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RAPID COMMUNICATION

Preliminary report of hepatitis B virus genotype prevalence in Iran

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Abstract

AIM: To determine the prevalence of hepatitis B virus (HBV) genotypes in Iranian hepatitis B surface antigen (HBsAg) carriers, chronic hepatitis B and cirrhotic patients.

METHODS: A total of 109 HBsAg-positive patients were included in this study. HBV genotypes were determined by using INNO-LiPA methodology which is based on the reverse hybridization principle.

RESULTS: The distribution of patients with different stages of liver disease was as follows: 95 (86.4%) chronic hepatitis, 11 (10%) liver cirrhosis, and 3 (2.7%) inactive carrier. Of the chronic hepatitis and liver cirrhosis patients, 26.4% were HBeAg-positive while 70% were HBeAg-negative. Genotype D was the only detected type found in all patients.

CONCLUSION: Classifying HBV into genotypes has to be cost-effective and clinically relevant. Our study indicates that HBV genotype D prevails in the Mediterranean area, Near and Middle East, and South Asia. Continued efforts for understanding HBV genotype through international co-operation will reveal further virological differences of the genotypes and their clinical relevance.

Key words: Hepatitis B virus; Genotype; Chronic Hepatitis B; Cirrhosis

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INTRODUCTION

Human hepatitis B virus (HBV), which is the prototype member of the family Hepadnaviridae, is a circular, partially double-stranded DNA virus of approximately 3200 nt[1]. This highly compact genome contains the four major open reading frames encoding the envelope (preS1, preS2 and surface antigen HBsAg), polymerase (HBPol) and X (HBX) proteins, respectively[2]. HBV is an etiologic agent of acute and chronic liver disease, including fatal fulminant hepatitis, liver cirrhosis and hepatocellular carcinoma[3-6]. Over 2 billion people worldwide have been exposed to HBV and 350 million are chronic carriers of HBV[7-9].

In 1988, HBV was classified into four genotypes by a sequence divergence in the entire genome exceeding 8%, and designated by capital letters of the alphabet from A to D[10,11]. In 1994, Norder *et al*[12] found an additional two HBV genotypes by the same criteria, and named them E and F, respectively. Genotype G was reported recently in 2000[2] and genotype H, which is phylogenetically closely related to genotype F, was proposed in 2002[11]. HBV genotypes have distinct geographical distribution[2,7,13,14].

In general, genotype A is pandemic, but most prevalent in North West Europe, North America, Central Africa[2,13] and India[7]. Genotypes B and C are prevalent in Asia[7,8,13,15], especially in populations of Eastern Asia and the far East origin[3,16]. Genotype D is also more or less pandemic, but is predominant in the Mediterranean area and the Middle East[2,3,16]. Genotype E is restricted to Africa and genotype F is found in Central and South America[7,8,13,16]. Genotype G has been recently identified in France and North America[7,8]. It has been reported that there are remarkable differences in the clinical and virologic characteristics between the patients with different genotypes[17,18,19]. According to Iranian studies[20], over 35% of Iranians have been exposed to HBV, approximately 2% are chronic carriers. Compared to the United States where HBV infection is responsible for 25% of chronic hepatitis, HBV accounts for up to 70%-80% of chronic hepatitis cases in Iran, indicating that HBV alone is the leading

cause of chronic liver disease in Iran[21]. Until now, to our knowledge, no data regarding HBV genotype is available, and also the genotypes distribution of HBV and genotype-related differences with the liver disease are still unclear in Iran. In this study, we therefore determined the prevalence of HBV genotypes in Iranian hepatitis B surface antigen (HBsAg) carriers, chronic hepatitis B and cirrhotic patients.

MATERIALS AND METHODS

This cross-sectional study was performed in Tehran Hepatitis Center in 2004. A total of 109 patients with hepatitis B surface antigen (HBsAg) positive for at least 6 mo were enrolled in this study. Of the 109 patients, 95 were classified as chronic hepatitis, defined as HBsAg positivite with or without the presence of HBeAg, high level of HBV-DNA (> 100 000 copies/mL) detected by Amplicor HBV monitor, persistent or intermittent elevation in ALT levels and compatible liver biopsy. Three were inactive carriers characterized by persistent HBV infection of liver without significant, ongoing necro-inflammatory disease. Eleven had liver cirrhosis characterized by clinical evidence (splenomegaly, ascitis) and paraclinical results, including low platelet count, prolongation of prothrombin time and esophageal varices on upper gastrointestinal endoscopy. Liver cirrhosis was confirmed by liver biopsy. Patients were excluded if they were co-infected with hepatitis C virus (HCV), hepatitis D virus (HDV) or human immunodeficiency virus (HIV).

The following parameters were recorded for each patient from patient's document in Tehran Hepatitis Center: sex, age, stage of liver disease, alanine amino-transferase (ALT) level, aspartate transaminase (AST) and presence of hepatitis B virus E antigen and anti-hepatitis B virus E antibodies.

HBV genotypes were determined by using INNO-LiPA methodology (LiPA, INNO-LiPA HBV genotyping assay, Innogenetics N.V., Ghent, Belgium). The INNO-LiPA HBV genotyping assay is a line probe assay designated to identify hepatitis B virus genotypes A to G by detection of type-specific sequences in the HBV-pol gene domain B to C. This method is based on the reverse hybridization principle. Biotinylated DNA material generated from the HBsAg open reading frame was hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, unhybridized DNA was washed from the strip, alkaline phosphatase-labeled streptavidin was added and bounded to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen resulted in a purple/brown precipitate. Amplification of appropriate the HBV genomic region was performed using the INNO-LiPA HBV DR amplification kit. The INNO-LiPA HBV genotyping strip contains 1 red marker line, 2 control lines, and 14 parallel probe lines. The conjugate control line is a control for the color development reaction and the amplification control line contains universal HBV probes to check for the presence of amplified the HBV genomic material.

Statistical analysis

Data were analyzed with SPSS 11.5 software (SPSS Inc. Chicago, Illions, USA) using Student's t test, χ^2 test and Fisher's exact test.

RESULTS

A total of 109 patients with a mean age of 37.17 ± 11.75 years, including 13% females and 87% males, were enrolled in this HBV genotype study. The distribution of patients in different stages of liver disease was as follows: 95 (86.4%) chronic hepatitis, 11 (10%) liver cirrhosis, and 3 (2.7%) inactive carrier. Of the chronic hepatitis and liver cirrhosis patients, 26.4% were HBeAg-positive and 70% were HBeAg-negative. The mean serum ALT, AST, and ALP levels were 126.08 IU/L (88.46-163.71), 86.46 IU/L (49.54-123.39), 173.34 IU/L (152.74-193.94), respectively.

Genotype D was the only detected type found in all patients. Mean age of patients was significantly higher in the anti-HBe-positive group as compared with the HBe-Ag-positive group (P = 0.000). Also, the number of the patients in the anti-HBe-positive group was significantly higher than the HBe-Ag-positive group (P = 0.019; Fisher' s exact test). Moreover, significant difference was found between the mean age of patients with different stages of liver disease. None of the patients in the HBe-Ag-positive group had a normal ALT level. Most of the patients in the chronic hepatitis stage had an abnormal ALT level in comparison with the liver cirrhosis stage (P = 0.024; Fisher's exact test).

DISCUSSION

Classifying HBV into genotypes has to be cost-effective and clinically relevant. It is imperative to collect more information on HBV genotypes from all over the world to reach a decision on their clinical utility[11].

Data on the relation among the HBV genotypes, their pathogenicity in chronic liver disease including hepatocellular carcinoma and their effect on therapy are awaited with great interest, especially in Asia which is an endemic region of blood-borne hepatitis viruses[15].

Presently, based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into eight genotypes A-H, and different HBV genotypes are dominant in various parts of the world[14,16]. The most important finding of our study was that the only HBV genotype D was detected in all patients. The pattern of distribution of genotypes seemed to be simpler and was predominantly centralized into genotype D in all forms of the chronic HBV infection. The results of this study concur with previous studies, indicating that HBV genotype D prevails in the Mediterranean area, Near and Middle East, and South Asia[3,4,16]. For example, the result of a similar study performed in Turkey showed all 44 patients studied had genotype D[16]. Another study in Yemen demonstrated that genotype D was the dominant genotype in a settled population, while genotype A was

found only in communities with continuing African links[22]. In addition, one study in Egypt revealed genotype D was the most prevalent HBV genotype[23]. On the contrary, genotypes A, B and C were found to be predominant in Pakistan[24]. According to recent studies, genotype D in Asia is associated with more severe disease and may predict occurrence of hepatocellular carcinoma in younger patients[9].

After all, only less than 1 000 of the 350 million persistent HBV infections have yet been genotyped. Continued efforts for understanding HBV genotypes through international co-operation will reveal further virological differences of the genotypes and their clinical relevance.

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