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[Objective]

Hepatitis B virus (HBV) is divided into nine serological types (subtype) and seven genotypes (A to G). The genotypes and subtypes have been suggested as useful clinical and epidemiological markers, and in addition genotype with subtype correlate strongly with ethnicity. On the other hand, analysis of mutations in surface (S) gene has provided important information for the improvement of diagnosis, potential vaccines and therapeutic antibody in HBV endemic area. Vietnam is one of the high HBV endemic countries with 14% to 26% hepatitis B surface antigen (HBsAg) positive carriers. However, sufficient information on the molecular epidemiology of HBV is not available except in Ho Chi Minh City (the southern Vietnam) and for refugees entering United States. We therefore undertook a study on sequencing of the 219 nucleotide of S gene of HBV for patients with chronic hepatitis in Hanoi, in order to determine the distribution of genotypes and subtypes and to analyze spectra of mutations. The site of 219 nt includes “a” determinant region that binds HBV antibody.

[Materials and Methods]

Blood samples were collected from hepatitis patients in Vietnam, December 2001 and subjected to examine HBsAg. Among HBsAg positive patients, 40 patients with HBV DNA positive in the sera were selected : 34 patients with chronic hepatitis, 4 with acute hepatitis and 2 with hepatocellular carcinoma. All the patients had not been vaccinated. They received traditional medication except hepatocellular carcinoma cases treated with anticancer medicine. DNA was extracted from the sera and PCR was performed for 219 nucleotides. The PCR products were used for direct sequencing. Genotypes were determined by phylogenetic analysis using MEGA program version 2.1 and by genotype-specific probe assay (GSPA) using SMITEST (HBV Genotype Detection Kit, Genome Science Laboratories Co. Ltd., Tokyo, Japan). Subtypes were classified by the deduced aa residues (Magnius and Norder, 1995).
[Results]
Among 30 samples determined by phylogenetic analysis, 23 and 7 were genotypes B and C, respectively. Of the remaining 10 samples, 7 were of genotype B and one was mixture of genotype B and C by GSPA method. Consequently, 75% (30/40) were genotype B, 17.5% (7/40) were C and 2.5% (1/40) was B and C mixture. Two samples were not analyzed because of insufficient amount of DNA. Among 40 samples, 25 were classified as ayw1 (63%), 7 as adr (18%), and 1 as adw2 subtype. Three samples were classified as mixture of subtypes ayw1 and ayr, ayw1 and adw2, and adw2 and adw3. As for the rest 4 samples having mixture of two nucleotides at amino acid residues for subtype determinations, it was not possible to determine a defined subtype.

The nt sequences of the samples with genotype B and C were compared to those of their reference ones. Surprisingly, 24 out of 40 samples (60%) have at least one mixture of two different nucleotides at certain nucleotide positions. Total numbers of base substitutions were 55. Missense mutations were five-fold more prevalent than silent mutations in the genotype B with adw2 and with mixture of adw2 and adw3, and C with adr, while the ratio of missense to silent mutation as 13 : 20 in genotype B with ayw1. Amino acid sequences of the samples with genotype B and of the samples with genotype C were compared with their reference aa sequences. In the 28 genotype B samples, except two samples, 19 aa substitutions were found. Three M133L (mutations) and one P127T (variation) were in the first and three T141I (variations) in the second loop of the “a” determinant. Other 4 mutations and 8 variations were outside of “a” determinant. In the 7 genotype C with adr samples, there were 5 aa changes ; two I126T (variations), one I126M (new mutation) and one T131N (variation) in the first loop and one A166G (mutation) at the outside of the “a” determinant.

[Conclusion]
1. On the basis of 219 nt sequence in the S gene, genotype B with ayw1 is the predominant genotype/subtype (63%) in Hanoi, Vietnam followed by genotype C with adr (18%).
2. Quasi-species nature of HBV was strongly reflected as overlapping two different nucleotides at certain nt positions in 24 (60%) samples.
3. Fifty five base substitutions have been detected. One new mutation and 3 others were found in the first loop of “a” determinant region, however no mutation was found in the second loop where most of the vaccine associated mutations were arisen. Five new variants were found in this study.