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Short Article

Determination of Hepatitis B Virus Genotypes in Yazd, Central Province of Iran

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Background and Aims: Hepatitis B virus (HBV) infects the liver and causes acute and chronic hepatitis, hepatocellular carcinoma and cirrhosis. HBV has been divided to eight genotypes (A–H) and subgenotypes of A, B, C and F. For the first time, we determined HBV genotypes in infected samples by INNO-LiPA method in Yazd, central province of Iran.

Materials and Methods: This study was performed on samples suspected of HBV infection. The sera of fifteen out of ninety-five samples that had shown positive results by RT-PCR were used for HBV genotyping by using INNO-LiPA HBV genotyping assay.

Results and Conclusions: Seven (46.7%) out of fifteen samples were female. The mean age of the patients was 37.8±14.3 years. The average number copy of HBV in infected samples was $1.04\times10^{6}\pm4.74\times10^{5}$ Copies/ml. All fifteen infected samples had genotype D. Our results showed that HBV genotype D was the only detectable genotype in Yazd, central province of Iran. A further study with a larger sample size in different provinces of Iran is needed to identify HBV genotypes in Iran.

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Introduction

The hepatitis B virus (HBV) which is classified in the virus family Hepadnaviridae was discovered in 1966 [1, 2]. HBV infects liver and may cause chronic hepatitis, cirrhosis and hepatocellular carcinoma [1]. It is estimated that four hundred million people are chronically infected and at risk for HBV related liver disease [3] and two billion people worldwide show serologic evidence of past or present HBV infection [2]. Some studies have shown that different HBV genotypes were determined in the patients with both acute and chronic HBV infections [4]. HBV consists of eight genotypes (A–H) and each genotype was broken down into several subgenotypes. Distribution of HBV genotypes in different regions of world is determined. Genotype A is reported in the northwestern Europe, North America and Africa while genotypes B and C are known to exist in different parts of Asia and Oceania. Genotype D has world-wide distribution and predominates in the Mediterranean area. Genotypes E, F and H are reported in Africans on the West Coast of Africa and Madagascar on the east, the aboriginal populations of South America, and the Amerindian populations of Central America, California and Mexico respectively. Genotype G is found in the carriers of HBV in France, Germany, United Kingdom, Italy and the United States of America. The kind of genotype may influence response to treatment and possibly vaccination against of hepatitis B virus infection [5]. Genotype D has been reported in the neighboring countries such as Afghanistan, Pakistan and Turkey as a predominant genotype [6-8]. INNO-LiPA method is a new and highly sensitive technique for determining the genotypes of hepatitis B virus and candidates are very convenient and reliable means of genotyping for clinical diagnostic laboratories [4]. In this study, we investigated HBV genotypes in infected subjects by INNO-LiPA method for the first time in Yazd, central province of Iran.

Materials and Methods

Patients

The present study was conducted on individuals suspected of HBV infection referring to Yazd Bou-Ali Pathobiology laboratory as a reference molecular laboratory from April 2013 to March 2014. All samples were tested for anti-hepatitis C virus and anti-human immunodeficiency virus which all were negative. The project procedure was approved by Ethics Committee of Yazd Shahid Sadoughi University of Medical Sciences. Five milliliter of each peripheral blood sample was obtained and tested for hepatitis B surface antigen (HBsAg) by a chemiluminescence assay (LIAISON DiaSorin SpA, Italy). Then, the positive sera of the samples for HBsAg were analyzed using Real-time polymerase chain reaction (RT-PCR) method.

HBV viral load assessment

The HBV load in serum samples were assayed according to the manufacturer instruction of the RoboGene HBV DNA Quantification Kit (AJ Roboscreen GmbH, Germany). We used

Rotor-Gene 6000 (Corbett Research, Sydney, Australia) to determine the serum viral load. Analytical sensitivity of the kit was 250 copies/ml.

DNA extraction, LiPA amplification and detection

DNA from fifteen samples who had showed RT-PCR positive were extracted from 150µl of serum by High Pure Viral Nucleic Acid Kit (Roche Diagnostics, USA). DNA extracts were immediately stored at -20°C until being used. DNA of samples were analyzed for HBV genotyping using INNO-LiPA HBV Genotyping assay (LiPA; INNO-LiPA HBV Genotyping assay, Innogenetics N.V., Ghent, Belgium). For amplification of the HBsAg region and in order to provide a biotinylated product, the extracted DNA was amplified by nested PCR and PCR products obtained with both sets of outer and nested primers which generate an amplicon of 409 bp and 342 bp respectively. For Genotyping, the PCR products were denatured by denaturation solution incubated with a test strip for hybridization of the denatured amplicon to genotype-specific probes immobilized as parallel lines on each strip. Following hybridization, the strips were stringently washed and incubated with a streptavidin conjugate to allow color development from the biotinylated DNA bound to the strip.

Results and Discussion

The demographic characteristics of RT-PCR positive samples for HBV are shown in Table1.

Table 1. The demographic characteristics of RT-PCR positive samples

Sex	Count (%)	Age (Mean±SD)	Viral load (Mean±SEM)
Male	71(74.7)	39.6±16.4	$3.31 \times 10^{11} \pm 3.1 \times 10^{11}$
Female	24(25.3)	41.8±13.2	$2.9 \times 10^{7} \pm 2.83 \times 10^{7}$
Total	95 (100)	41.3±14.1	$2.29 \times 10^{11} \pm 2.19 \times 10^{11}$

There was no significant difference between sex and age (p=0.4). Seven (46.7%) out of fifteen samples were female. The mean age of the patients was 37.8±14.3 (range 18-69 years). The mean of HBV was $1.04 \times 10^{6} \pm 4.74 \times 10^{5}$ copies/ml. For all fifteen specimens, genotype D was determined by INNO-LiPA method (Table 2).

Table 2. Characteristics of Studied Patients for HBV Genotyping

Patients Number	Sex	Age (yr.)	Viral load (Copies/ml)
1	F	34	422275
2	M	18	2446093
3	F	33	6116090
4	F	39	3977
5	F	69	281752
6	F	30	4305637
7	M	28	960132
8	F	28	108040
9	M	34	525750
10	M	21	1119
11	M	32	48046
12	M	57	365023
13	M	37	348
14	M	51	26719
15	F	56	1987
Total		37.8±14.3 (Mean±SD)	$1.04\times10^{6}\pm4.74\times10^{5}$ (Mean±SEM)

All samples had genotype D

F=Female, M=Male

A representation of the membrane strip with all the immobilized control and genotype-specific oligonucleotide bands and strip of one sample is shown in figure 1.

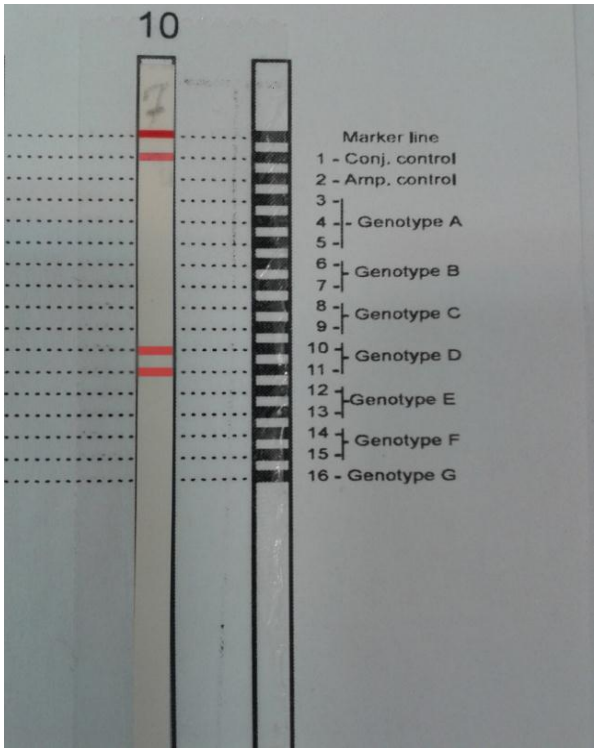

Fig.1. A representation of the INNO-LiPA HBV genotyping assay strip and strip of positive sample for genotype D.

According to the high mortality (500,000 deaths annually) occurring as a consequence of cirrhosis and hepatocellular carcinoma caused by chronic HBV infection, there is a need for further studies on HBV infection. Determine of patient strains of HBV can be useful for prognosis and response to treatment, the severity and activity of liver disease, determination of the virus origin and course of evaluating HBV [9, 10].

In this research using INNO-LiPA method, we showed that all the HBV infected samples were positive for genotype D. Attaullah et al. detected prevalence of HBV genotypes in HBsAg positive individuals in Afghanistan people. They found that genotype D (35.67%) is the predominant genotype. Genotype C was identified in 32.16% of samples followed by genotype A (19.3%), and genotype B (7.02%) [6]. A systematic review of the prevalence, risk factors, awareness status and genotypes HBV in the Pakistan population showed that genotype D (63.71%) is the most prevalent genotype in Pakistan [7]. In line with our results, Atalay et al. found genotype D in Turkish patients with chronic hepatitis B. Other genotypes were not detectable [8]. In China, Southeast Asia, and the Pacific Islands, genotypes B and C are dominant genotype; however in Africa, genotypes A, D, and E are the most common genotypes. In countries such as the USA and Western Europe with a low prevalence of chronic HBV infection and a multiethnic population, genotypes A and D are dominant although multiple genotypes are be expected to exist [11]. To date, the methods for determining of patient strains of HBV that are based on PCR with genotype-specific primers or PCR amplification of the pre-S region or S region are quick and simple, but the interpretation of the results in multiple infections and the genetic determination of all isolated types is troubled. INNO-LiPA, based on S-gene analysis can be compared with full genome analysis for genotyping purposes. Hussain et al. diagnosed HBV genotypes in patients with chronic HBV infection from China and the United States using INNO-LiPA assay and sequencing methods. They showed the results of the two methods being completely concordant [12]. In this study, we used INNO-LiPA assay for HBV genotyping and all samples were typed. Since several genotypes of HBV are highly closely associated with the severity, development of severe liver diseases and antiviral therapy and the INNO-LiPA HBV genotyping assay is a convenient and rapid method, we suggest INNO-LiPA HBV genotyping assay as a sensitive and reliable means of genotyping for clinical diagnostic laboratories.

Conclusion

Our finding showed that all HBV infected samples were positive for genotype D in Yazd, central province of Iran. Future studies are needed to work on the prevalence of HBV genotypes in different provinces of Iran and compare it to the neighboring as well as other countries.

Conflict of Interest

The authors confirm that this article content has no conflict of interest

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