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MINIREVIEW

Hepatitis B Virus: From Diagnosis to Treatment

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Abstract

Hepatitis B infection is still a global concern progressing as acute-chronic hepatitis, severe liver failure, and death. The infection is most widely transmitted from the infected mother to a child, with infected blood and body fluids. Pregnant women, adolescents, and all adults at high risk of chronic infection are recommended to be screened for hepatitis B infection. The initial analysis includes serological tests that allow differentiation of acute and chronic hepatitis. Molecular assays performed provide detection and quantification of viral DNA, genotyping, drug resistance, and precore/core mutation analysis to confirm infection and monitor disease progression in chronic hepatitis B patients. All patients with chronic hepatitis B should be treated with antiviral medications and regularly monitored for efficient treatment. The current treatment is based on nucleos(t)ide analogs and pegylated interferons that save lives by decreasing liver cancer death, liver transplant, slow or reverse the progression of liver disease as well as the virus infectivity.

K e y w o r d s: hepatitis B virus (HBV), serology, nucleic acid testing, antiviral treatment

Introduction

Although there are effective vaccines and treatment strategies against hepatitis B (HB), it is still a significant health concern worldwide that can present in acute, permanent, severe liver failure and cancer forms resulting in high morbidity and death. Globally, 2 billion people have been infected with HB. There is an estimated more than 292 million people living with chronic hepatitis B (CHB) infection worldwide. The global HB surface antigen (HBsAg) positivity was estimated to be 3.9% in 2016 (HBF 2018a; Razavi-Shearer et al. 2018). Annually, 887,000 deaths occur each year due to HB and related illnesses, which were mainly related to advanced liver fibrosis and cirrhosis (WHO 2019a). The risk and progression of chronic infection are age-dependent and occur mainly in immunocompromised individuals. It is known that the younger an infected person is, the higher the risk of developing CHB infection. Although acute infection is generally cleared in immunocompetent, chronic infection develops in approximately 90% of infants, 30–50% of children aged five years, and 5–10% adults (Jefferies et al. 2018; Terrault et al. 2018; Hyun Kim and Ray Kim 2018; CDC 2020a). CHB infection is classified in five different clinical stages according to the HBsAg positivity (i) hepatitis B e antigen (HBe Ag) positive infection; ii) HBe Ag-positive hepatitis; iii) HBe Ag-negative infection; iv) HBe Ag-negative hepatitis, and v) HBsAg-negative stages that reflect the interaction between HBV replication and the immune system. Occult hepatitis B infection (OBI) is another sub-category that is characterized by a detectable HBV DNA with undetectable HBs antigen or serological markers of the previous viral exposure in the plasma (Malagnino et al. 2018). OBI is associated with severe liver damage and hepatocellular carcinoma (HCC), and poses a risk for individuals, especially in blood transfusional infection, HBV reactivation, chronic liver disease, and HCC (Roman 2018; Wang et al. 2020).

HBV spreads from mother to child, after exposure to infected blood or body fluids or sexual contact. In addition, HBV can survive and remain infective for several weeks on moist surfaces at room temperature (de Almeida et al. 2015; Terrault et al. 2018; Than

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et al. 2019). Despite being transmitted vertically from infected mother to a child, having sex with an infected partner, contacting the infected needle sticks or sharp object injuries, HBV is not transmitted through breastfeeding, hugging, kissing, coughing, and sneezing, or sharing food and drink (CDC 2020b).

HBV vaccination is the main and the safest precaution from being exposed to the virus (WHO 2019a). HBV vaccine has been administered since 1982 and leads to a dramatic decline in HBV infections globally (Van Damme 2016; WHO 2017a). The vaccine against HBV is available and can be administered from birth to older ages. ENGERIX-B®, RECOMBIVAX HB®, HEPLISAV-™ are three single-antigen vaccines while PEDIARIX®, TWINRIX® are two combination vaccines that are licensed for use in the United States (CDC 2020c). The recommended schedules for HBV vaccine are as follows: three-dose vaccination at 0, 1–2, and 6–18 months of a monovalent HepB (Heplisav-B) vaccine for infants; three-dose vaccination at 0, 1–2, and 6 months for the unvaccinated person, and alternative two doses of Recombivax HB at 11–15 years; two dose vaccinations of HepB at 18 years, and three-dose vaccination with Twinrix. Twinrix is a combination of HepA and HepB vaccine to be administered at 18 years and older (Dynavax 2018; CDC 2020c).

To reduce the spread of infection, the World Health Organization (WHO) European Region recommends the Universal Hepatitis B vaccination programs for infants born from HBsAg-positive mothers, all infants within the first 24 hours after birth, children up to 18 years old, and adults from the groups of high risk for HBV infection, i.e., people with infected sexual partners, homosexual men, hemodialysis patients, injecting drug users, and healthcare workers (WHO 2019b). In May 2016, the WHO addressed the first Global Health Sector Strategy on viral hepatitis 2016–2021 to end new CHB infections by 90% and reduce the mortality rate by 65% by 2030 (WHO 2016).

One of the most severe forms of hepatitis infections is hepatitis delta, also known as hepatitis D. The infection can develop in people infected with HBV (Gilman et al. 2019). Globally, an estimated of 5% of HBV are also infected with the hepatitis D virus (HDV) (WHO 2020). Individuals with HBsAg positive, the elevated alanine aminotransferase (ALT) level with undetectable HBV DNA should be screened for HDV antibodies and HDV RNA (Gilman et al. 2019). HBV-HDV co-infection is severe, and the risk of liver disease progression, liver cancer, early decompensated cirrhosis, and liver failure is higher (WHO 2020). Although there is no effective vaccine against HDV, vaccination against HBV also plays a significant role in protecting delta infection.

HBV, is a partially double-stranded DNA virus of 3.2 kilobases, and it transforms from pregenomic ribonucleic acid (RNA) to DNA by reverse transcription during its life cycle. The genome consists of an outer lipid envelope and inner nucleocapsid core encoded by four overlapping open reading frames, named C, X, P, and S (McNaughton et al. 2019; Wang et al. 2019). Although it is known as a virus with high replication ability, due to the absence of the proofreading reverse transcriptase enzyme, the naturally occurring mutations may arise in different genome regions. These regions may encode for polymerase, surface antigen, core/precore promoter, and comprise the X genes that significantly influence HBsAg expression and progression of HCC (Shaha et al. 2018; Arikan et al. 2019). Additionally, due to the complete overlapping of *pol* and *S* genes, drug resistance and nucleos(t)ide resistance mutations occurring in the *pol* gene can lead to changes in its product HBsAg (Kırdar et al. 2019).

The mutations in the gene C that encode for precore and core proteins are significantly correlated with liver disease progression in CHB patients (Al-Qahtani et al. 2018). The changes in the amino acid sequences: W28*, G29D, G1896A, G1899A, G1862T in the precore proteins that affect HBeAg, and F24Y, E64D, E77Q, A80I/T/V, L116I, E180A in the core proteins mutations are commonly identified and related to clinical severity (Kim et al. 2016; Wu et al. 2018).

The global genotype distribution of HBV differs in different geographic regions and areas worldwide (Rajoriya et al. 2017). HBV is classified into ten genotypes (A-J), and 40 sub-genotypes till today, according to the phylogenetic analysis (Rajoriya et al. 2017). Genotype A is predominant in Northwest Europe, North America, and Africa; genotypes B and C prevail in East Asia and far East countries, while genotype D is widespread worldwide (Arikan et al. 2016; Kmet Lunacek et al. 2017). Genotype E occurs only in West Africa (Ambachew et al. 2018). Genotype F has been found in Central and South America, and genotype G has been reported in Turkey, France, Canada, Vietnam, Germany, and America. Genotypes H and I have been isolated in Central America, Mexico, Vietnam, and Laos; the recently identified genotype J has only been found in Japan (Mahmood et al. 2016). Fig. 1 illustrates the distribution of HBV genotypes (A-J) worldwide. Additionally, the rate of HBV infection also differs in geographic regions. According to the HBsAg positivity, the prevalence of HBV infection is classified into low (< 2), low-intermediate (2–4.9%), high intermediate (5–7.9%), and high (≥ 8) (Kim et al. 2018). HBsAg is of the main concern, especially for the Western Pacific regions with 6.25 seropositivity. The global prevalence of CHB infection in the Eastern Mediterranean Region, South-East Asia Region, and European Region is estimated at 3.3%, 2.0%, and 1.6% respectively (Fig. 2) (WHO 2019a). HBV genotypes and sub-genotypes have been reported to

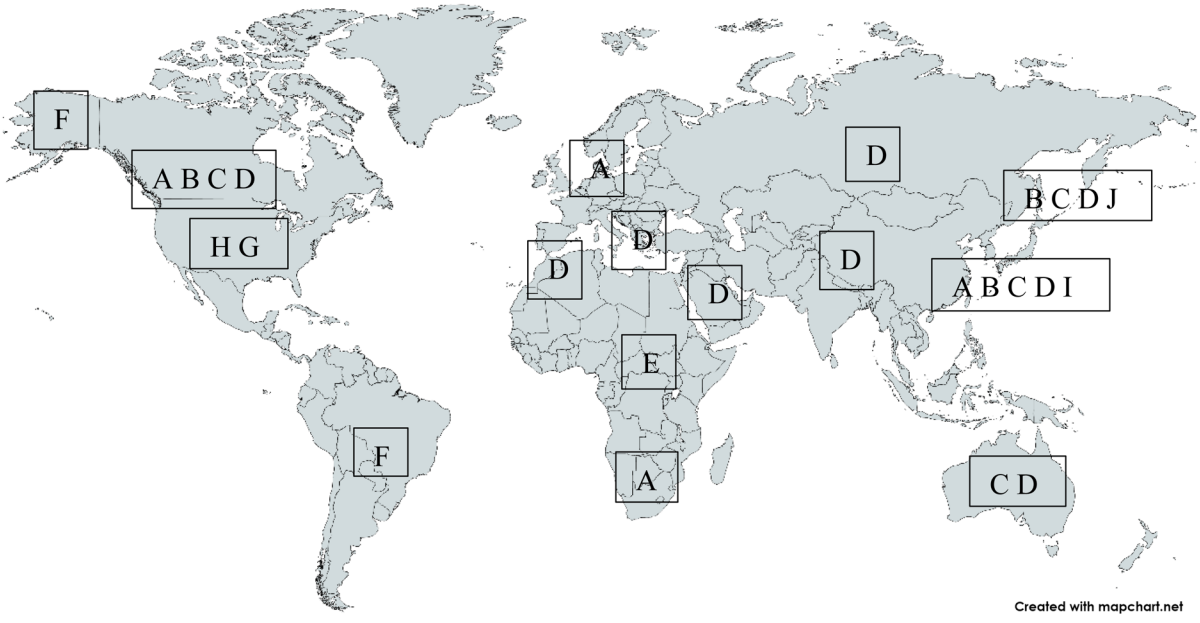

Fig. 1. Hepatitis B virus genotypes (A-J) (Paudel and Suvedi 2019).

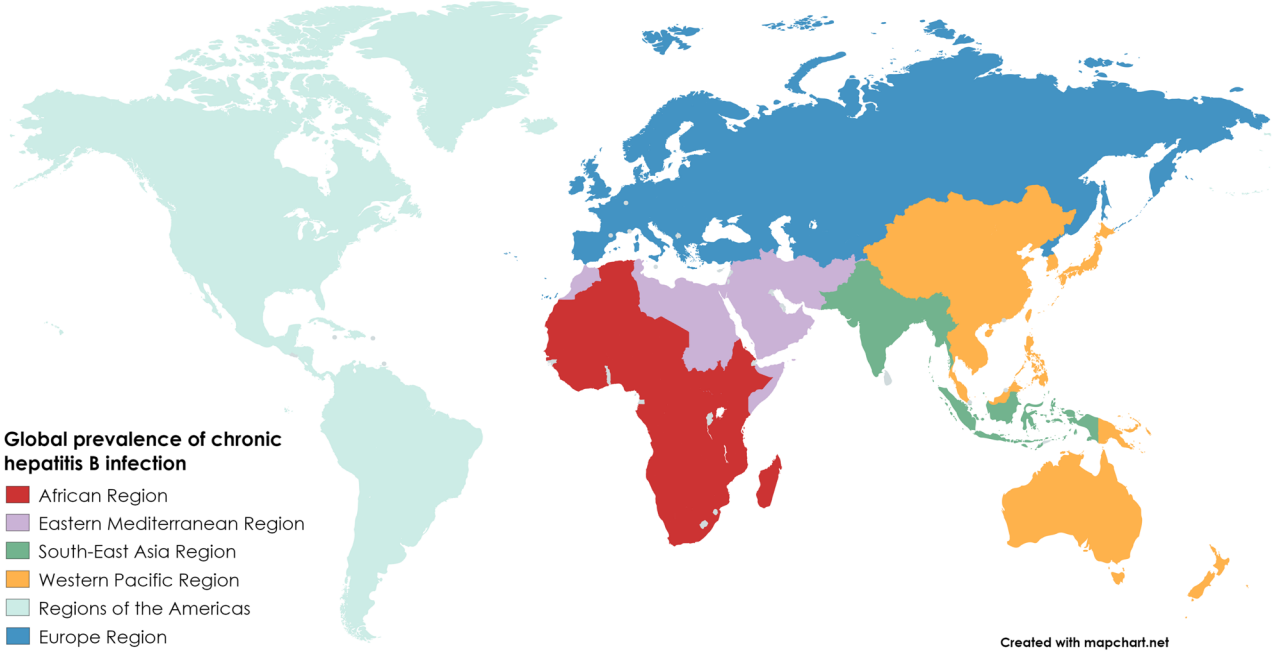

Fig. 2. Global prevalence of chronic hepatitis B infection (WHO 2019a).

effectively affect disease transmission, progression, and treatment outcome (Kmet Lunacek et al. 2017). Therefore, identifying HBV mutations and genotypes is essential for both disease manifestation and identification of individuals at risk of infection progression.

This review describes virological assays, including serological and molecular techniques for diagnosing HB infection and updates on the most effective treatment strategies against the virus for the prevention of liver progression and cirrhosis in chronic HBV carriers.

Laboratory diagnosis of hepatitis B virus

Initial assessment of HBV infection begins with patient history, physical examination, evaluation of liver disease activity, and interpretation of different hepatitis markers and/or their combinations such as HBsAg, HB core antigen (HBcAg), HBeAg, HB surface antibody (anti-HBs/HBsAb), HB core antibody (anti-HBc), anti-HBc IgM, HB e antibody (anti-HBe), and focus on the detection of antigens and antibodies (WHO 2017b). The

Hepatitis B Foundation (HBF) recommends screening all adults for HB with the triple serological marker panel that involves HBsAg, anti-HBs, and anti-HBc total (HBF 2018b). To classify the phases of the infection in HBV infected patients, the followings should be performed: i) the assays for HBsAg, HBeAg/anti-HBe, HBV DNA; ii) liver blood tests including aspartate aminotransferase (AST), alanine transaminase (ALT), and iii) transient elastography (Fibroscan) as a noninvasive test or needle liver biopsy as an invasive method for the presence of cirrhosis (EASL 2017).

HBV serological markers

Various serological assays can detect virus-specific antigens and antibodies which appear during and after HBV infection. These tests are used to determine whether a patient is susceptible to infection or immune due to passed infection or HBV vaccination (CDC 2020d). Currently, various serological diagnostic assays, including rapid diagnostic tests (RDTs) and laboratory-based immunoassays, such as enzyme immunoassays (EIAs), chemiluminescence immunoassays (CLIAs), electrochemiluminescence immunoassays (ECLs) are used (WHO 2017b). These tests can be performed with serum, plasma and/or capillary/venous whole blood and oral fluid specimens to detect the presence of antigens or antibodies against the virus with high analytical sensitivity, specificity, and accuracy (WHO 2017b). Dried blood spot (DBS) specimen may be an alternative type of specimen in settings where blood taking and RDTs laboratory testing are not available and/or accessible or from a person with poor venous access (WHO 2017b). The laboratory reports are given qualitatively or quantitatively as international units (IU) or signal per cutoff (S/Co) values (Terrault et al. 2018).

HBsAg. HBsAg is an envelope protein that is expressed on the surface of the infectious virion called Dane particles. The detection of HBsAg in the serum indicates the current HBV infection. The HBsAg positivity can be considered with a second surface antigen test before further evaluating HBV DNA in the regions with HBsAg prevalence < 0.4 (WHO 2017b). The incubation period for hepatitis is 90 days (60–150 days) after exposure to HBV, and HBsAg appears in the blood for about six weeks (1–10 weeks) after the first exposure to the virus (CDC 2005). During the immunological window period, HBsAg may disappear rapidly without the appearance of HB surface antibodies, and the IgM antibody is the only evidence of the infection during this period (Otero et al. 2018). If HBsAg positivity persists after six months, it implies the progression of a chronic infection. The quantitative immunochemiluminescence analysis is performed to evaluate HBsAg levels of CHB patients and is a useful marker for interferon alfa (IFN-α)-treated CHB patients with HBeAg negative (EASL 2017).

Anti-HBc. Detection of anti-HBc antigens confirms exposure to HBV and indicates acute, chronic, or resolved infection but not vaccine-induced immunity (Terrault et al. 2018). The presence of IgM antibodies, together with HBsAg positivity, generally indicates the acute infection that generally persists positive for not more than six months (Jackson et al. 2018). Individuals who are core-antibody positive and HB surface-antibody negative are chronically infected and show a decreased risk of HBV reactivation. There is also no clinical benefit of vaccination for the group of individuals who are positive only for core antibodies due to exposure to HBV or people who are positive for anti-HBc and anti-HBs due to immune control (Cholongitas et al. 2018; Ganczak et al. 2019).

HBeAg and anti-HBe. The presence of HBeAg correlated with active viral replication is indicative of the contagiousness of the patient. Whereas, the appearance of anti-HBe indicates the low level of viral replication and is strong evidence for infection resolution (CDC 2005). These tests are often used to determine the CHB infection phase (EASL 2017).

Anti-HBs or HBsAb. The presence of anti-HBs indicates the recovery and immunization against HB infection either by HB vaccine or prior infection. People whose first-degree relatives or sex partners are chronic carriers are recommended to be vaccinated if their triple serological screening tests are negative (EASL 2017). The anti-HBs titer should be ≥ 10 mIU/ml in order to be protective (Dini et al. 2017).

Biochemical parameters and fibrosis markers

The severity of liver fibrosis is assessed using biochemical parameters, including AST and ALT, which are enzymes released from the liver in response to damage and disease. The other biochemical parameters are gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, serum albumin gamma globulin, full blood count, and prothrombin time (PT) (EASL 2017). When biochemical and HBV markers are inconclusive, then invasive and noninvasive methods are used to assess the stage of liver damage (EASL 2017). Since liver biopsy is an invasive, costly, and painful procedure compared to other techniques, various non-invasive methods are preferred to predict the stage of liver fibrosis and the presence of cirrhosis in CHB patients. The WHO recommends AST to platelet ratio index (APRI) calculated according to the formula: APRI = [AST/AST ULN (upper limit of normal) $\times 100$/platelet count (10^9/l] to estimate the stage

of liver fibrosis (WHO 2017b). TE is another noninvasive method; however, due to its limitations such as high cost, inaccurate results with elevated ALT levels, restriction with liver necro-inflammation, and obesity, the WHO recommends the APRI index as a relatively accurate method for predicting advanced liver fibrosis (EASL 2017; WHO 2017b; Huang et al. 2019). It has been recommended that 40 IU/ml as ULN value should be used in the APRI formula (WHO 2017b). ALT levels should also be measured in CHB patients as it correlates with disease severity. According to the WHO guidelines, the ULN ALT level is below 30 U/l and 19 U/l for men and women, respectively (WHO 2017b).

Molecular assays

The molecular diagnostic techniques are used for HBV DNA quantification, genotyping, detection of drug resistance mutations, and precore/core mutation analysis (Villar et al. 2015) Currently, UltraQual HBV PCR Assay, COBAS AmpliScreen HBV Test, Procleix Ultrio Assay, Procleix Ultrio Plus Assay, and COBAS TaqScreen MPX Test are FDA approved nucleic acids amplification tests (NATs) used for diagnosis of HB infection (FDA 2019)

HBV DNA quantification. HBV DNA quantification. HBV DNA by NAT is used to determine the infectivity of individuals and infectivity of HBsAg positive pregnant women to prevent mother to child transmission risk and reach a decision whether to treat diseases. The HBV DNA measurement with molecular technologies enables early detection of people at risk before HBsAg emerges and rules out OBI (Aghasadeghi et al. 2020). The testing of HBV DNA is also used to monitor the treatment response in CHB patients (WHO 2017b). The viral load of HBV is usually measured either in IU/ml or copies/ml by ultraviolet (UV) spectrophotometry, real-time PCR (rt-PCR), digital PCR, loop-mediated isothermal amplification (LAMP), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA) as well as electrochemical, quartz crystal microbalance, microcantilever, and surface plasmon resonance biosensors (Liu and Yao 2015; WHO 2017b; Al-Sadeq et al. 2019; Sayan et al. 2019; Arikan and Sayan 2020). The HBV DNA level represents the disease progression, long-term results of CHB infection, and the treatment's achievement to prevent the progression of HCC. The measurement of the level of HBV DNA is recommended to be performed with a more sensitive rt-PCR assay with 10 IU/ml detection limit (EASL 2017).

HBV DNA genotyping, drug resistance, preC/core mutations. To date, ten genotypes of HBV, A to J, and more than 40 sub-genotypes that differ > 8% and 4–8% nucleotide divergence in the genome, respectively, have been identified (Al-Sadeq et al. 2019). Different genotypes and sub-genotypes show different geographical distribution and are correlated with persistence of viral load, risk of developing cirrhosis, HBsAg seroclearance, antiviral therapy response, and prognosis due to the presence of mutations (Paudel and Suvedi 2019; Wang et al. 2019). It has been known that patients infected with HBV genotype A are more likely to develop CHB infection than patients infected with genotype B, associated with the development of antiviral resistance or genotype C, associated with acute hepatitis (EASL 2017; Wang et al. 2019). HBV genotyping is not required for initial diagnosis; however, genome sequencing for evaluation of HBV genotypes and drug resistance mutations are useful parameters for patients at risk of developing HCC in order to monitor an efficient therapy (EASL 2017).

There are many genotyping systems, including reverse hybridization, restriction fragment polymorphism (RFLP), multiplex nested PCR or real-time PCR, oligonucleotide microarray chips, reverse dot blot, restriction fragment mass polymorphism (RFMP), and invader assay (Fletcher et al. 2019). Molecular identification of HBV genotypes could also be done by sequencing the whole HBV genome, followed by phylogenetic analysis. Phylogenetic analysis is performed by constructing a phylogenetic tree with nucleotide sequences of the entire HBV genome to characterize different HBV genotypes and subgenotypes. A web-based program available through the National Center of Biotechnology Information is used that enables us to make the comparison between the newly obtained HBV sequences with the reference sequences available in GenBank. BLAST or FASTA are tools for searching similar sequences available in the EBI web site (http://www.ebi.ac.uk/Tools/homology.html) (Schreiber 2007).

The whole-genome sequences of different HBV strains are aligned, and the phylogenetic tree is constructed using distance methods including neighboring joining (NJ), un-weighted pair-group using arithmetic averages (UPGMA) or character-based techniques including maximum parsimony (MP) and maximum likelihood (ML) (Rozanov et al. 2004; Schreiber 2007). The similarity method is considered the "gold standard" approach for genotyping and sub-genotyping and can be performed on individual genes on the HBV S gene instead of the complete genome. However, the partial sequencing (HBV S gene) allows determining only the HBV genotype, not the HBV sub-genotype (Pourkarim et al. 2014).

Apart from HBV genotyping, HBV drug resistance mutations are also tested by using sequence-based assays. Several sequence-based assays such as line

probe assay have been developed; however, due to its accuracy, the Sanger sequencing of the PCR amplicon from the HBV reverse transcriptase region is accepted as a "gold standard". Real-time PCR reduces the risk of contamination due to its applicability and speed. Therefore, it is widely used to detect drug resistance mutations (Mou et al. 2016).

Treatment of hepatitis B virus infection

The treatment's primary goal is to save lives by decreasing liver cancer death, liver transplant, slow or reverse liver disease progression, and infectivity (Terrault et al. 2018). Nowadays, there are currently seven approved drugs: two formulations of IFN-standard and pegylated interferon (Peg IFN), and five nucleos(t)ide analogs (NUC): lamivudine (LAM), telbivudine, entecavir (ETV), adefovir (ADV), and tenofovir (TDF) (Lok et al. 2016). Guidelines suggest either standard or Peg IFN-α (IFN-a) immunomodulators such as standard or Peg IFN-α (IFN-a), or NUCs such as LAM adefovirdipivoxil, ETV, TDF, or telbivudine as treatment alternatives for CHB patients (Manzoor et al. 2015).

IFN-α is a host defense against HBV infections by interferon-stimulated genes (ISGs), which have immoral antiviral functions against a variety of viruses (Liang et al. 2015). Some studies have shown that in 76–94% of individuals, the treatment response is extended and is associated with more confirmatory clinical outcomes in terms of liver-related complications and survival (Niederau et al. 1996).

IFN-α-2a/b was the first certified treatment choice for CHB infection, and it replaced the standard IFN-α-2b because of pharmacokinetic properties. The pegylation is used to increase the half-life of interferon (Lok and McMahon 2009). The study reported that the treatment accomplishment percentage of Pegasys is 24% compared to 12% standard interferon (Cooksley et al. 2003). LAM is a cytidine NUC that prevents the reverse transcriptase enzyme of HBV; however, the resistance rates due to mutations in the YMDD locus of HBV polymerase is high (Chan et al. 2007; Manzoor et al. 2015). Hepsera is the tradename for adefovirdipivoxil, and ADV is a NUC. Hepsera has some side effects, including rash, swelling of the throat, lips, tongue, face, difficulty breathing, and proximal kidney tubular dysfunction (Ho et al. 2015). Despite side effects, the resistance rate of ADV is lower compared to LAM (Innaimo et al. 1997). Baraclude or ETV is a potent inhibitor of HBV's DNA polymerase enzyme, and resistance is rarely observed (Lai et al. 2006; Manzoor 2015).

Recommendations for the treatment of HBV/HIV (Human immunodeficiency)-coinfected persons are based on the WHO 2013 combine guidelines, which was updated in 2015, on the use of antiretroviral drugs for treating and preventing HIV infection. Interferon or Peg IFN as antiviral therapy was eliminated from these guidelines because their use is restricted in LMICs due to its high cost and significant adverse effects that need careful monitoring (WHO 2015). In addition, Peg-IFN was found to have only about 20% sustained non-treatment response in terms of viral suppression and low HBsAg loss and seroconversion rates (Lin et al. 2016).

New generation NUCs act by inhibiting HBV DNA replication by normalizing ALT levels. Unfortunately, NUC's use relies on long-term therapy and induces drug-related mutant infection (Tsuge et al. 2013). NUCs rarely eliminate all of the chronic HBV infection and HBV replication (Jeng et al. 2010). In recent years, NUCs or IFN monotherapy or combination therapy in CHB treatment have been investigated to minimize the therapies (Scaglione and Lok 2012). Since the combination of NUCs and IFN can inhibit more than one step of the HBV lifecycle than mainly targeting the reverse transcriptase step by NUCs monotherapy (Wei et al. 2015). Benefits and limitations of antiviral drugs used against for HBV infection are given in Table I (Abdul Basit et al. 2017).

The chemical name of TAF is L-alanine, [(S)[[(1R)-2-(6-amino-9H-purine-9yl)-1 (methylethoxy]methyl] phenoxyphosphinyl]-1-methyl ethyl ester, (2E)-2-butenedioate (Gilead Sciences 2015). TAF pharmacokinetics are linear and dose-dependent. According to the 28-day phase 1b study, which assessed antiviral activity, safety, and pharmacokinetics in CHB patients, TAF was found to be well-tolerated and safe. However, some side-effects, including headache, nausea, fatigue, cough, and constipation, were also reported. The antiviral effect of TAF over the 4 weeks was demonstrated by changes in serum HBV DNA levels of the treated patient groups in the same study (Agarwal et al. 2015).

Conclusions

The review summarized the serological, molecular diagnosis techniques, and current treatment strategies for HBV infection. The initial diagnosis with the serological assays is used to detect HBsAg and other HBV antigens and antibodies. Next, molecular assays are performed to verify the first step of diagnosis, quantify HBV viral load, and identify HBV genotypes and determine drug resistance mutation. Although molecular assays are frequently preferred due to their high sensitivity, high cost, the need for experienced personnel, and numerous equipment for analysis are the main limitations of molecular analysis. In the future, there is a need for new technologies such as biosensors that provide faster time to result with not only high specific-

ity, sensitivity, and low cost but also low false positive/negative ratio that can play a significant role in screening, diagnosis, and management of HBV infection. Additional technologies may also help to develop new treatment targets. A combination of the HBV therapies and small-molecule drugs or biologics will be necessary to control the HBV infection effectively.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Abdul Basit S, Dawood A, Ryan J, Gish R. Tenofovir alafenamide for the treatment of chronic hepatitis B virus infection. Expert Rev Clin Pharmacol. 2017 Jul 03;10(7):707–716. https://doi.org/10.1080/17512433.2017.1323633

Agarwal K, Fung SK, Nguyen TT, Cheng W, Sicard E, Ryder SD, Flaherty JF, Lawson E, Zhao S, Subramanian GM, et al. Twenty-eight day safety, antiviral activity, and pharmacokinetics of tenofovir alafenamide for treatment of chronic hepatitis B infection. J Hepatol. 2015 Mar;62(3):533–540. https://doi.org/10.1016/j.jhep.2014.10.035

Aghasadeghi MR, Aghakhani A, Mamishi S, Bidari-Zerehpoosh F, Haghi Ashtiani MT, Sabeti S, Banifazl M, Karami A, Bavand A, Ramezani A. No evidence of occult HBV infection in population born after mass vaccination. Wien Med Wochenschr. 2020 Jun; 170(9–10):218–223. https://doi.org/10.1007/s10354-020-00748-z

Al-Qahtani AA, Al-Anazi MR, Nazir N, Abdo AA, Sanai FM, Al-Hamoudi WK, Alswat KA, Al-Ashgar HI, Khan MQ, Albenmousa A, et al. The correlation between hepatitis B virus Precore/core mutations and the progression of severe liver disease. Front Cell Infect Microbiol. 2018 Oct 22;8:355. https://doi.org/10.3389/fcimb.2018.00355

Al-Sadeq DW, Taleb SA, Zaied RE, Fahad SM, Smatti MK, Rizeq BR, Al Thani AA, Yassine HM, Nasrallah GK. Hepatitis B virus molecular epidemiology, host virus interaction, co infection, and laboratory diagnosis in the MENA Region: an Update. Pathogens. 2019 May 11;8(2):63. https://doi.org/10.3390/pathogens8020063

Ambachew H, Zheng M, Pappoe F, Shen J, Xu Y. Genotyping and sero-virological characterization of hepatitis B virus (HBV) in blood donors, Southern Ethiopia. PLoS One. 2018 Feb 20;13(2):e0193177. https://doi.org/10.1371/journal.pone.0193177

Arikan A, Şanlidağ T, Süer K, Sayan M, Akçali S, Güler E. [Molecular epidemiology of hepatitis B virus in Northern Cyprus] (in Turkish). Mikrobiyol Bul. 2016 Jan 7;50(1):86–93. https://doi.org/10.5578/mb.10292

Arikan A, Sayan M, Sanlidag T, Suer K, Akcali S, Guvenir M. Evaluation of the pol/S gene overlapping mutations in chronic hepatitis B patients in Northern Cyprus. Pol J Microbiol. 2019 Sep; 68(3):317–322. https://doi.org/10.33073/pjm-2019-034

Arikan A, Sayan M. Comparison of Qiagen and Iontek hepatitis B virus DNA polymerase chain reaction quantification kits in chronic hepatitis B patients infected with hepatitis B genotype D. Viral Hepat J. 2020 Apr 28;26(1):9–13. https://doi.org/10.4274/vhd.galenos.2020.2019.0024

CDC. Interpretation of hepatitis B serological test results [Internet]. Atlanta (USA): Centers for Disease Control and Prevention; 2005 [cited 2020]. Available from https://www.cdc.gov/HEPATITIS/HBV/PDFs/SerologicChartv8.pdf

CDC. Morbidity and mortality weekly report recommendations and report. Centers for Disease Control and Prevention. 2018;67(1):1–32.

CDC. Interpretation of hepatitis B serological tests results [Internet]. Atlanta (USA): Centers for Disease Control and Prevention; 2020a [cited 2020 Mar]. Available from https://www.cdc.gov/hepatitis/hbv/index.htm

CDC. Hepatitis B questions and answers for public health professionals [Internet]. Atlanta (USA): Centers for Disease Control and Prevention; 2020b [cited 2020 Mar]. Available from https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm

CDC. Immunization schedules [Internet]. Atlanta (USA): Centers for Disease Control and Prevention; 2020c [cited 2020 Feb]. Available from https://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fvaccines%2Fschedules%2Fhcp%2Fchild-adolescent.html

CDC. Interpretation of hepatitis B serological Tests results [Internet]. Atlanta (USA): Centers for Disease Control and Prevention; 2020d [cited 2020 Mar]. Available from https://www.cdc.gov/hepatitis/hbv/index.htm

Chan HL, Wang H, Niu J, Chim AM, Sung JJ. Two-year lamivudine treatment for hepatitis B e antigen-negative chronic hepatitis B: a double-blind, placebo-controlled trial. Antivir Ther. 2007;12(3): 345–353.

Cholongitas E, Haidich AB, Apostolidou-Kiouti F, Chalevas P, Papatheodoridis GV. Hepatitis B virus reactivation in HBsAg-negative, anti-HBc-positive patients receiving immunosuppressive therapy: a systematic review. Ann Gastroenterol. 2018;31(4):480–490. https://doi.org/10.20524/aog.2018.0266

Cooksley WGE, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwandee T, Chutaputti A, Chang WY, Zahm FE, Pluck N. Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat. 2003 Jul;10(4):298–305. https://doi.org/10.1046/j.1365-2893.2003.00450.x

de Almeida RW, Espírito-Santo MP, Sousa PSF, de Almeida AJ, Lampe E, Lewis-Ximenez LL. Hepatitis B virus DNA stability in plasma samples under short-term storage at 42°C. Braz J Med Biol Res. 2015 Jun;48(6):553–556. https://doi.org/10.1590/1414-431x20144040

Dini G, Toletone A, Barberis I, Debarbieri N, Massa E, Paganino C, Bersi F, Montecucco A, Alicino C, Durando P. Persistence of protective anti-HBs antibody levels and anamnestic response to HBV booster vaccination: A cross-sectional study among healthcare students 20 years following the universal immunization campaign in Italy. Hum Vaccin Immunother. 2017 Feb;13(2):440–444. https://doi.org/10.1080/21645515.2017.1264788

Dynavax. Hepatitis B Recombinant vaccine [Internet]. 2018 [cited 2018 Feb]. Available from https://investors.dynavax.com/static-files/f93ec0ea-c184-4afb-b2d6-98ebd0030a79

EASL. HBV 2017 Clinical practice guidelines on the management of hepatitis B virus infection [Internet]. Geneva (Switzerland): European Association for the Study of the Liver; 2017 [cited 2020 Mar]. Available from https://easl.eu/wp-content/uploads/2018/10/HepB-English-report.pdf

FDA. Hepatitis B [Internet]. Silver Spring (USA): Food and Drug Administration; 2019 [cited 2019 Apr 9]. Available from https://www.fda.gov/vaccines-blood-biologics/blood-donor-screening/hepatitis-b

Fletcher GJ, Eapen CE, Abraham P. Hepatitis B genotyping: the utility for the clinicians. Indian J Gastroenterol. 2019 Oct 15;39(4): 315–320. https://doi.org/10.1007/s12664-019-00995-y

Ganczak M, Topczewska K, Budnik-Szymoniuk M, Korzeń M. Seroprevalence of anti-HBc, risk factors of occupationally acquired HBV infection and HBV vaccination among hospital staff in Poland: a multicenter study. BMC Public Health. 2019 Dec;19(1):298. https://doi.org/10.1186/s12889-019-6628-1

Gilead Sciences. Prescribing information: VEMLIDY® (tenofovir alafenamide) [Internet]. Foster City (USA): Gilead Sciences; 2015 [cited 2020 Sep]. Available from https://www.gilead.com/-/media/files/pdfs/medicines/liver-disease/vemlidy/vemlidy_pi.pdf

Gilman C, Heller T, Koh C. Chronic hepatitis delta: A state-of-the-art review and new therapies. World J Gastroenterol. 2019 Aug 28; 25(32):4580–4597. https://doi.org/10.3748/wjg.v25.i32.4580

HBF. About the Hepatitis B Foundation. Doylestown (USA): Hepatitis B Foundation; 2018a [cited 2020 Sep 3]. Available from https://www.hepb.org/assets/Uploads/About-the-Hepatitis-B-Foundation-Fact-Sheet-final-8-28-18.pdf

HBF. Hepatitis B test [Internet]. Doylestown (USA): Hepatitis B Foundation; 2018b [cited 2020 Sep 3]. Available from https://www.hepb.org/prevention-and-diagnosis/diagnosis/hbv-blood-tests

Ho EY, Yau T, Rousseau F, Heathcote EJ, Lau GKK. Preemptive adefovir versus lamivudine for prevention of hepatitis B reactivation in chronic hepatitis B patients undergoing chemotherapy. Hepatol Int. 2015 Apr;9(2):224–230. https://doi.org/10.1007/s12072-015-9612-6

Huang D, Lin T, Wang S, Cheng L, Xie L, Lu Y, Chen M, Zhu L, Shi J. The liver fibrosis index is superior to the APRI and FIB-4 for predicting liver fibrosis in chronic hepatitis B patients in China. BMC Infect Dis. 2019 Dec;19(1):878. https://doi.org/10.1186/s12879-019-4459-4

Hyun Kim B, Ray Kim W. Epidemiology of hepatitis B virus infection in the United States. Clin Liver Dis (Hoboken). 2018 Jul;12(1): 1–4. https://doi.org/10.1002/cld.732

Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. Antimicrob Agents Chemother. 1997 Jul;41(7):1444–1448. https://doi.org/10.1128/AAC.41.7.1444

Jackson K, Locarnini S, Gish R. Diagnostics of Hepatitis B virus: standard of care and investigational. Clin Liver Dis (Hoboken). 2018 Jul;12(1):5–11. https://doi.org/10.1002/cld.729

Jefferies M, Rauff B, Rashid H, Lam T, Rafiq S. Update on global epidemiology of viral hepatitis and preventive strategies. World J Clin Cases. 2018 Nov 6;6(13):589–599. https://doi.org/10.12998/wjcc.v6.i13.589

Jeng WJ, Sheen IS, Liaw YF. Hepatitis B virus DNA level predicts hepatic decompensation in patients with acute exacerbation of chronic hepatitis B. Clin Gastroenterol Hepatol. 2010 Jun;8(6):541–545. https://doi.org/10.1016/j.cgh.2010.02.023

Kim H, Lee SA, Do SY, Kim BJ. Precore/core region mutations of hepatitis B virus related to clinical severity. World J Gastroenterol. 2016;22(17):4287–4296. https://doi.org/10.3748/wjg.v22.i17.4287

Kırdar S, Yaşa MH, Sayan M, Aydın N. [HBV pol/S gene mutations in chronic hepatitis B patients receiving nucleoside/nucleotide analogues treatment] (in Turkish). Mikrobiyol Bul. 2019;53(2):144–155. https://doi.org/10.5578/mb.67816

Kmet Lunacek N, Poljak M, Meglic-Volkar J, Rajter M, Prah J, Lesnicar G, Selic Kurincic T, Baklan Z, Remec T, Pal E, et al. Epidemiological, virological and clinical characteristics of hepatitis B virus genotypes in chronically infected persons in Slovenia. Hepat Mon. 2017 Mar 04;17(3):e43838. https://doi.org/10.5812/hepatmon.43838

Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, et al.; BEHoLD AI463027 Study Group. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2006 Mar 09;354(10):1011–1020. https://doi.org/10.1056/NEJMoa051287

Liang TJ, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT, Locarnini S, Zoulim F, Chang KM, Lok AS. Present and future therapies of hepatitis B: from discovery to cure. Hepatology. 2015 Dec;62(6):1893–1908. https://doi.org/10.1002/hep.28025

Lin CL, Yang HC, Kao JH. Hepatitis B virus: new therapeutic perspectives. Liver Int. 2016 Jan;36 Suppl 1:85–92. https://doi.org/10.1111/liv.13003

Liu YP, Yao CY. Rapid and quantitative detection of hepatitis B virus. World J Gastroenterol. 2015;21(42):11954–11963. https://doi.org/10.3748/wjg.v21.i42.11954

Lok AS, McMahon BJ, Brown RS Jr, Wong JB, Ahmed AT, Farah W, Almasri J, Alahdab F, Benkhadra K, Mouchli MA, et al. Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. Hepatology. 2016 Jan;63(1):284–306. https://doi.org/10.1002/hep.28280

Lok ASF, McMahon BJ. Chronic hepatitis B: Update 2009. Hepatology. 2009 Sep;50(3):661–662. https://doi.org/10.1002/hep.23190

Mahmood M, Anwar MA, Khanum A, Zaman N, Raza A. Distribution and clinical significance of hepatitis B virus genotypes in Pakistan. BMC Gastroenterol. 2016 Dec;16(1):104. https://doi.org/10.1186/s12876-016-0513-5

Malagnino V, Fofana DB, Lacombe K, Gozlan J. Occult hepatitis B virus infection: An old entity with novel clinical involvements. IDSA. 2018;5(10):ofy227. https://doi.org/10.1093/ofid/ofy227

Manzoor S, Saalim M, Imran M, Resham S, Ashraf J. Hepatitis B virus therapy: what's the future holding for us? World J Gastroenterol. 2015;21(44):12558–12575. https://doi.org/10.3748/wjg.v21.i44.12558

McNaughton AL, D'Arienzo V, Ansari MA, Lumley SF, Littlejohn M, Revill P, McKeating JA, Matthews PC. Insights from deep sequencing of the HBV genome-Inique,tiny, and misunderstood. Gastroenterology. 2019 Jan;156(2):384–399. https://doi.org/10.1053/j.gastro.2018.07.058

Mou Y, Athar MA, Wu Y, Xu Y, Wu J, Xu Z, Hayder Z, Khan S, Idrees M, Nasir MI, et al. Detection of anti-hepatitis B virus drug resistance mutations based o multicolor melting curve analysis. J Clin Microbiol. 2016 Nov;54(11):2661–2668. https://doi.org/10.1128/JCM.00439-16

Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, Häussinger D. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med. 1996 May 30;334(22):1422–1427. https://doi.org/10.1056/NEJM199605303342202

Otero W, Parga J, Gastelbondo J. Serology of hepatitis B virus: multiple scenarios and multiple exams. Rev Col Gastroenterol. 2018; 33(4):404–413. http://doi.org/10.22516/25007440.327

Paudel D, Suvedi S. Hepatitis B genotyping and clinical implications. London (UK): IntechOpen; 2019. http://doi.org/10.5772/intechopen.82492

Pourkarim MR, Amini-Bavil-Olyaee S, Kurbanov F, Van Ranst M, Tacke F. Molecular identification of hepatitis B virus genotypes/subgenotypes: revised classification hurdles and updated resolutions. World J Gastroenterol. 2014;20(23):7152–7168. https://doi.org/10.3748/wjg.v20.i23.7152

Rajoriya N, Combet C, Zoulim F, Janssen HLA. How viral genetic variants and genotypes influence disease and treatment outcome of chronic hepatitis B. Time for an individualised approach? J Hepatol. 2017 Dec;67(6):1281–1297. https://doi.org/10.1016/j.jhep.2017.07.011

Razavi-Shearer D, Gamkrelidze I, Nguyen MH, Chen D-S, Van Damme P, Abbas Z, Abdulla M, Abou Rached A, Adda D, Aho I, et al.; Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol. 2018 Jun; 3(6):383–403. https://doi.org/10.1016/S2468-1253(18)30056-6

Roman S. Occult hepatitis B and other unexplored risk factors for hepatocellular carcinoma in Latin America. Ann Hepatol. 2018 Jul; 17(4):541–543. https://doi.org/10.5604/01.3001.0012.0914

Rozanov M, Plikat U, Chappey C, Kochergin A, Tatusova T. A web-based genotyping resource for viral sequences. Nucleic Acids Res. 2004 Jul 01;32 Web Server:W654–W659. https://doi.org/10.1093/nar/gkh419

Sayan M, Arikan A, Sanlidag T. Comparison of performance characteristics of DxN VERIS system versus Qiagen PCR for HBV genotype D and HCV genotype 1b quantification. Pol J Microbiol. 2019;68(1):139–143. https://doi.org/10.21307/pjm-2019-008

Scaglione SJ, Lok AS. Effectiveness of hepatitis B treatment in clinical practice. Gastroenterol. 2012;142(6):1360–1368. https://doi.org/10.1053/j.gastro.2012.01.044

Schreiber F. Phylogenetic sequence analysis. Methods course bioinformatics. 2007/08 [cited 2020]. Available from http://gobics.de/fabian/molbio_course/data/handout_phylogeny.pdf

Shaha M, Sarker PK, Hossain MS, Das KC, Jahan M, Dey SK, Tabassum S, Hashem A, Salimullah M. Analysis of the complete genome of hepatitis B virus subgenotype C2 isolate NHB17965 from a HBV infected patient. F1000 Res. 2018;7:1023. https://doi.org/10.12688/f1000research.15090.3

Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018 Apr;67(4):1560–1599. https://doi.org/10.1002/hep.29800

Than TT, Jo E, Todt D, Nguyen PH, Steinmann J, Steinmann E, Windisch MP. High environmental stability of Hepatitis B virus and inactivation requirements for chemical biocides. J Infect Dis. 2019 Mar 15;219(7):1044–1048. https://doi.org/10.1093/infdis/jiy620

Tsuge M, Murakami E, Imamura M, Abe H, Miki D, Hiraga M, Takahashi S, et al. Serum HBV RNA and HBeAg are useful markers for the safe discontinuation of NA treatments in CHB patients. J Gastroenterol. 2013;48:1188–1204. https://doi.org/10.1007/s00535-012-0737-2

Van Damme P. Long-term protection after hepatitis B vaccine. J Infect Dis. 2016 Jul 01;214(1):1–3. https://doi.org/10.1093/infdis/jiv750

Villar LM, Cruz HM, Barbosa JR, Bezerra CS, Portilho MM, Scalioni LP. Update on hepatitis B and C virus diagnosis. World J Virol. 2015;4(4):323–342. https://doi.org/10.5501/wjv.v4.i4.323

Wang J, Zhang P, Zeng J, Du P, Zheng X, Ye X, Zhu W, Fu Y, Candotti D, Allain JP, et al. Occurrence of occult hepatitis B virus infection associated with envelope protein mutations according to anti-HBs carriage in blood donors. Int J Infect Dis. 2020 Mar; 92:38–45. https://doi.org/10.1016/j.ijid.2019.12.026

Wang W, Shu Y, Bao H, Zhao W, Wang W, Wang Q, Lei X, Cui D, Yan Z. Genotypes and hot spot mutations of hepatitis B virus in northwest Chinese population and its correlation with disease progression. BioMed Res Int. 2019 Dec 10;2019:1–9. https://doi.org/10.1155/2019/3890962

Wei W, Wu Q, Zhou J, Kong Y, You H. A better antiviral efficacy found in nucleos(t)ide analog (NA) combinations with interferon therapy than NA monotherapy for HBeAg positive chronic hepatitis B:A meta-analysis. Int J Environ Res Public Health. 2015 Aug 21; 12(8):10039–10055. https://doi.org/10.3390/ijerph120810039

WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection [Internet]. Geneva (Switzerland): World Health Organization; 2015 [cited 2020 Mar]. Available from https://www.ncbi.nlm.nih.gov/books/NBK305553/pdf/Bookshelf_NBK305553.pdf

WHO. Global Health Section Strategy on Viral Hepatitis 2016–2021 [Internet]. Geneva (Switzerland): World Health Organization; 2016 [cited 2020 Jun]. Available from https://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf;jsessionid=BB8238CF4BF67C1B4A8A93329842962F?sequence=1

WHO. Hepatitis B vaccination has dramatically reduced infection rates among children in Europe, but more is needed to achieve elimination [Internet]. Geneva (Switzerland): World Health Organization; 2017a [cited 2020 Mar]. Available from http://www.euro.who.int/en/health-topics/communicable-diseases/hepatitis/news/news/2017/04/hepatitis-b-vaccination-has-dramatically-reduced-infection-rates-among-children-in-europe,-but-more-is-needed-to-achieve-elimination

WHO. Guidelines on hepatitis B and C testing [Internet]. Geneva (Switzerland): World Health Organization; 2017b [cited 2020 Jun]. Available from https://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf;jsessionid=A0864CBC8CC4C869B57151EFE7359170?sequence=1

WHO. Hepatitis B Key Facts [Internet]. Geneva (Switzerland): World Health Organization; 2019a [cited 2020 Jul]. Available from https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

WHO. Hepatitis B in the WHO European region [Internet]. Geneva (Switzerland): World Health Organization; 2019b [cited 2020 Jul]. Available from http://www.euro.who.int/__data/assets/pdf_file/0007/377251/Fact-Sheet-Hepatitis-B_2019-ENG.pdf?ua=1

WHO. Hepatitis D [Internet]. Geneva (Switzerland): World Health Organization; 2020 [cited 2020 Jul]. Available from https://www.who.int/news-room/fact-sheets/detail/hepatitis-d

Wu IC, Liu WC, Chang TT. Applications of next generation sequencing analysis for the detection of hepatocellular carcinoma associated hepatitis B virus mutations. J Biomed Sci. 2018;25(51):1–12. https://doi.org/10.1186/s12929-018-0442-4