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Research paper

Genotype characteristic and phylogenetic analysis of hepatitis B virus in northeast-Iran

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ABSTRACT

Viral hepatitis is considered as a worldwide health problem and hepatitis B virus (HBV) infection is one of the major health concerns which are annually responsible for more than one million deaths. HBV can be classified into at least eight genotypes, A–H and four major subtypes. Predominant HBV genotype in Mediterranean and Middle East countries is genotype D, but there is a few studies have been performed on the HBV genotype in Iran. The genotype characteristic and phylogenetic analyses were determined in chronic HBV patients in the northeast of Iran. First, seventy-eight patients with chronic HBV infection were enrolled. Demographic features were reviewed and sera samples were collected. HBV genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and results were confirmed by sequencing. Finally, a phylogenetic tree was obtained using Geneious software. Sixty-two (79.48%) of patients were males (mean age: 36.82 years). Twelve out of 78 patients (15.4%) were hepatitis B envelope antigen (HBeAg)-reactive. There were no significant differences between the clinical and HBeAg-positive serological data and HBeAb positive individuals. RFLP DNA sequencing and phylogenetic analysis showed that genotype D was the only genotype which observed in Mashhad, northeast of Iran. This is the first report of HBV genotyping in Mashhad. The results revealed that genotype D was the only genotype detected in this area which was consistence with previous studies in the Middle East, Mediterranean countries, southwest and center of Iran.

1. Introduction

Hepatitis B virus (HBV) infection is one of the most significant global health problems and is annually responsible for more than one million deaths worldwide (Zuckerman and Zuckerman, 2003). It is estimated that there is currently more than 350 million people infected with HBV, approximately 5% of the world's population (www.who.int/vaccines-documents/).

The Middle East and Southeast Asian countries are recognized to be high endemic regions of HBV infection; however, there is a lack of data regarding the prevalence of HBV infection in some parts of these regions. In this region, the rates of hepatitis B carrier are also different, ranging from 2% to 18.5% according to different studies (Andre, 2000; Toukan, 1996). Notably, Iran has been known as a mid-endemic area with a prevalence of approximately 2%. In addition, more than 35% of Iranian population has been exposed to HBV infection (Malekzadeh et al., 1997; Merat et al., 2000).

Generally, HBV infection is related to a wide range of clinical manifestations, including: acute and fulminant hepatitis, inactive carrier state, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Chen, 1993). There are not clear study regarding the relation between HBV infection and liver cancer in Iran. According to the literature, it has been shown that HBV is a frequent etiology of hepatocellular carcinoma (HCC) in southern Iran (Alavian and Haghbin, 2016; Hajiani et al., 2005).

Several molecular epidemiology studies have reported a correlation between HBV genotypes and various epidemiological, virological, and clinical outcomes of infection (Duong et al., 2004). HBV can be

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Table 1
The demographic and laboratory characteristics of patients.

	HBsAg-positive	HBeAg-positive	HBeAb-positive
No. of patients (%)	78 (100%)	12 (100%)	42 (100%)
Age	36.83 ± (14–71)	28.61 ± (15–52)	36.83 ± (17–64)
T-Bilirubin (mg/dL)	1.49 ± 0.74	1.06 ± 0.92	1.08 ± 0.87
ALT (U/L)	79.22 ± 42.4	69.41 ± 58.7	62.83 ± 53.2
AST (U/L)	58.26 ± 43.1	44.81 ± 38.6	45.78 ± 39.4

Data are expressed as mean ± SD. T-Bilirubin, Total-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

classified into at least eight genotypes, designated A–H and four major subtypes, ayw, ayr, adw and adr which is related to geographic distribution (Arauz-Ruiz et al., 2002; Norder et al., 1992; Okamoto et al., 1988; Stuyver et al., 2000; Takahashi et al., 1998). Globally, predominant HBV genotype among patients with HBV infection in North America, Northwest Europe and Africa is A, while HBV genotype B and C are frequently found in China and Japan. Genotype D is known to be the most frequent genotype, distributed evenly worldwide but particularly in the Mediterranean countries, the near and the Middle East, and South Asia (Abdo et al., 2006; Haghshenas et al., 2014).

Genotype E is confined to West Africa, genotypes F and H, those so-called Amerindian genotypes, are more likely present in populations originated from Central and South America and Genotype G has been reported in France and the USA (Abdo et al., 2006; Arauz-Ruiz et al., 2002; Chu et al., 2003; Duong et al., 2004; Halfon et al., 2006; Suzuki et al., 2003).

Few studies have been carried out regarding the distribution of HBV genotypes in Iran. Two recently published studies have been reported that genotype D was the only genotype observed in Tehran and southwest of Iran (Amini-Bavil-Olyaee et al., 2005a; Amini-Bavil-Olyaee et al., 2005b; Mojiri et al., 2008). This was similar to other studies in the Middle East countries, such as Turkey (Bozdayi et al., 2005; Senturker Guldas and Abacioglu, 2004), Egypt (Saudy et al., 2003) and Yemen (Sallam and William Tong, 2004). However, other HBV genotypes have also been submitted to the GenBank database from Middle East countries, such as genotype A in Uzbekistan (Kato et al., 2002), genotypes B and C in Pakistan (Alam et al., 2007) and genotype E in Saudi Arabia (Abdo et al., 2006).

Razavi Khorasan is an important and big province located in Northeastern Iran, in the vicinity of Afghanistan, Turkmenistan, Pakistan and also near to Uzbekistan; countries with different reported HBV genotypes (Abdo et al., 2006; Alam et al., 2007; Kato et al., 2002). Historically, it was a famous trade place, because the Silk Road and Eurasian Route passed through this province and their cities, especially Mashhad.

Moreover, Mashhad is a holy city with a population of more than 2.5 million people, which is a holy city for Muslims; with a population of around 2.5 million people is the center and capital of this province and a very popular destination for pilgrimage tourists that accept more than 20 million visitors per year. Thus, studies on infectious agents particularly HBV infection have a high priority. In a comprehensive study, the prevalence of some viral infection such as Kaposi's sarcoma-associated herpesvirus (KSHV), Herpes simplex virus (HSV), and human T lymphotropic virus type 1 (HTLV-1) has been reported from Mashhad. The prevalence of HBV infection in Khorasan province is estimated to be 3.6% (Farhat et al., 2003). Currently, genotype D is the only genotype which has been reported in South Khorasan (Ghaziasadi et al., 2012; Ramezani et al., 2015); however, this is the first report of HBV genotyping in the northeast of Iran, Mashhad.

2. Materials and methods

2.1. Study population

The study protocol was reviewed, approved and supervised by the Mashhad University of Medical Sciences Ethics Committee (permit number: MUMS 84066) and a written informed consent was taken from all the participants. A total of 78 patients (62 men and 16 women; mean age = 36 ± 12.35 years old, ranged 14–71) with chronic HBV infection who had been referred from different parts of Razavi Khorasan province, to Gastroenterology Research Center, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad-Iran, were enrolled. All patients were positive for HBsAg for at least 6 months. Patients with evidence of co-infection with hepatitis C virus (HCV), hepatitis D virus (HDV), human immunodeficiency virus (HIV) and HTLV-1 were excluded.

2.2. Demographic, biochemical and serologic data

Demographic characteristics were reviewed. Sera were collected from all patients to investigate biochemical and serological measures

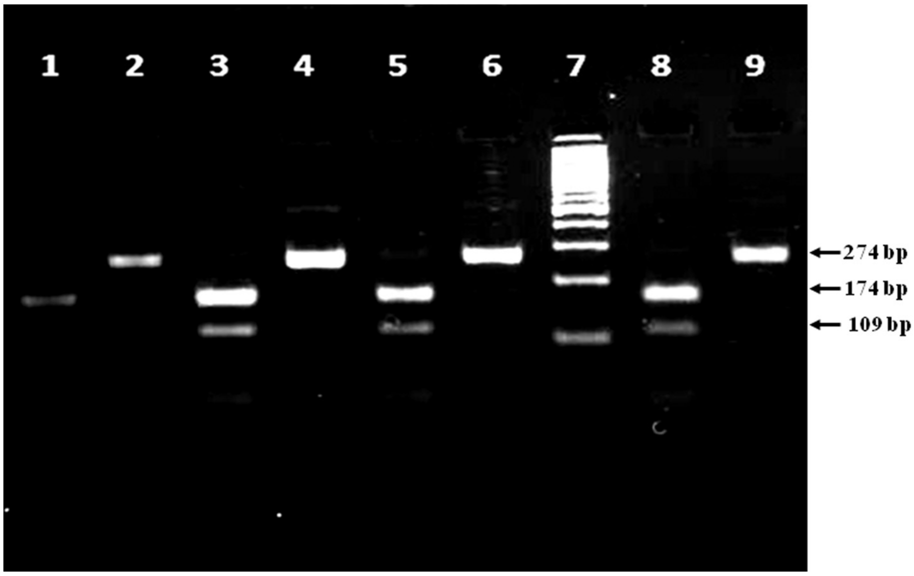

Fig. 1. The PCR-RFLP products on 2% agarose gel electrophoresis. Lanes 1, 3, 5, and 8 shows the digested products with *Tas*I (*Tsp*509I) (174 bp and 109 bp); lanes 2, 4, 6, and 9 digested products with *Hinf* I (274 bp); lane 7, 100 bp plus DNA marker.