

LABORATORY DIAGNOSIS OF HEPATITIS B

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ABSTRACT

Hepatitis B is a liver disease caused by a virus known as Hepatitis B virus (HBV) and is a major global health problem. The liver is susceptible to a wide variety of adverse effects caused by an excess of alcohol, drugs, infections such as viral hepatitis. Vaccination for children, adolescents and high-risk adults could also prevent hepatitis B. The prognosis is variable hepatitis, cancer and other metabolic disorders. Hepatitis B is the causative agent and belongs to the hepadnaviridae family of viruses. Transmission of HBV virus is through exposure to infected blood and body fluids. One of the important diagnostic criteria of Hepatitis B is by performing a thorough laboratory investigation.

Keywords: *Liver, hepatitis, vaccination, hepadnaviridae.*

INTRODUCTION

The liver is the second largest organ in the human body after skin.¹ It is the largest internal organ weighing about 1.2-1.5kg and it is in reddish brown in colour due to its great vascularity. The liver is located beneath the diaphragm in the epigastric and right hypochondriasis regions of the abdomen.^{1,2}

The liver is divided into right and left lobes which are separated by the middle hepatic vein. The right lobe is larger than the left lobe and the right lobe contains the caudate and quadrate lobe. For further breakdown, the liver is divided into eight segments by division of the right, middle, left hepatic veins. Each of the lobes contains a smaller unit

known as lobules. Most liver have 50000 to 100000 lobules that consist of a vein surrounded by the liver cells called hepatocytes.^{1,2}

The liver gets its supply from the hepatic artery and the portal vein. The liver gets 25% of the supply from the hepatic artery and the autoregulation of blood flow by the hepatic artery ensures a constant total liver blood flow. The remaining 75% of blood is supplied by the portal vein. The portal vein drains most of the gastrointestinal tract and the spleen. The liver receives parasympathetic innervations from the vagus nerve and sympathetic innervations from the thoracolumbar nerves through the celiac ganglia.²

The liver cells, hepatocytes have numerous functions, including synthesis, storage, and release of vitamins; storage , and release of glycogen; synthesis of blood proteins; phagocytosis of old red blood cells and certain bacteria; removal of toxic substances; and production of bile. Bile is stored in the gallbladder and is eventually secreted into the duodenum for the emulsification and absorption of fats.¹

As the liver is so complex, therefore it is susceptible to a wide variety of adverse effects caused by an excess of alcohol, drugs, infections such as viral hepatitis, cancer and other metabolic disorders. Hepatitis B virus (HBV) infection is the interest of this study. The term 'hepatitis' means inflammation of the liver and could be caused by medications, chemical toxins, alcohol, autoimmune diseases and viruses. In the case of hepatitis B, the disease is caused by the HBV and is the most serious type of viral infections.³

Around 2 billion of the world's population-alive today have been infected with HBV while 350 million still remain infected.⁴ National Centres for Disease Control (CDC)

estimated for some regions of Asia indicate that one-third to one-half entire population have been infected with HBV. The prevalent of HBV infection in Asia, sub-Saharan Africa is about 48% but less so in the Western Europe and North America which is less than 1%. In Asia, sub-Saharan Africa the virus is acquired through perinatal transmission (vertical) from the chronically infected mother or through infection in early childhood (horizontal transmission) whereas in southern and western Europe, the mode of transmission are perinatal transmission, needle sharing, tattooing and body piercing. In areas with low endemicity like North America and Australia, the transmissions are mainly through sexual contacts and needle sharing among drug users.^{4,5}

The prevalence rate for chronic HBV is related to differences in age at infection whereas for an acute infection will become chronic is 70-90% for perinatally acquired infection and 20-50% for horizontal infections acquired during early childhood. For adult-acquired HBV infection to develop into chronic infection ranges from 1% to 3%.⁴

Hepatitis B is an elusive disease to tract as the infection and the symptoms could be asymptomatic especially in children. Furthermore it does not cause any pain or discomfort. As a result, the disease was not diagnosed and never reported to the public health authorities until symptoms appears usually years to decade after the initial infection, epidemiologists reported. For example, in the United States in 2001, there were 7,844 acute clinical cases of HBV infection reported to the CDC but the CDC estimates that there were actually 78,000 new HBV infections nationwide that year. Therefore, this study was conducted to have better understanding regarding the diagnosis of the disease as well as the management.⁴

Hepatitis B is a liver disease caused by a virus known as Hepatitis B virus (HBV) and is a major global health problem.³ Earlier, hepatitis B is known as “long incubation” and “serum” hepatitis. Hepatitis B could be asymptomatic or have a self-limited acute illness. This may progress to acute liver failure and eventually develop chronic liver disease.⁶ Hepatitis B is the causative agent and belongs to the hepadnaviridae family of viruses. It is about 42 nanometres and is spherical in shape. The virus is composed of an inner core (nucleocapsid) and an outer envelope which has three hepatitis B surface antigens (HBsAg) that involves in the diagnosis of HBV infection. The nucleocapsid is further divided into two parts, the core antigen (HBcAg) and the pre-core antigen (HBeAg). The core of the virus has the genetic material which is important in the replication process.
4,7

Transmission of HBV virus is through exposure to infected blood and body fluids like semen, wound exudates, vaginal secretion and vagina. The highest concentration of the virus is found in the blood (10⁸- 10¹⁰ virions/ml) and serum while saliva contains the lowest.^{6,9} There are few common modes of transmission of HBV such as percutaneous and premucosal exposure to infectious body fluids, sharing or using nonsterilized needles or syringes in body piercing, tattooing, drug injection and acupuncture, sexual contact with an infected person, and perinatal exposure from an infected mother.^{4,6,9}

The premucosal transmission could be further divided into vertical transmission and horizontal transmission. In the regions of high endemicity, the vertical transmission is the major mode of transmission of HBV. Vertical transmission or also known as perinatal transmission is spread from mother to their babies at birth. Postexposure immunoprophylaxis

plays an important role in reducing hepatitis B in infants. Without this, many infants born with HBV-infected mothers will develop chronic hepatitis B. Horizontal transmission occurs through sexual contact and occurs mostly in the countries with low and intermediate prevalence of HBV infection.^{6,9}

Interfamilial transmission is rare but may occur in settings involving interpersonal contact over extended periods. This could take place when one person in the family is chronically infected with HBV resides in a household. The transmission may occur from child to child, and young children are at the highest risk of infection when they have frequent contact with the skin or mucous containing secretions or saliva. Besides, sharing things like washcloths, towels, razor or toothbrush could facilitate the transmission of HBV.¹⁰ Below are the list of group of people who are at the risk of infected by HBV: a) Health care workers and emergency personnel, b) Infants born to mothers who are infected at the time of delivery, c) Partners or individuals living in close household contact with an infected person, d) Individual with multiple sexual partners, e) Illicit drug users, f) Individuals who get tattoos or body piercing, g) Individuals who travel to endemic countries such as Africa and Asia, h) Individuals emigrating from countries where hepatitis B is common, or born to parents who emigrated from these countries, i) Families adopting children from countries where hepatitis B is common.

The incubation period of HBV is two to six months. After this period of time, a person is said to have acute hepatitis B. Most people are able to clear and recover from the virus, however if the body is unable to mount an effective immune response, a person may become chronic carrier of HBV. Liver cirrhosis and hepatocellular carcinoma (HCC) are

the complications that may occur in the chronic carriers of HBV. CDC estimated 25% of chronic carriers will suffer from the complication mentioned above.^{6,8}

The Pre-S1 and Pre-S2 of the virus attaches itself to the receptor on the surface of the hepatocyte and penetration occurs. The virus loses its coat and the virus core is transported to the nucleus. Transcription of HBV into mRNA occurs whereby HBV DNA is converted into circular form (cccDNA). This acts as a template for mRNA transcription.^{3,4}

Replication of the genome and translation into HBV protein occurs in the endoplasmic reticulum. Then they are packaged and exported from the cell. One point should be noted that HBV does not cause direct injury to the liver. The damage is caused by the cellular immune response of the host.³

The viral antigen on the infected hepatocyte is recognized by the cytotoxic T cell via HLA class I molecules. Th1 responses are linked with the clearance of the virus whereas Th2 responses are linked with the development of chronic infection and severity of the disease.³

The virus persists in a patient who has a poor cell-mediated response and leads to a healthy chronic HBV infective state. A better response, however, results in continuing hepatocellular damage and eventually chronic hepatitis.^{2,3,4,10}

There are two phases in the chronic HBV infection that is replicative and integrated phase. There is active viral replication, hepatic inflammation and the patient is highly infectious with HBeAg and HBV DNA positivity in the replicative phase. The viral genome becomes integrated into the host DNA at some point of time. The viral genes are

then transcribed along with those of the host. When this occurs, the level of HBV DNA in the serum is low and the patient is HBeAg negative and HBe antibody positive. From the liver histology, the liver is inflamed, often with cirrhosis. The aminotransferases is normal or slightly elevated. HCC develops at the late stage of the disease.^{2,3,4,10}

DIAGNOSIS OF HEPATITIS B

The diagnosis of hepatitis B is done through clinical symptoms and laboratory examination. In general, there are general considerations in the diagnosis of hepatitis B. A person's history, age, risk factors, vaccination status and previous tests results should be used to guide appropriate testing.^{9,12}

The diagnosis of HBV infection is made through blood testing. Serological test can be performed on either serum or plasma. HBV antigens and antibody are stable at room temperature for days, 4°C for months, and frozen at -20°C to -70°C for many years. Today, automated enzyme immunoassays that depend on colorimetric or chemiluminescence signal measurement, care should be taken to avoid haemolysis of the sample as it may interfere with the ability of the assay accurately detect these markers. Besides, measures should be taken to avoid the degradation of the viral nucleic acid in the specimen, which can result in falsely low or no measurable viral load. Therefore, serum should be removed from the clotted blood within 4 hours of collection and stored at -20°C to -70°C.¹²

Acute Hepatitis B

The average incubation period is 60 to 90 days (range is 40 to 160 days). At the beginning patient usually complain of fatigue, malaise, anorexia, right upper quadrant

discomfort, or flu-like symptoms. Besides, low-grade fever, jaundice, and mildly tender hepatomegaly are the most common signs. As jaundice sets in, the urine becomes darker and the stools paler. In acute hepatitis B the disease may last from one to six weeks but may be prolonged and can be fulminate. The disease cannot easily distinguished by history, physical examination or routine serum biochemical tests alone as the cute hepatitis B resembles other forms of acute hepatitis clinically.^{2,9,13}

Laboratory Criteria of Acute Hepatitis B

The laboratory diagnosis of acute hepatitis B is made through the presence of IgM antibody to HBV core antigen (IgM anti-HBc). IgM anti-HBc is rapidly followed by IgG anti-HBc. Even though this occurs, IgM may persist for months to years and may even reappear during flares of chronic HBV. In self-limiting cases, there are presence of antibody to the hepatitis B surface antigen (anti-HBs) which indicates recovery from infection. This usually appears weeks to months following disappearance of serum HBsAg. Markers of HBV replication- HBeAg and HBV DNA is also present during the initial phase of infection. They are also present in the chronically infected individual. HbsAg, HbeAg, and HBV DNA are not specific for acute infection.^{4,13}

Chronic Hepatitis B

The progression from acute to chronic infection is largely influenced by the age of the person who becomes in contact with the virus. A person is said to be chronically infected if the person's immune system is not able to clear the virus six months later after

the initial exposure of HBV. Usually, person with chronic infection is asymptomatic besides being fatigue, loss of appetite and nausea.

Table 1: Significance of viral markers in hepatitis B

| Significance of viral markers in hepatitis B | |
|---|---|
| Antigens | |
| HBsAg | Acute or chronic infection |
| HBeAg | Acute hepatitis B Persistence implies: continued infectious state, development of chronicity increased severity of disease |
| HBV DNA | Implies viral replication Found in the serum and liver |
| Antibodies | |
| Anti-HBs | Immunity to HBV; previous exposure; vaccination |
| Anti-HBe | Seroconversion |
| Anti-HBc | |
| IgM | Acute hepatitis (high titre) Chronic hepatitis (low titre) |
| IgG | Past exposure to hepatitis B (HBsAg-negative) |

No abnormalities would be found during physical examination. Muscle wasting, ascites, oedema, palmar erythema, encephalopathy and bruising, suggest advanced disease with cirrhosis.^{9,12}

Laboratory Criteria of Chronic Hepatitis B

A person is said to be chronically infected if HbsAg persist for more than 6 months. Besides, it is also determined by the presence of HBsAg, HBeAg, HBV DNA, HbcAb Ig G. HbAb is not found. It is found that HBsAg will clear spontaneously in approximately 2% of chronic carriers. Over 7 years of observation, HbsAg are also clears independent of HBsAg. Clearance of HbeAg, even with persistence of HBsAg is associated with resolution of the inflammatory process, recovery, and decreased infectivity. In addition, alanine aminotranferases (ALT) should also be measured. Patients with both HbeAg-positive and HbeAg-negative who has normal serum ALT (women < 20 IU/L and men < 30 IU/L) should be monitored life long as the condition may change over time although they remain asymptomatic. In individual with elevated serum ALT concentrations should be monitored closely.^{4,13}

Differential Diagnosis of Hepatitis B

The differential diagnosis for hepatitis B are as follows: a) Cholangitis, b) Cytomegalovirus, c) Hepatitis A, C, D, E and d) Wilson's disease.

CONCLUSION

The liver is the largest internal organ and the second largest organ after skin in the human body. It has numerous functions in maintaining the physiology of human body; therefore it encounters a variety of adverse effects. One such example is hepatitis B. Hepatitis is inflammation of the liver and is caused by the HBV. It is estimated that 2 billion people around the world is infected with HBV and 350 million still remain infected.

HBV spreads through percutaneous and premucosal exposure to infectious body fluids like semen, exudates, vaginal secretion, nonsterilized needles or syringes in body piercing, tattooing, drug injection, sexual contact with an infected person and perinatal exposure from an infected mother.

There are two phases of hepatitis B that is acute phase and chronic phase. The incubation period is about 60 to 90 days and a person is said to be in the chronic state if the person's body is not able to clear the virus after 6 months after the initial exposure to HBV. The diagnosis of hepatitis B is made from clinical manifestation and also laboratory findings. During acute period, the symptoms are similar to those of flu or cold. Later on, the symptom may develop like yellow skin and eyes, dark urine and light-coloured stool and itchy skin. Recovery period is from 2 to 3 months. The laboratory diagnosis of acute hepatitis B is made through the presence of IgM antibody to HBV core antigen (IgM anti-HBc). IgM anti-HBc is rapidly followed by IgG anti-HBc. During chronic period, the symptoms may be asymptomatic and those with chronic hepatitis B can develop chronic hepatitis, cirrhosis and HCC. A person is said to be chronically infected if HbsAg persist for more than 6 months. Besides, it is also determined by the presence of HBsAg, HBeAg.

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