Diagnosis of hepatitis B

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Abstract: Hepatitis B virus (HBV) infection is a major global health problems leading to severe liver disease such as cirrhosis and hepatocellular carcinoma (HCC). HBV is a circular, partly double-stranded DNA virus with various serological markers: hepatitis B surface antigen (HBsAg) and anti-HBs, anti-HBc IgM and IgG, and hepatitis B e antigen (HBeAg) and anti-HBe. It is transmitted by sexual, parenteral and vertical route. One significant method to diminish the burden of this disease is timely diagnosis of acute, chronic and occult cases of HBV. First step of HBV diagnosis is achieved by using serological markers for detecting antigens and antibodies. In order to verify first step of diagnosis, to quantify viral load and to identify genotypes, quantitative or qualitative molecular tests are used. In this article, the serological and molecular tests for diagnosis of HBV infection will be reviewed.

Keywords: Hepatitis B virus (HBV); serology; molecular diagnosis

Introduction

Hepatitis B virus (HBV) belongs to the Hepadnaviridae family. It shows a diameter of 30–42 nm and consists of outer lipid envelope containing hepatitis B surface antigen (HBsAg) and an icosahedral capsid core composed of protein (1). Viral capsid bears viral genome and DNA polymerase that has reverse transcriptase activity. HBV genome comprise a circular, partly double-stranded DNA and has four open reading frames overlapped: (I) S that encodes for surface proteins (HBsAg); (II) pre-C/C for hepatitis B e antigen (HBeAg) and core protein (HBcAg); (III) P for polymerase including reverse transcriptase; (IV) X that encodes for a transcriptional transactivator factor (HBxAg) (2). The covalently closed circular DNA (cccDNA) is the transcriptional template of HBV and stays inside the hepatocyte nucleus as a mini-chromosome (3). Reverse transcriptase involved in the replication of HBV is error susceptible, thus the mutation rate is high, similarly observed in retroviruses and RNA viruses (4,5).

HBV infection is responsible for the most of chronic liver diseases worldwide and is transmitted through parenteral, sexual and vertical route. About 240 million people are chronically infected by HBV, so have the risk of developing of liver cirrhosis and hepatocellular carcinoma (HCC). According to HBsAg prevalence, HBV endemicity is divided into three categories; high, intermediate, low. China, South East Asia, Indonesia, and sub-Saharan Africa are regarded as highly endemic areas because chronic HBV infection is reported in more than 8% of the population (6). Intermediate areas show chronic HBV infection rate between 2% and 7% of population, and include South America, South West Asia, Eastern and Southern Europe. Developed countries, such as North America and Western Europe are grouped as low endemic regions; in these areas, HBV prevalence rates range from 0.5% to 2%.

Along with active anti-HBV vaccination, one significant method to diminish the burden of this disease is the diagnosis of acute, chronic and occult HBV infection. In this article, the serological and molecular diagnosis of HBV will be reviewed.

Serological markers for HBV infection

Serological markers for HBV infection consist of HBsAg,

anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM and IgG. The identification of serological markers allows: to identify patients with HBV infection; to elucidate the natural course of chronic hepatitis B (CHB); to assess the clinical phases of infection; and to monitor antiviral therapy (7).

HBsAg is the serological hallmark of HBV infection. After an acute exposure to HBV, HBsAg appears in serum within 1 to 10 weeks. Persistence of this marker for more than 6 months implies chronic HBV infection (8). Several studies have reported the association between transcription activity of cccDNA in the liver and serum HBsAg levels (9-11). Differences in the serum HBsAg levels during the different phases of infection indicate the distribution of cccDNA during the respective phases of the disease. The serum HBsAg titers are higher in patients with HBeAg-positive CHB than in HBeAg-negative CHB (10-12). Monitoring of quantitative HBsAg levels predicts treatment response to interferon and disease progression in HBeAg-negative CHB patients with normal serum alanine aminotransferase levels (13,14).

Anti-HBs is known as a neutralizing antibody, and confers long-term immunity (15). In patients with acquired immunity through vaccination, anti-HBs is the only serological marker detected in serum. In the past HBV infection, it is present in concurrence with anti-HBc IgG. Occasionally, the simultaneous appearance of HBsAg and anti-HBs has been reported in patients with HBsAg positive (16). In most cases, anti-HBs antibodies are unable to neutralize the circulating viruses, thus these patients are regarded as carriers of HBV.

In the past, HBeAg and anti-HBe had been used to know infectivity and viral replication, but their use for this purpose has mostly been replaced by HBV DNA assay. HBeAg to anti-HBe seroconversion is related to the remission of hepatic disease (17), however, active viral replication is sustained in some patients with HBe seroconversion due to mutations in the pre-core and core region that inhibit or decrease the production of HBeAg (8).

HBcAg is an intracellular presence in infected hepatocyte, thus it is not identified in the serum. During acute infection, anti-HBc IgM and IgG emerges 1–2 weeks after the presence of HBsAg along with raised serum aminotransferase and symptoms. After 6 months of acute infection, anti-HBc IgM wears off. Anti-HBc IgG continues to detect in both patients with resolved HBV infection and CHB. Some HBsAg-negative individuals are positive for anti-HBc IgG without anti-HBs, in this situation, it should be considered isolated anti-HBc positive. It can be seen in three conditions. First, it can be predominantly seen as IgM class during the window period of acute phase. Second, after acute infection had ended, anti-HBs has decreased below the cutoff level of detection. Third, after several years of chronic HBV infection, HBsAg has diminished to undetectable levels. If the result of serological markers shows isolated anti-HBc positive, anti-HBc IgM should be checked in order to assess the possibility of recent HBV exposure. HBV DNA assays should be tested in chronic liver disease patients to find out occult HBV infection characterized by existence of detectable HBV DNA without serum HBsAg (18).

Molecular methods for HBV infection

HBV DNA is a direct measurement of the viral load, which reveals the replication activity of the virus. It is detectable at the early stage of infection (1 month after HBV infection) and increases up to peak level (more than 10^8 copies/mL) approximately 3 months after the exposure to HBV and then gradually diminishes in chronic infection or disappears at the recovery from HBV infection.

As the prevalence of serologically negative HBV infection (HBeAg-negative CHB and occult HBV infection) has increased, HBV-DNA detection has obtained more awareness in clinical medicine (19). The detection of HBV DNA is a reliable marker of replication activity, and higher titers of HBV DNA are related to the more rapid disease progression and higher incidence of HCC (20). Furthermore, HBV DNA testing is useful in routine clinical setting to determine patients who need antiviral therapy and monitor them for suitable treatment (21).

There are two principles of techniques to identify and quantify HBV DNA: signal amplification such as hybrid capture and branched DNA technology; target amplification such as polymerase chain reaction (PCR) (19,22). Real-time PCR can detect wide dynamic range of viral load (lower range, 10–15 IU/mL; upper range, 10^7–10^8 IU/mL). For this reason, it has come to be the standard method to detect and quantify HBV DNA in clinical setting. Furthermore, it can be fully automated and does not generate carry-over contamination (23). *Table 1* displays the comparison of assays for quantitative measurement of HBV DNA.

HBV genotyping

HBV has a high genetic heterogeneity because it reproduces via a reverse transcriptase that has insufficient proofreading

Table 1 Comparison of quantitative methods for HBV DNA

Methods	Commercial assay name	Manufacturer	Measurable range (IU/mL)	Limit of detection (IU/mL) (using WHO HBV standard)
Semi-automated qPCR	COBAS AmpliPrep/COBAS TaqMan HBV Test v2.0	Roche Molecular System, California, United States	$20–1.7\times 10^7$	20
Semi-automated real-time PCR	COBAS TaqMan HBV Test for use with high pure system	Roche Molecular System, California, United States	$29–1.1\times10^7$	6
Automated real-time PCR	Abbott RealTime HBV	Abbott Diagnostic, Chicago, United States	$10–1\times10^9$	10
Branched DNA	VERSANT HBV 3.0 Assay	Siemens Healthcare, United States	$2,000–1\times10^8$	2,000

WHO, World Health Organization; HBV, hepatitis B virus; PCR, polymerase chain reaction; qPCR, quantitative PCR.

Table 2 Methods of HBV genotyping

Methods	Advantages	Disadvantages	References
RFLP	Easily done, low cost, simple, rapid	Low sensitivity for typing samples with low HBV	(19,39)
Reverse hybridization	High sensitivity, automated systems	Relatively high cost	(40,41)
Genotype specific PCR	High sensitivity, automated systems, easy to perform, suitable for detecting mixed genotype infections	High cost	(41)
Sequence analysis	Gold standard method for genotyping, identification of patients infected with recombinant genotypes	Time consuming, technically demanded	(41)

HBV, hepatitis B virus; RFLP, restriction fragment length polypmorphism; PCR, polymerase chain reaction.

capability. According to the sequence divergence, HBV can be divided into ten genotypes, labelled A–J: they have distinct geographic distribution (24). Genotype B and C are restricted to Oceania and Asia, whereas genotype A and D are omnipresent but most common in Africa and Europe (25). Genotype I is unusual and can be observed in Vietnam, Laos, India and China, while genotype J has been reported in Japan and Ryukyu (26,27). Other genotypes such as E, F, G, and H are also occasionally found in Asia.

Evidences increasingly suggest that the HBV genotyping is significant to predict HBV disease progression and determine appropriate antiviral therapy. Acute infection with genotypes A and D leads to higher rate of chronicity than genotypes B and C (28-30). Genotype C generally is considered as a risk factor for perinatal infection (31) and related to severe liver disease, including cirrhosis and HCC (32-34). In the interferon therapy, patients with genotypes A and B have better treatment response than genotypes C and D (35). Recent studies reported that patients infected with genotype B or C had a lower opportunity to gain serological

response to tenofovir (36,37).

HBV genotyping can be confirmed using diverse methods: reverse hybridization, genotype-specific PCR assays, real-time PCR, restriction fragment-length polymorphism, sequence analysis, microarray (DNAChip) and fluorescence polarization assay (38). The characteristics of variable HBV genotyping methods are presented on *Table 2*.

Diagnosis of hepatitis B infection

Acute hepatitis B is a clinical diagnosis identified by the detection of HBsAg, symptoms, high serum aminotransferases. Usually anti-HBc IgM can be detected and HBV DNA is present. HBeAg can also be identified in most acute phase of infections, but has little clinical importance. The diagnosis of chronic infection is based on the persistence of HBsAg for more than 6 months. Patients with chronic HBV infection are commonly diagnosed by laboratory means but not by clinical presentations. Past HBV infection is defined by the coexistence of anti-HBs

and IgG anti-HBc.

Occult HBV infection is defined by persistence of low level of intrahepatic HBV DNA without detectable HBsAg (42,43). It is a serological situation defined by the presence of isolated anti-HBc with the absence of HBsAg and anti-HBs antibody (44,45). The detection of HBV DNA in the liver is the gold standard of diagnosis for occult HBV infection, since cccDNA remains in the hepatocytes and HBV DNA is occasionally identified in the liver but not in the serum. However, gaining hepatic HBV DNA is difficult in clinical setting since the procedure is invasive. Real-time PCR for serum HBV DNA detection have been shown with adequate sensitivity to identify occult HBV infection in many cases; thus, HBV DNA testing is widely used to diagnose occult HBV infection (43).

Occult HBV infection has some clinical importance. First, it can be transmitted via transfusion, solid organ transplantation including orthotopic liver transplantation (46,47), or hemodialysis (48,49). Second, reactivation of HBV infection may occur in patients receiving chemotherapy or immunocompromised state (50-52). Third, it may accelerate liver injury and lead to hepatic fibrosis in patients with chronic liver disease including chronic hepatitis C infection (53-55). Forth, it appears to be a risk factor for HCC by its carcinogenic effect and by leading to continuous hepatic inflammation and fibrosis (56-58).

Tests for occult HBV infection are considered in the following conditions: in patients with cryptogenic liver disease, especially when having anti-HBc in serum; in patients considering immunosuppression therapy or chemotherapy; and in solid organ transplantation donors, due to the possibilities for transmission (59).

Conclusions

In this article, we aimed to give informations about HBV serological and molecular diagnosis. First step of HBV diagnosis is achieved by using serological markers for detecting antigens and antibodies against this virus. In order to verify first step of diagnosis, to quantify viral load and to identify genotypes, qualitative or quantitative molecular tests are used. Diagnosis of HBV infection is an important tool to determine acute, chronic and occult cases of infection in order to establish preventive remedies and to initiate antiviral treatment.

Acknowledgements

Footnote

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