

Brief Report

Hepatitis B Virus Genotypes in Eastern Azerbaijan, Northwest Iran

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Abstract

Background: The aim of this study was to investigate, for the first time, the genotype of hepatitis B virus (HBV) among hepatitis patients in Eastern Azerbaijan Province, Northwest Iran.

Methods: A total of 100 HBV-infected patients were enrolled in this study. Among these, 40 samples were positive for both HBsAg and HBeAg, whereas 60 samples were HBsAg positive and HBeAg negative. Patients' sera were subjected to DNA extraction and the genotype determined by PCR using type-specific primers.

Results: The results of genotyping revealed that genotype D was the only identified genotype in both acute and chronic patients in the study region. Analysis of sequencing results showed 98% – 99% homology with the previously reported HBV genotype D sequences.

Conclusions: Genotype D was recognized as the predominant HBV genotype in the studied area. There was no significant relationship between genotype D of HBV and different types of infections.

Keywords: Genotype, Hepatitis B virus, Iran, Nested PCR

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Introduction

Hepatitis B virus (HBV) infection is a major health problem worldwide, accounting for 500,000 to 1.2 million deaths annually. The global prevalence of HBV chronic carriers is considered more than 350 million, of which 75% are Asians.¹ The Middle East is considered to be an intermediate endemic area for HBV infection; the carrier prevalence in this region is between 2% to 18.5%. Therefore, Iran is a region of intermediate endemicity for HBV infection. According to a report by the World Health Organization (WHO) and Center for Disease Control and Prevention (CDC), the overall prevalence of chronic hepatitis B infection in Iran is around 2% – 7%.^{2,3}

HBV strains have been classified into eight different genotypes (A–H) on the basis of a nucleotide diversity of > 8% in the complete genome sequences. Different HBV genotypes show distinctive geographical distributions which are important epidemiologically.⁴ It has been shown that disease prognosis and response to treatment varies depending on the genotype of the infecting virus. Hence, determination of the HBV genotype is valuable for clinical management of an infection.⁵ Genotype D has been reported as the predominant genotype in some locations in Iran,^{6–8} but to the best of our knowledge there is no data available about HBV genotype in Northwest Iran. In the present study we have investigated, for the first time, the genotype of HBV among hepatitis patients in Eastern Azerbaijan Province, located in Northwest Iran.

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Materials and Methods

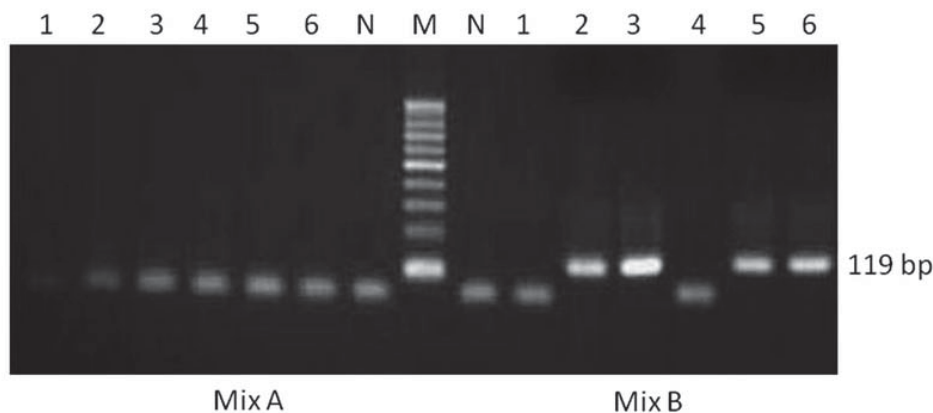
The study population consisted of hepatitis patients who referred to the referral laboratory of East Azerbaijan Province, between May 2009 and February 2010. Demographic information such as age, gender, occupation, disease and hospitalization history, surgery, blood transfusion or organ transplantation, and history of familial infection with HBV were collected for all patients. Serological markers for hepatitis B infection that included HBsAg and HBeAg were determined for all samples. DNA extracted from patients' sera were subjected to genotyping according to the method described by Naito et al. This method is based on the use of type-specific primers and nested multiplex PCR for genotyping of HBV (Table 1).⁹ To confirm the PCR genotyping results, randomly selected PCR products were subjected to direct sequencing.

Results

Of 100 samples that were screened, 40 were positive for both HBsAg and HBeAg, whereas 60 samples were positive for HBsAg and negative for HBeAg. According to the questionnaires, 30 HBsAg-positive HBeAg-negative patients had histories of chronic HBV infection, whereas the remaining 30 were recently infected. Of 100 analyzed samples, 85 (85%) demonstrated genotype-specific PCR bands. In all positive subjects the size of the specific band indicated the presence of HBV genotype D. An example of our PCR genotyping results are shown in Figure 1. Genotyping was unsuccessful in 15 cases; all were negative in both first and second PCRs. In the first group (40 HBsAg- and HBeAg-positive cases), 14 (35%) were positive in the first PCR and 39 (97.5%) samples were positive in the second PCR. In this group only one sample was unable to be genotyped. In the 30 HBsAg-positive, HBeAg-negative patients who had no histories of any chronic infection, 7 (23.3%) in PCR1, and 25 (83.3%) in PCR2 were positive. Finally,

Table 1. Primer sequences used for HBV genotyping

Primer	Nucleotide sequence	Position	Polarity	Genotype Specificity
First PCR				
P1	5>-TCA CCA TAT TCT TGG GAA CAA GA-3>	nt 2823–2845	Sense	Universal HBV primers
S1-2	5>-CGA ACC ACT GAA CAA ATG GC-3>	nt 685–704	Antisense	
Second PCR Mix A				
B2	5>-GGC TCM AGT TCM GGA ACA GT-3>	nt 67–86	Sense	Type A Type B Type C
BA1R	5>-CTC GCG GAG ATT GAC GAG ATG T-3>	nt 113–134	Antisense	
BB1R	5>-CAG GTT GGT GAG TGA CTG GAG A-3>	nt 324–345	Antisense	
BC1R	5>-GGT CCT AGG AAT CCT GAT GTT G-3>	nt 165–186	Antisense	
Mix B				
BD1	5>-GCC AAC AAG GTA GGA GCT-3>	nt 2979–2996	Sense	Type D Type E Type F
BE1	5>-CAC CAG AAA TCC AGA TTG GGA CCA-3>	nt 2955–2978	Sense	
BF1	5>-GYT ACG GTC CAG GGT TAC CA-3>	nt 3032–3051	Sense	
B2R	5>-GGA GGC GGA TYT GCT GGC AA-3>	nt 3078–3097	Antisense	

**Figure 1.** Electrophoresis pattern of PCR genotyping reactions. All positive samples showed a 119bp specific band which indicates genotype D in Mix B (lanes 2,3,5,6). Lanes 1 and 4 did not show any genotype-specific band. M: DNA ladder 100bp, N: No DNA template control.

in the third group (30 HBsAg-positive, HBeAg-negative patients with chronic infection), one (3.3%) sample in the first PCR, and 21(70%) samples in the second PCR were positive. The most untypable group was the chronic patients of which we were unable to determine the genotype of 9 (30%) samples. Genotype D was the only genotype found in both acute and chronic patients, as well as in all HBeAg-positive and negative patients.

Comparison of sequencing results with HBV sequences retrieved from the Genbank databases indicated that all sequences were consistent with genotype D. Alignment analysis showed 98% – 99% homology with the previously reported genotype D sequences. The sequence of a preS1/preS2/S gene from one patient sample was deposited in the Genbank under accession number JF773381.1.

Discussion

According to studies that have been performed in different parts of Iran, genotype D was the only type present in both the central and southern part of the country.⁶⁻⁸ However genotype B has been reported in a case in Kermanshah Province, which is located in Western Iran.¹⁰ Studies from Iran's neighboring countries such as Turkey,¹¹ Pakistan,¹² and Saudi Arabia,¹³ have shown genotype D

as the predominant HBV genotype, however lower rates of other genotypes have also been reported. According to our knowledge, this is the first report of an HBV genotype from East Azerbaijan Province in Northwest Iran. Results of this study have indicated that genotype D is the predominant genotype in this part of Iran. This finding is in agreement with most reports from other parts of Iran as well as other countries in the Middle East.^{6-8, 11-13}

Despite findings about the correlation between different HBV genotypes and clinical forms of infection, the clinical outcome of infection with genotype D is still controversial. The result of most worldwide investigations have demonstrated that HBV genotype D is found in all clinical forms of infection, including asymptomatic carriers, acute and chronic infections, fulminant hepatitis, and hepatocellular carcinoma.¹⁴ The results of our study have shown that genotype D is responsible for both acute and chronic forms of the infection, thus indicating the lack of any significant relationship between genotype D and different clinical forms of infection. This finding is in accord with previously published reports from other countries.

In conclusion, the results of this study indicate that genotype D is the predominant HBV type found in Northwest Iran.

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