

Short Report

Distribution of Hepatitis B Virus (HBV) Genotypes among HBV Carriers in Isparta

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ABSTRACT

Background: The aim of this study was to investigate the prevalence of hepatitis B virus (HBV) genotypes in Isparta, Southwest of Turkey, as well as the clinical features and transmission route for patients with HBV infections. **Methods:** Patients (n = 135) with HBV infection were included in the study. Epidemiological and clinical data were obtained. HBV genotypes were determined with a preS2 epitope ELISA kit. **Results:** Although the HBV transmission route remained unidentified in 51.1% of the patients, blood contact was determined as the most common probable transmission route (38.5%). One hundred twenty-four (91.8%) of 135 samples, could be genotyped. One hundred fifteen (85.1%) were genotyped as type D/E, six (4.4%) were genotyped as type A, two (1.4%) were genotyped as type C, and one (0.7%) were genotyped as type F. **Conclusion:** Genotype D/E is determined as the predominant HBV genotype circulating in Isparta, Southwest of Turkey. No relationship between genotypes and disease severity and transmission route has been detected.

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Keywords: Hepatitis B virus (HBV), genotype, transmission route

INTRODUCTION

Hepatitis B virus (HBV) is a well-known agent of acute and chronic hepatitis, with an estimated 350 million chronic carriers around the world [1]. The serological and genetic heterogeneity of HBV are well established [2]. Worldwide HBV isolates have been classified into six genotypes (A-F) and 8% divergence in the whole genome sequence or 4.1% divergence in the S gene sequence represents the basis for differentiating various genotypes [3]. In addition, two new genotypes, G and H, have been reported recently [4-6]. Based on the reactivity of hepatitis B surface antigen (HBsAg), the envelope glycoprotein of the virus, HBV isolates can be classified into four major subtypes, ayw, ayr, adw, and adr [7].

Previous studies have suggested that the natural

histories of HBV carriers, patients' responses to interferon therapy, and the development of chronic hepatitis and/or liver cirrhosis are associated with specific HBV genotypes [8-10]. Since genotyping previously required labor-intensive methods like HBV DNA sequencing or PCR plus restriction fragment length polymorphism analysis, little HBV genotyping of HBsAg-positive individuals has been performed. With the advent of an ELISA kit with monoclonal antibodies against the preS2 region [11], it became possible to genotype large numbers of samples with HBsAg more easily. Genotype ELISA is a better preferable, simple and rapid system for HBV genotyping than nucleic acid-based technologies. As Moriya *et al.* [8] have shown that, HBV genotype ELISA assay can not only determine HBV genotypes, but also can indicate possible preS2 mutations and other new genotypes in

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un-genotypeable cases as well. The correlation between the results of genotyping with this ELISA kit and those of nucleic acid-based technologies has been excellent [11].

The aim of this study was to investigate the prevalence of HBV genotypes in Isparta, Southwest of Turkey, as well as the clinical features and transmission route for patients with HBV infections.

MATERIALS AND METHODS

Study subjects. Patients (n = 135) with HBV infection who were admitted to Süleyman Demirel University Hospital (Isparta, Turkey) (Fig 1.) between January 2004 and October 2005 were included in the study. HBV infection was defined by the presence of positive HBsAg, anti-Hepatitis B core (IgM + IgG) and HBV DNA-positive. Epidemiological and clinical data were obtained. The possible transmission route of HBV, with regard to blood contact, sexual, or horizontal transfer (e.g., the presence of HBV in close contacts), was investigated with detailed interrogation. If the probable route of transmission could not be established, it was recorded as unknown.

Biochemistry and serology. Liver biochemical tests including ALT (alanine transferase, range: 10-35 U/L) and AST (aspartate amino transferase, range: 10-40 U/L) were carried out for all patients. Serological markers for hepatitis viruses were tested with commercially available kits (Axsym; Abbott Laboratories, North Chicago, IL, USA) for HBsAg, Hepatitis B early antigen (HBeAg), anti-HBc (IgM/IgM + IgG).



Fig. 1. Map of Isparta city in Turkey.

HBV DNA. DNA was extracted from serum by using the RTP DNA/RNA Virus mini kit (Roboscreen, Leipzig, Germany). Serum HBV DNA was measured with an ABI PRISM 7700 automated device (Applied BioSystems, Courtaboeuf, France) by means of real-time PCR (10^2 - 10^8 copy/ml ranges).

HBV genotypes. Genotypes were determined by using preS2 epitope ELISA kit (HBV Genotype; Institute of Immunology, Tokyo, Japan) by following the manufacturer's package insert procedure [11]. Briefly, at the first step, HBsAg in the serum was captured on a 96-well microplate coated with monoclonal antibody to the common determinant "a" of HBsAg. In the second step, each of four wells received enzyme-labeled monoclonal antibodies to a genotype-specific preS2 epitope (m, k, s, or u). Genotypes were determined by the combination of preS2 epitopes: su for genotype A, m for genotype B, ks for genotype C, ksu for genotypes D and E, and k for genotype F. In this assay, genotypes D and E have the same reactions with the defining monoclonal antibodies. When the sera reactive for HBsAg had no genotype-specific epitopes, antibody to epitope b, expressed on all HBV genotypes, was used in the second step to determine the presence of a preS2 product in the sample.

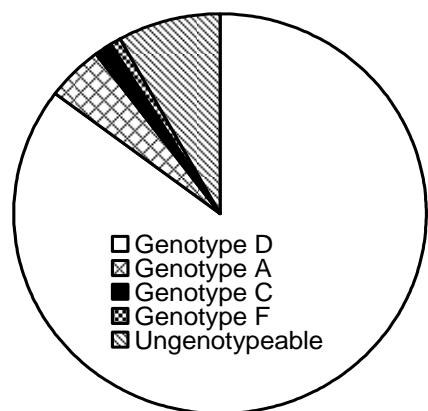
RESULTS

The mean age of the patients with HBV infection was 35.1 ± 14.8 years (81 men and 54 women; age range, 18 to 65 years). All patients were native residents of Turkey. Although the HBV transmission route remained unidentified in 51.1% of the patients, the most common probable transmission route was blood contact (38.5%), following recent surgery, dental treatment, tooth extraction or body piercing, followed by intra familial (5.9%) and heterosexual transmission (4.4%). Neither homosexual transmission nor a history of intravenous drug use was documented for any patient. The mean ALT and AST levels were 78.2 ± 75.5 U/L and 54.5 ± 50.8 U/L, respectively. ALT levels in 82 of 135 patients were higher than normal range. Of the 135 patients, 31 were positive for HBeAg and of 135 samples, 124 (91.8%) could be genotyped: 115 (85.1%) were genotyped as type D or E (preS2 subtype ksu), six (4.4%) as type A (subtype su), two (1.4%) as type C (subtype ks), and one (0.7%) as type F (subtype k) (Fig. 2). The distribution of transmission route, ALT

Table 1. The distribution of transmission route, ALT level, and HBeAg positivity according to HBV genotype.

	Genotype D (n = 115)	Genotype A (n = 6)	Genotype C (n = 2)	Genotype F (n = 1)	Ungenotype (n = 11)
Transmission route					
Blood contact (n = 52)	42	2	1	-	7
Intrafamilial (n = 8)	8	-	-	-	-
Sexual (n = 6)	5	1	-	-	-
Unidentified (n = 69)	60	3	1	1	4
ALT level					
Normal level (n = 53)	45	3	1	1	3
High level (n = 82)	70	3	1	-	8
HBeAg positivity (n = 31)	28	-	-	-	3

level, and HBeAg positivity according to HBV genotype are shown in Table 1.

**Fig. 2.** HBV genotype distribution in Isparta.

DISCUSSION

The present study showed that blood exposure is still the major means of acquiring HBV infection in our region. Intra-familial transmission and heterosexual contact were the other more important transmission routes, but this percentage was below the reported rates for other countries in Europe [9, 12]. Intravenous drug use is an important risk factor for transmission of HBV and HCV, but no drug use was identified in the present study. Especially in developing countries it is possible that sexual transmission and drug use may be under-reported because of social pressure. In Denmark, roughly, one-third of acute cases has been attributed to sexual transmission and one-third to injecting drug use, while the route of transmission in the remaining cases has been unknown [12].

This study demonstrates that genotype D/E is the predominant genotype circulating in Isparta,

Southwest of Turkey. Since, genotype D is reported to be the most common genotype also in the remaining parts of our country [13], we can suggest that our genotype D/E results may reflect genotype D. Predominance of this genotype has been reported from the Mediterranean region, the Middle East, and India [1, 2]. Genotypes B and C are prevalent in Asia and the Far East, while genotype A is prevalent in northwestern Europe, North America and Africa [14, 15]. Genotype F is found in Central and South America, while genotype E circulates in sub-Saharan Africa [1, 16]. Genotype G has been reported from France and North America [5, 6]. Genotype H has been described only recently, and its distribution is not understood yet [6]. Unfortunately, we could not look for genotype G and H in this study since the ELISA kit we have studied was not able to detect these genotypes and we had no other additional technical equipment.

In addition, we are not able to put forward a conclusion about the relationship between HBV genotype and transmission route since most of genotypeable samples were detected as genotype D.

Genotypes are considered as useful tools in understanding the epidemiology of HBV infection. Further, the possibility of their association with severity of liver disease has been actively pursued in recent years [17-19]. The important issue is whether a particular genotype is associated with certain clinical presentations of the disease [2]. Studies from Japan and Switzerland have shown clear association of genotype C and A, respectively, with severe clinical disease [10, 20]. Gandha *et al.* [2] reported that irrespective of the clinical category, i.e., patients with resolved acute viral hepatitis B, fulminant hepatitis B patients, asymptomatic HBsAg carriers with consistently normal serum ALT levels and patients suffering from chronic liver diseases,

genotype D were detected in most of the patients (91.93%). Therefore, they concluded that genotype D did not influence the clinical manifestations. In our study, irrespective of the laboratory findings like normal serum ALT levels, high serum ALT levels, HBeAg positivity, genotype D/E was detected in most of the patients (85.1%). So we can conclude that genotype D did not influence the clinical features. A study of 39 asymptomatic HBV carriers and 103 liver diseases patients from southern China showed circulation of A, B, C, and D genotypes with 78.9% being genotype C. Severe liver damage was observed in patients with genotype C as compared with those with genotype A or B [18].

In conclusion, genotype D is the predominant HBV genotype circulating in Isparta, Southwest of Turkey. No relationship between genotypes and disease severity and transmission route was detected.

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