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ORIGINAL ARTICLE

Genotype of Hepatitis B Virus Isolates from Iranian Chronic Carriers of the Virus

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Background and Aims: **Determination of the genotypes and subtypes of hepatitis B virus (HBV) provides epidemiological data, which can contribute further to vaccination and antiviral treatment strategies, diagnostic development, and prediction of the disease course. The aim of this study was to describe the molecular characterization and phylogenetic analysis of 60 HBV S-region isolated from native Iranian patients with chronic HBV infection.**

Methods: **HBV-positive sera were collected from Iranian patients with hepatitis B infection in Tehran Hepatitis Center. HBV-DNA was extracted and the partial HBV S ORF (677bp) were chosen for amplification and sequencing.**

Results: **By comparing the sequences of HBV isolated from Iranian patients with 50 complete sequences of HBV retrieved from the GenBank database, representing all other existing genotypes, the sequences of all 60 patients were consistent with that of genotype D. All HBV isolates from Iranian patients were clustered in genotype D branch with high bootstrap values.**

Conclusions: **Sixty HBV isolates from Iranian patients with chronic hepatitis B represent homogenous genotypic diversity. These sequences of Iranian HBV genomes may contribute to the information on the genetic diversity of HBV worldwide.**

Keywords: **Hepatitis B Virus, Genotype, Chronic Carrier, Iran**

Introduction

Hepatitis B virus (HBV) infection is a global health problem. Current estimates are that 2 billion people have been infected worldwide, of these; 360 million suffer from chronic HBV infection resulting in over 520000 deaths from acute hepatitis B and 470000 from cirrhosis or liver cancer [(1)]. Different studies have estimated that hepatitis B carriers rate varies widely from 0.1% to 20% through the world [(2)]. In the Middle East, the endemicity is intermediate, with a carrier rate of 2% to 7% [(3-5)].

The distribution of the genotypes of HBV varies in different geographical parts of the world. Genotype A is mainly detected in Northwestern Europe, North America, and Africa, whereas genotype B and C are found in Southeastern Asian populations. Genotype D is the commonest genotype in the world and the predominant one in Mediterranean basin. Genotype E and F are seen in East Africa and the New World, respectively. Genotype G is a recently determined genotype in a

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few patients in France, America, and Germany. Genotype H was reported in patients from Central America. Genotypes A and F have been divided into two subgenotypes [4, 6]. In addition, it was reported recently that each of B, C, and D genotypes could also be divided into four subgenotypes showing different geographical distribution [4].

It seems that the diversity of HBV genotypes may be related to different clinical patterns of infections [7, 8], liver disease severity [9, 10], development of cirrhosis and hepatocellular carcinoma [9, 11], viral persistence, and response to antiviral treatment [12]. It has been suggested that some of the molecular virological patterns, such as existence of basal core promoter and pre-core stop codon mutations are mainly related to certain HBV genotypes [8, 13-15]. Determination of the genotypes and subtypes of the HBV provides epidemiological data, which can contribute further to vaccination and antiviral treatment strategies, diagnostic development, and prediction of the course of the disease.

Iran has an intermediate rate of HBV infection with a reported prevalence around 3%. It is estimated that over 35% of Iranian population have been exposed to the HBV and about 3% are chronic carriers, ranging from 1.7% in Fars Province to over 5% in Sistan and Balouchestan [16, 17]. It has also been reported that 65% of all chronic hepatitis B patients in Iran are HBeAg negative [18]. The aim of this study is to describe the molecular characterization and phylogenetic analysis of 60 HBV S region isolated from native Iranian patients with chronic HBV infection.

Materials and Methods

Population Study and Sample Collection

HBV-positive sera were collected from Iranian patients with hepatitis B infection from Tehran Hepatitis Center. The collected sera were stored at -70°C. Serological markers of HBV infection were determined by commercial ELISA kits (Biokit, Spain).

Hepatitis Serology

HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HCV, and anti-HIV were determined by the microparticle enzyme immunoassay method and anti-HDV by the enzyme immunoassay method (Biokit, Spain). Patients who were co-infected with human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV) were excluded. The sample collection consisted of 60 chronic HBV carriers (10 women and 50 men with a mean age of 40 years). All patients were positive for hepatitis B surface antigen (HBsAg), hepatitis Be antibody (anti-HBe), and hepatitis B core antibody (anti-HBc). These sera samples were used for DNA extraction, polymerase chain reaction (PCR), and sequencing.

Amplification and Sequencing

HBV-DNA was extracted by a viral DNA extraction kit (Macherey-Nagel, Duren, Germany) proceeding with the manufacturer's protocol. The partial HBV S ORF (677bp) was chosen for amplification with specified primers [19]. All PCR contamination precautions were observed and negative controls using included sera from subjects with no HBV markers were included. The second-round PCR products of S ORFs were cleaned up using a PCR product purification kit (Macherey-Nagel, Duren, Germany). Then, PCR products were sequenced directly by a BigDye1 Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) with an ABI PRISM 3700 DNA analyzer automated sequencer at Sequence Laboratories MWG, Germany, using the inner primers.

Results

By comparing the sequences of HBV isolated from Iranian patients with 50 complete sequences of HBV retrieved from the GenBank database, representing all other existing genotypes, the sequences of all 60 patients were consistent with that of genotype D (Fig 1). The HBV S (677bp), and related references sequences were aligned with CLUSTAL W program by BioEdit software (The BioEdit Sequence Alignment Editor software, Department of Microbiology, North California State University) and confirmed by visual inception. Genetic distance was estimated using the Kimura two-parameter matrix [20]. Phylogenetic trees were constructed by the neighbor-joining (NJ) method [21]. Bootstrap resampling and reconstruction were carried out 1000 times for confirming the reliability of phylogenetic trees [22].

Discussion

Genotyping is the genetic characterization of a genome, which can classify the genomes based on nucleotide substitutions, deletions or insertions, and it is able to discriminate one individual strain from another. This information constitutes the molecular virological characteristics of the strain and may be useful clinically [23]. Eight different HBV genotypes,

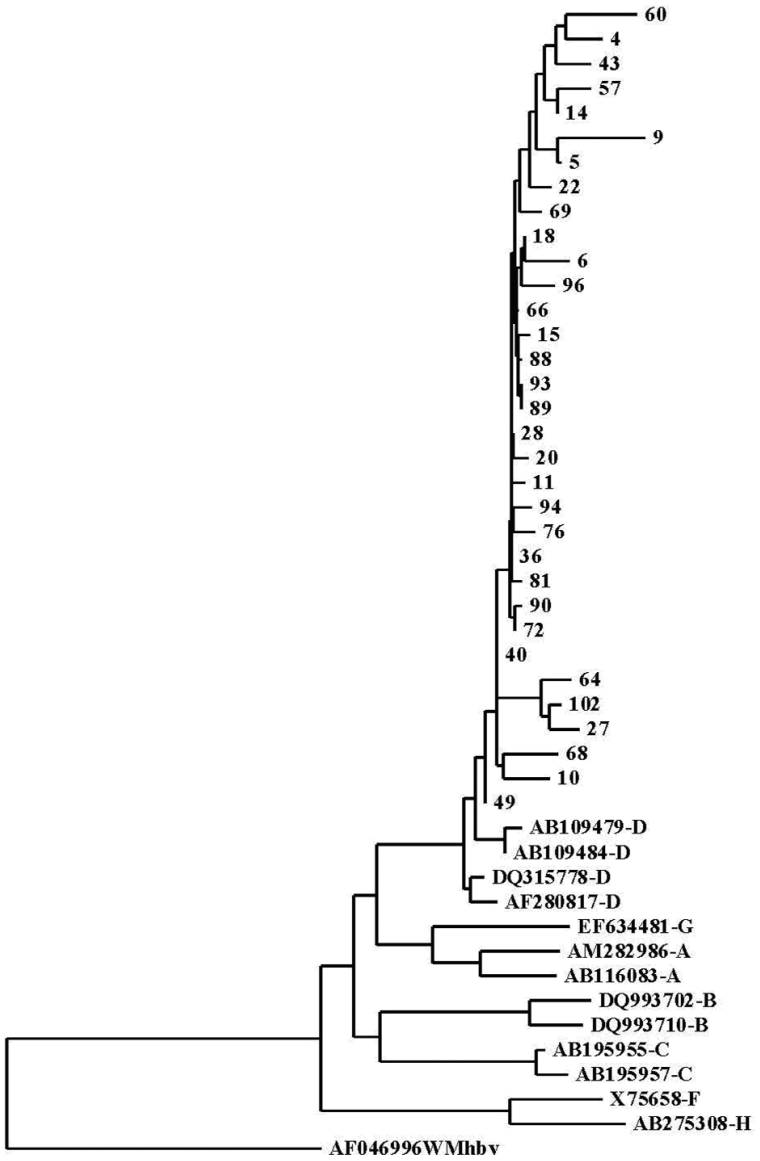

Figure 1. A neighbor joining phylogenetic tree based on the 1000bp combined partial HBsAg sequences of human HBV with 50 reference sequences and 40 Iranian isolates from this survey. Woolly monkey hepatitis B virus (WM-HBV) was utilized as an outgroup. The letters A to H designate to different genotypes of HBV. Iranian isolates are marked by numbers.

namely genotypes A-H, have been identified by genome sequencing of HBV strains obtained worldwide [24]. Genotypes can also be identified by reverse hybridization of polymerase chain reaction amplification products with genotype-specific probes [25].

The Middle East countries have known as an endemic region of HBV infection. The hepatitis B carrier rates in this area have been reported to range from 2% to 18.5%, which differs from country to country [26]. There is little information on the HBV genotype and its genome sequence in Iran and the Iranian population infected with HBV. In this study, we analyzed the S region of HBV-DNA isolated from 60 native patients with chronic HBV infection. The data obtained in this study indicate that Iranian patients with chronic hepatitis B, do not show genotypic diversity. All HBV sequences yielded molecular characteristics of genotype D genomes by being similar in length regarding to preliminary report of HBV genotype in Iran [27].

All the HBV isolates from Iranian patients were clustered in Genotype D branch with high bootstrap values. These results are the same as epidemiological studies from the Mediterranean region [13, 28-31], Iran [27, 32], and may be representative for neighboring countries. For instance 100% of the 109 patients with chronic hepatitis B patients investigated in Karachi have Genotype D and two patients had co-infection with genotype A [33]. In Russia [34] phylogenetic analysis of the HBV pre-S2 gene demonstrated two clear clusters of genotype D strains present that were clearly separate from strains of the same genotype isolated from other regions of the world. In India [35] only genotypes A and D were present and genotype D was dominant in chronic liver disease patients from New Delhi. Also there are some reports about HBV genotype D in Turkey [23], Afghanistan [36], and Egypt [37].

In conclusion, 60 HBV isolates from Iranian patients with chronic hepatitis B represent homogenous genotypic diversity. These sequences of Iranian HBV genomes may contribute to the information on the genetic diversity of HBV worldwide.

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