Viral Hepatitis: Chronic Hepatitis B

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To our families...

Preface

Chronic hepatitis B infection is a global health care problem and a significant disease burden across the world. Infection is seen in billions of people worldwide with possible consequences as serious as liver failure, cirrhosis, and hepatocellular carcinoma seen in many patients. Beyond just potentially serious outcomes of chronic infection, the degree of disease activity and rate of progression to fibrosis can vary widely among patients, requiring close monitoring of viral activity by the managing practitioner. Despite increasing implementation of vaccines and passive immunization, management of hepatitis B virus (HBV) infection remains a persistent challenge in many areas globally, frequently for variable and unique reasons when evaluating individual needs of a given population.

Fortunately, given the various challenges different communities face when confronting the goal of suppression and eradication of HBV, we have witnessed many advances in the field of HBV monitoring and treatment. Advances include improved understanding of the viral structure and genomics, broadened data collected regarding the virus' epidemiology, consequences of chronic and untreated infection across multiple populations studied, and development of potent and safe antiviral drugs suppressing viral replication as well as development of guidelines for appropriate use of these medications. Despite the multitude of advances, there is still significant work ahead regarding development and safe implementation of broad viral elimination and eradication strategies. We have had the privilege of gathering highly respected experts across several disciplines to review different aspects of chronic HBV infection in terms of the natural history of the virus, associated infections, epidemiology, and indications for antiviral therapy. We hope this book serves as a valuable and enjoyable source of information for practitioners managing HBV in any clinical setting, from inpatient centers managing critical sequelae of chronic infection to outpatient clinics managing diagnosis and monitoring disease activity of chronic HBV infection. Many thanks to the faculty for their excellent work and the editorial team for their great efforts.

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Ryan D. Heath and Veysel Tahan

1.1 Introduction

About 400 million persons worldwide currently are chronically infected with hepatitis B virus (HBV), increasing risk of progression to high mortality conditions such as cirrhosis and hepatocellular carcinoma (HCC) [1]. Disease activity and progression to fibrosis can occur at a variety of rates, with an unpredictable clinical course requiring close observation from the managing health practitioner. More specifically, an understanding of the natural history of HBV is important from the standpoint of deciding if and when to initiate antiviral treatment in a patient infected with HBV.

HBV is a member of Hepadnavirus, a small enveloped virus with partially double-stranded circular DNA replicating by reverse transcription [2]. DNA is delivered into host hepatocyte DNA at the time of infection, converted into a transcriptional template for mRNA which is subsequently transcribed into the hepatitis B surface antigen (HBsAg) and e antigen (HBeAg) [3]. The virus is transmitted parenterally via apparent or unapparent percutaneous or permucosal exposure to infected blood or other body fluids. Risk factors for infection include transfusion of unscreened, sexual promiscuity, sharing or reusing of syringes between intravenous drug users, tattoos, working in a health-care facility, living in a correctional facility, renal dialysis, and any long-term contact with an HBV-infected individual [4]. The likely means of infection varies considerably between high- and low-prevalence areas. Low-prevalence areas are associated with higher rate of infection via sexual behavior and sharing of syringes [5]. Low-prevalence areas are associated with the USA and Western Europe [1]. High-prevalence areas are associated with perinatal infection or exposure during early childhood. Ninety percent of HBeAg-positive mothers transmit HBV to their offspring; ~20% of HBeAg-negative mothers will

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transmit HBV to their offspring [6, 7]. Higher-prevalence areas have been observed to include Mediterranean countries, Southeast Asia, and Sub-Saharan Africa [1].

In cases of maternal-fetal transmission, rates of transition of the acute infection to chronic exceed 80%. Acute HBV infection in infancy and childhood has been observed at rates between 20 and 30% [8]. Data from sequencing of HBV have demonstrated that for an acute HBV infection to become chronic, the virus' core promoter region must produce HBeAg [9, 10]. Analysis of patients presenting with acute hepatitis B, among immunocompetent adults, is remarkable for a rate of progression to chronic HBV infection in <1% of cases [11]. The acute infection consists of an initial incubation period lasting 2–6 weeks, followed by acute hepatitis with elevated serum aminotransferases. Acute hepatitis resolves with normalization of aminotransferases, with HBsAg persisting for months before clearing from serum.

Acute infection is typically associated with varying degrees of jaundice, fatigue, nausea, vomiting, and abdominal pain, though many are asymptomatic despite a higher titer of HBV DNA [12]. In acute infection, clinical HBV becomes apparent after an incubation period of 45–180 days. HBV DNA clearance is mediated largely by antiviral cytokines produced by cells of the innate and adaptive immune response, including tumor necrosis factor alpha (TNFα), interferon α, and interferon β [13–15]. After viral DNA begins to decline, a cytolytic immune response with hepatocyte apoptosis and necrosis ensues, coincident with the onset of clinical hepatitis and rise in ALT. Cytotoxic T cells recruit various antigen nonspecific inflammatory cells into the liver by secreting cytokines, initiating a necroinflammatory response [16–18]. In individuals without sufficient CD4 and CD8 response, chronic HBV infection ensures after incomplete HBV clearance [19].

The first serologic marker to appear in acute HBV is HBsAg, 1–6 weeks before onset of symptoms. IgM anti-hepatitis B core (anti-HBc) appears 1–2 weeks after HBsAg, persisting for up to 6 months after HBsAg clears [20]. Previously undiagnosed chronic HBsAg carriers with acute exacerbations of HBV are seronegative for IgM anti-HBc. The presence of HBeAg for more than 10 weeks indicates a high likelihood of transition to persistent infection. Persistence of HBsAg for >6 months indicates chronic infection. HBV DNA tends to be higher in patients with HBeAg-positive chronic infection and lower in HBeAg-negative infection.

1.2 Phases of Chronic HBV Infection

The National Institutes of Health (NIH) had previously defined three phases of chronic HBV infection: the immune-tolerant phase, the immune-active phase, and the inactive hepatitis B phase [21, 22]. All phases have been linked to the level of HBV replication, the strength of the host immune reactivity against the replacing HBV, and the interplay between the host and virus [10, 23]. Subsequent literature has restructured the events of chronic infection in different ways; however, they all speak to an early period of high replication with active liver disease followed by a subsequent phase with low replication remission [24].

In developing chronic HBV, HBeAg is initially positive, concomitant with high levels of HBV DNA, remaining so for years to decades. Most patients acquiring

HBV lose HBeAg positivity and develop antibody to it, anti-HBe. Observed clearance of HBeAg is estimated to be ~10% per year [25–27]. This rate varies considerably by HBV genotype, with genotype C notably remaining HBeAg positive for years longer than other genotypes [28]. Below is a more detailed look at the natural history of chronic HBV infection.

1.3 Immune-Tolerant Phase

The immune-tolerant phase is observed to demonstrate positive HBeAg, normal ALT, and elevated HBV DNA—commonly above one million IU/mL. This state occurs most frequently in the setting of perinatal infection [28, 29]. In immunocompetent individuals, hepatocellular damage occurs from the host response toward eliminating HBV, occurring over years to three decades [30]. During this phase, there is no or minimal hepatitis nor fibrosis [31, 32]. Due to the HBV polymerase gene's reverse transcriptase properties, the virus integrates into host hepatocyte DNA. Thus, high levels of HBV DNA over many years likely increase risk of HCC over time even in the absence of inflammation and fibrosis. The length of this phase may be prolonged, as noted above in cases of vertical transmission; it is observed to be significantly shorter in children [11]. The rate of clearance does appear to differ between genders as well, with animal studies demonstrating the androgens can increase the rate of HBV transcription and suppress the tumor suppressor gene in early HCC [33, 34]. One Taiwanese prospective cohort study followed 240 patients over 10.5 years, finding that only 5% progressed to cirrhosis and none to HCC during this follow-up period [35].

1.4 Immune-Active Phase

This phase is characterized by elevated ALT levels and an elevated HBV DNA; patients may either be HBeAg positive or HBeAg negative with positive HBe antibodies [29, 36, 37]. HBV DNA levels may fall during this phase, typically preceded by a flare of hepatitis as infected hepatocytes undergo an immune-mediated cytolysis [27, 38]. Recurrence of flares is observed to occur more commonly in men, potentially correlating with the observation of relative increased risk of HCC in men compared to women with HBV-related cirrhosis [39]. Clearance of HBeAg occurring spontaneously or as a result of antiviral therapy reduces risk of decompensation and improves survival [40–44]. The annual rate of spontaneous clearance in this phase ranges from 3 to 12% [45]. Factors associated with seroconversion include age, higher aminotransferase levels, and certain HBV genotype [25, 46–49]. There are two presentations of anti-HBe Ab-positive chronic HBV. Ten to twenty percent remain in the immune-active phase after seroconversion from HBeAg to anti-HBe Ab [27, 50]. Others transition into inactive HBV with reactivation to the immune-active phase [39, 51–54]. Liver histology will likely appear normal on exam, though "ground glass" hepatocytes have been observed in this stage [55].

1.5 Inactive Carrier Phase

This phase is characterized by absent HBeAg, positive anti-HBe Ab, normal ALT, HBV DNA <2000 IU/mL, and improvement in liver fibrosis and inflammation over time [56–58]. It is important to note, however, that severe fibrosis may still be observed in this phase [59]. Around 2% per year will lose HBsAg positivity and enter complete remission [60]. Patients are not considered inactive carriers until at least three normal ALT levels and two to three unremarkable HBV DNA levels are obtained over a 12-month period.

There are four possible outcomes after conversation from HBeAg to anti-HBe Ab. Approximately 20% of patients experience at least one reversion to positive HBeAg, associated with hepatitis flares. Genotypes C and F are most associated with this outcome, accounting for ~40% of cases [28]. The remaining 70–80% enter into the inactive hepatitis B phase and remain so for life [25–27, 50, 53, 61]. Prognosis is generally favorable, potentially correlating with relatively improved prognosis with faster transition to this state [62]. Some will remain in the active immune phase manifested by elevated ALT and HBV DNA >2000 IU/mL [27, 39, 53]. Fourth, some will experience anti-HBe Ab-positive hepatitis characterized by elevated ALT and HBV DNA levels, though these levels may fluctuate significantly over time. Flares of patients in this state are estimated to occur at ~4% per year, occurring more often in patients >30 years of age, males, and with the presence of precode mutation [63]. HBV DNA may be detected in the liver and serum up to ~10 years after supposed recovery from acute HBV infection [64, 65]. This likely accounts for reports of chemotherapy-induced reactivation of HBV replication in patients with previous serology suggestive of recovered infection [50, 66, 67].

1.6 Clearance of HBsAg

It is observed that chronic HBV patients will clear HBsAg at a rate of 0.5–0.8% per year, associated with an improved clinical outcome and improved inflammation and fibrosis [25, 62, 68, 69]. This phase has also been termed the “recovery phase,” though it is not widely accepted given that HCC has been observed to occur in these individuals, albeit at a reduced rate than others with chronic HBV infection [21, 70–74]. Of note, HCC appears to be seen more commonly in patients with concurrent cirrhosis, HCV confection, or older age at the time of seroconversion [74, 75].

1.7 Reactivation of Chronic HBV

Reactivation is characterized by negative HBeAg, positive anti-HBe Ab, detectable HBV DNA, elevated aminotransferase, and continued inflammation on liver biopsy [10]. Most patients reach this phase after an inactive carrier state; however, some patients have been observed to progress directly from HbeAg-positive chronic hepatitis to HBeAg-negative chronic HBV infection [50]. Patients are generally older

Table 1.1 Typical lab/histological features in various phases of chronic HBV infection

	Immune tolerance	Immune clearance	Inactive carrier state	Reactivation
ALT	Normal	Likely elevated	Normal	Elevated
HBV DNA	Elevated	Variable, however, likely elevated	Very low	Variable, however, often seen elevated
HBV serology	HBeAg (+)	HBeAg (+)	HBeAb (+)	HBeAb (+)
Histology	Normal, can see mild inflammation	Moderate-severe hepatitis with possible fibrosis	Generally normal, can see mild inflammation	Moderate-severe hepatitis with likely fibrosis and possible cirrhosis

ALT alanine transaminase, *HBV* hepatitis B virus, *HBeAg* hepatitis B e antigen

and demonstrate signs of advanced liver disease. Aminotransferase levels are noted to fluctuate and can be seen at normal levels [54] (Table 1.1).

Eight genotypes are currently differing from one another in genome sequencing by ~8% [76]. There are further subtypes observed to differ among whole genome sequencing by ~4 to 8% [77]. Genotype A is found most frequently among Northern Europe and North American Caucasian populations. Genotypes B and C are found most commonly in Asian populations, including Asian immigrants to other countries. Genotype D is commonly observed in Southern and Eastern Europe as well as the Middle East and India. Genotype F is found in native North and South American populations. Genotypes E, G, and H comprise an uncommon cluster of HBV genotypes without well-established epidemiology. As mentioned earlier, familiarity of a patient's genotype and subtype carries clinical significance. Genotype A1 is associated with development of HCC in younger males with serology typically demonstrating negative HBeAg, positive anti-HBe Ab, low levels of HBV DNA, and rarely with advanced fibrosis. Genotype A2 is associated with HCC in persons of advanced age.

Genotype B is divided into two major groups, Bj, found in Japan, and the Ba subtype commonly seen in the rest of Asia. Bj has been further differentiated into a group containing B2–B5, which contains portion of the C genotype recombined into the core region of genotype B, and a group containing subtypes, B1 and B6, which do not. Ba is associated with older age at the time of HBeAg seroconversion, higher risk for HCC, and higher frequency of BCP mutations than genotype Bj [78]. In Taiwanese populations infected with HBV Bj genotype variant, HCC has even been noted in non-cirrhotic male patients [79–81].

Genotype C is associated with higher risk of HCC and slower rate of seroconversion than A2, Ba, Bj, and D [28, 82–85]. Mutations in the core promoter region appear to have significant correlations with HBeAg seroconversion, with mutations in three regions increasing significantly in the immune clearance phase compared to the immune-tolerant phase [86]. This increased proportion of mutations was observed with increased prevalence cirrhosis and HCC [87, 88].

Genotype D is associated with HBeAg-negative chronic hepatitis, and frequency harbors the PC variant [61]. It is also observed that persons infected with genotype

Table 1.2 Major HBV genotypes and associated clinical features

Genotype	Geography	Possible clinical presentation	Relative risk (if applicable)
A1	Sub-Saharan Africa	HCC noted in younger males without cirrhosis	N/A
A2	Northern Europe	HCC in older patients	Lower risk of HCC when compared to genotype D
Bj	Japan	HCC and cirrhosis generally noted in older patients	N/A
Ba	Eastern Asia	HCC and cirrhosis	Generally seen in younger age than Bj
C	Asia	HCC and cirrhosis	Clears HBeAg later than other genotypes, higher risk of sequelae
D	Middle East	HBeAg-negative, anti-HBe Ab-positive chronic hepatitis B	Lower risk of HCC and cirrhosis given generally prolonged inactive carrier phase

HBV hepatitis B virus, *HCC* hepatocellular cancer, *N/A* not applicable, *HBeAg* hepatitis B e antigen, *Anti-HBe Ab* anti-hepatitis B e antibody

D and found to be in the inactive hepatitis phase are likely to remain in this phase without developing complications of liver disease or HCC [58]. When active, however, this genotype is associated with relatively increased degree of severe liver damage than genotype A, as well as higher incidence of HCC in Indian populations [89] (Table 1.2).

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Management of Chronic HBV Infection in Children

2

Md Rukunuzzaman, Md Benzamin, and Kaniz Sultana

2.1 Introduction

Hepatitis B virus (HBV) infection is prevalent worldwide. The lowest rates (0.2–0.5%) of HBsAg carrier rate is in countries having a high standard of living such as Britain, Canada, the USA, Scandinavia, and some other parts of Europe. In Southeast Asia, the prevalence of HBV infection is 8–20% [1]. Millions of people are chronically infected with HBV in Bangladesh, and most infections occur during childhood [2]. Studies showed that the overall prevalence was about 3% in Bangladesh [3]. In another study the prevalence of HBV infection among the students of a Girls' School of Dhaka city was found to be 2.3% [4], 7% in multi-transfused thalassemic patients [5], and 3.6% in pregnant women of Dhaka city [6]. About 350 million people are chronically infected globally. Annually there are over four million acute cases of HBV infection, and among them about 25% are carriers [1].

HBV infection has different clinical manifestations depending on the patient's age at infection, immune status, and the stage at which the disease is recognized. Children mostly remain asymptomatic and active. Jaundice or growth failure is rare, and liver damage is usually mild during childhood. Serious sequelae, like cirrhosis and hepatocellular carcinoma, may develop at any age [7]. HBV vaccination is included in EPI since 2005. Though the vaccine is available both commercially and through EPI, HBV infection is still a health problem, and every year new cases are reported throughout the country [8]. Identification of risk factors and routes of its transmission will help to prevent global spread of the disease, especially in endemic regions [9]. In Bangladesh prevalence of HBsAg is highest among children between 5 and 9 years of age [3]. Boys are affected more than girls, probably due to a higher risk of exposure [10]. According to existing reports, there is no seasonal variation

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for primary HBV infection, and it is more common among urban children than in rural children [11]. HBsAg is found in all body secretions and excretions. Transmission by percutaneous and permucosal exposure includes transfusion of unscreened blood or blood products, sharing of unsterilized injection needles for intravenous medication, hemodialysis, acupuncture, tattooing, and injuries from contaminated sharp instrument by hospital personnel [12]. Sexual and perinatal HBV transmission usually results from abraded mucous membrane exposure to infectious blood and body fluids [8]. About 70–90% of infants infected in their first few years of life become chronic carriers [13]. Perinatal transmission is more common in hyperendemic areas of Southeast Asia, especially when HBsAg carrier mothers are also HBeAg positive [14]. Infection may also be transmitted between household contacts [7]. HBV is stable on environmental surfaces for at least 7 days. Indirect inoculation of HBV can occur via inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment, and other objects by contact with mucous membranes or open skin wounds [1]. Breastfeeding has not been shown to contribute significantly to HBV transmission from infected mothers to infants who have received active and passive immunoprophylaxis [15, 16].

Several drugs are used for the treatment of chronic infection. Lamivudine, adefovir, entecavir, tenofovir, and interferon are commonly used in children. Treatment of chronic HBV infection by antiviral drugs is very costly. According to local market price, total treatment cost of oral antiviral drugs is about 20, 000 taka; that of interferon is about 200,000 Taka. Moreover, outcome of treatment is also guarded. Seroconversion (disappearance of HBeAg and appearance of anti-HBe) occurs in about 17–32% of cases if treated with oral nucleot(s)ide analogue and 58% of cases if treated with interferon [17].

2.2 Natural History of Chronic Hepatitis B (CHB) Infection

CHB infection evolves through five phases. All patients may not experience all phases, and phases may not be sequential. Duration of phases varies, and reversion of phases may occur. Phases are immune-tolerant phase, immune-reactive phase, inactive carrier state, HBeAg-negative CHB phase, and HBsAg-negative phase [18, 19] (Tables 2.1 and 2.2).

Immune-Tolerant Phase It is characterized by host immune tolerance, though there is active viral replication. This phase is long in perinatally acquired infection and may even be 40 years or more. In this phase, HBeAg is positive and anti-HBe is negative, serum HBV DNA is >20,000 IU/ml, and there is persistently normal ALT. Liver biopsy shows mild or no necro-inflammation, and there is no or minimal fibrosis. This phase is highly contagious because of high viremia [20].

Immune-Reactive Phase In this phase the host immune response is strong and reacts against virus-infected hepatocytes. Here HBeAg is positive and begins to clear. HBeAg clearance rate is 10–15% per year. Anti-HBe begins to become positive in the later part of this phase. Episodic flare of anti-HBcIgM occurs that may

Table 2.1 Phases of chronic hepatitis B (CHB) infection

Different phase	HBsAg	HBeAg	Anti-HBe	HBV DNA	ALT	Necro-inflammation
Immune tolerant	+	+	–	High	Normal	Mild/no
Immune reactive	+	+	–	High	Raised	Moderate/severe
Inactive carrier	+	–	+	Low/undetectable	Normal	Mild/no
HBeAg –ve CHB	+	–	+/–	Fluctuating	Fluctuating	Active
HBsAg –ve	–	–	–	Undetectable of very low	Normal	No

Table 2.2 Management of drug resistance [37]

Lamivudine resistance	Adefovir resistance	Entecavir resistance
– Switch to tenofovir – Add adefovir	– Switch to tenofovir – Switch to or add Entecavir – Add lamivudine in the absence of previous lamivudine resistance	– Switch to tenofovir

cause confusion with acute hepatitis [18]. Serum HBV DNA is >2000 IU/ml, and there is persistent or intermittent elevation of ALT. Liver biopsy shows features of chronic hepatitis (HAI ≥ 4), and there is more rapid progression to hepatic fibrosis. This phase may last from several weeks to several years [21].

Inactive Carrier State These phase also known as low replicating phase. In this phase patients are HBeAg negative and anti-HBe positive and have undetectable or low serum HBV DNA, and there is persistent normal ALT. Liver biopsy shows the absence of significant hepatitis. Here patients are asymptomatic. A minimum of 1-year follow-up with normal ALT and low serum HBV DNA is needed to declare a patient as inactive HBV carrier. This phase has favorable long-term outcome with low risk of cirrhosis and HCC. But about 10% of patients of this phase may reactivate to HBeAg-positive or HBeAg-negative CHB infection [18, 19].

HBeAg-Negative CHB Phase This phase follows seroconversion from HBeAg to anti-HBe during immune-reactive phase or may develop many years after inactive carrier state. It represents the reactivation of CHB. It may be due to pre-core mutation. Patient may be HBeAg positive or HBeAg negative. There is persistent or intermittent elevation of ALT. Liver biopsy shows features of chronic hepatitis (HAI ≥4). Patients of this phase have active liver disease and may progress to cirrhosis, hepatic decompensation, and HCC [21].

HBsAg-Negative Phase This phase is characterized by the absence of both HBsAg and HBeAg in blood. HBV DNA becomes undetectable. Though HBV DNA is cleared in the blood, it may present in hepatocytes. Such occult HBV infection may

reactivate after immunosuppressive therapy. Mean annual rate of seroconversion of HBsAg is 0.5–1% in seroconverted cases [18].

2.3 Clinical Presentations of CHB Infection

Patients with CHB are mostly asymptomatic. In one study, history and clinical examination of patients with CHB showed that 56.7% were asymptomatic and 40% had nausea or vomiting, 35.5% abdominal pain, 15.3% jaundice, 21.1% hepatomegaly, 7.8% splenomegaly, 5.6% hematemesis or melena, and 6.7% ascites [22]. Clinical manifestations of CHB can be described in four overlapping stages. These are early or slowly progressive liver disease, progressive liver disease, advanced liver disease with complications, and extrahepatic manifestations. In early or slowly progressive liver disease stage, symptoms are nonspecific. Individuals frequently complain of anorexia, nausea, tiredness, abdominal discomfort, and right upper quadrant pain. Physical examination reveals no finding or only hepatomegaly. Some of the stigmata of chronic liver disease may be present. In the stage of progressive liver disease, there may be episodic hepatic flare along with symptoms of early disease. In this stage, common signs are hepatomegaly, mild jaundice, and peripheral stigmata of chronic liver disease. Ultimately, CLD progress to advanced liver disease when different complications develop. Jaundice, ascites, coagulopathy, encephalopathy, and fetor hepaticus may present. Complications like infection, portal hypertension, hepatorenal syndrome, and hepato-pulmonary syndrome may develop in this stage. Extrahepatic manifestations involve hematological, renal, rheumatological, dermatological, endocrine, and neurological systems [23].

2.4 Investigations

Complete blood count (CBC) is usually normal. Macrocytic anemia is typically found in chronic liver disease, but microcytic or normocytic anemia may also present. In the case of hypersplenism resulting from portal hypertension, pancytopenia may be found. Liver function tests (LFTs) may be normal in early CHB infection. Commonly done LFTs are serum alanine aminotransferase (ALT), prothrombin time (PT), serum bilirubin, and serum albumin. ALT is raised in immune clearance phase and in HBeAg-negative CHB cases. Viral markers including HBsAg, anti-HBcIgM, HBeAg, anti-HBe, and HBV DNA should be evaluated. In CHB infection HBsAg is positive, but anti-HBcIgM is usually negative. HBeAg is always positive in immune-tolerant phase, and HBeAg is usually negative in HBeAg-negative CHB cases. Anti-HBe becomes positive when HBeAg is negative. Patients infected with genotype D and infected with pre-core mutant virus tend to be HBeAg negative but with high HBV DNA titer [21]. Ultrasonography of hepatobiliary system is usually normal in early stage, but increased echogenicity and evidence of portal hypertension may be found as the disease progresses. Liver biopsy findings composed of summation of four individual scores: periportal ± bridging necrosis, intralobular degeneration and focal necrosis, portal inflammation, and fibrosis. On the basis of histological activity index

score, cirrhosis may be classified as minimal, mild, moderate, and severe [23–25]. Before and after giving antiviral drugs, creatinine should be checked routinely.

2.5 Treatment of CHB

Goals of treatment: Goals of therapy are to reduce viral replication, to minimize liver injury, to reduce consequence of liver injury like cirrhosis and hepatocellular carcinoma (HCC), and to reduce infectivity of HBV [21]. Predictors of positive response include high pretreatment ALT level, low pretreatment HBV DNA <20,000 IU/ml, late acquisition of HBV infection, and higher hepatocellular inflammation [21]. Treatment is successful when there is low or undetectable HBV DNA, negativization of HBeAg, seroconversion to anti-HBe, normalization of aminotransferase, and reduction of necro-inflammation. A case is considered cured when there is absence of HBsAg, undetectable HBV DNA, and absence of HBeAg [26].

Indications for antiviral therapy—The following criteria should be fulfilled to start antiviral therapy:

1. Chronic HBV infection: (a) HBsAg positive for >6 months or more; (b) HBsAg positive and anti-HBcIgM negative in one occasion
2. Active hepatic inflammation: (a) raised ALT for 6 months >1.5 ULN or >60 IU/l whichever is lower; (b) histological evidence of chronic hepatitis: moderate to severe inflammation and fibrosis
3. Viral replication: (a) HBV DNA >2000 IU/ml and/or (b) HBeAg positive

There are some special circumstances where treatment of CHB can be given in the absence of standard criteria. These conditions are cirrhosis (compensated/decompensated), chemotherapy, immunosuppression, the presence of coinfection (HBV–HIV), family history of HCC, and pregnant women with high viral load [24, 25].

In patient with cirrhosis, the goals of antiviral therapy are to prevent liver disease progression to decompensated cirrhosis, development of HCC, and liver-related death [23]. Antiviral treatment in cirrhotic patients is not based on ALT because ALT may be normal in advanced liver disease. Treatment in cirrhotic children can be started even if the HBV DNA is low. Treatment with interferon can't be given in decompensated chronic liver disease patients because interferon may precipitate sepsis and liver failure. Treatment with nucleot(s)ide analogues is the preferred drug therapy. Here drugs are continued for indefinite period of time [24]. Five-year survival is 25% without therapy and 85% with therapy.

Drugs currently recommended to treat CHB:

1. Nucleot(s)ide analogues:
   - Lamivudine
   - Adefovir
   - Entecavir
   - Tenofovir
2. Conventional interferon Alfa (IFNα)

2.5.1 Lamivudine

Lamivudine is the most commonly used antiviral drug. It is an oral drug. These drugs are cheap. It can be used in decompensated chronic liver disease and has no significant side effects. Seroconversion occurs in 23% of cases following 52 weeks of treatment. Recommended duration of treatment is at least 1 year and should be continued for 6 more months after HBeAg seroconversion [7, 20]. Long-term lamivudine therapy does not significantly increase seroconversion rate; in fact, it can increase the chance of developing mutant strains. Chance of development of mutant strains and chance of relapse following stoppage of therapy is increased with lamivudine. Viral resistance develops in 16% of cases after 1 year of therapy and 76% after 5 years of therapy [27]. Therefore the use of lamivudine is limited due to occurrence of resistance.

Dose: 3 mg/kg/day; highest dose is 100 mg/day.

Advantages:

- Cheap
- Less side effects
- Oral administration
- Usable in the third trimester of pregnancy
- Can be used in decompensated chronic liver disease

Disadvantages:

- High resistance rate (increased if more time of treatment)
- Seroconversion rate is low

2.5.2 Adefovir

Adefovir is also an effective antiviral drug in children. This drug is cheap and safe but nephrotoxic. Mutations associated with adefovir resistance are less common; lamivudine-resistant mutant strains appear more susceptible to adefovir. As a single drug antiviral therapy, it is not suitable because of its modest antiviral suppression effects and its renal toxicity. It is commonly used alone or in combination with lamivudine in lamivudine-resistant cases. Drug resistance develops in 29% of cases after 5 years of treatment with adefovir [28, 29]. This drug causes seroconversion in 20% of cases.

Dose: 0.3 mg/kg/day in <6 years, 0.25 mg/kg/day in >6 years, and 10 mg/day if age is >12 years [15]

Advantages: Cheap, oral administration, and effective in lamivudine-resistant cases

Disadvantages: Nephrotoxicity; seroconversion rate is low

2.5.3 Tenofovir

Tenofovir is a nucleotide reverse transcriptase inhibitor that is more potent than adefovir in suppressing lamivudine-resistant HBV. Tenofovir has been reported to achieve much higher biochemical, virological, and histological responses in both HBeAg-positive and HBeAg-negative patients, compared with adefovir and lamivudine. It has some side effects like renal insufficiency, Fanconi syndrome, and osteomalacia, but no bone disease was detected at 3-year follow-up. Dose adjustment is required in patients with renal impairment. Tenofovir demonstrated safety and efficacy in patients with liver cirrhosis, and regression of cirrhosis during treatment with tenofovir was observed in 71 (74%) of 96 patients treated for 5 years [30]. Tenofovir was also found to be safe during pregnancy as pregnancy category B.

Dose: 300 mg once daily

Advantages: High response rate, few side effects, oral administration, and usable in the third trimester of pregnancy

Disadvantages: Not approved for children <12 years and reduced mineral density in children

2.5.4 Entecavir

Entecavir is recommended in children after 2 years of age [31]. It is a potent antiviral drug causing undetectable HBV DNA after 1 year of therapy and in 91% of cases after 3 years of therapy. Chance of resistance is 0.8% after 3 years of entecavir therapy [32].

Dose: 0.015 mg/kg/day; highest dose is 0.5 mg/day.

Advantages: Oral administration and low resistance rate

Disadvantages: Abdominal discomfort, diarrhea, tachycardia, and chest tightness

2.5.5 Interferon

Interferon produces its effect by antiviral effects and immune-modulatory action. Its efficacy is more than that of other oral drugs. Among the interferon, interferon alpha 2a is used to treat CHB infection. Pegylated interferon is used in adult but not recommended in children. Polyethylene glycol is linked to interferon molecule to make it long lasting. With interferon therapy there is 58% chance of HBV DNA loss, 38% chance of HBeAg/anti-HBe seroconversion, and 33% chance of HBsAg loss [33]. It is costly and associated with many side effects. It cannot be used in decompensated state of liver disease because it may cause infection and hepatic failure. HBeAg seroconversion may occur at any time during or within 1 year of ending treatment with interferon alpha. Patients should not be declared as treatment failure or should not start another drug until 1 year of treatment [26].

Dose: 6 MIU/m^2 thrice weekly by subcutaneous injection.
Advantages:

- More effective antiviral drug
- Recommended for young children
- Short treatment (6 months treatment)

Disadvantages:

- Some side effects like liver failure, infection, flu-like symptoms, depression, bone marrow suppression, and hypothyroidism
- Hazardous parenteral administration
- Not suitable to use in decompensated cirrhosis or liver transplantation

Predictive of positive response
High pretreatment ALT level
Low pretreatment HBV DNA, <20,000 IU/ml
Younger age
Viral genotype B
Late acquisition of HBV infection
Higher hepatocellular inflammation
When to stop antiviral drugs

Duration of interferon therapy is 6 months. Oral antiviral drugs should be continued at least for 1 year and maintained for at least 12 months after HBeAg seroconversion if there is no evidence of resistance or any severe adverse drug reaction. Children with HBeAg-negative chronic hepatitis B and cirrhosis and who do not undergo HBeAg seroconversion may need longer duration or even lifelong therapy [26].

2.5.5.1 Recommendations

1. *HBeAg-positive patients with HBV DNA levels >20,000 IU/ml and elevated ALT for 3–6 months should be considered for treatment.*
2. *HBeAg-negative patients with HBV DNA levels >2000 IU/ml and elevated ALT levels for 3–6 months should be considered for treatment.*
3. *Cirrhotic child should also be treated irrespective of the ALT level, even if the viral load is below 20,000 IU/ml in HBeAg-positive patients or below 2000 IU/ml in HBeAg-negative patients.*
4. *Tenofovir and entecavir are considered first-line therapies for treatment-naïve HBV patients because they are the most potent agents available with no or very low rates of antiviral resistance.*
5. *Tenofovir is the first-line therapy for lamivudine-resistant HBV case. Entecavir should not be used in this setting due to the risk of development of entecavir resistance.*
6. *In HBeAg-positive patients, nucleos(t)ide analogue therapy should be continued until 12 months after HBeAg seroconversion with close monitoring of HBV DNA and ALT levels following treatment withdrawal.*

7. *In HBeAg-negative patients, nucleos(t)ide analogue therapy should be continued indefinitely or until HBsAg loss.*
8. *HBV DNA should initially be monitored every 3 months to enable early detection of antiviral resistance and every 6 months once aviremia is achieved.*

2.5.6 Special Populations

2.5.6.1 Cirrhosis Due to CHB (Compensated or Decompensated)

In the case of cirrhotic patient, to prevent disease progression and HCC and to reduce liver-related death, antiviral drugs should be given. Nucleot(s)ide analogues are the drug of choice and should be continued lifelong.

2.5.6.2 Immunocompromised Children

Antiviral therapy is recommended in patient with CHB getting cancer chemotherapy or immunosuppressive therapy. Reactivation of HBV may occur following immunosuppressive or cancer chemotherapy. Antiviral therapy should be started 2 weeks before initiation and continued for up to 6 more months after stoppage of chemotherapy or immunosuppressive therapy. Lamivudine or adefovir alone or in combination can be used [24].

2.6 Pregnant Woman

Most women with chronic HBV infection have mild disease during pregnancy; however, hepatitis may flare up after delivery, so close monitoring is warranted.

Based on the risk of teratogenicity as assessed during preclinical evaluation, the nucleos(t)ides are listed by the US Food and Drug Administration (FDA) as pregnancy category C drugs (lamivudine, adefovir, and entecavir) and category B drugs (telbivudine and tenofovir). There is a considerable amount of safety data on pregnant HIV-positive women who have received tenofovir, lamivudine, and/or emtricitabine [34]. In these women, tenofovir is preferred because it has a better resistance profile and more extensive safety data when used during pregnancy [33].

2.7 Treatment in Immune-Tolerant Phase

Most of the authors do not recommend any treatment in immune-tolerant phase. But some studies recommend to start therapy with interferon and lamivudine than lamivudine alone to break the chain of long immune-tolerant phase especially in vertically transmitted cases [35, 36].

2.7.1 HBV–HCV Coinfection

In the case of coinfection of HBV and HCV, HCV infection is to be treated first with 6 months course of interferon and ribavirin. Pegylated interferon was also found safe and effective in children [27]. If seroconversion of HBeAg does not occur after interferon therapy, long-term treatment with lamivudine or adefovir can be started [24].

2.7.2 HBV–HIV Coinfection

In HBV–HIV coinfection, both the infections should be treated simultaneously. Lamivudine and adefovir have anti-HIV action. So lamivudine plus adefovir along with a third agent against HIV can be used. There are two reasons for considering HBV therapy in HBV–HIV coinfection. Firstly, liver disease progresses more rapidly in coinfected patients, and secondly, there is high risk of developing hepatotoxicity following antiretroviral therapy in coinfected patients than in patients infected with HIV alone [24].

2.8 Occult Hepatitis B Infection (OBI)

It is the state of HBV infection in which there is absence of serum HBsAg, presence of low level of HBV DNA <200 IU/ml, and presence of markers of previous infection, e.g., anti-HBc total/or anti-HBs positivity. OBI occurs due to persistence of cccDNA, and transmission occurs through blood transfusion and organ transplantation. OBI needs no antiviral therapy usually. Antiviral drug should be started to OBI if chemotherapy or immunosuppressive therapy is to be given, especially in the absence of anti-HBs and continued up to 12 months after stoppage of immunosuppressive therapy.

2.9 Antiviral Resistance

We should suspect antiviral resistance if there is inability to reduce HBV DNA 1 log10 IU/ml or more after 3 months of therapy, rise of HBV DNA at least 1 log10 IU/ml following treatment, and rise of ALT following treatment and detection of gene mutation. To prevent antiviral resistance, we should initiate treatment only when indicated. Drug of optimal antiviral potency and low resistance should be used, and sequential monotherapy and interruption should be avoided.

2.9.1 Recommendations

1. *All patients undergoing chemotherapy or treatment with other immunosuppressive therapies should be screened for HBsAg.*

2. *Patients testing positive for HBsAg should receive antiviral prophylaxis 2 weeks before starting treatment and continuing for at least 6 months after the last dose of immunosuppressive drug with close monitoring during and after therapy.*
3. *Patients with isolated anti-HBc who are immunosuppressed should have close HBV DNA monitoring and should be considered for antiviral therapy.*
4. *All pregnant women should be screened for HBsAg and, if positive, tested for HBV DNA, HBeAg, and ALT.*
5. *HBV treatment should be considered in high-risk mothers to reduce the risk of vertical transmission in cases of high viral loads.*
6. *Initiation of therapy should be in the third trimester.*
7. *Patients should be monitored during pregnancy and postpartum for withdrawal flare-ups after nucleos(t)ide analogue treatment is stopped.*
8. *The recommended first-line treatment during pregnancy is tenofovir (FDA category B).*
9. *Sequential monotherapy and interruption should be avoided to overcome drug resistance*.

2.10 Persons Who Are HBsAg-Positive

- Breastfeeding is to be continued.
- Screen family members and vaccinate when indicated.
- Cover open wounds and scratches.
- Clean blood spills with detergent or bleach.
- Can share food and utensils.
- Can participate in all activities including sports.
- Should not be deprived of schools.
- Should not be isolated from other children.
- Should not share razors and toothbrushes.
- Should not donate blood or organs.

2.11 Follow-Up

Patients getting antiviral drugs should be under regular follow-up. Follow-up should be given in respect to clinical and laboratory parameters. Fever, fatigue, depression, flu-like symptoms, thyroid dysfunction, bone marrow depression, gastrointestinal disorder, mood disorder, and personality changes are common side effects of interferon therapy. Lamivudine rarely causes lactic acidosis. Adefovir may cause nephrotoxicity. Clinically patients should be monitored for the abovementioned side effects of drugs. Drug compliance is an important issue for effective therapy. Features of decompensation like jaundice, ascites, coagulopathy, encephalopathy, and fetor hepaticus should be searched because interferon cannot be given in decompensated chronic liver disease. CBC is to be checked time to time for any neutropenia. Thyroid function test is to be done for hypothyroidism. Evaluation of renal

function through serum creatinine, to assess adefovir toxicity. Serum ALT should be checked to assess drug response and posttreatment flare [26]. HBeAg and anti-HBe should be checked, too, monthly for seroconversion. Serial HBV DNA assay is needed to see the drug response. HBsAg status is checked in seroconverted patients. Ultrasonography of hepatobiliary system and alpha-fetoprotein is done yearly to see any malignant changes in the liver [22].

Conclusion

Management of chronic HBV infection is difficult. Treatment outcome is guarded, and seroconversion occurs in 10–60% of patients. Moreover, commonly used drugs are costly. In densely populated countries where education is low, awareness of people through mass media may be considered as an effective way to prevent the spread of disease. Children are worst sufferer, and they are the future of the nation. Special precaution should be taken to prevent transmission of the virus to them. Health education and vaccination at birth are the logical and practical approach to safeguard the children.

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Epidemiology of Hepatitis B

3

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and Hakan Leblebicioglu

3.1 Prevalence of Hepatitis B in the World

Chronic hepatitis B virus (HBV) is one of the globally spread infections despite of existing effective anti-HBV vaccine, antivirals, and worldwide initiatives on the safety of health-care injections and blood product transfusions in medical facilities in the past 20–25 years [1, 2].

An approximate number of HBV-infected people is 257 million; approximately 378 million people in the world are chronic HBsAg carriers. About 620,000 deaths each year are related to HBV. Sixty-eight percent of HBV-infected individuals live in Africa and in the Western Pacific Region [3].

The chances of chronic hepatitis B developing depend on the age at which the individual was infected. It reaches 90% from the perinatal period up to 6 months old and decreases to 20–60% between 6 months and 5 years of life [4, 5]. Cirrhosis or primary liver cancer in adults develops usually in 25% of people who were infected with HBV in childhood [6]. Most people living with chronic HBV infection were born before the anti-HBV vaccine became widely available and used in newborns and infants [1].

Global Burden of Disease Study 2013 indicates a high morbidity and mortality due to chronic HBV, despite a decrease of these indicators over the past decades [7].

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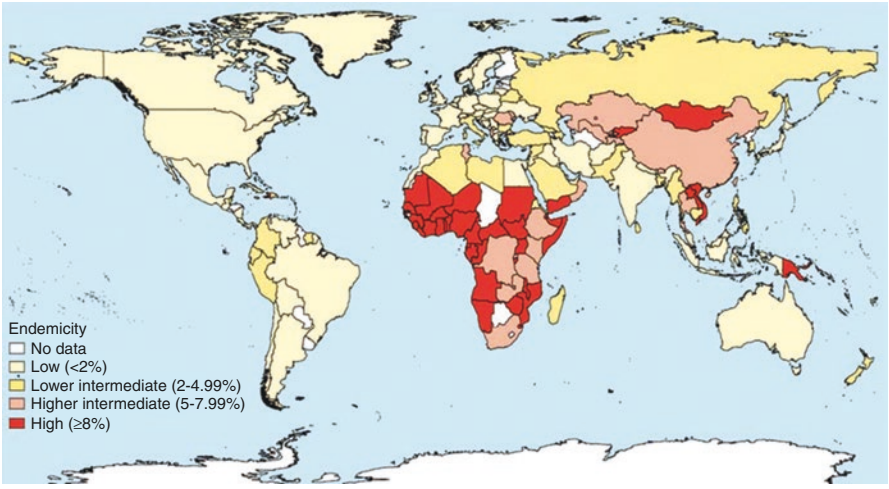

Fig. 3.1 Global prevalence (%) of HBsAg. *Source*: Schweitzer A., et al. The Lancet 2015; 386.10003: 1546–1555

In 2015, hepatitis B led to 887,000 deaths, generally from severe consequences and complications of infection (including cirrhosis and hepatocellular carcinoma) [1].

Globally, HBsAg prevalence is about 3.61% [4], but it widely varies in different WHO regions and countries (Fig. 3.1).

Over 75 million individuals with HBsAg live in the African region. The highest HBsAg prevalence in the general population is in Sub-Saharan Africa (8.8% according to meta-analysis). It was primarily observed in vertical transmission from mother to fetus and infant, low level of anti-HBV vaccination routine campaign (including vaccination of newborns), and unsafe medical procedures and products [30]. Almost all countries in Africa (with exception of Algeria, Eritrea, and Seychelles) have higher intermediate or high level of HBsAg prevalence (5–7.99% and ≥ 8%) [4, 8].

The WHO region of the Americas has the lowest number of individuals living with HBsAg (more than 7 million people). Several countries in the WHO region of Americas (USA, Mexico, Guatemala) have low endemic level (<2%), with the exception of Haiti where the level of HBsAg prevalence is high [4].

More than 17.4 million individuals with HBsAg live in the Eastern Mediterranean WHO Region. It has generally lower intermediate level of endemicity (2–4.99%), but Somalia, Sudan, and Djibouti have a higher level of HBsAg prevalence than many other countries in this WHO region [4].

The European WHO Region in general has lower intermediate level of HBsAg prevalence and little less than 18.5 million individuals with HBsAg. But the level of endemicity significantly rises moving from west to east: from low level in Western Europe (lowest 0.01% in the UK) to high in the republics of Central Asia (10.32% in Kyrgyzstan, 13% in Uzbekistan) [4, 9].

The Southeast Asian region has low endemicity level (<2%) in Indonesia, Nepal, and India. Other countries have endemicity levels from low intermediate to high intermediate. In total there are more than 34 million individuals with HBsAg living in this region [4].

The highest number of people living with chronic hepatitis B are in the Western Pacific WHO Region (>95 million persons). This region generally has a high intermediate level of HBsAg prevalence (5–7.99%), with the highest level of endemicity in the Pacific Island States [4].

The three countries with the highest level of HBsAg prevalence in population are China (74 million, 7.2%), India (17 million, 1.46%), and Nigeria (15 million, 13.6%) [4, 10, 11]. The highest endemic territories in the world are Sub-Saharan Africa, Southeast Asia, Central Asia, and the Amazon basin, where the prevalence of HBsAg carriers is more than 8% [8].

3.1.1 HBV Prevalence and Income

Low- and middle-income countries have the greatest burden of hepatitis B and face a significant problem in screening for disease and treatment of affected population. Low-income countries have prevalence of HBV infection 7.4 times greater than high-income countries. Meanwhile, the proportion of diagnosed individuals in high-income countries is 18%, while in low-income countries, it is only 0.8%. This situation reflects a problem in the testing for HBV in countries with insufficient income. The proportion of patients who receive antiviral therapy also varies depending on the income from 9% in low-income countries to 14% in high-income states [3].

Chronic hepatitis B prevalence according to income (GNI, World Bank, 2016) is reflected in Fig. 3.2. A clear-cut negative trend is shown between the level of income

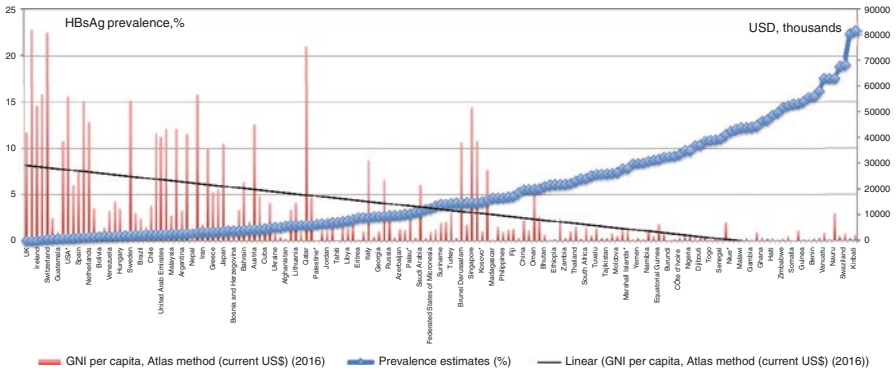

Fig. 3.2 Prevalence (%) of HBsAg worldwide according to income (GNI, USD thousands). *Source*: (1) GNI—World Bank, 2016 or the latest available year; (2) prevalence of HBsAg—Schweitzer A., Horn J., Mikolajczyk R. T., Krause G., & Ott J. J. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. The Lancet 2015; 386.10003:1546–1555

in a country and the prevalence of HBV in the population. It is clear that upper intermediate and high income can ensure total vaccination of newborns and safe medical manipulations and lead to decreased prevalence of HBsAg among the general population. But traditions of behaviors and culture in the country and prevalence of particular risk groups for viral hepatitis (migrants, intravenous drug users, people living with HIV/AIDS, etc.) can influence the level of HBV endemicity in the country.

The HBV carrier rate is over 8% in countries of Sub-Saharan Africa, Southeast Asia, Central Asia, and the Amazon basin which belong to the high-endemic regions with the lowest level of income. In countries belonging to the low-endemic regions, HBsAg prevalence is less than 2% (USA, part of South America, Northern Europe, Australia), where the level of income varies from upper intermediate to high. The countries of the Mediterranean basin and the Middle East and some Eastern European countries have intermediate HBV carrier rate that varies between 2% and 8% in predominantly middle-income levels.

Infection contracted under 5 years of age composes the main basis for hepatitis B burden of disease [1, 12]. Transmission of HBV infection from mother to newborn during delivery often leads to chronic hepatitis B. Therefore, prevention of hepatitis B focused on children under 5 years is the cornerstone of the vaccination campaign. The hepatitis B vaccine era started between the 1980s and early 2000s in different countries. In general, vaccination with 3 doses of anti-HBV vaccine in children is achieved 84% globally. Unfortunately, vaccination of newborns reached only 39% of infants in the world [3]. The vaccination campaign, which has been implemented on a permanent basis, has allowed the reduction of transmission of HBV to infants. Between the pre-vaccine era and 2015, the prevalence of children under 5 years old with chronic hepatitis B decreased from 4.7% to 1.3%. It varies from very low (0.2%) to low in the WHO region of the Americas and in the European region accordingly. Meanwhile, the prevalence remains high enough in Africa (3%) and moderate in Eastern Mediterranean WHO Region (1.6%) (Fig. 3.3) [3]. Reduction of the prevalence of chronic hepatitis B among children in the long-term perspective should lead to decreased hepatitis B epidemic globally. The United Nations chose as an indicator of the Sustainable Development Goal target for "combating hepatitis" the cumulative incidence rate of chronic hepatitis B at 5 years of age [13].

At present time the worsening situation regarding the prevalence of chronic hepatitis B in adults and children exists in the African region in which there are 6.1% adults and 3.0% children infected. Only if vaccination companies will be well developed together with safe medical manipulations in extremely long-term perspective in this region can the situation improve (Fig. 3.4). The highest number of people living with HBV (115 million persons) is observed in the Western Pacific Region, but a relatively low number of infected children (0.9%) make positive prognosis in regard to improving the situation in the future (Fig. 3.4). The Eastern Mediterranean region took the intermediate position according to estimated parameters. Among all WHO regions, the Eastern Mediterranean region took the third place in the number of infected adults (3.3%), but second place in endemicity in

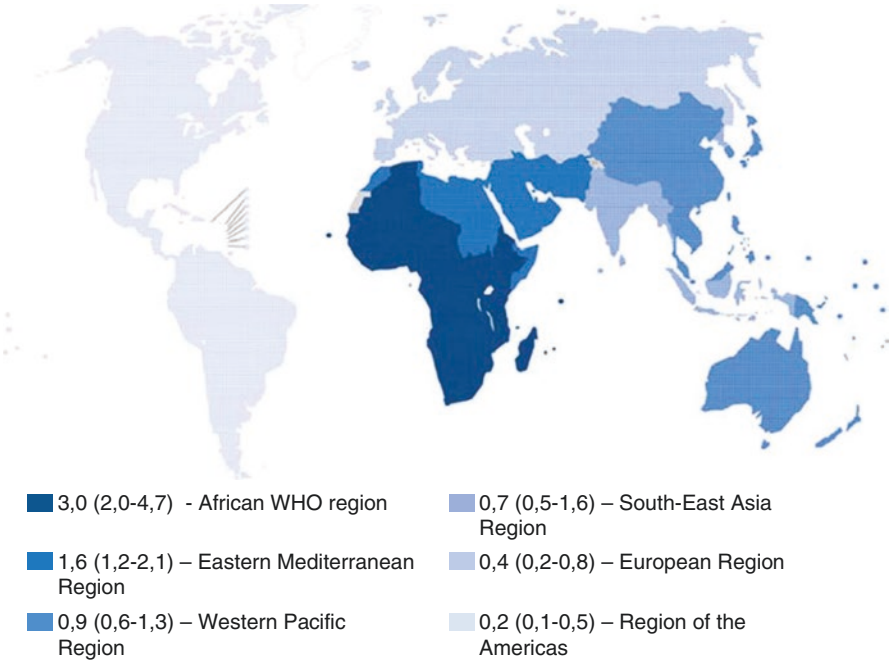

Fig. 3.3 Prevalence (%) of HBsAg in children under 5 years after the use of the vaccine by WHO region 2015. *Source*: World Health Organization. Global hepatitis report 2017. World Health Organization, 2017

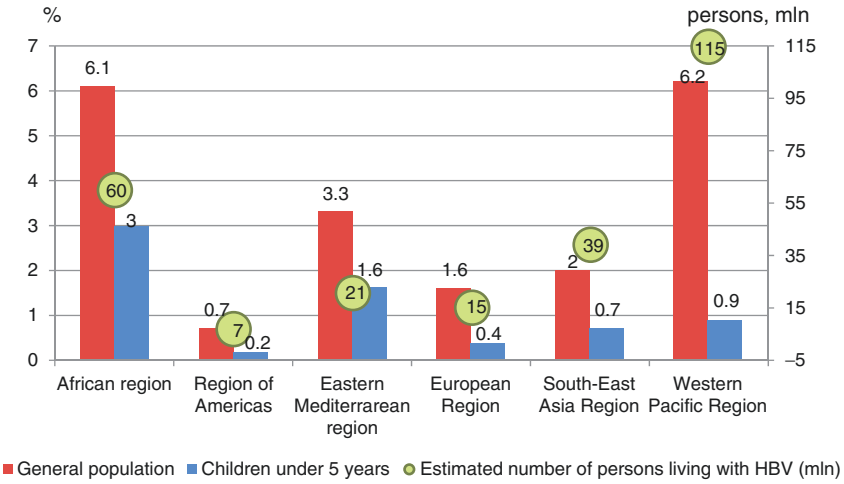

Fig. 3.4 Prevalence (%) of HBsAg in the general population and children under 5 years after the use of the vaccine and estimated number of persons living with HBV (millions) by WHO region 2015. *Source*: World Health Organization. Global hepatitis report 2017

children (1.6%) and only fourth place in the number of infected people (21 million). It also allows to consider the total situation in the region as unfavorable.

The region of the Americas ranked second after the European Region of WHO in regard to the lowest prevalence of HBsAg among adults and children and total number of infected people in the world (Fig. 3.4). But this picture reflects only the average situation in these regions. Inside the regions the endemicity varies from favorable situations in the USA and Canada to much worse in Latin American countries (with the exception of Brazil), especially in Peru and the Dominican Republic where HBsAg prevalence among children 5–9 years old is more than 5%. European regions vary geographically from demonstrating relatively low prevalence of HBsAg in Western Europe compared to high endemicity in Central Asian countries. Southeast Asian region in common also has enough good position for all three parameters (prevalence of HBsAg among adults and children and total number of infected people).

3.2 Prevalence of Hepatitis B in Risk Groups

There are several risk groups for chronic hepatitis B and carriers of HBsAg. Their distribution in the world and prevalence among them HBV-infection is depend first of all from common situation with this infection in country, but also from specific political, economic, national features, situation with sexual-transmitted diseases and drug abusers, and quality of health care.

The main risk groups for chronic HBV infection are:

- Intravenous drug users (IDUs)
- Men who have sex with men
- Indigenous peoples and minorities
- Prisoners
- Migrants
- Blood donors
- Health-care workers (clinicians, laboratory workers)
- Coinfection

3.2.1 Prevalence of HBsAg in IDUs

The fullest systematic review about the prevalence of chronic HBV infection among IDUs was published in 2011 by Nelson et al. [14].

The authors measured the prevalence of HBsAg in 59 countries where 73% of the global number of IDUs live (Fig. 3.5). The prevalence of HBV infection among IDUs is usually correlated with the prevalence of HBV infection in the general population. The highest rates of HBsAg carriers among IDUs are in countries with high prevalence of HBsAg in the general population (mainly in Asia). In countries

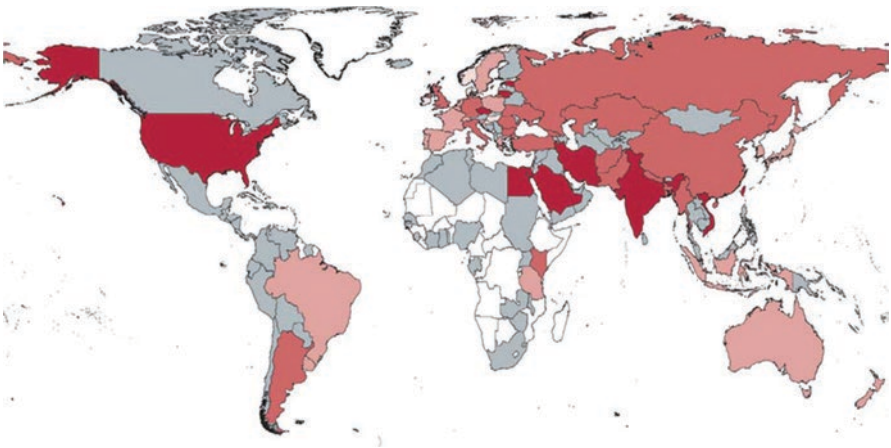

Fig. 3.5 Prevalence of hepatitis B surface antigen in injecting drug users. *Source*: Nelson P. K., et al. The Lancet 2011, 378(9791), 571–583.

with a low and intermediate level of HBsAg carriers, the prevalence of HBV infection among IDUs is usually less than 10%.

In countries where the level of HBV in the general population is high, the prevalence of HBsAg in IDUs is around 10–20% (USA, Southeast Asia, East Asia, Egypt, Arabian Peninsula). The prevalence of chronic HCV infection in IDUs is relatively higher than HBV level, and HBsAg detection in IDUs usually shows HBV + HCV coinfection [14].

Decreased HBV cases in IDUs are observed in the past decade in many high-income countries. Meanwhile, addicts using intravenous drugs continue to be at significant risk of HBV transmission, especially in Eastern Europe [15].

3.2.2 Men Who Have Sex with Men

HBV continues to be an important sexually transmitted infection (STI) among men who have sex with men (MSM) for many years. Individuals who have homosexual sex have a relatively higher risk of HBV and HAV infection than the general population. The HBV incidence among MSM is 20 times higher than in the heterosexual population. It makes MSM a group more vulnerable to becoming infected with HBV than the general population. The higher prevalence and increased transmission rate in MSM are associated with unprotected anal intercourse [16]. It is especially clearly seen in countries where transmission of HBV in newborns and children under 5 years old is rare [17]. Genotype A strain of HBV circulates among MSM in many high-income countries around the globe for many years, for example, in the UK, the Netherlands, and Japan [18–20]. Six to ten percent of HBV-infected MSM have HBV + HIV coinfection [21].

3.2.3 Indigenous Peoples and Minorities

Minorities and indigenous people usually have poor access to health-care services or are out of range to adequate services. This is due to the fact that they live in remote, hardly accessible areas, that they are discriminated against for a variety of cultural reasons, or that the health-care structure is not adapted to their cultural differences [3]. This has led to higher levels of HBV in these specific groups of population. In the Italian population of Europe, over 12.5% of children are HBsAg carriers in Slovakia and 4% in Greece [22, 23]. The prevalence of HBsAg among different indigenous groups in the Amazon region of the Americas varies from 1% to over 14% [24]. In the Nicobar and Andaman Islands of India (Indian Ocean), the level of HBsAg among local populations varies from 23% to 66%. The prevalence among pregnant women is 20.5% [25]. In Australia (Aboriginal and Torres Strait Islander peoples), HBsAg prevalence is 2.25% among adults and 3.96% among pregnant indigenous people and only 0.90% in non-indigenous pregnant women [26].

In the Arctic the total prevalence of HBV infection in indigenous populations varies from 3% in Canada to 12% in Siberia [27]. There are no data about Maori population (New Zealand).

Indigenous groups usually also have a high level of HBV + HDV coinfection/superinfection. It varies from 7% to 42% among individuals with chronic hepatitis B [24, 28].

3.2.4 Prisoners

Usually the level of HBV and HCV detection among prisoners is higher than in the general population of the country. The level of HBV- and HCV-infected patients depends on the prevalence of IDUs inside the prison [29]. According to a global review of HBV infection in prisoners, the medium level based on data from 43 countries is 4.8%, but varies significantly among countries (Fig. 3.6). Chronic hepatitis B in prisoners in Central and Western African countries has an extremely high level (23.5%). Significant levels of HBsAg prevalence are also indicated in Central Asia and Eastern Europe (10.4%) and in East and South African countries (5.7%) [30]. In other regions, HBsAg level among prisoners is less than 5%. Unprotected sex and unsafe injection drug use remain important modes of transmission. For example, the prevalence of antibodies to hepatitis B core Ag in prisons of England and Wales is 6% [31]. 0.9% of the prisoners had hepatitis B surface antigen, and 29.5% had one or more serum markers for hepatitis B virus in Tennessee, USA [32]. The prevalence of chronic hepatitis B in Pakistan is 5.9% [33] and in Iran 1.2%, where HBsAg level is significantly and positively associated with the duration and frequency of imprisonment [34, 35]. In Australia, the prevalence of HBV infection is 1.0% in the general population and 2.3% in prisoners. Despite a reduction in the proportion of Australian IDUs in prisons, evidence of HBV infection remains high, because many prisoners are frequently unaware of the status of their infectious diseases [36].

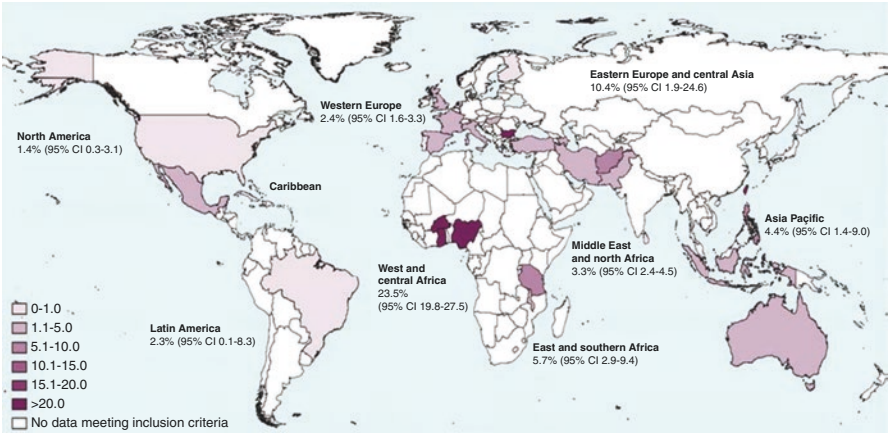

Fig. 3.6 Prevalence of hepatitis B surface antigen in prisoners between 2005 and 2015. *Source*: Dolan K. et al. The Lancet 2016, 388(10049), 1089–1102

3.2.5 Migrants

Individuals can relocate from countries with a high level of HBsAg among the general population to countries with a low HBV prevalence. The prevalence of chronic HBV infection in migrants from different countries reflects the prevalence of HBsAg in their countries of origin [37]. Twenty-five percent of migrants in the European Union are HBsAg carriers. This is observed to be greater than in the general population; the proportion of migrants from countries with intermediate and high HBsAg prevalence is only 5% [3].

According to 110 studies included in a systematic review, about 3.5 million immigrants living in immigrant-receiving countries are HBsAg carriers (Figs. 3.7 and 3.8) [37]. Globally the prevalence of HBV infections in migrants varies from 3.7% to 9.7%. The largest number of migrants with chronic hepatitis B lives in the USA (1.6 million), Canada (285,000), Germany (284,000), Italy (201,000), UK (193,000), and Australia (176,000). The frequency of HBsAg detection is highest in migrants who arrived from Sub-Saharan Africa (10.3%), East Asia, and the Pacific Islands (11.3%); intermediate in migrants from South Asia (4.6%), Eastern Europe, and Central Asia (5.8%); and low in migrants from the Middle East and North Africa (2.0%) and Latin America and the Caribbean (1.7%). Migrants who arrived from countries with intermediate or high HBsAg prevalence and live in immigrant-receiving countries with low level of HBV infection in the general population are included in the risk group for HBV infection [37]. Restrictions in health care for migrants in different countries and population groups can deteriorate the situation and lead to the development of HBsAg in migrants and the general population.

3.2.6 Blood Donors

Despite the lower prevalence of transfusion-transmitted infections among donors, 1.6 million units of blood components are discarded annually in the world due to the

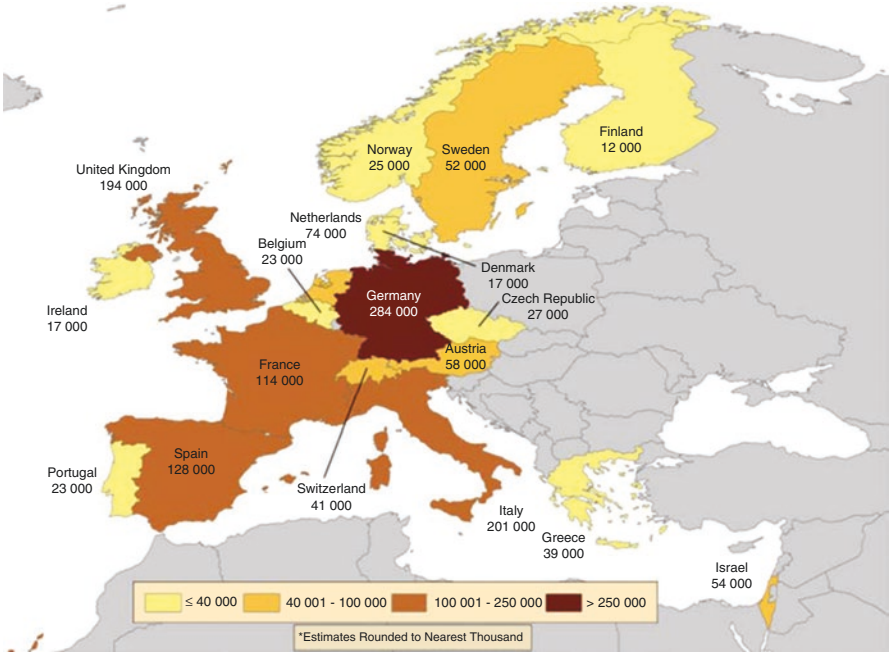

Fig. 3.7 Estimated number of migrants with chronic hepatitis B infection living in Western Europe. *Source*: Rossi C. et al. C. PloS one. 2012; 7(9), e44611

presence of markers of HBV, HCV, HIV, and syphilis [38]. Meanwhile, donors with positive tests are not always observed and treated after detection of transfusion-transmitted diseases, including HBV infection [39].

3.2.7 Health-Care Workers (Clinicians, Laboratory Workers)

Health-care workers (HCWs), especially clinicians, laboratory workers, etc., are at high risk of contamination with HCV and HBV due to exposure with body fluids. Usually this happens via needlestick or other sharp medical instruments injury or direct unprotected contact with body fluids, especially blood [40]. Obligated HBV immunization, together with modern principles of infection control, should be implemented for all HCWs in purpose to form a safe work environment. HCWs often have a higher prevalence of HBsAg than the general population, which reflects a high risk of contamination during work [41].

According to Gerberding the prevalence of chronic hepatitis B among clinical HCWs in the USA was 21.7% in spite of the availability of vaccination and high infection control quality [42]. HCWs in low-resource countries with unobligated vaccination against HBV, poor quality of infection control, and high level of HBsAg in the general population are significant risk factors of HBV infection. According to Prüss-Üstün et al., an estimated 66,000 cases of HBV infection and 261 related

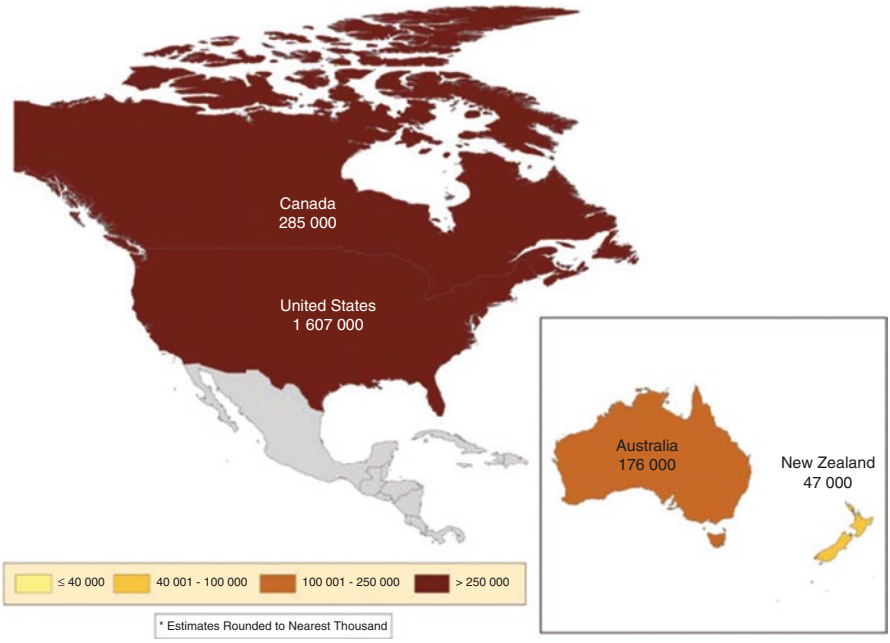

Fig. 3.8 Estimated number of migrants with chronic hepatitis B infection living in North America and Oceania. *Source*: Rossi C. et al. C. PloS one. 2012; 7(9), e44611

deaths in the world annually are caused by sharp medical instruments. But HCWs infected with HBV due to occupational activity have a more favorable prognosis than HCWs infected with HCV in connection with the implementation of post-exposure prophylaxis and vaccination [40].

3.2.8 Coinfection

Individuals with HBV + HIV or HBV + HCV + HIV coinfection have higher risk of more rapid progression of liver injury and, sometimes, discredited access to medical aid. People with coinfection may also belong to stigmatized groups of population due to sexual behavior or injection drug use [3].

The global prevalence of HBV + HCV coinfection is difficult to measure due to the absence of comprehensive population-based studies. Testing only for HBsAg without other markers of HBV infection doesn't allow the estimation of the real prevalence of HBV + HCV coinfection. Additionally, clinically silent or occult chronic hepatitis B with positive HBV DNA in serum and undetectable level of HBsAg has been described in HCV-infected individuals [43].

Due to common modes of transmission, HBV + HCV coinfection is frequently observed in persons with a high risk of parenterally transmitted infections and in highly endemic regions. Coinfected patients constitute a heterogeneous group with

different immunity profiles and various viral replication. Individuals with HBV + HCV coinfection usually have more rapid progression of liver injury and higher incidence of cirrhosis and hepatocellular carcinoma.

About 2–10% of HCV-infected persons are HBsAg positive, and 5–20% individuals with chronic HBV infection have positive anti-HCV antibodies. HBV + HCV coinfection is most often found in several groups with a high risk of blood transfusion-transmitted or sexually transmitted infections (IDUs, patients on hemodialysis, β-thalassemia patients, HIV-infected persons, patients undergoing organ transplantation) [44, 45].

According to the presented data, the epidemiology of HBV infection has been well studied in countries of the European Union, USA, Canada, and Australia. Information about the distribution of HBV among migrants, prisoners, and drug addicts is available in numerous studies. Also the data on the prevalence of hepatitis B among children under 5 years is well documented, which allows to predict the spread of the infection among the general population in the future. Mostly these countries have high and middle levels of income and a well-developed preventive medicine. Meanwhile, data on the prevalence of HBV among the general population and risk groups in the countries of Eastern Europe, Central and Southeast Asia, Latin America, Africa, Pacific and Caribbean basins, and Middle East are presented in few, if any, studies, allowing only indirect inference for conclusions regarding the real epidemiological situation with hepatitis B in a number of countries. Despite the existence of effective anti-HBV vaccine and antivirals and blood and injection safety programs which are implemented in the majority of countries, HBV infection continues to be the widely spread infection. Generally, the prevalence of HBV-infected people has a positive relationship with country income. Other reasons (religious and national traditions, behavior, culture, traditions, density of population, prevalence of HBV risk groups among population, etc.) can influence this situation. Migrants from countries with high HBV prevalence can deteriorate the situation in countries with high income and low HBV prevalence in the general population in the future. Due to high number of HBV-infected people in other risk groups in relatively prosperous countries in regard to the prevalence of HBV among the general population, migration can lead to the worsening of epidemiological situations prospectively. Most frequently people became infected during perinatal period, newborn period, and the first years of life. Therefore detection of infection and treatment during planning of pregnancy and prevention of vertical transmission are essential.

At the present time, elimination of HBV from organism is possible only in rare cases; it is easier to transform HBV infection in a latent form and maintain this condition for a long time. But persistence of the virus in the human body, even in latent form, significantly increases the risk of hepatocellular carcinoma. Therefore, active screening and treatment of patients with HBV infection, prevention of vertical transmission, and safe medical procedures only will not bring the desired effect in combating the spread of hepatitis B. In this regard, only a well-built vaccination campaign of the entire population from newborn to adults worldwide can lead to the reduction of the burden of hepatitis B and its elimination in the long-term perspective.

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Genetic Diversity of the Hepatitis B Virus and Its Epidemiological Significance

4

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4.1 Genotypes, Subgenotypes, and Serological Subtypes of Hepatitis B Virus

In the 1970s of the last century, nine different serological subtypes (serotypes) of HBsAg (ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq−, adrq+) were identified in a number of studies using monoclonal antibodies [1–3]. This HBsAg subtype classification has been applied to investigate the geographical distribution of HBV variants [4]. Further studies have shown that the subtypes of HBsAg do not reflect the true genetic diversity of HBV [5]. As the classification was based on a limited number of amino acid substitutions, in some cases, the subtype of HBsAg could change as a result of only one point mutation in the gene that encodes this protein. Thus, two amino acids encoded by the 122 and 160 S-gene codons determined the HBsAg's assignment to d/y and w/r subtypes, respectively [6–8]. By 1988, the complete genomes of 18 HBV strains of various subtypes were sequenced, which paved the way for the development of the first genetic classification of HBV. Okamoto et al. originally divided the available isolates of HBV into four genetic groups designated A, B, C, and D [8]. Two new groups, designated E and F, were identified based on S-gene variability of ayw4 and ayd4 subtypes [9, 10]. Later, three more

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genotypes of HBV (G, H, and I) were described [11–13]. Recently, a tentative genotype J strain was reported and isolated from a single individual in Japan [14]. Current HBV genotype classification is based on intergroup divergence greater than 7.5% in phylogenetic analysis. Genotypes A, B, C, D, F, and I are further classified into subgenotypes-subgroups with nucleotide divergence between 4% and 7.5% and high phylogenetic bootstrap support [15, 16] (Fig. 4.1).

For genotype A four subgenotypes, A1, A2, A3, and A4, were described [16–18]; however, subgenotype A3 is often referred to as quasi-subgenotype because it does not meet the criteria for subgenotype classification [19]. Previously misclassified subgenotypes A5 and A7 belong to subgenotype A4, and A6 belongs to subgenotype A3 [19]. Genotype B is divided to five subgenotypes [16, 20, 21]. Comprehensive analysis of genotype B has led to the reclassification of several subgenotypes: strains preliminary assigned to B5, B7, B8, and B9 were defined as subgenotype B3, and subgenotype B6 was reclassified to B5 [19, 22]. Within genotype C subgenotypes C1–C5 are well characterized [16, 23]. Eleven new genetic variants of HBV, designated as subgenotypes C6–C16, have recently been described in a series of studies from Southeast Asia [24–28]. Later, some strains of subgenotype C14 were reclassified to subgenotype C2 [19]. Genotype D is divided into six subgenotypes, D1–D6 [22]; however, its classification is still disputable. Previously described subgenotypes D7–D9 were found to be recombinant genetic variants [19, 28–30]. The nucleotide divergence between D1 and D3 strains is less than 4%,

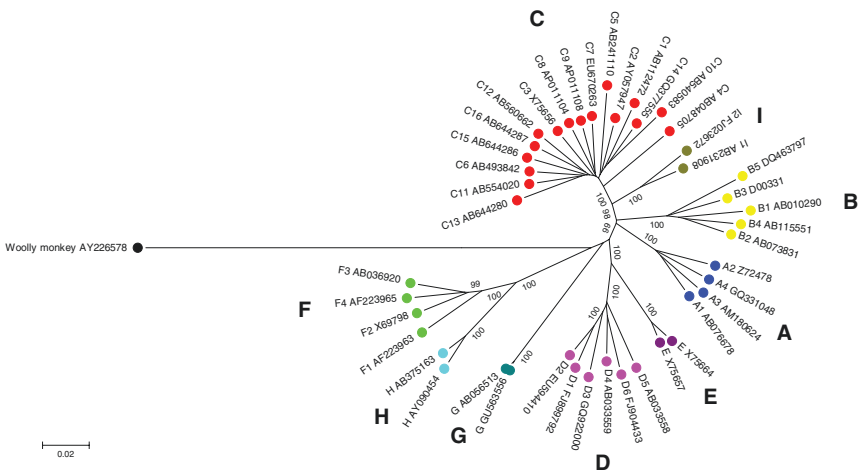

Fig. 4.1 Phylogenetic relationships of HBV genotypes and subgenotypes. The tree was constructed by the neighbor-joining method. The evolutionary distances were computed using the maximum composite likelihood method. Bootstrap analysis values greater than 95% are shown. The analysis involved 44 full-length HBV genomes from GenBank. Subgenotype and accession numbers are shown in taxa names. Woolly monkey strain was used as an out-group. Evolutionary analyses were conducted in MEGA7 software

and subgenotypes D4–D6 also show signs of intergenotype recombination [19]. Genotype F was originally divided into two subgenotypes, F1 and F2 [16], and in subsequent studies, two new genetic variants, subgenotypes F3 and F4, were identified [31, 32]. Genotype I strains identified so far are shown to be recombinants of unknown genotype (first part of the genome) and genotype C (second part of the genome from 1600 to 3000 bp). However, extensive analysis of their genetic and phenotypic characteristics agreed to group these variants into a separate genotype [13, 33]. Two subgenotypes I1 and I2 deviate less than 4% from each other; however, they belong to different subtypes (adw2 and ayw2), thus can be distinguished as unique subgroups [33].

4.2 Geographical Distribution of HBV Genotypes and Subgenotypes

HBV genotypes and subgenotypes show characteristic geographical distribution (Fig. 4.2). Genotypes A and D have been found throughout the world, although in some regions, they have higher prevalence and their subgenotypes often have distinct distribution.

Genotype A circulates in Europe, Africa, and the Americas. Subgenotype A1 prevails in countries of Southern and Eastern Africa, Southern Asia, and South America [16, 34, 35]. It was hypothesized that it originally was exported from Africa with a slave trade [22]. Subgenotype A2 is found mainly in Europe and North

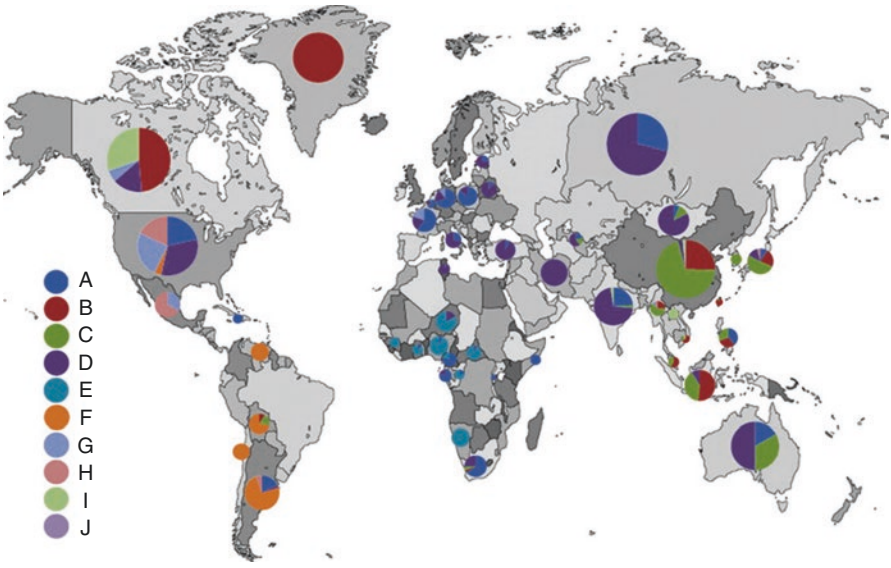

Fig. 4.2 Geographic distribution of HBV genotypes. Source: Shi W. et al. Infect Genet Evol 2013; 16:355–361

America [16, 22]. Subgenotype A3 is isolated in patients from Cameroon, Gabon, Rwanda, and Nigeria and from African population of Haiti [17, 18, 36, 37]. Subgenotype A4 is also found in Africa (Mali, Cambia, Congo, Rwanda) [18, 38].

Genotypes B and C are common in Asia. Subgenotype B1 is found predominantly in Japan; B2 in China; B3 in Indonesia, Philippines, and China; B4 in Vietnam and Cambodia; and B5 in Inuits of Arctic region [20, 21, 24, 39]. Subgenotype C1 prevails in Mainland Southeast Asia, C2 is mostly from East Asia, C3 is predominant in Oceania, C4 is exclusively found in aborigines from Australia, and subgenotypes C5–C16 circulate in Indonesia and Philippines [16, 24, 25, 28, 40, 41].

Genotype D is the second most common genotype and dominates in Mediterranean countries, India, Eastern Europe, and North America [16, 42, 43]. Subgenotypes D1–D3 cocirculate in many parts of the world. D1 is the most common in Mediterranean region (Greece, Turkey, North Africa), Russia, Iran, and Pakistan [16, 44, 45]; D2 in Eastern Europe and Turkey [16, 46]; and D3 in Canada, Alaska, and Russia [45, 47, 48]. Subgenotype D4 was found in aboriginal population of Papua New Guinea and Australia [10], D5 in some Indian tribes [49], and D6 in Indonesia [28].

Genotype E prevails in West Africa [50]. Genotype F is distributed in South and Central America, as well as in Alaska. Subgenotype F1 was found in Argentina, Chile, Costa Rica, Salvador, and Alaska; F2 in Venezuela and Brazil; F3 in Columbia, Panama, and Venezuela; and F4 in Brazil, Argentina, and Bolivia [51]. Genotype G is reported from Central and North America, as well as from Europe [11, 52, 53]. Genotype H was mostly found in Central America and Mexico [12, 52]. Both subgenotypes of genotype I (I1 and I2) are found in Laos and Vietnam. I1 strains were also reported from China and I2 from some Indian tribes. Geographical distribution of the main genotypes, subgenotypes, and serotypes is presented in Table 4.1.

Migratory processes that are gaining strength with each passing year lead to a gradual erasure of clear boundaries of the geographical spread of certain genotypes. In a large-scale study that included 17 hepatological centers in the United States, 7 HBV genotypes were registered: A (33%), B (21%), C (34%), D (9%), E (1%), F (1%), and G (1%). A reliable relationship between race and genotype of HBV was revealed. Thus, genotype A was detected in Caucasians and African Americans, while genotypes B and C prevailed among Asians. In Americans, born in the United States, Europe, the Far East, and Southeast Asia, the most common HBV genotypes were A, D, C, and B, respectively [54].

4.3 HBV Genotypes and Vaccination

The most widespread recombinant HBV vaccine is derived from subgenotype A2 that is found predominantly in Europe and North America [55]. The early research demonstrated cross-protection between HBsAg subtypes in chimpanzees [56, 57] as well as in a field studies [58]. Although global experience suggests that there is a high degree of cross-protection among the subtypes of the virus, there is convincing

Table 4.1 Geographical distribution of HBV genotypes, subgenotypes, and subtypes

Genotype	Subgenotype	Serological subtype	Geographic distribution
A	A1	adw2/ayw2	Africa
	A2	adw2	Europe/North America
	A3	ayw1	Africa, Haiti
	A4	ayw1	Africa
B	B1	adw2	Japan
	B2	adw2	China
	B3	adw2	Indonesia, Philippines, China
	B4	ayw1/adw2	Vietnam, Cambodia, France
	B5	adw2	Eskimos/Inuits
C	C1	adr	Thailand, Myanmar, Vietnam
	C2	adr	Japan, China, Korea
	C3	adr	New Caledonia, Polynesia
	C4	ayw2/ayw3	Australian aborigines
	C5	adw2	Philippines, Indonesia
	C6–C12	adr	Indonesia, Philippines
	C13–C15	adr	Indonesia
	C16	ayr	Indonesia
D	D1	ayw2	Middle East, Central Asia
	D2	ayw3	Europe, Japan, Lebanon
	D3	ayw2/ayw3	Worldwide
	D4	ayw2	Australian aborigines, Micronesians, Papua New Guineans, Arctic Denes
	D5	ayw3/ayw2	India
	D6	ayw2	Tunisia, Nigeria
E	–	ayw4	Western/Central Africa
F	F1	adw4	Argentina, Costa Rica, El Salvador, Alaska
	F2	adw4	Nicaragua, Venezuela, Brazil
	F3	adw4	Venezuela, Colombia
	F4	adw4	Argentina
G	–	adw2	USA, Mexico, Germany, Italy, UK, France
H	–	adw4	Mexico, Japan, Nicaragua, USA
I	I1	adw2	Laos, Vietnam, China
	I2	ayw2	Laos, India, Vietnam

Modified from: Kramvis A. Intervirology, 2014; 57:141–150

evidence that protection between homologous variants of HBV is much stronger than between heterologous variants. Two well-documented cases of acute and chronic hepatitis B caused by HBV genotype F in fully vaccinated individuals with protective titers of antibodies have been described in Europe [59, 60]. In a large-scale study among 2.13 million US blood donors, nine cases of occult (HBV DNA positive, anti-HBc negative) HBV infection were identified. In three cases of non-vaccinated individuals, HBV genotype A only was found; however, in the majority of vaccinated cases, non-A genotypes prevailed (five out of six cases) [61]. All these vaccinated donors remained asymptomatic but were HBV DNA positive for several weeks before the infection was resolved. In the cohort of 2028 vaccinated Chinese blood donors, 24 cases of HBsAg-negative HBV DNA-positive cases were found.

Among 15 cases with known HBV genotype, 14 had genotype B and 1 genotype C [62]. None of the described above breakthrough infections in vaccinated individuals were caused by vaccine escape mutants, but almost all had HBV genotype heterologous to vaccine strain. Available data suggests that symptomatic HBV infection after successful vaccination is a rare event; however, asymptomatic transient infection is quite frequent, and protection depends on HBV genotypes [55, 63].

4.4 HBV Genotypes and Transmission Route

The way of transmission of HBV infection depends on many factors: prevalence of HBV in the region, level of socioeconomic development of the country, cultural and ethnic characteristics, lifestyle, occupation, HIV co-infection, etc. In highly endemic regions, such as Southeast Asia, the most common route is a mother-to-child transmission. It is well known that genotypes B and C prevail in these regions [22]. The most significant association with mother-to-child transmission is revealed for HBV genotype C [64]. It is possible that the highest genetic heterogeneity of genotype C (16 subgenotypes) is in part a consequence of the evolution of the virus under the dominant transmission route. For Europe, where genotypes A and D predominate, sexual and nosocomial routes of HBV transmission are the most significant. By penetrating into the risk groups, certain HBV genetic variants can get predominant spread within these populations. Thus, there are reports of transmission of HBV genotypes A and G among men who have sex with men (MSM) [53, 65, 66]. The predominant distribution of the genotype D (mainly subgenotype D3) is described in acute hepatitis B among people who inject drugs (PWID) in Canada, where this genotype is less prevalent in general population [67]. Interestingly, the connection of subgenotype D3 to transmission with drug use and unsafe injections, in contrast to the subgenotype A2, for which sexual transmission was more characteristic, was also reported from Europe [68–71]. Another example of association between route of transmission and genotype is a report from Argentina where two simultaneous epidemics were identified: one among PWID caused by genotype A and the other among MSM caused by genotype F [72]. The data on association between particular transmission routes and HBV genetic variants can be useful in an epidemiological investigation [73]. From the evolutionary point of view, the route of transmission can be an important factor influencing the rate of genetic changes and, thus, facilitating the acquisition of new phenotypic properties by HBV genotypes.

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Laboratory Diagnosis of HBV

5

Şükran Köse and Tuba Dal

5.1 Introduction

Hepatitis B virus (HBV) is an enveloped virus with a genome composed by small (3.2 kb) partially double-stranded DNA and four open reading frames (ORFs) with overlapping region: PreC/C that encodes for hepatitis B e-antigen (HBeAg) and core protein (HBcAg); P for polymerase (reverse transcriptase) and S for surface proteins; three structures of HBsAg, small (S), middle (M), and large (L); and X for a transcriptional transactivator protein [1, 2]. Hepatitis B virus can be transmitted by parenteral route. Approximately 360 million patients are chronically infected with HBV [2]. In high endemic regions such as Southeast Asia, sub-Saharan Africa, China, Indonesia, and Nigeria, HBV chronic infection can be present in more than 8% of the population; in intermediate areas including South America, Eastern and Southern Europe, and Southwest Asia, infection rates are between 2% and 7% of population; and in low endemic areas including Western Europe and North America, chronic infection rates range from 0.5 to 2.0% of population [3–5]. The diagnosis of HBV infection is generally based on serological and molecular assays [3]. The serological markers identify virus-encoded antigens and their corresponding antibodies: hepatitis B surface antigen (HBsAg), anti-HBs, hepatitis B e-antigen (HBeAg), anti-HBe, and antibodies to hepatitis core antigen (anti-HBc). The molecular tests focus on the detection of quantitative viral load, drug resistance mutations, genotyping, and core promotion/precore mutation assays [6].

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5.2 Serological Markers for HBV Diagnosis

5.2.1 HBsAg

Since the discovery of HBsAg, it has served as a biomarker for the diagnosis of HBV infection. It indicates active infection and it appears 1–3 weeks before the onset of symptoms. The persistence of this marker for more than 6 months (24 weeks) is considered as chronic infection. HBsAg detection methods include electron microscopy, radioimmunoassay, and enzyme immunoassays [1, 7].

5.2.2 Anti-HBs

Anti-HBs is a neutralizing antibody which indicates immunity to HBV infection. It appears 1–3 months after HBV vaccination or after recovery of HBV acute infection. In HBV carriers the simultaneous anti-HBs and HBsAg reactivity may be observed due to the incapability of anti-HBs antibodies to neutralize the circulating virions [1].

5.2.3 HBeAg

HBeAg appears as a marker before the onset of symptoms and indicates viral replication independent of the phase of the infection. HBeAg is an indicator of viral replication and shows higher transmission risk of infection.

5.2.4 Anti-HBe

Seroconversion of HBeAg to anti-HBe indicates remission of liver disease [7]. Some anti-HBe reactive subjects continue to have active viral replication and hepatic disease due to the precore and core region mutations not producing the protein “e” [1].

5.2.5 HBcAg

HBcAg is an intracellular marker so it is not detected in the serum of infected individuals.

5.2.6 Anti-HBc

Approximately 1 month after the onset of HBsAg, the IgM anti-HBc antibody is detected during acute infection which is a marker of recent infection. IgM anti-HBc disappears after 6 months of infection. Although the IgM anti-HBc indicates acute

infection, it remains detectable in 10–20% of chronic patients with reactivation or flares, and isolated anti-HBc IgM can be detected in window period of acute phase. Anti-HBc IgG can be found in cured patients of HBV and chronic patients [1]. Isolated anti-HBc IgG can be detected in years after acute infection had finished, and anti-HBs had decreased to undetectable levels: when the titer of HBsAg has decreased thereunder the detection level after many years of chronic HBV infection [1].

In recent years, quantitative methods for HBsAg and HBeAg have been used to determine the response to anti-HBV treatments and predict therapy outcome when determined early during treatment or at baseline. HBsAg quantification is associated with the concentration of covalently closed circular DNA (cccDNA), the persistent intrahepatic form of HBV DNA [4]. Chan et al. suggested that serum HBsAg levels correlated with HBV ccc DNA and intrahepatic HBV DNA and low pretreatment HBsAg level was better than viral load to predict a good response to combination therapy of peg-interferon (IFN) plus lamivudine [8]. Fried et al. reported that quantitative HBeAg level is a more useful marker than serum HBV DNA level for predicting HBeAg seroconversion in patients with peg-IFN therapy [9]. In HBsAg quantification, two fully automated assays were available for the most prevalent HBV genotypes. The first assay was the Architect HBsAg QT (Abbott Laboratories, Abbott Park, IL, United States) which is a chemiluminescent microparticle immunoassay method. This assay can detect as low as 0.2 ng/mL HBsAg with a dynamic range of 0.05–250.0 IU/mL (1 IU/mL is equivalent to 1–10 ng/mL HBsAg) [10]. The second assay Elecsys HBsAg II (Roche Diagnostics, Indianapolis, IN, United States) is a "sandwich" assay [11]. Additionally, hepatitis B core-related antigen (HBcrAg) has been suggested as an additional marker of HBV infection. In 2017 Chen et al. reported that serum qHBcrAg level was well correlated with intrahepatic cccDNA level in chronic hepatitis B (CHB), and they suggested that qHBcrAg was a good candidate to be a satisfactory surrogate marker, and measurement of serum qHBcrAg may be clinically useful for monitoring the viral status of intrahepatic HBV and predicting the long-term prognosis of CHB patients [12]. Serological markers and their clinical significance were stated in Table 5.1 [9].

5.3 Serological Techniques for HBV Diagnosis

5.3.1 Radioimmunoassay (RIA)

Radioimmunoassay was the first technique used for HBV diagnosis where one of reactants is conjugated to radioisotopes. This method has good sensitivity but high cost and risk to operator [9].

5.3.2 Enzyme Immunoassay (EIA)

In enzyme immunoassay, enzymes are attached to one of the reactants, and after the addition of a suitable substrate/chromogen, the colored product is monitored visually or by spectrophotometer. This technique has advantages including highly

Table 5.1 Serological markers and their significance

Marker	Clinical significance
HBsAg	Hallmark of infection Positive in early phase of acute infection and persistently positive in chronic infection
Anti-HBs	Neutralizing antibody. It indicates recovery and/or immunity to HBV. After immunity conferred by HBV immunization, it is the only detectable marker
HBeAg	It is a marker of active replication of HBV. It shows high transmission risk
Anti-HBe	Low replicative phase if HBV DNA is low It indicates decrease of HBV infectivity and remission of disease Some anti-HBe reactive subjects continue to have active viral replication and hepatic disease due to the precore and core region mutations
IgM anti-HBc	Appears with the onset of symptoms and disappears after 6 months of infection Presence with high-index value during acute HBV infection but 10–20% of chronic hepatitis B patients with hepatitis flares also positive for IgM anti-HBc with low-index value
IgG anti-HBc	It is not a neutralizing antibody. It indicates an exposure to HBV. In occult HBV infection, isolated IgG anti-HBc may be seen

anti-HBs HBsAg antibody, *HBc* hepatitis B core antigen, *HBe* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBV* hepatitis B virus, *Ig* immunoglobulin

reproducible results, automation, and low cost. In a study, 70 HBsAg test kits were evaluated comparatively for their clinical sensitivity, analytical sensitivity, sensitivity to HBV genotypes and HBsAg subtypes, and specificity. This study reported that specificity of the HBsAg assays was ranged from 96.4 to 99.5%. However, they detected reduced sensitivity for HBsAg with genetic diversity of HBV occurred with genotypes/subtypes D/ayw3, E/ayw4, F/adw4 and by S gene mutants [13].

5.3.3 Chemiluminescent Microparticle Immunoassay (CMIA) and Electrochemiluminescence Immunoassay (ECLIA)

Chemiluminescent immunoassays use light-generating molecules as labels (luminol derivatives, acridinium esters, or ruthenium complex) for electrochemiluminescence. Architect HBsAg Qualitative 2 assay uses anti-HBsAg antibody conjugated with acridinium as a chemiluminescent compound to detect HBsAg; on the other hand, Elecsys HBsAg 2 assay uses ruthenium complex conjugated antibodies to form a sandwich complex for the HBsAg determination. In a study from Turkey, İnan N et al. compared technical performance of the chemiluminescent microparticle immunoassay (CMIA) and electrochemiluminescence immunoassay (ECLIA) for the detection of HBsAg. They found the sensitivity of HBsAg tests to be 100 and 98% and the specificity of HBsAg tests to be 99 and 97% for ECLIA and CMIA methods, respectively. There was a 75% correlation between the assay results of the two methods. They suggested that these methods were suitable and reliable for routine HBsAg screening [14].

5.3.4 Rapid Point-of-Care Tests (RPOCTs)

Rapid point-of-care tests (RPOCTs) are based on particle agglutination, immunochromatography, immunodot, or immunofiltration. The device contains a solid support such as cellulose or nylon membranes, latex microparticles, or plastic cards where viral antigens or antibodies are fixed. They are developed to make the diagnosis more rapid (10 min) and accessible to patients [15]. Point-of-care (POC) tests are easier to use and inexpensive compared with ELISA, but the performance of these assays is poor in seroconversion panels and among individuals infected by several HBV mutants [1]. In addition, some POC tests accommodate not only serum or plasma but also whole blood collected by finger stick, which can avoid a phlebotomy [16]. The sensitivity of these methods for HBsAg detection varies from 43.5 to 100%, while specificity varies from 95.8 to 100% [17–20]. Njai et al. conducted a cross-sectional study to assess the diagnostic accuracy of three POC tests (Determine, Vikia, and Espline) for the detection of HBsAg in the field or a laboratory setting in the Gambia. In the field, they used finger-prick whole blood for the Determine and Vikia tests and dried blood spots for the reference standard test (AxSYM HBsAg enzyme-linked immunosorbent assay [ELISA]). In the laboratory, they used serum for the Determine, Espline, and reference test (Architect chemiluminescent microparticle immunoassay). The sensitivity and specificity of the POC tests were ranged from 88.5 to 100%. In their study most of the patients with false-negative results [18, 19] were classified as inactive chronic carriers. They suggested that the three point-of-care tests had acceptable ranges of diagnostic accuracy [17].

5.3.4.1 Immunosensors

Immunosensors are solid-state affinity ligand-based biosensing apparatus that combines immunochemical reactions to proper transducers. An immunosensor comprises of a sensing element and a transducer. The sensing element is composed based on the immobilization of antigens or antibodies, and the binding event is transformed into a measurable signal by the transducer [21]. An immunosensor is used to produce a signal proportional to the concentration of the analyte [22]. Wang et al. [23] developed a gold nanorod-based localized surface plasmon resonance biosensor. The detection limit of their biosensor was 40 times lower than the detection limit of the EIA method and had the ability to quantify HBsAg until 0.01 IU/mL.

5.4 Quantitative Molecular Methods for HBV Diagnosis

Recently, due to the presence of HBeAg-negative CHB, occult HBV infection, and escape mutants, nucleic acid-based HBV DNA assays have gained importance in clinical settings. In addition to this, nucleic acid-based methods have

become alternative to serological methods because of the developments in technology, cost reduction, and increased availability of this system. These systems are not only used for the diagnosis of HBV infection, but also they are used for evaluating the efficacy of antiviral therapy and to monitor HBV patients [24]. HBV DNA is a direct measurement of the viral load and indicates the replication activity of the virus. Its detection is beneficial in routine clinical practice to identify individuals who need antiviral treatment and provide them the most suitable therapy. The higher titers of HBV DNA are associated with more rapid disease progression and higher hepatocellular carcinoma (HCC) (incidence). HBV DNA becomes detectable after HBV infection of 1 month, and it increases up to peak level (more than 10^8 copies/mL) approximately 3 months after the exposure to HBV. HBV DNA levels decreases in chronic infection, and at the recovery from HBV infection, HBV DNA disappears. Nowadays, a variety of molecular technologies have been used in HBV DNA quantification, and the techniques to identify and quantify HBV DNA were divided into two groups including signal amplification methods such as hybrid capture and branched DNA technology and target amplification methods including polymerase chain reaction (PCR) [6]. Comparison of the commonly used quantitative methods for HBV DNA was indicated in Table 5.2 [1].

Table 5.2 Comparison of commonly used quantitative methods for HBV DNA

	Assay name	Manufacturer	Measurable range (IU/mL)	Limit of detection (IU/mL) (WHO HBV standard was used)	Conversion factor (IU/mL to copies/mL)
Semiautomated qPCR	COBAS AmpliPrep/ COBAS TaqMan HBV test v2.0	Roche Molecular System, California, United States	20–1.7×10^7	20	5.82
Semiautomated real-time PCR	COBAS TaqMan HBV test for use with high pure system	Roche Molecular System, California, United States	29–1.1×10^7	6	5.82
Automated real-time PCR	Abbott RealTime HBV	Abbott Diagnostic, Chicago, United States	10–1×10^9	10	3.41
Branched DNA	VERSANT HBV 3.0 assay	Siemens Healthcare, United States	2000–1×10^8	2000	5.6

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

5.4.1 Real-Time PCR

PCR techniques have rapidly evolved over the last few years. The advantages of real-time PCR are the detection of amplified DNA as the reaction progresses and a broader dynamic range compared to conventional PCR. The most commonly used reagents for real-time PCR are TaqMan probes which is an oligonucleotide probe that has a fluorescent reporter at the 5′ end and a quencher attached to 3′ end [25]. The close proximity of the reporter to the quencher prevents fluorescence emission and hydrolyzation of the TaqMan probes by the 5′ to 3′ exonuclease activity of the Taq polymerase releasing the reporter and thus allowing emission of fluorescence. An increase of the product targeted by the reporter probe at each PCR cycle causes a proportional increase of fluorescence, and this fluorescence can be measured by a real-time PCR device. Fully automated real-time PCR methods are considered as standard methods to detect and quantify HBV DNA because of their high capacity to detect wide dynamic range of viral load (lower range, 10–15 IU/mL; upper range, 10^7–10^8 IU/mL) and due to lack of carry-over contamination [9]. The World Health Organization established a universal standard for HBV DNA quantification measured in IU/mL, with the purpose of correlating different results using a single reference unit [26]. However, significant variability can be observed in the quantification among different assays. Hence, patients are suggested to be monitored by a single assay [6].

5.4.2 Digital PCR

Digital PCR can be used to quantify and clonally amplify nucleic acids directly, including DNA, cDNA, and RNA [27]. In digital PCR, a sample is partitioned so that individual nucleic acid molecules within the sample are localized and concentrated within many separate regions. Huang JT et al. [28] developed a droplet digital PCR using formalin-fixed paraffin-embedded HCC tissue with copy numbers ranging from 1.1 to 175.5 copies/μL. These studies indicated that digital PCR methods were suitable methods for HBV DNA quantification.

5.4.3 Ligase Chain Reaction (LCR)

Ligase chain reaction (LCR) is a target amplification technique for amplifying shorter DNA targets [29]. It uses the DNA ligase to join two same-strand targeting oligonucleotides, which are designed to hybridize at adjacent positions of the template nucleic acid. In this technique, if the target sequence is present in the reaction mix, oligonucleotides hybridize to adjacent sequences of the target sequence, and the gap between the oligonucleotides is ligated by a DNA ligase, and a continuous fragment is generated. This ligation product will be a template for a PCR-like reaction resulting in amplification of the target nucleic acid molecule [30]. The

Gap-LCR was a modified reaction that allowed amplification of longer DNA stretches by inclusion of a polymerase extension [31]. Additionally, LCR can also be adapted to real-time detection, and it can be used for HBV S gene and PreC mutations [32] and semiquantitative detection of HBV [33].

5.4.4 Isothermal Amplification Strategies

PCR-based assays are the most widely used methods for HBV DNA quantification, but they need a thermocycling machine to separate DNA strands and amplify the fragments. In recent years, isothermal amplification-based methods such as LAMP, loop-mediated isothermal amplification; NASBA, nucleic acid sequence-based amplification; RCA, rolling circle amplification; and TMA, transcription mediated amplification have also been developed for HBV DNA detection and quantification [34]. Isothermal amplification methods are carried out at a constant temperature, do not require a thermal cycler, and have significant potential for the detection and quantification of HBV DNA. These techniques can be adopted to biosensors, so they may become future diagnostic devices in medicine. Here, we described the isothermal amplification methods that have been used to quantify HBV DNA.

5.4.4.1 Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a single-tube technique for DNA amplification which was developed by Eiken Chemical Co. Ltd., Japan. In LAMP, the target sequence is amplified at a constant temperature of 60–65 °C using either two or three sets of primers and a polymerase with high activity of strand displacement and replication activity. An additional pair of loop primers can accelerate the amplification [35]. This technique also allows simple detection either by agarose gel electrophoresis, visual inspection of turbidity, or visualizing fluorescence under ultraviolet light and may be combined with a reverse transcription step to allow the detection of RNA [36]. Due to the specific nature of the primers, the amount of DNA products in LAMP is considerably higher than in PCR-based amplifications, and LAMP was less sensitive to inhibitors in complex samples, such as the blood than PCR due to the use of a different DNA polymerase. However because it requires complex primer design, it has less application in molecular biology practice [37]. The highly sensitive real-time fluorogenic (RtF-LAMP) protocol was also developed with a lower detection limit of 48 IU/mL, broad dynamic range, low intra-assay and inter-assay variability (4.24–12.11%), and an excellent correlation with real-time PCR. This method may be useful in the future as a low-cost alternative for HBV DNA quantification [38].

5.4.5 Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA is also an isothermal process and is particularly suited to the detection of RNA viruses, but this can be used for the amplification of DNA templates. The

amplification process is based on a modified primer including the T7 promoter sequence, which forms a modified cDNA after hybridizing with the template. This is subsequently amplified into ssRNA amplicons in a process catalyzed by *T7 RNA* polymerase (T7 RNA Pol) [39]. Compared with PCR, major advantages of NASBA are as follows: it works under isothermal conditions and it is more rapid and sensitive than PCR [6]. Recently, Deiman et al. [40] reported the amplification of HBV DNA by NASBA and found it to be capable of detecting even 10 IU/mL with a dynamic detection range of 10^2–10^9 IU/mL. Yates et al. [41] developed an HBV DNA quantification system based on the amplification with NASBA and real-time detection with molecular beacon technology. The detection range of the assay was 10^3–10^9 copies/mL in plasma or serum. This technology also has great potential for application in future detection devices.

5.4.6 Rolling Circle Amplification (RCA)

The rolling circle amplification (RCA) is a simple, reliable, and isothermal amplification method, which is driven by DNA polymerase to generate a long tandem repeat product based on a circular DNA template [24, 42]. The rolling circle amplification reaction is initiated by the annealing of primers to the circular ssDNA, followed by elongation of the new strand up to the point of initiation, displacing the strand and continuing again and again. This repeated elongation due to strand displacement generates a continuous catenated ssDNA even up to 10^9-folds [34]. Margeridon et al. [43] used the RCA for amplification of full genome of HBV DNA with low viral loads, from sera as well as from the liver. They could amplify as low as 13 copies of cccDNA from liver biopsy samples. Martel et al. [44] developed a RCA-based method for complete genome amplification of HBV rcDNA from sera, with viral loads ranging from 10^3 to 10^8 IU/mL. The literature data showed that the main advantages of RCA include the following: this technique does not require advanced laboratory equipment or experimental expertise compared with PCR; it is resistant to inhibitors present in clinical samples and requires little or no assay optimization; it can amplify targets on solid support or in solution, offering opportunity for microarray and biosensor application; and it has been used for amplification of rcDNA (with some enzymatic modification) as well as for direct amplification of cccDNA [6].

5.4.7 Transcription-Mediated Amplification (TMA Reaction)

Transcription-mediated amplification (TMA reaction) is a RNA transcription-mediated amplification system using two enzymes to drive the reaction including RNA polymerase and reverse transcriptase. Transcription-mediated amplification can amplify either DNA or RNA and produces RNA amplicon, in contrast to most other nucleic acid amplification methods that only produce DNA. Since RNA is more labile in a laboratory environment, TMA reaction reduces the possibility of carry-over contamination. While PCR can produce two copies per cycle, TMA can

produce 10^2–10^3 copies, which result in a ten billion-fold increase in nucleic acid products within 15–30 min [6]. Kamisango et al. [45] developed a sensitive and quantitative assay using TMA and hybridization protection assay, which could detect 5×10^3 to 5×10^8 genome equivalents/mL of HBV.

5.5 Biosensors, Microarrays, and Microfluidic Devices

Biosensors are analytical devices used for detection, which combine a biological component with a physicochemical detector. Clinical researches on biosensors were based on immunological reactions or DNA hybridization, and the biosensors always yielded rapid results with high sensitivity [34, 45]. Earlier biosensors especially integrated enzymes with transducers convert the biological reaction into a measurable electrical or electronic signal. Later, biosensors utilized other biological materials including antibodies, receptors, and more recently nucleic acids. Most of the developed biosensors utilize antigen-antibody or receptor-ligand interaction, nucleic acid hybridization-based interaction for generating biological responses. Nucleic acid- or signal amplification-based biosensors are more specific and sensitive [24]. The assembly of numerous DNA biosensors onto the same detection platform results in DNA microarray-based diagnostic systems which were used for HBV detection, genotyping, and detection of mutants [24]. However, due to such systems being time consuming and requiring sensitive instrumentation for detection, microfluidic devices (lab-on-chip) which were capable of sample and reagent processing as rapid micro total analysis system were developed. Recently, microfluidic devices have been used for the study of HBV detection, replication, and genotyping [34, 45].

5.5.1 HBV Genotyping

HBV has a high genetic heterogeneity because it reproduces via a reverse transcriptase with insufficient proofreading capability. According to the sequence divergence, it has been classified in ten genotypes (A to J). Genotype B and C are restricted to Oceania and Asia, whereas genotype A and D are most common in Africa and Europe. Genotype I has been reported in Vietnam, Laos, India, and China while genotype J in Japan and Ryukyu and genotype E, F, G, and H in Asia. The patients with genotypes A and B have better treatment response than genotypes C and D in the interferon therapy, and patients infected with genotype B or C had a lower opportunity to gain serological response to tenofovir. These evidences indicated that the HBV genotyping is significant to predict progression of HBV disease and determine appropriate antiviral therapy [46]. Therefore, genotyping methods gained importance in HBV infections. Genotyping methods for HBV include reverse hybridization, genotype-specific PCR assays, real-time PCR, restriction fragment length polymorphism, sequence analysis, microarray (DNA chip), and fluorescence polarization assays. The characteristics of commonly used HBV genotyping methods were indicated in Table 5.3 [1].

Table 5.3 HBV genotyping methods

Methods	Advantages	Disadvantages
Restriction fragment length polymorphism (RFLP)	Low cost, simple	Low sensitivity for genotyping HBV in samples with low HBV
Reverse hybridization	High sensitivity, automated systems	Relatively high cost
Genotype-specific PCR	High sensitivity, automated systems, easy to perform This method is robust on detecting mixed genotype infections	High cost
Sequence analysis	Gold standard method This method can identify recombinant genotypes	Time consuming, technically demanded

HBV hepatitis B virus, *PCR* polymerase chain reaction

5.6 Next-Generation Sequencing

Gene sequencing has allowed the identification and confirmation of HBV mutants/variants, genotypes, and subgenotypes, and it has an important role in patient management, in drug resistance testing, and for the epidemiological analysis [34]. Initially, two DNA sequencing technologies were available: (1) Sanger sequencing (DNA sequencing with chain terminating inhibitors) and (2) Maxam-Gilbert sequencing (chemical cleavage technique). Pyrosequencing was further developed into an array-based massively parallel microfluidic-sequencing platform for extremely high-throughput sequencing. Then clonal bridge amplification and sequencing-by-ligation technologies which have allowed simultaneous sequencing of millions of templates were developed [47]. These technologies are known as next-generation sequencing technologies, and they can produce several giga bases (Gb) of high-quality sequence data in a single run. These methods allow whole genome sequencing, ultra-deep sequencing, amplification, and identification of previously unknown microbes [34]. Margeridon-Thermet et al. [48] performed ultra-deep pyrosequencing of hepatitis B virus quasispecies from nucleoside and nucleotide reverse transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. They detected coinfection and recombination among two different HBV genotypes in some of the patients. Ultra-deep pyrosequencing technologies require very high level computational instrumentation for analysis of such huge sequence data and complex pipeline of software, and they are still expensive for using in routine clinical practice. In the near future, the use of these technologies in medical researches will clarify many unknown features of HBV.

Conclusion

Enzyme immunoassays are the most important serological assays used for HBV and detection due to their simplicity, automation, and broad detection range. In recent years, HBsAg and HBeAg quantitation methods were developed to

determine the response to anti-HBV treatments and predict therapy outcome, but further studies are needed for standardization and validation of these quantitative assays to monitor the treatment response of HBV patients. Recently, due to HBeAg-negative chronic hepatitis B (CHB), occult HBV infection, and escape mutants, nucleic acid-based HBV DNA assays gained importance in clinical settings. Although many sensitive isothermal amplification assays are available for HBV detection and quantitation, none of them have become so popular, as real-time PCR. In the near future, biosensors and biochips seem to be useful technologies for serological diagnosis of HBV and HCV. For DNA sequencing, ultra-deep sequencing will be helpful for the analysis of HBV mutants in order to study the dynamics of viral variants.

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Prevention of Mother-to-Child Transmission of HBV

6

Nilay Karaca and Çetin Karaca

Abbreviations

APR	The Antiretroviral Pregnancy Registry
HBeAg	Hepatitis B e-antigen
HBIG	HBV immunoglobulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IUT	Intrauterine transmission
MTCT	Mother-to-child transmission
TDF	Tenofovir disoproxil fumarate

6.1 Introduction

The World Health Organization estimates that 2 billion people worldwide have been exposed to hepatitis B virus (HBV). Among them, 370 million people have chronic infection. Chronic HBV infection remains a significant health issue and a leading cause of cirrhosis and hepatocellular carcinoma worldwide. Transmission of the HBV, despite the availability of the vaccine, still occurs, particularly in the perinatal setting [1]. Up to 50% of new cases of HBV infection are due to mother-to-child transmission (MTCT). Perinatal (vertical) transmission of HBV is defined as positivity at 6–12 months of the hepatitis B surface antigen (HBsAg) or of HBV-DNA

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in an infant born to an infected mother. In the absence of standard active-passive immunoprophylaxis treatment, approximately 70–80% of infants born to HBsAg- and hepatitis B e-antigen (HBeAg)-positive mothers became chronically infected with HBV, and about 90% of these children who are exposed remain chronically infected. HBeAg-negative mothers carry a 10–40% transmission risk, and of those infants who are infected, 40–70% may remain chronically infected. The transmission rate was reduced to 5–10% when newborn received appropriate active-passive immunoprophylaxis at birth. Mothers with highest transmission risk should be assessed for relatively new and safe oral antiviral treatments. Recent prospective studies with oral antivirals in late pregnancy showed promising results in reducing the MTCT rate and the safety profiles of these oral antiviral agents [2–5].

6.2 Mechanisms of Mother-to-Child Transmission

Mother-to-child transmission of HBV can occur via three mechanisms: intrauterine transmission (IUT), intrapartum transmission, and postpartum transmission.

6.2.1 Intrauterine Transmission

Hepatitis B virus has been found in sperm, oocytes, and embryos. The risk of HBV transmission during assisted reproductive treatment is theoretically possible. Germ cell-like sperm and oocytes could be infected by HBV and transferred the virus to the embryo (antenatal transmission) [1, 5–8]. IUT is defined as HBsAg in the neonatal blood 1–30 days after birth or detectable HBV-DNA in neonatal peripheral venous blood [9]. IUT accounts for only a minority of cases of HBV transmission. HBeAg is the only structural HBV protein that can pass through the placenta. Other mechanisms for IUT include serum/body fluid transmission, which usually occurs in conditions of placenta damage caused by contraction of the uterine muscle such as threatened abortion; invasive procedures into the uterus like amniocentesis during pregnancy; cellular transmission, which refers to transmission of HBV from the maternal side to the fetal side trough placenta cells; and transfer of infected peripheral blood mononuclear cell from the material circulation system into the fetal circulation system. IUT is not prevented by active-passive immunoprophylaxis [10–12].

6.2.2 Intrapartum Transmission

MTCT during intrapartum stage is the most common. Intrapartum infection could occur as a result of maternal-fetal micro-transfusion during delivery or swallowing of infective fluid. Partial placental leakage occurring during delivery and trauma from instrumentation can result in the mixing of fetal and maternal circulation and cause to an increase in the risk of HBV infection [13]. HBsAg was detected 96% of vaginal fluid samples. The benefit of cesarean delivery in protecting against HBV

transmission has not been clearly established in well-conducted controlled trials [14, 15]. Cesarean section should not be routinely recommended for reducing MTCT.

6.2.3 Postpartum Transmission

Postpartum transmission is defined as infection of HBV due to contact with maternal breast milk, body fluids, blood or other close contacts between newborns, and mothers after delivery [5]. Breastfeeding has been a major concern in postpartum HBV transmission. HBsAg was found in 72% of breast milk samples [16]. Newborns who received HBV immunoglobulin (HBIG) and first dose of HBV vaccine at birth can be breasted [17]. Bleeding from cracked nipples may cause HBV transmission. Mothers should avoid breastfeeding during this period to prevent transmission of HBV. HBV-infected mother should not donate breast milk.

6.3 Strategies for Prevention

Preventing MTCT involves screening pregnant women, providing antiviral treatment to women with high HBV-DNA levels, and administering passive-active immunoprophylaxis to newborns of mothers who are HBsAg positive.

6.3.1 Active-Passive Immunoprophylaxis

The hepatitis B vaccine is used safely in all trimesters of pregnancy. Nonimmune pregnant women at risk should be vaccinated. Vaccination of mother has the potential benefit of providing passive immunoprophylaxis to the fetus. The standard vaccination strategy for infants born to mothers who test positive for HBsAg includes the administration of 100 IU HBIG and hepatitis B vaccine within 12–24 h of birth. The vaccination series is later completed with 2 additional doses (1 at 4 weeks to 2 months of age and 1 at 6 month of age). In mothers with unknown HBsAg status at the time of birth, newborns should receive the hepatitis B vaccine within 12 h of birth, and, if subsequently found on admission screening to be HBsAg positive, newborns should then be administered HBIG as soon as possible (within 7 days of birth) [18]. This combination scheme has been demonstrated to be more effective than the administration of either the HBV vaccine or HBIG. Despite adequate active-passive immunoprophylaxis, there is a much greater risk of transmitting HBV that mothers have high viral load and HBeAg positivity. Detectable HBV-DNA in the infant's serum at birth comes the most important predictor for immunoprophylaxis failure. Maternal HBeAg positivity is strongly correlated with high levels of maternal viremia. A maternal viral load below 10^6 IU/mL is not associated with perinatal transmission, whereas the risk of transmission is about 3% in cases of a maternal viral load 10^6–10^7 copies/mL, about 7% for a viral load 10^7–10^8 copies/mL, and about 8% for a viral load >10^8 copies/mL [19].

6.3.2 Antiviral Prophylaxis

Antiviral treatment is suggested for HBsAg-positive mothers with high HBV-DNA levels, in addition to standard passive-active immunoprophylaxis of the infant. First and second trimesters are more important than the third trimester for the development of fetal organs, so starting of antiviral treatment is recommended in the third trimester to minimize the effect of these drugs to fetal development. Antiviral treatment is started at the beginning of the third trimester, so this time is sufficient to reduce HBV-DNA viral load [5]. Using antiviral agents in third trimester of pregnancy for prophylaxis of MTCT showed in various studies. Currently available antivirals against to active HBV infection are interferon alpha, pegylated interferon alpha and the nucleoside/nucleotide analogs such as lamivudine, adefovir, telbivudine, entecavir, and tenofovir. None of the seven antivirals currently approved for HBV are classified as Food and Drug Administration pregnancy category A. Interferon and pegylated interferon are classified in pregnancy category X and strongly contraindicated during pregnancy. Lamivudine, entecavir, and adefovir are in pregnancy category C. Finally, telbivudine and tenofovir are in pregnancy category B [20]. Although none of the medications are approved in pregnancy, both lamivudine and tenofovir have been used commonly in HIV-positive pregnant patients. The Antiretroviral Pregnancy Registry (APR), a prospective registry that collects information on drug exposures to assess potential teratogenicity, has collected information on nearly 2000 pregnancies with exposure to tenofovir and over 4000 pregnancies with lamivudine exposure with no evidence of increases in birth defects over baseline [21]. The first drug tested in late pregnancy for prophylaxis of MTCT was lamivudine. In a randomized controlled study, the use of lamivudine 100 mg per day in HBeAg-positive mothers with DNA > 9 log10 copies at the third trimester of pregnancy showed a significant reduction in immunoprophylaxis failure (intention-to-treat analysis; 18% vs 39% in the treated group vs untreated group, respectively, $p < 0.01$) [22]. According to a meta-analysis, lamivudine use in late pregnancy has prevented MTCT in highly viremic mothers [23]. The rates of birth defects for lamivudine exposure did not differ from those born to others without exposure to antiviral therapy [4].

Telbivudine is a nucleoside analog that has a higher antiviral power than lamivudine. Recent prospective studies showed the efficacy of telbivudine 600 mg per day in preventing MTCT when used during the second or third trimester in highly viremic HBeAg-positive mothers with HBV-DNA >6 log10 copies (200,000 IU)/mL. MTCT rate was 0 and 8% in the treated group and the control group, respectively ($p < 0.001$). In this trial, no measurable differences in congenital deformities were observed for newborns when the treat and control groups were compared up to 28 weeks after birth [24]. The efficacy and safety of telbivudine administration in high viral load mothers in pregnancy to prevent MTCT were confirmed in a meta-analysis of those six studies for a total of 576 pregnant women [25].

Although there are few case reports in the APR on the other antivirals agents including entecavir and adefovir, they are not recommended for pregnant women [21].

Tenofovir disoproxil fumarate (TDF) has a high genetic barrier to resistance. The use of TDF during the third trimester to prevent MTCT might be an appropriate option for these young mothers who may require antiviral treatment after delivery [26]. Two retrospective studies evaluated the efficacy of TDF administrated in the third trimester of pregnancy at a dose of 300 mg once daily. All newborns received active/passive immunoprophylaxis. None of them were HBsAg positive at 28 weeks of age. The newborns of two groups did not differ in terms of adverse events and birth defects [27, 28]. Tenofovir alafenamide is a newer formulation of tenofovir. It should not be used because there is no data related to the use of TAF in pregnancy.

The safety data on the use of antiviral treatment during breastfeeding is unclear. Antivirals are excreted into the breast milk. However, only low levels of antivirals are detected in the breast milk, and these are unlikely to have any biologic effects on the nursing infant [29, 30]. The decision to breastfeed should be based upon patient choice.

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Current Management of Chronic HBV Infection

7

Nese Inan and Fehmi Tabak

7.1 Introduction

Hepatitis B virus (HBV) is one of the major health burdens that causes chronic infection to about 240 million people around the world [1]. Chronic hepatitis B (CHB) is one of the main causes of end-stage liver diseases including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [2]. Each year, around 780,000 people are lost to HBV-related complications [1, 3]. Even with the development of a successful vaccination and effective treatments, because of the lack of awareness among general population and the people under risk of hepatitis B, CHB continues to be one of the main health problems [3, 4]. HBV infection can be either acute or chronic and observed with different illness manifestations ranging from asymptomatic infection or mild disease to severe or rarely fulminant hepatitis. CHB is defined as detection of hepatitis B surface antigen (HBsAg) in the blood for a period of more than 6 months [5]. CHB shows the relationship between the replication of HBV and the host's immune response, which has a dynamic process where not all chronically infected patients end up with chronic hepatitis. The development of cirrhosis and HCC depends on viral, host and environmental factors [6] (Table 7.1).

Comprehending the phases of CHB is essential to make treatment decisions and monitorization of complications such as cirrhosis and HCC [5–7] (Table 7.2). Natural history of CHB is a dynamic process in which a duration of phases is variable and not all patients have to go through the progress of all phases [6]. Patients in some cases after being inactive carriers from immune-active phase still can become active and redevelop chronic hepatitis [8] (Fig. 7.1). According to a study

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Host	Viral/Disease	Environmental
•>40 years of age •Male sex •Immuno comprimised	• **High serum HBV DNA** • Elevated ALT levels • Prolonged time to HBeAg seroconversion •HBeAg-negative CHB development •Genotype C	•Concurrent viral infections (HCV, HIV, HDV) •Heavy alcohol use •Metabolic syndrome (obesity, diabetes)

Table 7.1 Host, viral/disease and environmental factors associated with cirrhosis and HCC

done by Hepatitis B Research Network, based on viral load and liver enzymes, more than one third of patients (38%) couldn't enter defined phase groups and are determined as inconclusive. Even the end result of serial monitorization of HBV DNA and ALT, majority of patients ended up indeterminate (68%) [9]. Even with effective and safe treatment options available for CHB, due to persistency of cccDNA (covalently closed circular DNA) which is the stable form of HBV in nucleus of hepatocyte, it seems inconceivable for now to eradicate the virus and find a cure with the current medical establishments [3, 10].

7.2 Goals of Treatment

The goals of CHB treatment are as follows: to reduce morbidity and mortality rates which are associated with progressive liver diseases; improve quality of life and survival; to decrease necroinflammatory changes and hepatic fibrosis which can cause progressive liver disease, cirrhosis, decompensate cirrhosis, liver failure, and even death; and to reverse fibrosis by suppression of HBV DNA to undetectable levels [5, 6]. Prevention for the transmission from mother to a child and hepatitis B reactivation and HBV-related extrahepatic manifestations are options within our grasp due to recent advances in CHB treatment [5]. Prevention of recurrent or new HCC also can be counted among the treatment goals, because it's shown that control of HBV infection not only decreases the incidence of cirrhosis and HCC but also decreases incidence of recurrent and new HCC in previously diagnosed patients [11]. During the treatment process, decisions are usually made based on serum HBV DNA and ALT levels, HBeAg status and liver histology. Nevertheless, the success of therapy is determined according to serological status of HBsAg and HBeAg, as well as levels of HBsAg and HBV DNA [12, 13].

7.3 Diagnosis and Initial Evaluation

The majority of patients with CHB especially in immune-tolerant or inactive carrier phases are asymptomatic and therefore are usually unaware of their infection. Hence, the risk of transmission to other people and severe liver-related

Table 7.2 Phases of CHB

	HBeAg-positive		HBeAg-negative	
	Immune-tolerant phase	Immune-active phase	Inactive CHB phase	Reactivation phase
New terminology	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBV DNA	Elevated	Elevated	Low or undetectable	Elevated
	>1 million IU/mL	≥20,000 IU/mL	<2000 IU/mL	≥2000 IU/mL
ALT[a]	Normal	Elevated	Normal	Elevated
Liver histology	Minimal inflammation/ fibrosis	Moderate-severe inflammation/ fibrosis	Minimal necroinflammation but variable fibrosis	Moderate-severe inflammation/ fibrosis

[a]ALT alanine aminotransferase, normal(<19 U/L for females, <30 U/L for males for AASLD or < 40 U/L for EASL, APASL guidelines)

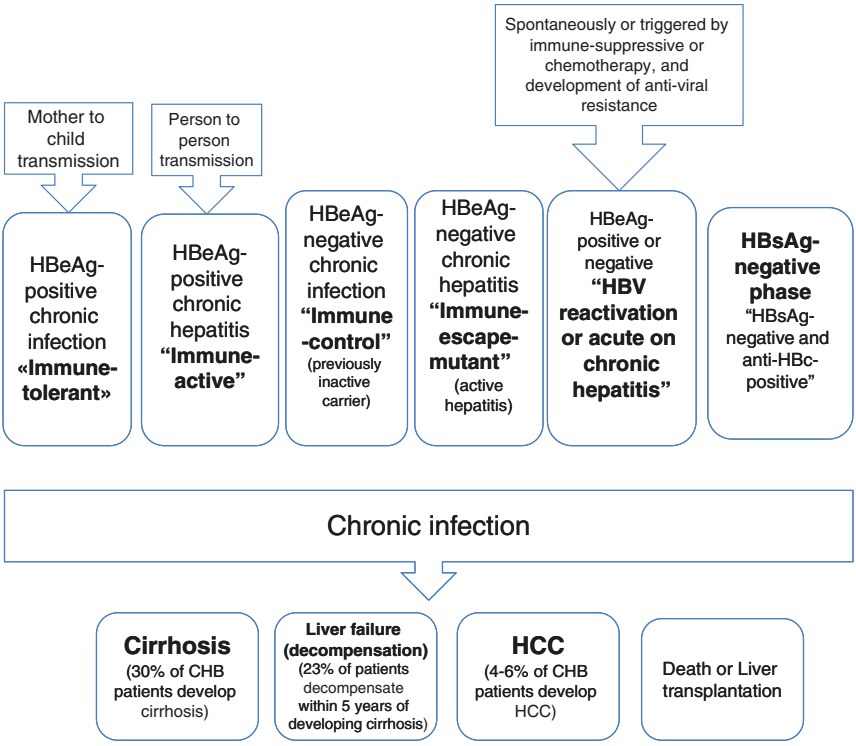

Fig. 7.1 Natural history of chronic hepatitis B virus infection [1, 5, 6, 8]

complications increases. Screening of HBV provides early diagnosis and treatment opportunities, as well as reduction of the transmission of virus by vaccination [14]. Diagnosis of CHB is made by using a combination of biochemical, serological, virological and histological markers [15]. Serum HBsAg is the primary marker of infection. Besides, hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe) and hepatitis B core antibody (anti-HBc IgM/IgG) are other serological indicators of HBV infection. After diagnosis of HBV based on positivity of HBsAg, HBV DNA, ALT and presence of HBeAg and liver fibrosis (liver biopsy or FibroScan) also have to be evaluated. Screening of coinfections such as HIV, HCV and HDV also should be recommended [1, 6].

7.4 Candidates for Treatment

Treatment of patients with CHB has been improved with development of sensitive methods that detect HBV DNA (IU/mL) quantitatively in the blood and potent antiviral drugs. Currently approved treatment modalities include pegylated interferon (Peg-IFN)-α and nucleos(t)ide analogues (NAs). These treatment options are effective on suppression of HBV, reduction of liver inflammation/fibrosis and prevention

of progression of liver diseases. Included with this is the known fact that since both antiviral treatment options cannot eradicate HBV, long-term control of infection is almost impossible after cessation of treatments. For this reason, it is a key question that which patient should start treatment and which one should be monitored. The population of patients with CHB who are quite heterogeneous since natural history of chronic hepatitis needs different treatment and follow-up strategies. To optimize the treatment of individual patient, various characteristics such as age, gender, genetic polymorphism, lifestyle, clinical status, liver histology, presence of coinfection, positive family history of HCC, occupational necessities, family planning and patient preference along with viral properties such as levels of HBV DNA and ALT and status of HBeAg should be considered [12, 13]. Factors that depend on drugs like efficacy, safety, ratio of resistance, duration, way of administration and treatment cost also play a role in the choice of treatment [16].

Professional associations such as AASLD (American Association for the Study of Liver Diseases), EASL (European Association for the Study of the Liver) and APASL (Asian Pacific Association for the Study of the Liver) are developed clinical guidelines for clinicians to diagnose the patients with CHB and to help optimize treatment. According to aforementioned guidelines, treatment decisions should be made based on clinical status, ALT, levels of HBV DNA, presence of HBeAg and liver histology. Before the decision of treatment, series of evaluations for levels of HBV DNA and ALT are important and should be checked more than once.

Patients with life-threatening liver diseases such as acute liver failure, decompensated cirrhosis or severe exacerbation of CHB should be recommended to start treatment promptly. Noncirrhotic patients with serum HBV DNA levels higher than 20,000 IU/mL and persistently high ALT levels and/or who present with moderate/severe inflammation or fibrosis on histology should be treated. It's highly recommended that patients with HBeAg-positive CHB should be monitored for a possibility of spontaneous seroconversion of HBeAg before starting a treatment period of 3–6 months. However, for HBeAg-negative CHB patients who meet the treatment criteria, monitorization period is not recommended before treatment [1, 6, 7, 12, 17].

According to EASL guideline, all compensated cirrhotic patients with detectable DNA level should be treated regardless of ALT levels, whereas AASLD and APASL guidelines recommend treatment of compensated cirrhotic patients with serum HBV DNA level >2000 IU/mL. ALT cut-off values also differ according to guidelines. Definition of upper limit of normal (ULN) is >40 U/L for APASL and EASL guidelines, whereas it is >19 U/L for women and >30 U/L for men according to AASLD. The AASLD and APASL guidelines recommend treatment for patients with an ALT level >2 times the ULN, but for the EASL guideline, ALT level > the ULN is enough for a treatment decision [6, 7, 17].

Patients with CHB who receive immunosuppressive treatment or cancer chemotherapy and also pregnant women with high vertical transmission risk to their neonates should be treated [8, 17]. It's known that viral suppression decreases the risk of virological relapse after transplantation in patients who require liver transplant [12].

Details of guidelines for recommendations to start the CHB treatment are listed in Table 7.3 [6, 7, 17]. Our simple recommendations are shown in Fig. 7.2.

Table 7.3 Guideline recommendations for the indication for treatment of CHB [6, 7, 12, 17]

	AASLD	APASL	EASL
Noncirrhotic patients			
HBeAg-positive	*HBV DNA > 20,000 IU/mL, ALT > 2×ULN*	*HBV DNA > 20,000 IU/mL, ALT > 2×ULN*	*HBV DNA > 2000 IU/mL, ALT > ULN*
	Monitor for 3–6 months Treat if no spontaneous HBeAg loss Liver biopsy before treatment is optional	Monitor for 3–6 months Treat if no spontaneous HBeAg loss Liver biopsy before treatment is optional	Monitor for 3–6 months Liver biopsy(or noninvasive markers of fibrosis) is recommended Treat if no spontaneous HBeAg loss and biopsy shows moderate/severe inflammation and/or at least moderate fibrosis
HBeAg-negative	*HBV DNA > 20,000 IU/mL, ALT ≤ 2×ULN*	*HBV DNA > 20,000 IU/mL, ALT1–2×ULN*	*HBV DNA > 20,000 IU/mL, ALT < ULN*
	Monitor every 3–6 months Consider biopsy in patients >40 years, with ALT persistently 1–2×ULN or with family history of HCC Treat if biopsy shows moderate/severe inflammation or significant fibrosis	Monitor every 1–3 months Consider biopsy in patients >40 years, with ALT persistently 1–2 × ULN or with family history of HCC Treat if biopsy shows moderate/severe inflammation or significant fibrosis	Monitor every 3–6 months Consider biopsy in patients >30 years, with ALT persistently 1–2×ULN or with family history of HCC Treat if biopsy shows moderate/severe inflammation or significant fibrosis
	HBV DNA > 20,000 IU/mL, ALT > 2×ULN	*HBV DNA > 2000 IU/mL, ALT > 2×ULN*	*HBV DNA > 20,000 IU/mL, ALT > 2×ULN*
	Treatment is clearly indicated; liver biopsy is optional	Treatment is clearly indicated; liver biopsy is optional	Treatment is clearly indicated; liver biopsy is optional
	HBV DNA 2000–20,000 IU/mL, ALT 1–2×ULN	*HBV DNA > 2000 IU/mL, ALT 1–2×ULN*	*HBV DNA > 2000 IU/mL, ALT > ULN*
	Consider liver biopsy Treat if liver biopsy shows moderate/severe inflammation or significant fibrosis	Monitor ALT and HBV DNA every 1–3 months Consider liver biopsy if patient is ≥40 years Treat if biopsy shows moderate/severe inflammation or fibrosis	Liver biopsy (or noninvasive markers of fibrosis) is recommended Treat if no spontaneous HBeAg loss and biopsy shows mod/severe inflammation and/or at least moderate fibrosis
	HBV DNA ≤ 2000 IU/mL, ALT ≤ ULN	*HBV DNA ≤ 2000 IU/mL, ALT ≤ ULN*	*HBV DNA ≤ 2000 IU/mL, ALT ≤ ULN*
	Monitor	Monitor	Monitor
Cirrhosis compensated	*HBV DNA > 2000 IU/mL*	*HBV DNA > 2000 IU/mL*	*HBV DNA detectable*
	Treat, any ALT level	Treat, any ALT level	Treat, any ALT level

Table 7.3 (continued)

	AASLD	APASL	EASL
Decompensated	*HBV DNA < 2000 IU/mL*	*HBV DNA < 2000 IU/mL*	
	Consider treatment if ALT>ULN Treat any HBV DNA or ALT level and refer for liver transplantation	Consider treatment if ALT>ULN Treat any HBV DNA or ALT level and refer for liver transplantation	Treat any HBV DNA or ALT level and refer for liver transplantation
ALT cut-off level, U/L	30 for men, 19 for women	40 U/L	40 U/L

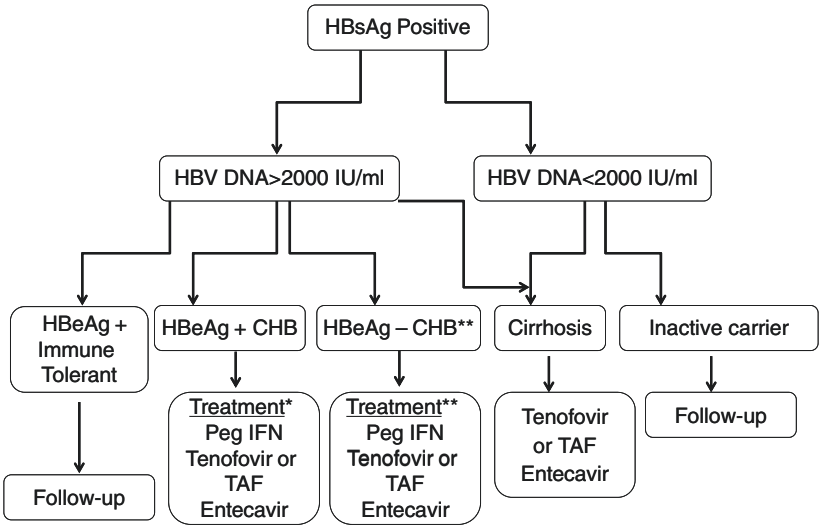

Fig. 7.2 Treatment algorithm. *High ALT **qHBsAg >1000 IU/ml and Fibrosis score >2 (biopsy or fibroscan)

7.5 What Are the Factors that Predict the Treatment Response?

Several factors are associated with treatment response, and long-term remission may help to guide treatment decisions. Pretreatment factors that predict response to Peg-IFN and NAs are similar. Low HBV DNA levels and high ALT levels are the most reliable predictors of response for both antiviral treatment strategies. Genotype is an important predictor factor for only patients who receive Peg-IFN treatment.

Patients with genotype A show significantly higher rates of HBeAg and HBsAg seroconversion than patients with genotype B, C or D [18]. For HBeAg-positive patients, decrease in HBsAg level at 12 weeks of Peg-IFN treatment is associated with high chance of HBeAg seroconversion [4]. Either spontaneous- or treatment-induced seroconversion of HBeAg is associated with a HBV DNA decline, normalization of ALT levels and improvement of necroinflammation on liver biopsy [10].

Predictive factors in patients treated with Peg-IFN:

- *HBeAg-positive CHB*
- Decline of HBsAg levels below 1500 IU/mL at 12 weeks is a strong predictor of anti-HBe seroconversion.
- HBsAg levels >20,000 IU/mL or lack of decline of HBsAg levels at 12 weeks is associated with a very low probability of subsequent anti-HBe seroconversion.
- *HBeAg-negative CHB*
- A combination of no HBsAg decline and <2 log10 IU/mL decline of HBV DNA seems to be a predictor of non-response in European HBeAg-negative patients with genotype D [19, 20].

Predictive factors associated with HBsAg loss:

– Baseline factors: genotype A, tenofovir (TDF) + Peg-IFN treatment (48 weeks)
– Factors during treatment: increase in ALT level within the first 12 weeks (M:>400 U/mL; F:>300 U/mL) and >3.5 log decrease in HBsAg level at week 24 (a positive predictive value of 85% and a negative predictive value of 99% for HBsAg loss at week 72) [21]

Predictive factors for entecavir (ETV) therapy are low viral load, low fibrosis score, negative HBeAg and absence of prior IFN therapy [22].

HBeAg level is also an important predictive factor for NAs. In patients with HBeAg-positive CHB, HBeAg decline at week 24 of TDF treatment is associated with seroconversion of HBeAg and HBsAg loss at 8 years of treatment [23].

In patients treated with LAM, HBV DNA level at 6 months of therapy is a very important predictor of LAM resistance [11].

Hepatitis B core-related antigen (HBcrAg) which has a good correlation with cccDNA and HBV DNA levels is a very useful new marker to predict response to treatment of CHB and natural course of HBV. Beyond that, it is a promising marker to predict seroconversion HBeAg (whether spontaneous or treatment induced), sustained response to NA therapies and risk of HBV reactivation and development HCC [24, 25].

7.6 Measuring Response to Antiviral Therapy

Antiviral therapy responses can be defined as biochemical, serological, virological and histological at the several time points during and after therapy [17]. The definitions of responses to antiviral therapy are listed in Table 7.4.

Table 7.4 Antiviral therapy response criteria and definitions (adapted from [17])

Response to antiviral therapy	Definition
Biochemical response	Normalization of serum ALT levels
Serologic response	
For HBeAg	HBeAg loss and seroconversion to anti-HBe in patients with HBeAg-positive CHB
For HBsAg	HBsAg loss and seroconversion to anti-HBs
Virological response to Peg-INF	
Virological response	HBV DNA <2000 IU/mL
Sustained virological response	HBV DNA <2000 IU/mL at least 12 months after end of therapy
Virological response on NA therapy	
Virological response	Undetectable HBV DNA
Complete response	Sustained virological response with HBsAg seroconversion
Partial virological response	Reduction of HBV DNA >1 loq IU/mL but still detectable at 24 weeks of oral NA therapy
Sustained off-treatment response	No clinical relapse during follow-up after stopping treatment
Primary non-response	Reduction of HBV DNA <1 loq IU/mL at 12 weeks of oral NA therapy
Virological breakthrough	Increase HBV DNA > 1 loq IU/mL from nadir of initial response during therapy
Viral relapse	HBV DNA > 2000 IU/mL after cessation of therapy in patient with virological response
Clinical relapse	Viral relapse along with ALT level > 2×ULN
Histological response	Decrease in histology activity index by at least two points and no worsening of fibrosis score compared to pretreatment liver biopsy or fibrosis reduction by at least one point by METAVIR staging

7.6.1 Biochemical Response

It is defined as normalization of ALT levels, evaluated at several time points during therapy, at the end of therapy and after therapy. Biochemical responses do not always correlate with DNA responses [17]. To confirm sustainability off-treatment biochemical response, ALT levels should be monitored at least once every 3 months during 1 year follow-up period after the treatment since ALT levels fluctuate within time [6].

7.6.2 Serological Response

7.6.2.1 HBeAg Seroconversion

It is defined as the loss of HBeAg and development of anti-HBe (seroconversion) in patients HBeAg-positive CHB [17]. Although seroconversion of HBeAg is a milestone and treatment end point in the treatment of patients who had seroconversion

by NA treatment, only very low number of patients sustained their virological, serological and biochemical responses after consolidation treatment. In majority of patients, flares of ALT accompanies to recurrent viremia and in one third of the patients HBeAg-re-seroconverts and becomes positive [26].Treatment with Peg-IFN is still a rational first-line alternative therapy because of its finite duration and immunomodulation action, which provides higher rates of HBeAg/HBsAg seroconversion compared with NAs. Besides, in patients who respond to Peg-IFN treatment, rates of HBeAg/HBsAg loss or seroconversion increase in time. Low HBV DNA and HBeAg levels predict HBeAg loss and seroconversion. In HBeAg-positive patients with genotype A and B, significantly higher rates of HBeAg seroclearance are sustained more than 3 years after the end of Peg-IFN treatment, compared to patients with genotype C and D [27].

7.6.2.2 HBsAg-Seroconversion

It is defined as a HBsAg loss and development of anti-HBs. HBsAg seroconversion is associated with remission activity and improvement of long-term treatment outcomes. Nevertheless, HBsAg disappears in only <10% of patients. In both antiviral treatment strategies, maintenance of cccDNA within hepatocytes explains rapid DNA rebound after cessation of treatments [13, 17].

7.6.2.3 Virological Response

Virological responses are different for Peg-IFN and NA therapy. Virological response is defined as HBV DNA level < 2000 IU/mL at 6 months, end of treatment, and 6 and 12 months after completion of Peg-IFN therapy, whereas it is defined as undetectable HBV DNA level when it is evaluated every 3 or 6 months during NA treatment.

Accompaniment of HBsAg loss to sustained virological response is defined as complete response.

7.6.2.4 Histological Response

It is defined as at least two-point decrease in histological activity index without worsening of fibrosis or at least one-point decrease in fibrosis by METAVIR staging compared to biopsy results before treatment.

HBV DNA suppression, ALT normalization, HBeAg seroconversion, HBsAg loss and cirrhosis prevention or regression in liver histology are surrogate end points for current antiviral treatments [10, 17].

7.7 Monitoring

7.7.1 Monitoring of Patients Who Are Not Being Treated

Periodic monitorization of the patients who are not deemed to be treatment candidates at presentation is recommended because of the dynamic nature of CHB. Monitorization of the patients in immune-tolerant phase is recommended

every 3–6-month intervals to decide whether patients enter the immune-active or inactive carrier phases. More frequent monitoring of serum ALT levels and HBV DNA levels is necessary if ALT levels increase. HBeAg status is evaluated every 6–12 months in patients with HBeAg-positive CHB. Monitoring of ALT levels every 3–6 months and HBV DNA every 6–12 months is recommended in inactive carriers [6, 7, 10, 17].

7.7.2 Monitoring of Patients During and After Treatment

Regular monitoring of serum ALT levels, complete blood count and thyroid function is recommended in patients who are treated with Peg-IFN therapy. In addition, monitorization of all patients for potential side effects of Peg-IFN treatment like autoimmune disorders, ischemic conditions and neuropsychiatric and infectious complications is critical. HBeAg, anti-HBe and HBV DNA should be monitored every 6 months during treatment and 1 year after completion in patients who are HBeAg-positive. HBV DNA level should be monitored every 6 months during treatment and 1 year after treatment completion in patients who are HBeAg-negative. HBsAg should be tested once a year; in case of HBsAg loss, appearance of anti-HBs should be monitored. If treatment is stopped in patients with cirrhosis, close monitoring is recommended once a month for the first 6 months and then once in every 3 months [6, 7, 10, 17].

The aims of monitorization during NA therapy are to determine treatment response and to detect persistent viremia, virological breakthrough and complications of treatment. HBV DNA levels in patients who are treated with NAs have to be monitored every 3–6 months. Patients who are treated with ETV or TDF should be monitored every 6 months. Serum ALT, HBeAg and anti-HBe should be monitored every 3–6 months. HBsAg should be tested once a year in patients with HBeAg seroconversion. Patients treated with TDF should undergo periodical monitoring of renal function and bone profile. In patients at risk of renal failure, creatinine clearance, serum phosphate, urine glucose and protein should be tested every year. In patients who stop antiviral therapy, monitorization is recommended every 3 months in case recurrent viremia, ALT flares, seroconversion and clinical decompensation occur. If treatment is stopped in patients with cirrhosis, close monitoring is recommended in 1-month intervals for the first 3–6 months and then once every 3–6 months [4, 6, 7, 10, 17].

7.7.3 Monitoring of Liver Fibrosis and HCC

Staging of hepatic fibrosis degree is necessary and important since in patients with stage 2 ≥ fibrosis, treatment is indicated. METAVIR, Knodell and Ishak scores are the most widely used liver biopsy scoring systems. Even though liver biopsy is the gold standard to detect the degree of hepatic fibrosis, one should consider, besides high ratio of sampling errors, the risk of complications during liver biopsy like pain

and bleeding. For this reason, noninvasive methods such as *APRI* (aspartate aminotransferase-to-platelet ratio index) and *FIB-4* (fibrosis index based on the four factors) which have moderate diagnostic accuracy are developed as alternatives to liver biopsy to evaluate liver fibrosis.

Transient elastography (FibroScan), which is another reliable technique especially for detecting cirrhosis, uses ultrasound elastography to assess the liver stiffness as a surrogate of hepatic fibrosis [1, 5].

All patients with cirrhosis and severe fibrosis and patients with risk of severe HCC (high HBV DNA level, male gender, coinfection with HCV, HIV, HDV, comorbidities, age >40 year, long duration of infection, significant inflammation) even without cirrhosis and severe fibrosis should be monitored for the development of HCC every 6 months by USG [4, 6, 7, 10, 17].

7.8 Current Treatment Options

There are two current treatment options: a finite duration of treatment with Peg-IFN-α or long-term treatment with nucleot(s)ide analogues (NA). Peg-IFN, ETV and TDF are recommended as first-line treatment options because these therapies can halt progression of liver damage and prevent other liver-related complications. NA should be the treatment of choice in patients with severe liver diseases, who are non-responsive to IFN therapy, who have contraindications to IFN and who are unwilling to have IFN therapy. NA therapy provides sustained viral suppression, biochemical remission and histological improvement. NA therapy also prevents decompensation, but HBsAg seroclearance rates are low. Long-term administration necessities of NA are associated with high treatment cost, poor treatment compliance, unknown adverse events and risk of resistance [28]. Current NA drugs which are approved for treatment of CHB are contained into two groups: nucleoside (lamivudine, telbivudine and entecavir) and nucleotide analogues (adefovir dipivoxil, tenofovir dipivoxil fumarate and tenofovir alafenamide) [29, 30]. The long-term administration of potent NAs with high barrier to resistance, entecavir, tenofovir disoproxil or tenofovir alafenamide, is recommended in the treatment-naive patients. In contrast less potent NAs with low barrier to resistance, lamivudine, adefovir and telbivudine, are not recommended [6].

7.8.1 Interferons

Interferon approach offers a choice with a finite duration therapy and no risk of drug resistance, but low response rates, risk of decompensation and its unfavourable safety profile limit its use within certain patient population [8].

7.8.1.1 Standard IFN-α

It was the first successful treatment approved for CHB in 1991, and its major mechanism of action is immunomodulation, although it has a week antiviral effect [11].

7.8.1.2 Peg-IFN-α

Only Peg-IFN-α2a is approved for the treatment of CHB, and it replaced standard IFN-α in 2005 due to its improved pharmacokinetic properties and convenience. Peg-IFN treatment in patients with HBeAg-positive CHB provides higher ratio of HBeAg seroconversion (32%, 6 months after discontinuation of therapy) and HBsAg seroconversion (6%, 6 months of posttreatment follow-up) than NAs, whereas it is less effective in HBeAg-negative CHB patients. HBsAg disappeared in 12–65% of patients within 5 years of HBeAg loss even after discontinuation of IFN treatment [4, 11]. In HBeAg-negative patients with mild to severe liver diseases, 48 weeks of Peg-IFN-α treatment provides SVR in 25% of patients, ultimately leading to loss of HBsAg in approximately 30–50% of patients with SVR. Peg-IFN response rates further increase with careful patient selection based on baseline HBV DNA, ALT levels and age and early implementation of on-treatment rules based on HBV DNA and HBsAg levels [28]. In patients with higher ALT level (>5× ULN), acute renal failure, decompensated cirrhosis or severe exacerbations of CHB, Peg-INF is not recommended [12].

7.8.2 Nucleoside Analogues

7.8.2.1 Lamivudine(LAM)

LAM was the first nucleoside analogue reverse transcriptase inhibitor that was approved for CHB in 1998. LAM has been the most experienced oral antiviral in CHB patients; it has played a major role on HBV-related complications such as cirrhosis and HCC in the past, but currently, it has been replaced with other antiviral drugs which are more potent and have higher genetic barrier to resistance. By the fifth year, 50% of patients achieved seroconversion of HBeAg; nevertheless, it is not considered as a first-line agent in the treatment of CHB in many guidelines any more, since approximately 70% of the patients develop resistance after 5 years [2, 11, 13].

7.8.2.2 Telbivudine (LdT)

It is a thymidine NA that is structurally similar to LAM and has similar resistance profile. It is another nucleoside analogue approved for CHB in 2006. Although it is more potent than LAM, its cross-resistance with LAM limits its use only for treatment of pregnant female patients [2, 11, 13]. In one study, the efficacy and safety of LdT in combination with Peg-IFN, Peg-IFN monotherapy and LdT monotherapy were compared. Combination therapy provided higher virological response rates as well as rapid reduction in HBsAg levels compared to monotherapies. However, the unexpected high rates of peripheral neuropathy risk have been reported up to 20% in combination therapy and have resulted in early termination of this study [31].

In one open-label randomized trial, the efficacy of LdT monotherapy and continuation therapy with ETV was investigated in patients who had virological response to previous ETV treatment. At week 48, higher virological response (98% vs 63.8%) and HBeAg seroconversion rates (15.2% vs 5%) were achieved with continuation of

ETV therapy than with switching treatment to LdT. Besides, switch strategies to LdT have resulted with high ratio of virological breakthrough (23.4%) and resistance (14.9%) than continuation therapy with ETV. In accordance with this, switch strategies to LdT were not associated with a greater reduction in serum HBsAg levels. These results showed that a switch strategy to antiviral drugs with low genetic barrier in patients who had previous virological response has failed [32].

7.8.2.3 Entecavir (ETV)

ETV is a guanine nucleoside drug, with active triphosphate form potently inhibiting HBV polymerase. ETV inhibits initiation of HBV polymerase, synthesis of minus strand DNA from the pregenomic RNA template and also synthesis of HBV DNA chain [2]. It provides rapid and sustained suppression of viral load in HBV-infected patients [33]. ETV is considered to be an effective treatment option for patients with HBV-related cirrhosis, due to its demonstrated efficacy on suppression of viral load, improvement of liver functions and activation of complement system. Treatment with ETV for 96 weeks has resulted in significantly higher improvements in the compensated group compared with the decompensated one ($P < 0.05$) [34].

7.8.3 Nucleotide Analogues

7.8.3.1 Adefovir Dipivoxil (ADV)

It was the first nucleotide analogue of adenosine monophosphate approved for the treatment of CHB in 2002. It is effective in viral suppression for both treatment-naive and LAM-resistant CHB patients. On-year ADV treatment provides 12% HBeAg seroconversion rate and histological improvement of 53% in patient with HBeAg-positive CHB. ADV which has high resistance rate and risk of nephrotoxicity is not recommended as a first-line monotherapy after approval of more potent drugs such as ETV or TDF [11, 35].

7.8.3.2 Tenofovir Disoproxil Fumarate (TDF)

It is the second nucleotide analogue which was approved for CHB in 2008. In a study, 48 weeks of TDF treatment is compared with ADV treatment in HBeAg-negative and HBeAg-positive patients. TDF therapy resulted in a higher rate of viral suppression than ADF therapy in HBeAg-negative (93% vs 63%) and in HBeAg-positive patients (76% vs 13%), respectively. Furthermore, TDF treatment provided significantly higher amount of patients to achieve primary end point of study (HBV DNA < 400 copy/ml plus histological improvement) compared to ADV treatment among HBeAg-negative patients (71% vs 49%) and among HBeAg-positive patients (67% vs 12%). By these results, TDF showed superior efficacy than ADV in terms of viral suppression and histological improvements. Viral suppression is maintained in 99% of patients with HBeAg-positive CHB and 100% of patients with HBeAg-negative CHB after 4 years of treatment with TDF. HBeAg loss and HBeAg

seroconversion rates were 41% and 29% after 4 years of TDF treatment, respectively. TDF had similar efficacy in LAM-experienced patients with treatment-naive ones. It's shown that regression of cirrhosis occurred in 74% of patients who had cirrhosis at baseline after 5 years of TDF treatment. After 7 years of follow-up of patients, no resistant mutations were detected [11, 36, 37].

7.8.3.3 Tenofovir Alafenamide (TAF)

It is phosphonamide prodrug of tenofovir that shares the same intracellular active metabolite with TDF, tenofovir diphosphate, which is effective against both HBV and HIV-1 infections. TAF has greater plasma stability than TDF; therefore, lower plasma concentration than TDF is enough for more efficient uptake by hepatocytes. For this reason, TAF compared to TDF provides better safety profiles especially on renal and bone dysfunction. The efficacy and safety of TAF are compared to TDF in phase III trials. According to the results of these trials, TAF achieved similar virological response rates compared to TDF both in HBeAg-negative and HBeAg-positive patients. For 96 weeks TAF treatment in patients with HBeAg-positive patients reached similar rates of virological response, HBeAg and HBsAg seroconversion (73%, 18% and 1%) compared to TDF arm (75%, 12% and 0%), respectively. Similarly, there was also no significant difference in the rate of virological response (90% vs 91%) between the TAF and TDF arms at 96 weeks of treatment in HBeAg-negative patients. Further studies are needed to show long-term efficacy and safety of TAF treatment in patients with CHB [30].

The efficacy and resistance patterns of approved preferred antiviral therapies in patients with CHB is are shown in Tables 7.5 and 7.6.

Table 7.5 Efficacy of approved preferred antiviral therapies in patients with CHB (not to head to head comparisons) [6, 10]

	Peg-IFNα2a[a]	ETV	TDF	TAF
Dose	180 μg percutaneous injection/week	0.5 mg oral tablet/day	300 mg oral tablet/day	25 mg oral tablet/day
HBeAg-positive CHB				
HBeAg seroconversion	32%	21%	21%	10%
HBV DNA < 60–80 IU/mL	14%	67%	76%	64%
ALT normalization	41%	68%	68%	72%
HBsAg loss	3%	2%	3%	1%
HBeAg-negative CHB				
HBV DNA < 60–80 IU/mL	19%	90%	93%	94%
ALT normalization	59%	78%	76%	83%
HBsAg loss	4%	0	0	0

[a]Assessed 6 months after completion of 12 months of therapy. NAs assessed after 2–3 years of continuous therapy

Peg-IFNα pegylated interferon alfa, *ETV* entecavir, *TDF* tenofovir disoproxil fumarate, *TAF* tenofovir alafenamide, *ALT* alanine aminotransferase

Table 7.6 Cross-resistance patterns of selected hot spot mutations in the HBV polymerase conferring NA resistance (adapted from [6])

HBV variants	LAM	LdT	ETV	ADV	TDF
Wild type	S	S	S	S	S
M204V	R	S	I	I	S
M204I	R	R	I	I	S
L180M + M204V	R	R	I	I	S
A181T/V	I	I	S	R	I
N236T	S	S	S	R	I
L180M + M204V/I ± I169T ± V173L ± M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

S sensitive, *I* intermediate/reduced susceptibility, *R* resistant, *LAM* lamivudine, *LdT* telbivudine, *ETV* entecavir, *ADV* adefovir, *TDF* tenofovir

(a) First-Line Treatment

Selection of a first-line antiviral drug should be based on the efficacy and safety of drug, risk of resistance development, treatment cost and patient preference [12, 18]. Peg-IFN, ETV and TDF/TAF monotherapies were recommended as first-line starting antiviral treatment options [6, 7, 17]. IFN treatment that induces long-term immunological control with a finite duration treatment results in high ratios of HBeAg and HBsAg loss. Parenteral administration and its unfavourable safety profile are main disadvantages of IFN treatment [6, 18].

ETV and TDF are recommended as the first-line therapies to treat HBV infection due to their specific activity, the high genetic barrier to resistance and the low incidence of associated complications [33]. ETV and TDF have comparable efficacy in the treatment of CHB; several studies have shown that they have no superiority in between in terms of virological response, biochemical response and seroconversion of HBeAg [38, 39].

(b) Combination Treatment

A combination of Peg-IFN plus NA therapies is not recommended since superiority of combination treatment over monotherapies has not been proven in the long term [4, 21, 28, 40]. Sequential combination therapy adding 48 weeks of Peg-IFN-α-2a to long-term ETV treatment provided higher rates of HBeAg seroconversion and more likely to experience HBsAg loss than from continuing ETV monotherapy (44%, 4% vs 6%, 0%). Sequential combination therapy can be guided by baseline HBeAg/HBsAg levels [41].

(c) Long-Term Treatment

Long-term NA monotherapies are recommended due to high virological and clinical relapse rates after discontinuation of NA monotherapies. Long-term NA monotherapies have been shown to prevent or delay the development of complications such as liver decompensation and HCC- and CHB-related death compared to no treatment. In patients with compensated cirrhosis, risk of occurrence of complications is higher than noncirrhotic patients, but it is still low in patients treated with

NA compared to no treatment. A potent NA with high barrier to resistance is recommended especially for patients with decompensated cirrhosis, since virological breakthrough during treatment increases the risk of development of HCC and other complications [42].

According to a real-world cohort study, for 7 years of ETV treatment outcomes, ALT normalization, HBeAg seroconversion, undetectable HBV DNA and HBsAg seroclearance results were found to be 98.3%, 81.2%, 98.2% and 2.5%, respectively. Genotypic resistance was 1.2% [25].

Seven years after TDF treatment, 99.3% maintained viral suppression and 80% achieved ALT normalization with no evidence of TDF resistance [43].

The long-term efficacy and safety of ETV in treatment-naive CHB patients are similar compared to TDF as a first-line treatment monotherapy. One study suggested that TDF might be more effective in a subgroup of HBeAg-positive patients with high viral load [44].

Long-term ETV treatment provides histological improvement, regression of fibrosis or cirrhosis as well as a reduction in incidence of HCC in treatment-naive patients [45, 46].

Long-term TDF and ETV monotherapies showed comparable efficacy in the treatment of patients with CHB- and HBV-related cirrhosis, considering the impact on viral suppression and improvement in liver functions [47].

(d) Management of Treatment Failure

Approach to patients with antiviral treatment failure due to several reasons should be focused on the reason of treatment failure. Since primary non-response is very rarely observed with ETV and TDF monotherapies, it is very important to check patient's compliance. In case of partial virological response (low-level viremia), ETV or TDF monotherapy should be continued regardless of ALT levels. Furthermore, to prevent the development of antiviral resistance in compliant patient, addition of other first-line drugs should be considered based on an expert opinion. In compliant patients who receive ETV or TDF, when the virological breakthrough is observed as an indicator of antiviral resistance, usually switching to other high barrier to resistance antiviral monotherapy or adding second antiviral drug without cross-resistance is recommended [6, 7, 10].

In patients with partial virological response to ETV therapy, switching to TDF monotherapy or TDF + ETV combination treatment resulted in similar complete response rates in both groups [48, 49].

Accordingly, recent meta-analysis results showed that TDF-based combination therapy had no significant efficacy or safety advantage compared to TDF monotherapy in patients with suboptimal responses to previous multiple NAs [50].

Earlier initiation of appropriate rescue therapy after emergence of VB is critically important, since rescue therapy has better response rates in low HBV DNA levels [10].

Peg-IFN retreatment may be effective in HBeAg-positive patients with treatment-experienced (IFN or NA), even increasing HBsAg loss in HBeAg-negative patients with treatment-experienced [51].

7.9 Safety of Antiviral Drugs

Peg-IFN treatment is associated with higher prevalence of adverse effects compared to NA therapy. Peg-IFN treatment is contraindicated in patients with HBV-related decompensated cirrhosis, autoimmune disease and severe depression/psychosis and in patients who are pregnant [4].

Although CHB patients are treated with low dose of ADV, 30% of patients developed proximal renal tubular dysfunction and 10% of patients had symptomatic osteomalacia. These side effects were found to be associated with age at the initiation of ADV and treatment duration [35].

The unexpected high rate of peripheral neuropathy risk has been reported up to 20% in combination with LdT with Peg-IFN-α2a; hence, decision on the initiation of combination therapy should be made carefully [21].

The most common adverse events of TDF and TAF are nasopharyngitis, upper respiratory infection and headache. TDF and ETV treatment may induce the reduction in renal functions. The development of nephrotoxicity of TDF and ETV depends on various mechanisms such as renal tubular damage, apoptosis and mitochondrial toxicity. It is also associated with previous renal failure, age, co-morbidities (HT, DM) and usage of concomitant nephrotoxic drugs. Interestingly, one study showed that the combination of TDF therapy with LdT led to the improvement in renal functions. TAF is associated with better bone safety profile besides renal profile. TAF provides lower systemic exposure to tenofovir than in those treated with TDF and thus has lower risk for tenofovir-associated renal toxicity. Monitorization of eGFR and phosphorus pre- and during treatment period and with appropriate dose adjustment or switch can minimize the impact of NA renal dysfunction [30, 52, 53].

7.10 Resistance Problem in Treatment

HBV is a partially double-stranded DNA virus, with four overlapping genes, and has a unique life cycle which involves the generation of an RNA template for replication via reverse transcription. NAs are competitive inhibitors of HBV reverse transcriptase as they are incorporated into the DNA strand, causing chain termination. HBV polymerase which has no proof-reading function results in a high number of mutant viral genome in infected persons. Particular selection pressure of NAs readily selects escape mutants and strongly influences the predominant HBV quasispecies in patients with chronic hepatitis. Regular monitorization of HBV DNA and ALT levels is very important, since resistance can result in primary non-response, partial virological response or virological breakthrough in patients treated with antiviral agent [17, 37].

HBV resistance against NAs is characterized with the selection of variants that can reduce susceptibility of the applied NA [17]. Clinical relation of drug resistance is understood by the usage of LAM which is the first NA with low genetic barrier to resistance. LAM resistance is associated with higher risk of liver dysfunctions, increase in ALT level as an indicator of liver damage and increased risk of cirrhosis

and HCC compared to without LAM resistance. In addition, in patients with resistance, virological rebound and hepatic decompensation were detected [37]. ADV resistance is also associated with exacerbation of disease and liver failure in some cases [54]. Virological breakthrough due to resistance to antiviral agent is associated with more rapid progression to liver failure, liver transplantation, development of HCC and death [33].

Genotypic resistance is defined as the detection of mutations in HBV genome which causes the development of resistance during antiviral treatment.

Phenotypic resistance is defined as reduced susceptibility to inhibition of antiviral drugs in relation with genotypic resistance [17].

The risk of selection of resistance to antiviral therapy is associated with several factors such as HBV DNA level, selection of antiviral, treatment duration and previous NA treatments [6, 37].

The currently approved NA drugs are classified into two group as high barrier to resistance (ETV, TDF,TAF) and low barrier to resistance (LAM, ADV, LdT). To prevent the development of resistance, potent NAs with high barrier to resistance should be selected as a first-line treatment option. Combination therapies with low barrier to resistance drugs such as LAM, ADV and LDT should be avoided since it can cause the occurrence of multidrug resistance strains and insufficient virological response [6].

In primary mutations that typically affect reverse transcriptase domain of HBV polymerase, any steric changes on polymerase protein result in the viruses escaping from inhibitory effects of NAs [37]. LAM resistance developed because of mutation on YMDD locus of HBV polymerase (rtM204I/V/S) and can cause cross-resistance of nucleoside analogues such as LdT and ETV, but it does not confer cross-resistance to ADV or TDF. The primary drug-resistant mutations against LdT and ADV are found to be rtM204I, rtA181T/V and rtA181V/T and rtN236T, respectively [17, 54]. ETV resistance requires YMDD mutations (rtM204V/I, rtL180M) plus the addition of other ETV 'signature' substitutions in the B domain (rtI169T or rtS184G), C domain (rtS202G/I) or E domain (rtM250V) [54]. Although no TDF resistance has been reported up to 7 years, ADV-resistant mutations (rtA181V/T, rtN236T) can decrease the efficacy of TDF (Table 7.7) [17, 37, 54].

Resistance rate against LAM is 23% after 1 year; it increases up to 80% after 5 years. Resistance rate against LdT is 25.1% for HBeAg-positive patients and 10.8% for HBeAg-negative patients after 2 years. Although ADV is an effective treatment option for the treatment of LAM-resistant patients, resistance rate against ADV is 30% after 5 years. Resistance rates against ETV are 1.2% in NA-naive patients and more than 50% in LAM-resistant patients after 5 years. ETV is effective in the treatment of patients with ADV resistance [17, 37].

Primary non-response is only seen with ADV treatment due to very low antiviral potency. Rapid switch to TDF or ETV is recommended. In patients with partial virological response, compliance of patient to treatment should be checked. If patients use low barrier to resistance drug (LAM, LdT, ADV), switching to more potent without cross-resistance drugs is recommended. It should be remembered that partial virological response of ETV and TDF treatment may be associated

with higher viral load at baseline. If HBV DNA levels draw a plateau, consider to switch to other treatment or combination of ETV plus TDF (TAF) especially in patients with severe liver disease. Development of resistance against antiviral drug causes virological breakthrough in compliant patients. Treatment should be considered to avoid further increase in viral load and ALT level and prevent liver failure [6].

TDF mono-rescue treatment has similar efficacy compared to TDF plus ETV combination rescue treatment in the treatment of patients with LAM plus ETV resistance (85.4% vs 89.2%, $P = 0.068$). TDF plus ETV combination rescue treatment is significantly more effective compared to TDF mono-rescue treatment in patients with high baseline HBV DNA level (92.9% vs 68.3%, $P < 0.001$) [55].

In case of LAM, LdT and ETV resistance, rapid switch to TDF monotherapy is recommended. TDF monotherapy or ETV-TDF combination therapy has similar efficacy on ETV resistance. In case of ADF resistance, switching to ETV monotherapy or TDF monotherapy or TDF + ETV combination therapy is recommended. In possible risk of TDF resistance, switching to ETV monotherapy or TDF + ETV combination therapy and for multiple drug resistance TDF + ETV combination therapy are recommended (Table 7.7) [6, 37].

Table 7.7 Management of patients who develop NA resistance (adapted from [6, 7, 17])

Resistance pattern	Rescue strategies (EASL2017)	Switch strategy (AASLD 2016)	Add strategy (AASLD 2016)	Rescue strategies (APASL 2015)
LAM resistance	Switch to TDF(TAF)	Switch to TDF	Add TDF to LAM or emtricitabine-TDF	Switch to TDF or add ADV
LdT resistance	Switch to TDF(TAF)	Switch to TDF	Add TDF to LdT	Switch to TDF or add ADV
ETV resistance	Switch to TDF(TAF)	Switch to TDF	Add TDF to ETV or emtricitabine-TDF	Switch to TDF or add ADV
ADV resistance	If LAM-naive: switch to ETV or TDF(TAF)	Switch to ETV	Add ETV to ADV	If LAM/LdT-naive: switch to ETV or TDF
	If LAM-resistant: switch to TDF(TAF)			If LAM/LdT-resistant: switch to TDF or LAM/TDF
	If HBV DNA plateaus: add ETV or switch to ETV			
TDF(TAF)[a] resistance	If LAM-naive: switch to ETV			
	If LAM-resistant: add ETV			
Multidrug resistance	Switch to ETV plus TDF(TAF)	Switch to TDF		Switch to ETV/TDF or Peg-IFN

[a]Not seen clinically so for, do genotype and phenotype tests

7.11 Treatment Duration and Stopping Rules

Currently approved treatment options have no effect on cccDNA which has template function for transcription of pregenomic RNA, virological relapse seen in majority of patients after discontinuation of therapy. For this reason, lifelong treatment with NA is required.

- *IFN*: finite duration or response-guided therapy for HBeAg-positive patients

48–52 weeks of Peg-IFN treatment is recommended for both HBeAg-positive patients and HBeAg-negative patients. A decline in HBsAg level at 12 or 24 weeks predicts good treatment response to Peg-IFN. No decline in HBsAg level at 12 or 24 weeks predicts no response to treatment (85–95% negative predictive value).

- *NAs*: indefinite treatment vs trial of stopping treatment
- HBeAg-positive: after HBeAg seroconversion and 12 months' consolidation therapy
- HBeAg-negative: after 3–5 years' treatment with persistently undetectable HBV DNA

After seroconversion of HBeAg in HBeAg-positive patients after 6–12 months of consolidation, therapy stopping of treatment is recommended. In patients with advanced fibrosis (fibrosis score >3) and cirrhosis, to avoid flares related to viral relapse, continuous treatment until HBsAg loss is recommended [6, 18].

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8 Hepatitis D Virus Infection: Role of Hepatitis B and the Current Updates on Management

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8.1 Introduction

Hepatitis D virus (HDV) was first recognized in 1977 in Italy. A new antigen-antibody system was detected by immunofluorescence staining in patients that were carriers of hepatitis B infection. It was given the term of delta antigen [1]. Further studies later carried out by the National Institutes of Health demonstrated that this detected antigen was linked to an entirely different virus, requiring hepatitis B virus (HBV) for the infection. It was given the name of hepatitis D virus and classified under the genus of *Deltavirus*, where it is the only member given its unique structure [2]. By the end of the 1980s, HDV prevalence was estimated to be about 5% of HBV infection, approximately 15 million people worldwide [3]. Early reports suggested the coinfection rate to be as high as 20–30% with subsequent reduction in the rate largely attributed to the effective HBV vaccination implementation. The accurate estimation of HDV current prevalence is limited as data is not available from many parts of the world. Endemic reports emerged from the Eastern Mediterranean, the Middle East, Turkey, Central America, and parts of South America [4–7]. The high prevalence of HBV does not necessarily correlate with a similar prevalence of the HDV. A clear example for this is the high prevalence of endemic HDV in Taiwan and Okinawa and low prevalence in Japan and Korean nearby. All of aforementioned countries reported to have high prevalence of HBV infection [4, 8]. In the United States, a retrospective study published in 2013 collected from patients in California showed 8% of total 499 chronic hepatitis B (CHB) patients were coinfected with HDV. Cirrhosis rates were higher in the coinfected patients. Interestingly, one half of the coinfected patients also tested positive for HCV antibodies proposing tri-infection with HCV [9]. Multiple studies across Europe showed that more than 70% of HDV-infected patients were injection drug users [7].

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Table 8.1 Geographic distribution and presentation severity of hepatitis D genotypes

Genotype	Geographic distribution	Clinical presentation
1	Worldwide	Wide range of severity
2 and 4	Asia	Not reported
3	Amazon region	Severe disease
5–8	Africa	Mild disease

8.2 Genotypes

Eight different genotypes are currently recognized with narrow range difference in the length but with up to 35% difference in nucleotide sequence listed in Table 8.1. Initially, HDV was classified into three genotypes, I–III. However, identification of more HDV isolates with different sequences led to the identification of further genotypes, and classification was made into eight clades, Table 8.1 [10]. Genotype 1 is the most common worldwide and associated with wide range of clinical severity. Genotypes 2 and 4 have been reported in Asia. Genotype 3 is reported in the Amazon region of South America and associated with severe clinical outcome. Genotypes 5–8 that are reported in Africa are associated with a milder form of the disease [11–14]. In addition, some of the certain HBV genotypes might be more associated with HDV infection than others. A study conducted from patients in the Brazilian Amazon showed that HBV genotype A was associated with lower viral load than that of genotype D or F [15]. HBV genotype C was associated with lower remission rate and worse adverse outcomes [11].

8.3 Hepatitis D Virus Structure and Life Cycle

HDV is a spherical virus that is about 36 nm in diameter, which makes it the smallest among all known viruses that infect human [16, 17]. Its genome is a single-stranded circular RNA, containing 1700 nucleotides. Within the HDV genome organization, there are several open reading frames (ORFs) with only one known ORF to be actively transcribed. Two forms of hepatitis D antigens (HDAg) are produced from a single ORF located in the anti-genomic HDV RNA strand. They are known as small HDAg (S-HDAg) and large HDAg (L-HDAg) that consist of 195 and 214 amino acids, respectively. S-HDAg has an important role in the HDV RNA replication. L-HDAg is involved in the virion assembly but also inhibits HDV genome replication [18–20]. The envelope surrounding the genome is derived from HBV surface antigen. More specifically, it is derived from small hepatitis B surface antigen (S-HBsAg), medium hepatitis B surface antigen (M-HBsAg), and large hepatitis B surface antigen (L-HBsAg) [19].

As the HDV envelope is derived from HBsAg, HDV targets the human hepatocyte with similar mechanism of entry. Initially, HDV attaches with low affinity to the receptor heparan sulfate proteoglycans (HSPGs). Next, with high affinity, the pre-S1 domain—located in L-HBsAg—binds to the hepatitis B-specific receptor sodium taurocholate cotransporting polypeptide (NTCP) [17, 21, 22]. Once inside the cell, HDV is uncoated and moves to the nucleus where it is able to provide RNA

and ribonucleoprotein (RNP) synthesis with no help from HBV. Uniquely compared to other RNA viruses, HDV does not encode RNA polymerase to replicate its genome. It is able to replicate by a rolling circle mechanism while utilizing the host RNA polymerases to replicate. The remark here is that the cellular RNA polymerases are DNA-dependent, mainly RNA polymerases I and II, aiding the replication process to make genomic and anti-genomic circular RNA. Also, linear anti-genomic RNA is made that will serve as messenger RNA (mRNA) and eventually translated to HDAg [23, 24]. After translation, several modifications of both S-HDAg and L-HDAg take place with the utilization of host cell's enzymes as the HDV lacks the enzymes needed for this step. Those modifications are believed to confer diverse functions which aim to enhance replication and assembly process. One of the recognized modifications involved is the addition of 19 amino acids at the carboxy-terminal domain of the L-HDAg through using farnesyltransferase. Farnesylated L-HDAg facilitates the assembly of the virion through binding the HDV RNA and HBsAg. It is also an inhibitor of the HDV replication. HBV synthesizes HBsAg in abundance, more than it requires to its own assembly. The HBsAg synthesis occurs at the endoplasmic reticulum membrane and envelopes the HDV prior to its release from the hepatocytes [25–29] (Fig. 8.1).

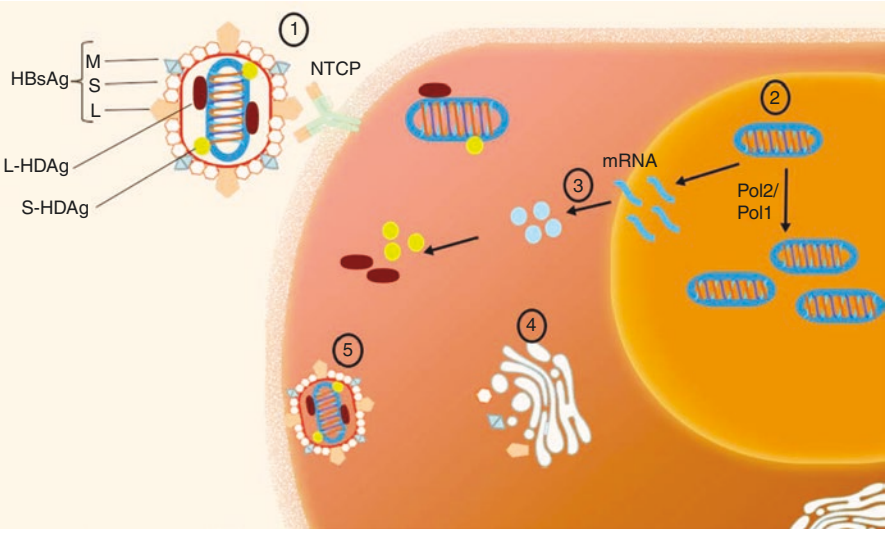

Fig. 8.1 Schematic diagram of HDV life cycle. (1) HDV attaches to the NTCP receptor through L-HBsAg and becomes uncoated once inside the hepatocyte. (2) RNA and RNP then move to the nucleus where it uses the cells' polymerases I and II for replication and synthesis of mRNA that will be (3) translated to HDAg and have further posttranslational modifications of L-HDAg and S-HDAg. (4) Parts of HBsAg, which present in abundance after its synthesis in the endoplasmic reticulum, will envelope (5) the HDV virion at the assembly step to be released from the hepatocyte. *HBsAg* hepatitis B surface antigen, *S* small, *M* medium, *L* large, *L-HDAg* large hepatitis D antigen, *S-HDAg* small hepatitis D antigen, *NTCP* sodium taurocholate cotransporting polypeptide, *RNP* ribonucleoprotein, *mRNA* messenger RNA, *Pol* polymerase

8.4 Role of Hepatitis B Virus in HDV Infection

As discussed above, HBV plays a vital role in the life cycle of HDV. While HBV is not involved in the replication of the HDV virion, it is needed for the hepatocyte attachment and the assembly given that HBsAg is the source of the HDV envelope. There are two forms of the HDV infections that patients present with: coinfection and superinfection. Coinfection occurs when both hepatitis B and D infections concomitantly occur in a patient leading to acute delta hepatitis infection. In this case, testing will be positive for HBV acute markers including anti-HBV immunoglobulin (Ig)M and also anti-HDV IgM. Typically, incubation period will take 3–7 weeks prior to patient presentation. Jaundice is presenting feature only in some patients [4, 30]. Early studies showed that coinfection is more associated with fulminant hepatitis compared with monoinfection with HBV. Study done in the United States by the mid-1980s showed 34% of HBV-infected patients had coinfection with HDV with close results in Europe [31, 32]. Superinfection is when HDV infect patients who are already with chronic HBV infection. This acute hepatitis can be hinted by worsening of previously stable chronic HBV infection or less often can present with acute liver failure [4]. HDV has a suppression role of HBV replication, yet production of HBsAg is maintained to serve as HDV envelope [33].

8.5 Chronic Hepatitis D

Progressing from acute to chronic hepatitis D (CHD) infection can result from either coinfection or superinfection. However, it is estimated that only 5% of coinfection will become chronic, while it is up to 90% of the case of superinfection cases. CHD progression to cirrhosis rate is more aggressive compared with CHB. Early reports showed progression of 80% of patients to cirrhosis over 6 years of observation [34]. However, a later study observed progression of 30% of HDV to cirrhosis over 28 years of follow-up. Persistent HDV replication was the only independent for mortality [35]. Hepatocellular carcinoma (HCC) risk is believed to be increased in HDV infection. One study suggested HDV increases HCC risk by threefold, while other study showed 15% end up with HCC diagnosis out of total 299 patients [35, 36].

8.6 Treatment

For the last 30 years, interferon alpha (INF) has been the treatment for CHD. With the pegylated interferon (peg-IFN), the frequency of injection changed from thrice to once weekly but has not made significant difference in the viral response rates. Conventionally, the treatment should not be shorter than 1 year as suggested by the initial studies done in the1990s followed by trials in the 2000s with overall virological response rate around 25% [37–39]. Losing HBsAg would be an ideal marker for treatment efficacy, but this is rarely achieved. Hence, currently quantitative HDV

Table 8.2 Recently proposed hepatitis D treatment options with their mechanisms of action and adverse effects

Medication	Mechanism of action	Adverse reactions
Myrcludex B	Cell entry inhibitor by blocking L-HDAg and NTCP binding	Transient elevated in bile acid and lipase
Lonafarnib	Inhibits farnesyltransferase and therefore inhibits virion assembly	Dose-dependent gastrointestinal side effects: nausea, diarrhea, bloating
REP 2139-Ca	NAP that might inhibit HBV attachment/entry and lead to reduction of HBsAg	Weakness, hair loss, dysphagia, and dysgeusia

L-HDAg large hepatitis D antigen, *NTCP* sodium taurocholate cotransporting polypeptide, *NAP* nucleic acid polymerase, *HBsAg* hepatitis B surface antigen

RNA has been used. Quantitative measurement of HBsAg was studied and was not shown to be a better surrogate marker of treatment response compared to quantitative HDV RNA [40]. However, using quantitative HDV RNA has its own limitations and low reliability. Hep Net International Delta Intervention Trial (HIDIT)-1 showed 9 out of 16 patients tested positive for HDV RNA during 5 years of follow-up after having peg-IFN therapy followed by being negative for HDV RNA. Based on these results, authors suggested avoiding term "sustained virological response" [41]. Additionally, standards for HDV RNA quantitative measurement assay were lacking until standards were published by the World Health Organization (WHO) in 2013. Such lacking might explain discrepancy in data regarding virological responses reported between studies [40].

Addition of HBV antiviral to the peg-IFN has been explored with disappointing results. This included combination of peg-IFN with ribavirin, adefovir, lamivudine, and tenofovir with no better viral response rate compared with peg-IFN [39, 42–44].

Other medications have been explored recently for effective HDV treatment, summarized in Table 8.2. Myrcludex B is a cell entry inhibitor that has been studied. It is a synthetic N-acylated pre-S1 lipopeptide that inhibits the cell entry by blocking the binding between the N-terminal pre-S1 domains of the L-HBsAg to NTCP [45]. Myrcludex B value was evaluated through phase Ib/IIa, randomized, open-label trial that included 24 patients randomized to three cohorts: the first received myrcludex B for 24 weeks followed by peg-IFN for 48 weeks, the second received myrcludex B combined with peg-IFN for 24 weeks followed by 24 weeks of peg-IFN alone, and the third received peg-IFN alone for 48 weeks. HDV RNA decreased at week 24 in 6 of 7 patients and became negative in 2 patients during the treatment in the myrcludex B group alone. Similar findings are observed in the group of peg-IFN. The group had combined peg-IFN and myrcludex B that had HDV RNA declined in all seven patients with five of them becoming HDV RNA negative at week 48. The study suggests synergetic effect when combining myrcludex B with peg-IFN [46]. It is generally well tolerated with reported asymptomatic elevated bile acid as well as transient elevation of serum lipase [47].

Lonafarnib (LNF), a farnesyltransferase inhibitor, is another medication that has been studied. It does its action through inhibition of farnesyltransferase that is involved in one of the posttranslational lipid modifications with prenylation of the L-HDAg and subsequently prevents the virion particle assembly [48]. In

proof-of-concept trial, LNF was given for 14 CHD patients for a duration of 28 days randomly assigned into two groups. They were given 100 mg and 200 mg twice daily for the first and second group, respectively. The decline of virus levels was significantly correlated to the drug level. Also, side effects were noted to be correlated to LNF dose. While some of the lower-dose group reported side effects, all of the higher-dose group reported side effects. Side effects were gastrointestinal related with diarrhea, nausea, and abdominal bloating [49]. Adding ritonavir led to boosting of LNF serum concentration up to fivefold. The combination of LNF, ritonavir, and peg-INF led to the reduction of the HDV RNA to more than three logs from baseline after total treatment of 8 weeks [50]. In LOWR HDV2 study, LNF 25 mg twice daily, ritonavir, and peg-IFN for 24 weeks led to the highest rate of HDV RNA negativity; 3/5 patient had negative HDV RNA. The results suggested synergistic activity of the combination with better tolerability given low dose of the LNF [50].

Nucleic acid polymers (NAP) have been evaluated for treatment of HDV. Its mechanism of action is unclear, but it is thought to utilize sequence-independent properties of phosphorothioate oligonucleotides to target viral replication with possible inhibition of attachment and/or entry of HBV. NAP REP9-AC was evaluated for duck HBV infection in the preclinical trials [51]. Further studies showed that NAP medications target HBsAg leading to decrease in its level or even negative HBsAg [52, 53]. REP 2339-Ca was studied in 12 CHD patients where it was given once weekly for 15 weeks as monotherapy and then combined with peg-IFN for additional 15 weeks. Peg-IFN monotherapy was then given for additional 33 weeks. Six patients showed >5 logs reduction in the HBsAg levels, and three patients showed around three logs reduction in HBsAg [54]. One-year follow-up showed that at least 4/5 patients with HBsAg negative loss at 24 weeks are maintaining HBsAg, HDV RNA, and HBV DNA loss [55]. Reported possible side effects included weakness, hair loss, dysphagia, and dysgeusia that were also suggested to be caused by heavy metal exposure endemic at the center site [52].

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Future Drugs in the Treatment of HBV

9

Aslıhan Demirel and Resat Ozaras

9.1 Introduction

Chronic hepatitis B (CHB) virus infection is a global public health problem. Although an effective vaccine is available over 30 years, the coverage rate remains low in endemic areas. There are currently 240 million chronic carriers of the virus who are at a high risk of developing hepatocellular carcinoma (HCC) [1], and hepatitis B is the major cause of cirrhosis and HCC globally, accounting for more than 750,000 deaths annually [2].

A new era has begun by the use of the first oral antiviral drug, lamivudine (LAM), to treat HBV infection in 1997. Long-term LAM treatment has provided viral suppression, liver enzyme normalization, fibrosis regression, and cirrhosis prevention [3]. However long-term use also caused the emergence of resistance and loss of sustained benefits of lamivudine monotherapy [4]. The introduction of newer antiviral agents, which are more potent, and with higher resistance barrier, entecavir and tenofovir, has provided lifelong suppression of HBV replication [5, 6]. However, HBV rebound remains a major challenge in more than 90% of patients after discontinuation of the drug [7]. Long-term use of HBV antivirals does not provide eradication [8]. The main favorable outcome of long-term antiviral use is the loss of HBsAg, which may allow the interruption of therapy, and is associated with a decreased risk of developing HCC, especially when it occurs at a young age [9, 10]. Unfortunately, current treatments achieve HBsAg clearance only in 10% of patients [10, 11]. We aimed to review new molecules against HBV using virus- or host-mediated mechanisms (Tables 9.1 and 9.2).

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Table 9.1 Drugs for HBV and their targets at preclinical level

Mechanism of action	Drug	Target
Core inhibitor	AT-61, Bay 41-4109	Nucleocapsid assembly
DNA cleavage	ZFN, TALEN, CRISPR/cas	cccDNA
cccDNA formation inhibition	CCC-0975, CCC-0346	cccDNA
cccDNA degradation		Lymphotoxin-β receptor agonist
Antisense oligonucleotides	ASOs	RNA

cccDNA covalently closed circular DNA, *ZFN* zinc-finger nuclease, *TALEN* transcription activator-like effector nuclease, *CRISPR/cas* clustered regulatory interspaced short palindromic repeats/CRISPR-associated system, *ASO* antisense oligonucleotide

Table 9.2 Drugs for HBV and their targets at clinical level

Mechanism of action	Drug	Target	Clinical phase
Polymerase inhibition	Tenofovir alafenamide	HBV polymerase	Phase 3
	Besifovir		Phase 3
HBV entry inhibition	Myrcludex	NTCP	Phase 2a
Core inhibition	NVR 3-778	Nucleocapsid assembly	Phase 2
Apoptosis induction	Birinapant	CIAPs	Phase 1
Inhibition of HBsAg release		REP-2139	Phase 2
Exogenous interferon stimulation	TLR agonist-GS9620	TLR7	Phase 2
Stimulation of interferon response	RIG-I	SB 9200	Phase 2
Therapeutic vaccination	Adaptive immunity	Tarmogen (GS4774)	Phase 2b
		ABX203	Phase 2b

NTCP sodium taurocholate cotransporting polypeptide, *CIAP* cellular inhibitor of apoptosis proteins, *TLR* toll like receptor, *RIG* retinoic acid-inducible gene

9.2 Aims of HBV Therapies

Current concept of long-term viral suppression has not eradicated the virus, and there appears a need to develop new antiviral strategies achieving more than viral suppression. The new strategy defines an "HBV cure" more than "functional":

- *The functional cure* is defined as HBsAg loss with or without anti-HB seroconversion, with undetectable serum DNA, but persistence of cccDNA [12]. Lowering liver cccDNA levels, inactivating cccDNA-directed transcription to prevent viral replication, and inducing a remission of liver disease are the endpoints of functional cure. These endpoints can also allow treatment interruption. This cure definition has been a major aim in the clinical trials. However, aiming an "inactive carrier" status by this concept is probably not enough to get free of developing HCC risk. Individuals who clear HBV infection after a chronic infection still have a greater risk of developing HCC than individuals who have not been infected with HBV [13, 14]. Additionally, patients that resolved an HBV infection may experience reactivation following immunosuppressive therapy for cancer, autoimmune disease, or organ transplantation [15].

- *Complete cure* is described as the physical elimination of cccDNA, in addition to functional cure. Current antiviral treatment strategies do not provide a complete cure. This strategy may facilitate the implementation of antiviral treatments for a finite period of time, may reduce their cost, may increase drug accessibility to populations in highly endemic areas, and may have an impact on development of cirrhosis and HCC.

9.3 HBV Life Cycle

HBV is a virus from the *Hepadnaviridae* family and can only infect primates and humans. It is a partially double-stranded circular DNA surrounded by the envelope that interacts with hepatocytes. It binds to heparin sulfate proteoglycans on hepatocytes, and then the pre-S1 lipopeptide of the large envelope protein binds to its receptor on the hepatocyte, the bile acid transporter sodium taurocholate cotransporting polypeptide (NCTP). Subsequently, the virus enters the cytoplasm by endocytosis [16, 17].

Following identification of NCTP as a critical mediator of HBV entry, entry inhibitors were developed and tried in HBV inhibition [18, 19]. Myristoylated preS peptide (myrcludex B), a lipopeptide derived from the preS1 domain of the HBV envelope, was shown to prevent HBV infection in hepatocyte cultures, as well as in vivo in liver humanized uPA/SCID mice [20]. The drugs that inhibit the function of NTCP, such as cyclosporine, were shown to also decrease viral infectivity in cell culture models [19].

The virus uncoats within the cytoplasm, and then the nucleocapsid containing the viral genome goes to the nucleus where the relaxed DNA is converted to covalently closed circular DNA (cccDNA). cccDNA is stable within hepatocytes, and it represents a template for producing mRNAs mediated by host polymerase II. Approximately, 1–5 cccDNA copies are found in each infected hepatocyte [21].

The pregenomic RNA acts as a template for the replication of full genome. It encodes the viral core protein (HBcAg) and the viral polymerase, which is a reverse transcriptase.

In the presence of sufficient envelope molecules in the hepatocyte, nucleocapsids are directed to their secretory pathway, and cccDNA amplification decreases. Mature virion exits hepatocyte and may infect other cells or goes to the nucleus for replenishing the cccDNA pool [16, 17]. HBsAg is synthesized much more than needed for viral enveloping purposes and released. Immune tolerance is suggested to be mediated by this disproportionate anti-HBs response.

9.4 HBsAg Release Inhibitors

HBsAg is a critical antigen, and its clearance is a key step in the control of HBV infection. It is clear that development of anti-HBs means the control of the disease. Presence of HBsAg protein provides an immunosuppression against HBV. Clearance of HBsAg is a target of future hepatitis B therapeutics [16, 22].

Synthetic oligonucleotides added a sulfur atom gain high stability and may interact with glycoproteins. These hydrophobic nucleic acid polymers (NAPs) function as broad-spectrum viral attachment/entry inhibitors. NAPs are taken up by hepatocytes and block entry and formation of subviral particles but not virion production [23], and several NAPs (REP-2055, REP-2139, and REP-2165) were shown to block the release of HBsAg [24].

A randomized, controlled trial assessed the safety and efficacy of NAP HBsAg release inhibitors REP 2139 and REP 2165 (Replicor) in combination with tenofovir disoproxil fumarate (TDF) and Peg-IFN-α-2a in treatment-naive HBeAg-negative CHB patients. More than 3–4 $\log_{10}$ reductions were noted in 7 out of 9 patients who were given REP 2139 and in 4 of 9 patients who received REP 2165 in combination with TDF and Peg-IFN [25]. Serum HBsAg decline continued over time from baseline >1 log in 29, >2 log in 25, and >4 log in 19 patients. In the polymer groups, most patients showed HBsAg clearance and nearly half developed anti-HBs. The study showed that NAPs in combination with antivirals might allow the control of HBV infection. It appears a promising option though a longer administration would be needed to show their sustained efficacy and safety in HBV treatment.

Besides NAPs, some other molecules that can inhibit HBsAg secretion have been studied: a benzimidazole compound BM601 [26] and triazol-o-pyrimidine derivatives [27].

BM601, a secretion inhibitor functioning at the level of virion, and HBsAg secretion pathway inhibit intracellular relocalization of the HBV surface protein to the Golgi apparatus, thus decreasing HBsAg and HBV release without affecting HBeAg secretion. Immunofluorescence analysis suggests that BM601 might inhibit virion and HBsAg secretion by interfering surface protein aggregation in trans-Golgi apparatus. It has no effect on transcription, protein production, nucleocapsid formation, or intracellular HBV DNA synthesis.

A triazol-o-pyrimidine derivative, HBF-0259, is a selective, potent inhibitor of secretion of both subviral and DNA-containing viral particles that targets the secretion of particles bearing HBV structural glycoproteins [27]. Its inhibitory activity was also confirmed by transfection of HBsAg, showing that the action of it is not dependent on those of other viral proteins. HBF-0259 had no effect on HBV DNA synthesis, demonstrating that inhibition is independent of viral genomic replication.

9.5 HBV Entry Inhibitors

After the identification of NCTP, as a key bile acid transporter expressed on hepatocytes and a critical mediator for HBV cell entry [17], HBV entry blockers are studied as a promising target. Following binding of the pre-S1 region of the HBsAg to NTCP on the hepatocyte surface, HBV-receptor complex is endocytosed into the cytoplasm of the hepatocyte.

Small molecules that block the binding to the NTCP receptor have been studied in patients with CHB. Myrcludex B is a lipopeptide that mimics the pre-S1 domain

and blocks the new infection of hepatocytes by HBV throughout inhibitory competition [21]. Myrcludex B is a HBV pre-S1-derived lipoprotein polypeptide that competes with HBV for binding of the pre-S1 protein of HBsAg to the NTCP, preventing HBV entry. Since hepatitis D virus (HDV) is a defective virus that uses the HBV surface proteins to enter and exit the hepatocyte, myrcludex was studied in HBV and HDV patients.

A phase 1b/2a study has been reported in 24 CHB patients with elevated levels of ALT and HBV DNA [28]. The patients with chronic hepatitis D (CHD) infection received myrcludex B or Peg-IFN-α-2a or their combination and were evaluated for virological and biochemical response and tolerability of the study drugs at weeks 12 and 24. Myrcludex was well tolerated. HBsAg levels remained unchanged; however HDV RNA significantly declined at week 24 in all groups. HDV RNA became negative in two patients, each in the myrcludex B and Peg-IFN-α-2a groups, and in five patients of the myrcludex B + Peg-IFN-α-2a group. HBV DNA was significantly reduced at week 24 in the myrcludex B + Peg-IFN-α-2a cohort suggesting a strong synergistic effect of myrcludex B and Peg-IFN-α-2a on both HDV and HBV. Myrcludex B has a significant effect on HDV RNA serum levels and induced ALT normalization. Synergistic antiviral effects on HDV RNA and HBV DNA in the myrcludex-IFN group indicated a benefit of the combination of entry inhibition with Peg-IFN-α-2a in the treatment of CHB patients.

9.6 Stimulation of Antiviral Effector Cells

Toll-like receptors (TLR) are a class of proteins expressed on macrophages and dendritic cells that recognize structurally conserved molecules derived from microorganisms. TLR-7 is a pattern-recognition receptor signaling which induces the expression of inflammatory genes of host defense including antiviral cytokine response and engagement of innate and adaptive effector cells [29]. Short-term administration of GS-9620, an oral agent of TLR-7, induced the production of select chemokines and cytokines including interferon-alpha. In chimpanzees, 8-week administration of GS-9620 reduced HBV-DNA levels more than 2 logs and also decreased the levels of HBeAg and HBsAg more than 50% [30]. Numbers of HBV antigen-positive hepatocytes decreased as hepatocytes' apoptosis increased.

In woodchucks, 4–8 weeks' treatment of GS-9620 induced rapid and sustained decrease in serum woodchuck hepatitis virus (WHV) DNA (6.2 logs), WHV-cccDNA, and WHV-RNA [31]. It caused loss of WHVsAg, and in a subset of animals, it induced a sustained antibody response against WHVsAg. The treatment reduced the incidence of HCC from 71% (in placebo group) to 8%. The use GS-9620 was associated with reversible increases in liver enzymes and thrombocytopenia and induced intrahepatic CD8+ T cells, B cells, NK cells, and interferon response transcription signals.

GS-9620 appears to induce interferon intrahepatically without systemic interferon induction. Induction of interferon response at the infected site may augment the effect of interferon without systemic side effects. However TLR agonists do not

appear to provide a successful control of HBV infection as monotherapy. Its place in combination treatments needs to be clarified.

Modulators of innate immunity other than TLR agonists are under investigation. SB 9200, an activator of viral sensor proteins, retinoic acid inducible gene I (RIG-I), and nucleotide-binding oligomerization domain-containing protein 2 (NOD2), leads to IFN-mediated antiviral immune responses in virus-infected cells. SB 9200 treatment (30 mg/kg, 12 weeks) in woodchuck hepatitis model resulted in 3.7 log reduction in WHV DNA and 1.6 log reduction in WHVsAg levels [32]. It was associated with lower hepatic levels of WHV nucleic acids and reduced liver inflammation. The antiviral effects are correlated with the induction of interferon-alpha and interferon-beta and IFN-stimulated genes in the blood and liver.

Promoting elimination of infected cells may have a therapeutic role. Cellular inhibitors of apoptotic proteins (cIAPs) prevent clearance of HBV-infected cells through TNF-mediated death of the infected cells. Inhibitors of cIAPs are known as second mitochondrial-derived activators of caspase (SMAC) mimetics. Birinapant, a SMAC mimetic, rapidly reduced serum HBV DNA and HBsAg levels and promoted the elimination of HBcAg-containing hepatocytes in immunocompetent mouse model [33]. With combined use, birinapant enhanced the ability of entecavir to reduce viral DNA production.

9.7 Therapeutic Vaccines

Therapeutic vaccination is a potential treatment option researched in some cancers and viral infections. The aim of therapeutic vaccination in chronic HBV infection is to restore exhausted T-cell response to HBV antigens. Vaccine strategies include (a) surface antigen, (b) HLA-A2-restricted HBV peptides, and (c) DNA vectors [34].

Despite inducing strong immune response in unaffected individuals, HBV vaccines failed to show a benefit in HBV-infected patients. This failure has been explained by the exhaustion of both CD4+ and CD8+ cells in HBV patients which is caused by prolonged exposure to HBV antigens [35]. High level of antigens during a chronic active infection may be decreasing the effectiveness of a therapeutic vaccine [36].

Reducing viral antigens by antiviral treatment (nucleoside or nucleotide analogs) may increase the efficacy. However long-term effective suppression of viral replication may not associate with a significant decrease in HBsAg levels (and HBeAg levels in HBeAg-positive patients).

Therapeutic vaccination studies have used optimized antigens. GS-4774 is a recombinant heat-killed yeast-based immunotherapy engineered to express HBV proteins (HBsAg, HBcAg, and hepatitis B × protein) [37]. In an open-label study, differing doses of GS-4774 were administered to healthy individuals. It was safe and well tolerated. It provided HBV-specific immune responses (determined by interferon gamma, ELISPOT, and lymphocyte proliferation assay) at all doses evaluated [37].

In a phase II clinical study, GS-4774 was given to 178 chronic hepatitis B patients without cirrhosis who were suppressed on an oral antiviral ≥1 year [38]. GS-4774 was well tolerated; however, it did not provide significant reduction in serum HBsAg levels.

Five HBeAg-positive patients receiving GS-4774 experienced HBeAg loss versus none in the control group. These patients given GS-4774 had HBsAg decline which was ≥0.5 log IU/mL; however no patients experienced loss of HBsAg.

9.8 DNA Vaccines

The goal of DNA vaccine is to boost the host's immune system to control the infection. DNA-based therapeutic vaccines are being tested, and promising results have been obtained in animal models [39, 40]. DNA vaccines induce both humoral and cellular immune responses, including cytotoxic and Th1 responses [41]. Genetic immunization of HLA-A2 transgenic mice generated immune response similar to that observed in patients with resolved HBV infection and self-limited disease, suggesting that responses induced by DNA immunization may have the same immune potential as those developing during natural HBV infection [42]. The first phase I clinical trial of therapeutic DNA vaccination was performed in chronic HBV patients to test whether HBV DNA vaccine encoding HBV envelope proteins could restore T-cell responsiveness [43]. Ten patients with chronic hepatitis B nonresponder to approved treatments for HBV infection were given four injections of DNA vaccine. HBV-specific T-cell responses were evaluated by proliferation, ELISPOT assays, and tetramer staining. A decrease in viral load was observed in six patients, and HBeAg seroconversion was noted in two patients. These two seroconverted patients had the lowest viral load at the beginning of the trial, emphasizing the role of low HBV DNA titers for successful immunotherapy. HBV DNA vaccination was found safe and led to the induction of IFN-gamma-secreting T cells specific to envelope-derived epitopes of HBV and cytotoxic CD8+ T cells.

A pilot study showed that a DNA vaccine against the hepatitis B virus (HBV) is safe and effective in 39 HBeAg-positive patients with chronic hepatitis B and that the HBV-specific T-cell responses induced by DNA vaccination under LAM treatment showed a correlation with the suppression of viral replication in patients with CHB [44].

A phase IIb trial assessed the efficacy and safety of HBV DNA vaccine versus placebo for sequential combination therapy with LAM in patients with chronic hepatitis B. Two hundred and twenty-five patients were randomized to receive either LAM + vaccine or LAM + placebo [45]. LAM treatment lasted 72 weeks. The primary endpoint was the rate of undetectable HBV DNA or HBeAg seroconversion (loss of HBeAg and presence of HBeAb). Although more patients had a decrease in HBV DNA >2 log IU/mL in the vaccine group at week 12 after end of treatment (EOT) compared with the control group, the primary endpoint was not achieved using the HBV DNA vaccine. Among patients with a viral load <1000 copies/mL at week 12, more patients achieved HBeAg seroconversion in the vaccine group than

among controls at week 36 after EOT, as well as less virological breakthrough and YMDD mutations suggesting that the HBV DNA vaccine could only be beneficial in subjects that have achieved initial virological response under antiviral treatment.

9.9 Anti-cccDNA Agents

Long-term complete suppression of viral replication with potent HBV polymerase inhibitors showed that episomic cccDNA or chromosomic integrated HBV DNA is still transcribed into RNA and/or sustained in HBsAg secretion. The eradication strategies should target the episomic and integrated forms of the HBV genome in the infected hepatocytes [46].

Control of acute hepatitis B infection is maintained by cytotoxic T cells. These cells destroy infected hepatocytes and also secrete antiviral cytokines that inhibit HBV gene expression and replication. On the other hand, clearance of viral cccDNA mechanisms includes secretion of interferon gamma [47], through upregulating the apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) deaminases, which promote non-hepatotoxic degradation of cccDNA [48].

DNA cleavage enzymes targeting the cccDNA in chronic hepatitis B include TAL effector endonucleases (TALENs), homing endonucleases or meganucleases, zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated system 9 (Cas9) proteins [49].

The CRISPR/Cas9 system was used to target the HBV genome and efficiently inhibit HBV infection, synthesizing four single-guide RNAs (sgRNAs) targeting the conserved regions of HBV [50]. The expression of sgRNAS with Cas9 decreased the viral production in Huh7 cells and also in HBV replication cell HepG2.2.15. CRISPR/Cas9 direct cleavage and cleavage-mediated mutagenesis occurred in HBV cccDNA of transfected cells. In mouse model carrying HBV cccDNA, injection of sgRNA-Cas9 plasmids resulted in the low level of cccDNA and HBV protein, suggesting that CRISPR/Cas9 system can target HBV cccDNA and inhibit HBV replication. HBV cleavage using CRISPR/Cas9 has provided reduction of cccDNA by about 92% in culture cell [51].

A potential challenge using CRISPR/Cas9 system is that HBV DNA can be found in various cells other than hepatocytes and many cell lines are permissive for HBV replication [52]. For eradication of HBV, it is essential to deliver the nucleases to every last infected cell in hepatic and extrahepatic viral reservoirs. The second challenge is the potential off-target effects with CRISPR/Cas9 system, which seems to occur in a high rate [53]. The third concern is the integrated linearized HBV DNA, that may be cut by the CRISPR/Cas9. The subgenomic HBV DNA fragments can be integrated into host genome commonly in patients with hepatocellular carcinoma or chronic infection, and the cleavage of integrated viral DNA can cause indels in the host genome, which may disrupt the host gene function.

The fourth concern is selecting certain target sites in the HBV genome: some patients under nucleos(t)ide analog treatment develop therapy-resistant HBV variants. Since the CRISPR/Cas9 system relies on precise sequence recognition, in high

viral load settings, de novo mutations cause loss of the CRISPR/Cas9 recognition sites, and a therapy-resistant pool of viruses potentially can reestablish the infection.

9.10 New Antivirals

9.10.1 Tenofovir Alafenamide

Tenofovir alafenamide (TAF), a prodrug of tenofovir (TFV), was approved for the treatment of CHB infection. It is a potent inhibitor of HBV replication at a low dose and showed high intracellular concentration and more than 90% lower systemic TFV concentration than TDF. In two identically designed, randomized, double-blind, phase 3 studies for HBeAg-positive [54] and HBeAg-negative [55] patients (primary analysis, 48 weeks), TAF 25 mg orally once daily was noninferior to TDF 300 mg in achieving an HBV DNA level <29 IU/mL at week 48. Once-daily TAF 25 mg provided effective and sustained viral suppression (120-week analysis) and was well tolerated. No resistance to TAF was seen through week 96. The frequency of HBeAg or hepatitis B surface antigen loss was also comparable. TAF provided a significantly higher ALT normalization rate than TDF, based on the American Association for the Study of Liver Diseases criteria (male: ALT ≤30 U/L and female: ALT ≤19 U/L). TAF has associated with a significantly lower decrease in the estimated glomerular filtration rate level than did patients treated with TDF. Furthermore, the decrease of hip and spine bone mineral density was significantly less in the TAF group.

Longer term follow-up data are needed to assess the better safety data of TAF.

9.11 Besifovir

Besifovir (LB80380) is a new oral acyclic nucleotide phosphonate. It is effective in suppressing HBV replication at doses above 60 mg. It reaches maximal concentration in plasma in 2 h, and its elimination half-life is 3 h. Besifovir and its metabolites are mainly excreted through the kidneys [56] and are safe in terms of renal and bone toxicity. The main adverse event is carnitine depletion, which affects the majority of the patients on besifovir requiring carnitine supplementation.

In a study comparing the safety and antiviral activity of two doses of besifovir with entecavir 0.5 mg daily in CHB patients, 114 patients were randomized to receive besifovir 90 mg daily ($n = 36$), besifovir 150 mg daily ($n = 39$), or entecavir 0.5 mg daily ($n = 39$). At week 48, the proportion of patients achieving undetectable HBV DNA, the serum mean log10 HBV DNA changes from baseline for the HBeAg-positive and the HBeAg-negative patients were comparable. There were no differences in the proportions of patients achieving normalization of alanine aminotransferase and HBeAg seroconversion among groups. In 94% of patients on

besifovir, serum L-carnitine had decreased and returned to normal with carnitine supplement [57].

In the follow-up study, patients receiving besifovir 90 mg (n = 31) and 150 mg (n = 28) and entecavir 0.5 mg (n = 30) were monitored for liver biochemistry, viral serology, HBV DNA levels, development of drug resistance mutations, and adverse events throughout 96 weeks of treatment [58]. Virological and biochemical responses were comparable, and no patient developed drug-resistant mutations. Besifovir was well tolerated and also had a good clinical safety profile.

Its antiviral activity and safety was studied in chronic hepatitis B (CHB) patients with lamivudine-resistant virus for a period of 12 weeks [59]. Sixty-five patients with lamivudine-resistant virus were given five ascending daily doses (30, 60, 90, 150, 240 mg) of besifovir. It was given combined with lamivudine for the first 4 weeks, followed by 8 weeks of besifovir monotherapy, and then followed by another 24 weeks of adefovir. The extent of the HBV DNA reduction at week 12 was dose-dependent. In 93% of patients, HBV DNA decreased by >2 log [10] copies/mL by week 12, and HBV DNA suppression was maintained during the 24 weeks of adefovir treatment. Besifovir at doses of up to 240 mg was found safe, well tolerated, and effective at reducing viral load in CHB patients with lamivudine-resistant virus for a period of 12 weeks [59].

Besifovir is an acyclic nucleotide phosphonate effective in HBV DNA suppression for both treatment-naive and lamivudine-resistant CHB patients. More data are needed for long-term use and particularly for special populations.

Conclusion

Beginning with IFN and Peg-IFN, HBV treatment improved with the use of potent and safe antivirals. Long-term use of antivirals is associated with viral, biochemical, and clinical improvement. However, the next step, a "complete cure," is not possible with these drugs. Combinations of viral- and host-mediated drugs are promising to sustain this goal in the near future.

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