

Chronic hepatitis B Virus Infection: Diagnosis and Therapy

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Abstract: Hepatitis B virus (HBV) infections are prevalent worldwide, some 240 million individuals have chronic HBV, with greater rates in Asia, and Africa, and chronic infection cause more than one million deaths annually. Vertical transmission during child birth. Risk factors include healthcare workers, blood transfusion, dialysis, parenteral drug uses, heterosexuals, homosexuals, recipients of plasma-derived products, and living in the endemic areas. HBV infection causes both hepatocellular damage and viral clearance. Chronic infection may result due to failure of initial innate and adaptive immune responses. HBV infection begins with general ill-health, nausea, vomiting, body aches, fever, dark urine, jaundice and itchy skin has been the possible symptoms of hepatitis. HBV is linked to liver cancer, and link between viral infection and the development of hepatocellular carcinoma (HCC) have been debated. Hepatitis B surface antigen (HBsAg) is most frequently used screen test. Individuals who remain HBsAg positive for at least six months are considered to be hepatitis carriers. Hepatitis B surface antigen negative hepatitis B virus infection has been reported. WHO recommended a combination of tenofovir and entecavir as first line agents? Asia-Pacific Practice guidelines on the management of hepatitis include achievement of HBeAg seroconversion, and suppression of HBV DNA below the level of detection, and prevent progression to cirrhosis and HCC, and prolong survival. Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer. Prevention with hepatitis B vaccines,

Keywords: Hepatitis B virus, Chronic hepatitis, Hepatocellular carcinoma, Diagnosis.

I. Introduction

Hepatitis B virus (HBV) infects more than 500 million people worldwide. It is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), and these sequelae of chronic infection account for more than one million deaths annually [1], and about 300,000 of these are due to liver cancer [2]. In 2004, an estimated 350 million individuals were infected, and worldwide some 240 million people have chronic HBV, with highest rates of infection in Africa and Asia [3]. National and regional prevalence ranges from over 10% in Asia to under 0.5% in the United States and northern Europe [4]. Areas of greater than 8% prevalence, Southeast Asia, China, Middle East except Israel [1]. Routes of transmission include vertical transmission, such as childbirth, early life horizontal transmission (bites, lesions, and sanitary habits), and adult horizontal transmission (sexual contact intravenous drug use) [4]. Other risk factors include working in healthcare, blood transfusion, dialysis, and living with an infected person, travel in countries where infection rate is high, and living in an institution [5]. HBV cause both acute and chronic infections. Many people have no symptoms in the initial infection. Some develop a rapid onset of sickness with vomiting, yellowish skin tiredness, dark urine and abdominal pain [3]. It may take 30 to 180 days for symptoms to begin [5]. Diagnosis is typically by testing the blood for parts of the virus and for antibodies against the virus [5]. It is one of the five known hepatitis viruses, A, B, C, D, and E [5], the infection has been preventable by vaccination since 1982 [5]. World Health Organization (WHO) recommended a combination of tenofovir and entecavir as first line agents [6]. Those with early cirrhosis are in most need of treatment [6]. This review describes recent advances in the pathogenesis, epidemiology, diagnosis, and management of HVB and impact of HVB infection on the development of HCC.

II. History

Hippocrates recognized the spread of jaundice by infectious agents as early as 4000 BC. The early cases of HVB infection was linked to the use of conventional viral vaccines, which were prepared from or contained human serum. In 1885, Luman described the appearance of jaundice in 15% of 1289 shipyard workers who received smallpox vaccine prepared from human lymph [7]. Luman's paper, now regarded as a classical example of an epidemiological study, proved that contaminated lymph was the source of the outbreak. Later, numerous outbreaks were reported following the introduction, in 1909, of hypodermic needles that were used, and more importantly, reused, for administration Salvarsan for the treatment of syphilis. The virus was not discovered until 1966 when Baruch Blumberg, then working at the National Institute of Health (NIH), discovered

the Australian antigen (later known to be hepatitis B surface antigen, or HBsAg) in the blood of Australian aboriginal people [8]. Although a virus had been suspected since the research published by MacCallum in 1947 [9]. D.S. Dane and associates discovered the virus particle in 1970 by electron microscopy, also visualized the presence of 22-nm HBsAg sub viral particles along with the complete 42-nm virus particles in the blood of hepatitis B patients [10]. Baruch Blumberg received the Nobel Prize in Physiology and Medicine in 1976 [10]. By the early 1980s the genome of the virus had been sequenced, and the first vaccine were being tested [11,12]. World hepatitis Day, observed July 28, aims to raise global awareness of hepatitis B and hepatitis C and encourage prevention, diagnosis and treatment. It has been led by World Hepatitis Alliance since 2007 and May 2010, it got global endorsement from the World Health Organization (WHO) [13].

III. Viral Etiology

HBV primarily infects hepatocytes. It enters hepatocytes via unidentified liver cell-specific receptor, consistent with the strict hepatotropism exhibited by this and other members of *Hepadnaviridae*. Candidate receptor molecules include endonexin, carboxypeptidase, and serum apolipoproteins, but none appear to fill the criteria for a liver cell-specific receptor that confers hepatotropism to HBV and other related viruses [14]. HBV is a member of hepadnavirus family [15]. The virus particle (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30-42-nm in diameter. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity [16]. The outer envelope contains embedded proteins that involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses, and the 42 nm virions, which are capable of infecting liver cells known as hepatocytes, are referred to as "Dane particles" [17]. In addition to the Dane particles, filamentous and spherical bodies lacking a core can be found in the serum of infected individuals. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigens (HBsAg), and is produced in excess during life cycle of the virus [18].

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020-3320 nucleotides long (for the full length strand) and 1700-2800 nucleotides long (for the short length-strand) [19]. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by the gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three frame "start" (ATG) codons that divide the gene into three sections, pre-S2, and because of the multiple start codons, polypeptides of three different sizes called (the order from surface to the inside: pre-S1, Pre-S2, and S), middle pre (pre-S2, S), and small are produced (S) [20, 21]. The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules [22].

IV. Transmission

Hepatitis B replicates to higher titer in the blood (10^8 to 10^{10} virions/ml), especially during the acute phase of illness. Any parenteral or mucosal exposure to infected blood thus represents a potential risk for acquisition of hepatitis B, and accounts for the 100-1000 times more efficient transmission of HBV compared to HIV after needle stick exposure [23]. Possible forms of transmission include sexual contact, blood transfusion and transfusion with other human blood products [24,25], re-use of contaminated needles and syringes [26]. The vertical transmission from mother to child (MTCT) during child birth. Without intervention, a mother who is positive for HBsAg has 20% risk of passing the infection to her offspring at time of birth. The risk is as high as 90% if the mother is also positive for HBeAg. HBV can be transmitted between family members within households, probably by contact of non-intact skin or mucous membrane with secretions or saliva containing HBV [27]. However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor [28]. Breast feeding after proper immunoprophylaxis does not appear to contribute to mother-to-child transmission (MTCT) of HBV [29].

Persons at high risk of acquiring HBV infection include members of the following groups: parenteral drug users, heterosexual men and women and homosexual men with multiple partners, household contacts and sexual partners of HBV carriers, infants born to HBV infected mothers, patients and staff in custodial institutions for the developmentally disabled, recipients of certain plasma-derived products (including patients with congenital coagulation defects), hemodialysis patients, health and public safety workers who have contact with blood, and persons born in areas of high HBV endemicity and their children [23,1].

V. Pathogenesis

The life cycle of hepatitis B virus is complex. Hepatitis B virus is one of a few known pararetroviruses: non-retroviruses that still use reverse transcription in their replication process. The virus gains entry into the cell binding to NTCP[30], on the surface and being endocytosed. Because the virus multiplies via RNA made by the host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded by viral polymerase and transformed into covalently closed circular DNA (cccDNA). This cccDNA serves as a template for transcription of four viral mRNAs by host RNA polymerase. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions that are released from the cell or returned to the nucleus and re-cycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where virion P protein (the DNA polymerase) synthesizes DNA via its reverse transcriptase activity[20,31].

Viral serotype and genotypes

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. The serotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination [32]. Genotypes differ by at least 8% of their sequence and were first reported in 1988 when six were initially described (A-F)[33]. Two further types have since been described (G and H)[34]. Most serotypes are now divided into sub-genotypes with distinct properties[35].

Immune system in pathogenesis

The exact mechanisms by which chronic liver injury occurs in HBV infection are not known, although most studies suggest that hepatitis virus is not directly cytopathic to hepatocytes[36]. Hepatitis virus primarily interferes with the functions of the liver by replicating in hepatocytes. A functional receptor is NTCP[30]. There is evidence that receptor in the closely related duck hepatitis B virus is carboxypeptidase D[37]. The virions bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-specific receptors are expressed primarily on hepatocytes, however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells[38].

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response in particular virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are used to purge HBV from viable hepatocytes[39]. Although CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver[40].

Acute and chronic hepatitis

Natural recovery from acute HBV probably depends on multiple components of cellular immune responses, including natural killer (NK) cells, natural killer T (NKT) cells, and viral specific CD4⁺T cells and CD8⁺ cytotoxic T lymphocytes (CTL). Both NK and NK T cell contribute to clearance through production of IFN- α / β , which mediates non-cytopathic control of viral replication[41]. Acute HBV infection is also accompanied by a strong and transient expansion of CD4⁺ T cells directed against multiple epitopes within the HBV. HBc is the dominant antigen recognized by CD4⁺ T cells in most cases of acute, resolving HBV infection[42].

Chronic (persistent) infection may result because of the failure of initial innate and adaptive immune responses. Among the infants who become infected at birth, both viral and host factor play a role in the development of chronic infection. The presence of HBeAg and the viral titer in the mother are both directly related to the likelihood of infant infection. In animal models HBeAg may be toleragenic, and because HBeAg and HBcAg are cross-reactive at the T-cell level, deletion of the CD4⁺ HBc-specific T-cell responses results in ineffective responses to HBcAg[43].

VI. Clinical Manifestations

Acute infection with hepatitis B virus is associated with acute viral hepatitis—an illness that begins with general ill-health, loss of appetite, nausea, vomiting, bodyaches, mild fever, and dark urine, and then progresses

to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result. The infection may entirely asymptomatic and go unrecognized [44].

Chronic infection with hepatitis B virus may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Across Europe hepatitis B and C cause approximately 50% of hepatocellular carcinomas [45]. Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B has been linked to the development of membranous glomerulonephritis (MGN) [46].

Symptoms outside liver are present in 1-10% of HBV-infected people and include serum-sickness-like syndrome, acute necrotizing vasculitis (polyarteritis nodosa), membranous glomerulonephritis, and popular acrodermatitis of childhood (Gianotti-Crosti syndrome) [47,]. The serum-sickness-like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice [48]. The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after onset of jaundice, but can persist throughout the duration of acute hepatitis B [49]. About 30-50% of people with acute necrotizing vasculitis (polyarteritis nodosa) are HBV carriers [50]. HBV-associated nephropathy has been described in adults but is more common in children [51]. Membranous glomerulonephritis is the most common type [49]. Other immune-mediated hematological disorders, such as essential mixed cryoglobulinemia and aplastic anemia [29].

Hepatocellular carcinoma (HCC)

Epidemiological evidence supports the role of HBV as a causal agent of liver cancer, the molecular mechanisms addressing the link between viral infection and the development of HCC have been debated [52, 53]. Despite the large body of work on this subject, a clear view of how HBV infection triggers events that lead to liver oncogenesis remains elusive. HCC development, like other cancers, proceeds in multiple steps that correlate with specific lesions associated with livers of patients with HCC. These include altered hepatic foci, dysplastic (neoplastic) nodules, and low- and high grade HCCs. These lesions are characterized as exhibiting different levels of cell differentiation. Similar to other well characterized human cancers, HCC progresses through these individual stages. The molecular switches associated with each step of HCC development still need to be identified [54].

HBV-related HCCs are derived from the clonal expansion of a single transformed cell or cancerous cells [52]. Whereas 80% of HBV-associated HCC tumors contain integrated viral DNA, most of the HBV genes are either truncated or transcriptionally inactivated [45]. As opposed to retroviral replication HBV DNA integration is not an obligatory part of the viral life cycle but may instead serve as an insertional mutagen [56]. Following integration into the host chromosomes, the HBV DNA control elements such as enhancers or promoters can act in cis to activate a cellular oncogene. HBV integrates randomly at multiple sites in the host chromosomes and molecular analyses of the HBV integration junction sites have not revealed specific integration adjacent to or neighboring cellular oncogenes [56].

Finally, HBV-associated HCC may be due to the repeated cellular division associated with the inflammatory response [45]. HCC associated with HBV nearly always arises in the context of cirrhosis, although a few cases of HCC without cirrhosis have been reported [57]. Cirrhosis is the result of years of inflammation and associated repair processes, during which there is considerable cell killing and repeated hepatocyte regeneration. In all types of liver damage, there is evidence of enhanced production of free radicals or significant decrease of antioxidant defense, or both [58]. Oxidative stress over a long period may give rise to high mutation rates in infected hepatocytes. Mutations, which confer in a cell a proliferative advantage and provide opportunities for achieving a transformed phenotype, are perpetuated. Therefore, according to this model, the role of HBV, if any, is merely to induce liver injury and the subsequent events are all secondary to the host immune response [1].

VII. Diagnostic Workout

Hepatitis B surface antigen (HBsAg) is most frequently used screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 to 240 copies of core protein, alternative known as hepatitis B core antigen, or HbcAg. Therefore most hepatitis B diagnostic panel contain HBsAg and anti-Hbc (Both IgM and AgG) [59]. Shortly after the appearance of the HBsAg, another antigen called Hepatitis B e antigen (HBeAg) will appear. During the natural course of infection, the HBeAg may be cleared, and antibodies to "e" antigen (*anti-HBe*) will arise immediately afterwards [60].

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis carriers [61]. Carriers of the virus may have chronic hepatitis B, which may be reflected by elevated serum alanine aminotransferase (ALT), more than 20 times normal [62], levels and inflammation of the liver, if they are in the immune clearance phase of chronic infection [63]. PCR tests have been developed to detect and measure the amount of HBV DNA, called viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment. Individuals with high viral loads, have ground glass hepatocytes on biopsy [64]. Krikke and associated reported 23 cases of surface antigen-negative hepatitis B virus infection in Dutch blood donors, and recommended the screening of blood for HBV DNA and HBV core antibodies to cover all stages and variants of HBV infection [65].

VIII. Therapy

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously [66]. Chronically infected individuals with persistently elevated alanine aminotransferase, a marker for liver damage, and HBV DNA levels are candidates for therapy [67]. Treatment lasts from six months to a year, depending on medication and genotype [68]. World Health Organization recommended a combination of tenofovir and entecavir as first line agents. Those with current cirrhosis are in most need of treatment [6]. The use of interferon, which requires injections daily or thrice weekly, has been supplanted by long-acting PEGylated interferon, which injected only once weekly [69]. Response to treatment differs between the genotypes. Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases. Sustained antigen loss after treatment is 45% in types A and B but only 25-30% in types C and D [70]. Adrian and associates conclude that prolonged antiviral therapy may reduce the risk of hepatocellular carcinoma among certain patients with chronic hepatitis B [71]. Sung and colleagues suggest that clinicians from Asia Pacific region use Asia Pacific consensus criteria beyond those advocated in treatment guidelines when deciding whether to initiate treatment in HBV-infected patients [72]. Asia Pacific clinical Practice guidelines on the management of hepatitis B, [3], covers the full spectrum of care of patients infected with hepatitis B, that include:

- (a) Achievement of HBeAg seroconversion and/or sustained suppression of HBV DNA to levels below the level of detection using PCR-based methods and ALT normalization.
- (b) Achievement of a durable response to prevent hepatic decompensation, reduce or prevent progression to cirrhosis and HCC, and prolong survival.

IX. Prognosis And Prevention

Children are less likely than adults to clear the infection. More than 95% of people who become infected as adults or older children will stage full recovery and develop protective immunity to the virus. However, this drops to 30% for younger children and only 5% of newborns that acquire the infection from their mother at the time of birth [73]. This population has a 40% lifetime risk of death from cirrhosis or hepatocellular carcinoma [69]. Of those infected between the age of one to six, 70% will clear the infection [74]. Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer. Polyarteritis is more common in people with hepatitis B infection. [75]. HBV DNA persists in the body after infection, and some people the disease recurs (reactivation) [76]. Although rare, reactivation is seen most often following alcohol or drug abuse [77]. Test of choice to determine the degree of cirrhosis, e.g., Transient elastography (Fibroscan), but it is expensive. Low cost test Aspartate aminotransferase to platelet ratio index may be used [6].

Prevention

Vaccines for the prevention of hepatitis B have been routinely recommended for infants since 1991 in the United States [78], and in other countries. Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 mIU/ml in the recipient's serum. Vaccination at birth is recommended for all infants of HBV infected mothers [79]. All those with a risk of exposure to body fluids such as blood should be vaccinated, if not already [78]. In assisted reproductive technology, sperm washing is not necessary for males with hepatitis B to prevent transmission, unless the female partner has not been effectively vaccinated [80]. In females with hepatitis B, the risk of transmission from mother to child with IVF is no different from the risk in spontaneous conception [80].

X. Conclusion

Hepatitis B virus (HBV) is the cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. Healthcare workers, intravenous drug users, individuals handling blood and blood products, are at high risk of HBV infection. HBV infection is preventable by vaccination. Treatment with a combination of antiviral drugs.

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