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

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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 adw1 (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 acids, 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 [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 HBBe 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 regions for distinguishing which HBV genotypes. The region selected for amplification also included the amino acid loop corresponding to the a, d/y, and w/r allelic subtypes determinants as well as mutations that have been shown to be related to the HBlg 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'-GAG TCT AGA CTC GTG GTG GAC TTC-3'; 3) RHBS1, 5'-GCC ARG AGA AAC GGR CTG AGG CCC-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...
corresponded were as follows: 1) HBBS1F (positions 244 to 267), 2) HBBS2F (positions 255 to 278), 3) HBBS2R (positions 648 to 671), and 4) HBBS1R (positions 668 to 691). Serum samples were treated as described above and subjected to two rounds of amplification sequentially with outer (FHBBS1 and RHBBS1) and inner (FHBBS2 and RHBBS2) 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 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 GenoType system (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 Amplificor 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 χ2 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.
different among different geographic areas. In our study it was very low (4.4%). Huy et al. [19] reported that the prevalence of HBV pre-S mutants ranged from 0% to 36% in an analysis of HBV-DNA-positive serum samples from individuals residing in 12 countries (2003). Either no cases or fewer cases with such a mutant were seen in countries with low HBV prevalence and in countries with low prevalence of HBV genotype C.

Interestingly, our data only showed pre-S mutations in patients infected with genotype C. Taking into consideration that these mutations were predominantly found in genotype C, it is possible that this genotype may be more prone to develop such mutations. Moreover, the mean age of patients with pre-S mutations was significantly higher than that of those without the mutants. This observation also confirmed previous data suggesting that the prevalence of pre-S mutants tends to increase in direct relation to the patient's age [15,35,19].

Regarding the site of mutation, our report showed that pre-S2 deletion was the most common mutation type. This result is also in agreement with those of recent reports from Japan and Korea [35,19].

In conclusion, our study demonstrates that the HBV genotypes D and A were the predominant strains circulating in the UAE. We also found that the HBV pre-S mutant is very rare in our area and is only in genotype C.

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