, and S. Depending on the translated initiation site among S, pre-S2, or pre-S1, three different sized proteins are produced: a small hepatitis B surface protein (small HBs) containing 226 amino acid residues; a middle hepatitis B surface protein (middle HBs) containing 55 additional amino acid residues; and a large hepatitis B surface protein (large HBs) containing 108 or 119 additional amino acid residues, depending on the serotype. The pre-S region mediates hepatocyte attachment of the virus (amino acids 21 to 47 in pre-S1) [1-4], contains B-cell and T-cell epitopes [5-7] and a binding site for neutralising anti pre-S2 antibody (amino acids 120 to 145) [1,3,4] and has an S promoter for controlling the production of middle HBs and small HBs.

In the late 1990s, two major types of pre-S deletion mutant LHBS were identified and highly associated with Hepatocellular carcinoma (HCC) [8,9]. LHBS is expressed primarily at the late stage of chronic HBV infection, after the viral genome has integrated into the host chromosome [10-13]. Pre-S mutant LHBS was first isolated from ground glass hepatocytes (GGH), the histological hallmarks of the late stages of chronic HBV infection, and is often seen in the liver sections of HCC patients [14]. Pre-S mutant LHBS is highly associated with advanced liver diseases, including cirrhosis and HCC, which suggests that it contributes to hepatocellular carcinogenesis [15-23].

After pre-S mutant LHBS was discovered, various geographically diverse studies [15-23] screening for pre-S mutations invariably reported that they were prevalent in chronic HBV carriers. In addition, pre-S mutant LHBS, especially the pre-S2 type, is highly correlated with the severity of HBV-related liver diseases, including HCC [15-18,20]. Therefore, it is important to screen for pre-S deletion mutations in chronic HBV carriers. This type of screening should be done in combination with the detection of other HBV markers, such as viral titers and HBe antigen (Ag), to estimate an HBV carrier's relative risk for HCC.

In this study, we investigated the prevalence and characteristics of the pre-S gene mutations predominant in the United Arab Emirates population as well as its association with HBV genotypes and both precore and core mutants.

Materials and Methods

A total of 120 consecutive serum samples from HBs Ag-positive patients were evaluated in this study. These samples were derived from 98 males and 22 females with a mean age of 36.4 ± 12.6 years (range: 18 to 70 years). All of these patients were UAE citizens. Serum samples were stored at -20°C and thawed immediately before use. The samples were evaluated for the presence of several serological markers of HBV infection (including HBe Ag, anti-HBe Ag, and HBs Ag) using the bio-Mérieux ELISA kit according to the manufacturer's instructions.

Detection of HBV-DNA by PCR (Polymerase Chain Reaction)

The extraction and amplification of HBV-DNA was carried out by nested PCR using the methods described by Kaneko et al. [24].

Analysis of HBV sequences from different genotypes

We used selected primers that have been described previously [25] and that corresponded to conserved regions of the various HBV genotypes that flank heterogeneous intervening regions to distinguish between the HBV genotypes. The region selected for amplification also included the amino acid loop corresponding to the a, d/y, and w/r allelic subtypic determinants as well as mutations that have been shown to be related to the HBsAg antibody, the anti-HBs monoclonal antibody, and vaccine resistance. The following primers were selected: 1) FHBS1, 5'-GAG TCT AGA CTC GTG GTG GAC TTC-3'; 2) FHBS2, 5'-CGT GGT GGA CTT CTC TCA ATT TTC-3'; 3) RHBS1, 5'-AAATKG CAC TAG TAA ACT GAG CCA-3'; and 4) RHBS2, 5'-GCC ARG AGA AAC GGR CTG AGG CCC-3'. The positions in the HBV genome (strain HBVADW; GenBank accession number V00866) to which the primers

***Corresponding author:** Mubarak S. Alfaresi, Department of Pathology and Laboratory Medicine, Zayed Military Hospital, Abu Dhabi, UAE, Tel: +9712-4055489; Fax: +9712-4492075; E-mail: uaenow@eim.ae

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corresponded were as follows: 1) HBS1F (positions 244 to 267), 2) HB-S2F (positions 255 to 278), 3) HBS2R (positions 648 to 671), and 4) HBS1R (positions 668 to 691). Serum samples were treated as described above and subjected to two rounds of amplification sequentially with outer (FHBS1 and RHBS1) and inner (FHBS2 and RHBS2) primers. The amplification conditions for the two rounds of the nested PCR were as follows: initial denaturation at 94°C for 20 s, followed by 30 cycles of amplification at 94°C for 20 s, 56°C for 20 s, and 72°C for 30 s, followed by a final extension step at 72°C for 1 min in a PTC-200 Thermocycler (MJ Research, Watertown, Mass).

Detection of BCP and precore mutants

For the detection of BCP and precore mutants, HBV-DNA-positive samples were amplified by using the primers described by Takahashi et al. [26].

Amplification of HBV pre-S region and sequencing analysis

The pre-S1 and pre-S2 regions were amplified as described previously [19] by heminested PCR with 5'-TCA CCA TAT TCT TGG GAA CAA GA-3' (P1, sense, nucleotides 2817 to 2839) and 5'-GGC ACT AGT AAA CTG AGC CA-3' (S2-2, antisense, nucleotides 668 to 687) for the outer primer pair (1,085 bases) and P1 and 5'-AGA AGA TGA GGC ATA GCA GC-3' (S4R, antisense, nucleotides 415 to 434) for the inner primer pair (834 bases). First-round PCR was performed with the following parameters: preheating at 95°C for 10 min to activate the AmpliTaq DNA polymerase, then 40 cycles of amplification at 94°C for 20 s, annealing at 55°C for 20 s, and extension at 72°C for 1 min, and elongation for 7 min in the last cycle. The second-round PCR was performed in the same conditions for 40 cycles except the annealing temperature was at 60°C.

The pre-S amplicons were isolated by 1% agarose gel electrophoresis and purified with the QIA quick gel extraction kit (Qiagen Inc., Chatsworth, Calif.). Recovered PCR products were then subjected to direct sequencing with primers of P1 and S4R, with an ABI Prism Big Dye terminator cycle sequencing ready reaction kit (Perkin Elmer). Sequences of amplified DNA were determined with a sequencer (ABI model 377 and 310; Applied Biosystems, Foster City, Calif.).

Sequence analysis

Genotyping, BCP, and precore mutant and pre-S region analysis were carried out by sequence comparison with known sequences from different HBV genotypes that have been previously described and were aligned as described above. The Geneious program (Biomatters, Inc.) was used for genotyping as well as for phylogenetic and molecular evolutionary analyses.

HBV DNA quantification

All samples were submitted to HBV DNA quantification using the commercial TaqMan Amplicor HBV assay (Roche Diagnostics), which has a lower limit of detection of 12 IU/L.

Statistical analysis

For statistical analysis, we used the PASW Statistics software package, version 18.0. Either the χ^2 test with the Yates correction or Fischer's exact test was used to analyze quantitative data and to compare proportions. All calculated P-values were two-tailed and all P-values <0.05 were considered to be statistically significant.

GenBank accession numbers

Sequences from the S gene that were acquired during this study were deposited in the GenBank under numbers GU594063-GU594150.

Results

Distribution of HBV genotypes

Of the 120 HBs Ag-Positive subjects, HBe Ag was detected in nine serum samples (7.5%). 90 (75%) of the 120 HBs Ag-Positive subjects were positive for HBV-DNA in the sera, detected by PCR. The mean age of the subjects was 36.4 ± 12.6 years and 82.2% were male. Of those positive for HBV-DNA, 70 cases (77.8%) were determined to be genotype D, 16 cases (17.8%) belonged to genotype A, and 4 cases (4.4%) belonged to genotype C. The distribution of the HBV antigen subtypes among these subjects was: ayw2 (78.9%), adw2 (14.4%), and adw (2.2%). In this study, all cases with genotype D belonged to the subtype ayw2. The obtained sequences were submitted to GenBank under accession numbers GU594063-GU594150. The sequences were also aligned with those of the isolates of known genotype and subjected to phylogenetic analysis (Figure 1).

Prevalence and characterisation of pre-S mutations

Based on direct sequencing, pre-S mutations were detected in only four of the 90 cases (4.4%). All of these cases belonged to genotype C. As for the prevalence of pre-S mutations according to site, pre-S2 deletion was the most common (50%), followed by both pre-S2 and pre-S1 deletion (25%), and start codon mutation (25%). The mean age of patients with a pre-S mutation was significantly higher than that of patients without the mutations ($P < 0.05$). In addition, the mean HBV load in patients with a pre-S mutation was significantly higher than in those without mutants ($P < 0.05$). The alignment of amino acid sequences of the entire pre-S1/pre-S2 region of the four samples is shown in Figure 2.

Discussion

HBV infection is an important global health problem that places a continuously increasing burden on developing countries like the UAE. Molecular epidemiological studies provide valuable information on understanding the prevalence and characteristics of HBV genotypes and mutations from different areas of the world. In this study we confirmed the predominance of genotypes D and A among the HBV strains in the UAE, which accounted for more than 95% of cases. These findings are not surprising; they reflect the typical genotypes circulating in the area.

Genotype C was not a prevalent genotype in this area. HBV genotype C is more commonly associated with severe liver diseases and the development of cirrhosis [27].

The pre-S1 and pre-S2 regions, together with the major hydrophilic region exposed at the surface of HBV particles, are highly immunogenic and potentially under selective pressure by the immune system [28]. The pre-S1 region contains the binding site for HepG2 cells in the liver membrane [2]. The pre-S2 region bears the binding site for polymerised human serum albumen, which is believed to be involved in the attachment of HBV to the human hepatocyte membrane [29]. Therefore, mutations at the pre-S gene might significantly modify the course of HBV infection [30].

Although the occurrence of HBV with the pre-S deletion mutation in liver diseases patients has recently been reported by several groups, its clinical significance has remained obscure [31-34].

The prevalence of pre-S mutations is variable and considerably

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