

Detection of Mediterranean Hepatitis B in a 45 Years Old Man in Mahdiah Clinical Laboratory, Isfahan, Iran

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ABSTRACT

Three major hepatitis B virus (HBV) antigens include HBcAg, HBeAg and HBsAg. HBeAg is the extracellular form of HBcAg, and is seen almost exclusively in people who have circulating serum HBV DNA. Presence of HBsAg in serum indicates that the individual has contracted HBV infection. Chronic hepatitis HBeAg-negative/anti-HBe—positive is known as an important form of chronic hepatitis B in the Mediterranean region. In this report, we used Real-Time PCR and ELISA for detection of HBV and HBeAg/HBsAg, respectively. In our investigation on 4243 HBV cases referred to the Mahdiah Clinical Laboratory between 2008 and 2016, we found a 53-year-old man with clinical symptoms of hepatitis and abnormal molecular and serological features. Despite the presence of clinical symptoms and high viral load (128×10^5 iu/ml), the patient was HBsAg-positive and HBeAg-negative. Identifying this type of HBV could indicate spread of this type of hepatitis in Isfahan, Iran.

Keywords: Hepatitis B, HbsAg, HBeAg.

INTRODUCTION

Hepatitis B virus (HBV) is a double-stranded DNA virus from the *Hepadnavirus* family [1]. Approximately 350 to 400 million people worldwide are infected with the virus [2]. There are three major HBV antigens including hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg) and hepatitis B envelope antigen (HBeAg), each of which is of clinical importance in diagnostic tests (Table 1). HBcAg surrounds the viral genome and its expression on the surface of hepatocytes induces an immune response for elimination of infected cells. HBeAg is the extracellular form of HBcAg, and is a viral replication marker similar to HBcAg. HBeAg is found almost exclusively in people who have circulating serum HBV DNA. Elevated level of HBsAg can be detected in serum of patients during acute and chronic phases of HBV infection. Therefore, the presence of HBsAg in serum indicates that the individual is infected (3).

Individuals exposed to HBV generally fall into three phases: immune tolerant phase, chronic hepatitis B phase (CHB), and inactive hepatitis B (or asymptomatic hepatitis B) carrier phase (2). Immune tolerant phase is characterized by presence of HBeAg and high levels of HBV DNA in serum, but minimal or no inflammation on liver biopsy. CHB phase is characterized by high levels of HBV DNA ($>10^5$ copies/mL), presence of HBeAg or anti-HBe and chronic inflammation on liver biopsy. Inactive hepatitis B carrier phase is characterized by presence of anti-HBe with HBV DNA levels but not as high as in chronic hepatitis B (2).

A mutation in the HBV precore region (G to A conversion at position 1896) leads to inhibition of HBeAg production. Moreover, the levels of viral replication, viral genotype and probably the genetic variability of the virus influence the expression of viral antigens [4-6]. In the early 1980s, a mutant form of CHB was reported with replication ability and negative HBeAg [7-9]. We hereby report for the first time, a case with anti-HBe-positive but HBeAg-negative CHB infection in Isfahan, Iran.

CASE REPORT

We examined 5800 suspected hepatitis B cases referred to the Mahdiah Clinical Laboratory in Isfahan (Iran) from 2008 to 2016. Diagnosis was made based on the number of HBV DNA copies and presence of HBsAg and HBeAg in the serum of individuals. Viral DNA was extracted using QIAamp DNA Mini Kit (Cat No: 51304, Qiagen, Germany). Real-Time PCR was performed using Rotor gene 6000 Corbett thermal cycler (Australia) and Artus HBV RG PCR Kit (Cat No: 4506265, Qiagen, Germany). ELISA was performed using Autobio kit (Cat No: E0315, China) to detect HBeAg and HBsAg.

According to the results, 4243 cases were HBV-positive. Among these cases, there was a 53-year-old man with primary clinical symptoms of hepatitis (vomiting, jaundice, tiredness and pain) and high viral load (128×10^5). Interestingly, the HBsAg and HBeAg of this case were positive and negative, respectively. To ensure the accuracy of the results, all experiments were repeated several times.

Table 1- Laboratory markers for HBV infection and its interpretation marker

Antibody/Antigen	Importance
HBsAg	Exposure to HBV. Present in acute or chronic infection
Anti-HBs antibody	Immunity acquired through natural infection or immunization
HBeAg	Marker of infectivity. Correlates with high level of viral replication
Anti-HBe antibody	Correlates with low rate of viral replication
Anti-HBc IgM antibody	Infection in previous 6 months
Anti-HBc IgG antibody	Distant HBV infection or chronic HBV infection
Hep B DNA $>10^5$ copies /mL	Rapid viral replication

DISCUSSION

Approximately thirty years ago, CHB patients with replicating HBV were reported from the Mediterranean region, while having positive anti-HBe and negative HBeAg [10]. Molecular investigations on HBeAg-negative HBV led to the detection of CHB. To date, eight HBV genotypes (A-H) have been identified [2]. Genotype D has been associated with anti-HBe-positive CHB infection in the Mediterranean region [11]. HBeAg-negative CHB indicates that the patient was exposed to HBV strains that are not able to produce HBeAg [10]. In clinical practice, the term HBeAg-negative CHB is appropriate for patients with chronic HBV infection who are HBeAg-negative but usually anti-HBe-positive and have elevated serum HBV DNA levels. The most important and reliable methods of detecting this antigen is hybridization methods, while PCR is a more sensitive technique [3]. Therefore, choosing the most suitable and effective therapy relies on the detection of HBV DNA. Given the rising prevalence of CHB, it is recommended to

inform laboratories and education systems about CHB cases similar to our case in order to maintain quality in detection and therapeutic approaches as well as monitoring the disease and its distribution. Studies have shown that the prevalence of this type of hepatitis is higher in some countries compared to Iran [10]. In addition, other studies in Iran have shown that this type of hepatitis is more common in Mashhad and Tehran (12, 13).

CONCLUSIONS

Considering the detection of this case in Isfahan, it seems that the prevalence of this type of hepatitis in Isfahan is increasing. Therefore, it is highly recommended to provide a comprehensive plan to prevent the spread of this type of hepatitis.

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