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Hepatitis B: Advanced Clinical Diagnosis Techniques and Treatment Lines-An Updated Review

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Abstract

Background: Chronic hepatitis B (CHB) remains a major global health challenge, affecting over 292 million people worldwide. The virus, often contracted in early childhood or during pregnancy, can lead to serious liver conditions like cirrhosis, hepatocellular carcinoma, and liver failure. As part of the World Health Organization's strategy to reduce the incidence and mortality of CHB by 2030, improving treatment outcomes through advanced diagnostic and therapeutic strategies is essential. Chronic infection often persists due to the immune system's failure to mount an effective response, leading to lifelong viral persistence.

Aim: This review aims to explore advanced clinical diagnosis techniques for hepatitis B, focusing on the role of pharmacists and laboratories in improving patient management through novel biomarkers and antiviral treatments.

Methods: The article examines current antiviral therapies, including nucleos(t)ide analogues (NUCs) and pegylated interferon alpha (PEG-IFNa), and their mechanisms of action. It also discusses the role of viral biomarkers, such as HBV DNA, HBV RNA, and HBsAg, in monitoring treatment response. The review highlights how laboratory tests can aid in assessing the effectiveness of these therapies, predict prognosis, and help pharmacists in tailoring individualized treatment plans.

Results: Antiviral treatments like NUCs are highly effective in reducing viral replication and improving patient outcomes, though complete eradication of the virus remains challenging due to the persistence of cccDNA. New viral biomarkers, such as HBV pgRNA and HBV DNA levels, offer insight into treatment efficacy and long-term prognosis. These biomarkers provide a non-invasive means to evaluate ongoing viral replication and guide treatment decisions, supporting more personalized care.

Conclusion: Advances in hepatitis B treatment are significantly enhanced by the integration of laboratory-based biomarker assessments. Pharmacists play a crucial role in the management of CHB by using these biomarkers to optimize therapeutic regimens and enhance patient outcomes. Laboratories are essential in the early detection of viral markers and in monitoring therapeutic responses, which are vital for improving treatment strategies and achieving functional cure in CHB patients.

Key words: Chronic Hepatitis B (CHB), Antiviral Treatment, Biomarkers, Pharmacists, Laboratory Diagnostics, Hepatitis B Virus (HBV), Viral Nucleic Acids

1. Introduction

A global health concern, chronic hepatitis B (CHB) infection affects over 292 million people globally. Hepatocellular carcinoma (HCC), cirrhosis, and liver failure are among the liver-related conditions for which it is a major cause [1]. The World Health Organization (WHO) has set goals to lower the incidence and mortality of CHB by 2030 because to its significant influence on public health [2]. Most CHB-infected people contract the virus during pregnancy or early childhood [3]. Chronic infection results from the immune system's underdevelopment at this point, which makes it unable to create a sufficient defense against the hepatitis B virus (HBV). Most of the time, this leads to a permanent infection. Fifteen to forty percent of people with CHB may develop liver-related problems if they do not receive the proper treatment.

Approved antiviral treatments, which mainly function by either inhibiting virus replication or modifying the immune response, can greatly reduce the clinical effects of CHB. As scientists look for novel pharmaceutical possibilities to treat CHB, these pathways are being thoroughly studied. The goal of the current research is to improve patient outcomes and therapy alternatives. Additionally, the discovery of new viral biomarkers has improved the capacity to evaluate the effectiveness of antiviral therapy and forecast the prognosis of patients receiving it. These

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indicators give doctors important tools for managing each patient individually by tracking the disease's course and assessing how well antiviral treatments work. The features and possible uses of several blood-based viral biomarkers in CHB patients undergoing antiviral treatment will be investigated in this review. Understanding how these indicators can help assess treatment responses and offer information about the long-term prognosis of treated individuals will be the main goal. Clinical results for individuals with CHB can be improved by further refining treatment options through the use of advanced biomarker-based assessments.

HBV Viral Cycle

Because of the special mechanisms that are part of its viral cycle, the hepatitis B virus (HBV) is still difficult to eradicate from the liver (**Fig. 1**). The hepatotropic, encapsulated virus known as HBV has a genome that is largely double-stranded in deoxyribonucleic acid (DNA). Encapsidated, a mature HBV virion has relaxed circular (rc) DNA that is roughly 3.2 kilobase pairs long. The virion facilitates cellular entrance into hepatocytes by binding to the sodium taurocholate co-transporting polypeptide [4]. The host's DNA repair systems [6,7] fix the rcDNA once it reaches the nucleus [5]. It is subsequently transformed into covalently closed circular DNA (cccDNA), which serves as the template for viral transcription [8]. The four overlapping open reading frames that make up the HBV genome are the source of several viral transcripts, such as messenger RNAs and pre-genomic RNA (pgRNA). Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antigen (HBcAg), X protein (HBx), and the HBV polymerase are among the important viral proteins that are subsequently translated from these transcripts.

In order to promote reverse transcription into rcDNA and, to a lesser extent, double-stranded linear DNA (dslDNA), the pgRNA is encapsulated in a capsid made of HBcAg. The resultant viral genomes are released as infectious virions after being encapsulated. In order to ensure the infection's survival, the encapsidated rcDNA can subsequently be guided to the nucleus to refill the cccDNA pool [9,10]. One important aspect impeding the virus's removal is the existence of the cccDNA pool in hepatocytes, which acts as a stable reservoir for continuous reproduction. Replication-deficient dslDNA can integrate into the host genome at locations of chromosomal DNA breaks, despite being present in only a small percentage of mature HBV virions. This integration may contribute to carcinogenesis by creating stable templates for the synthesis of HBsAg and HBx [11,12].

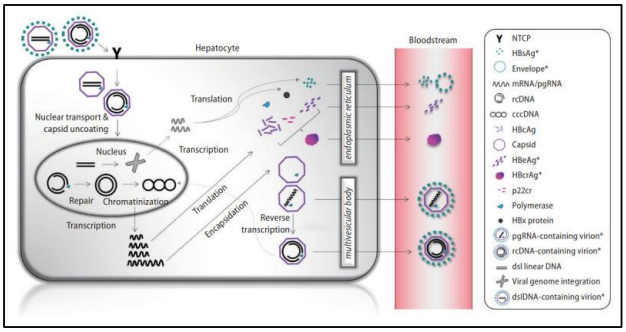

Figure 1: Viral Cycle of Hepatitis B.

Types of Antiviral Treatment

For the treatment of chronic hepatitis B (CHB), two main classes of antiviral medications are now authorized: pegylated interferon alpha (PEG-IFNa) and nucleos(t)ide analogues (NUCs). The reverse transcription stage of the HBV replication cycle is the main target of NUCs, which are DNA polymerase inhibitors. The effect on upstream events is minimal since only one stage of the replication process is blocked. As a result, although viral suppression takes place at the DNA synthesis level, cccDNA is mostly unaffected and takes a long time to reduce. NUCs must therefore be taken continuously, as stopping them too soon increases the chance of a virological comeback [13,14]. Entecavir, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide are among the first-line NUCs that are now advised. These medications are very efficacious, have a high barrier to resistance, and are typically well-tolerated by patients.

On the other hand, PEG-IFNa's actions are less well understood, despite the fact that it is thought to have direct antiviral and immunomodulatory effects. By controlling gene expression and protein synthesis, PEG-IFNa causes hepatocytes to enter a non-cytolytic antiviral state. One important mechanism is the overexpression of APOBEC3, a cytidine deaminase that causes G-to-A hypermutations in the HBV genome, which potentially results in the destruction of cccDNA [16] and inhibits viral replication [15]. Furthermore, PEG-IFNa reduces the binding affinity of STAT1/STAT2 transcription factors by hypoacetylating histones attached to cccDNA, which in turn results in less pgRNA transcription from the cccDNA template. [17]. In contrast to the lifelong treatment required with NUCs, PEG-IFNa can be delivered for a limited time (usually a 48-week course), but its efficacy in decreasing HBV DNA is frequently inadequate. Additionally, PEG-IFNa is injected subcutaneously and has a number of adverse effects, which restricts its use as a CHB therapy option.

Serum Viral Markers for Evaluating Treatment Response

Numerous viral biomarkers are measured in the blood as signs of continuous viral replication in order to evaluate therapy responsiveness in chronic

hepatitis B (CHB) (**Fig. 1**). In the CHB care cascade, well-known indicators like HBsAg and HBV DNA are essential parts of the treatment endpoint structure. More than 90% of patients receiving nucleos(t)ide analogue (NUC) therapy can achieve on-treatment virological suppression, commonly referred to as incomplete cure, which is the most achievable treatment objective. Approximately 20% of patients who finish a limited course of treatment get partial cure, which is defined as virological suppression without HBsAg seroclearance after medication. Although it is linked to better clinical outcomes, functional cure—defined as sustained HBsAg seroclearance and undetectable HBV DNA for at least six months—is only attained by only 1% of patients receiving antiviral therapy each year. With existing therapeutic approaches, sterilizing cure—which involves clearing integrated viral DNA—and complete cure—which refers to eliminating cccDNA—remain unreachable. With a threshold of 30% HBsAg decrease six months after stopping investigational drugs, functional cure has thus emerged as the preferred treatment goal and is considered an acceptable response rate in phase 3 clinical studies of new CHB treatments [18]. Because it is necessary to evaluate transcriptionally active intrahepatic cccDNA, which usually necessitates invasive liver biopsy for tissue sample collection, blood-based viral indicators are often used. Quantification of cccDNA is still mostly limited to research settings due to the invasive nature of the operation, as well as possible problems including sampling mistakes, intra- and inter-observer variability, and the absence of established measurement protocols [19,20]. As a result, a number of blood-based HBV biomarkers have been investigated as stand-ins for cccDNA, which can be roughly divided between HBV translational products and viral nucleic acids.

Viral Nucleic Acids

HBV DNA

Encapsulated, encapsulated rcDNA makes up the majority of detected serum HBV DNA [21]. Serum HBV DNA and intrahepatic cccDNA in untreated patients had a moderate to good connection (correlation value r 0.36–0.49) [22–25]. With lower detection limits that are close to or greater than 1-2 log, the in vitro nucleic acid amplification techniques used for HBV DNA detection are extremely sensitive. Serum HBV DNA quickly drops to undetectable levels under NUC treatment. First-line NUC therapy causes undetectable HBV DNA in 90–94% of HBeAg-negative patients and 64–76% of HBeAg-positive individuals after 48 weeks of treatment [26]. However, only 14% of HBeAg-positive patients and 19% of HBeAg-negative patients achieve undetectable HBV DNA following a full 48-week treatment of PEG-IFNa [26].

HBV pgRNA

Encapsidated pgRNA enclosed in virus-like particles makes up circulating HBV RNA [27]. Serum HBV pgRNA and intrahepatic cccDNA in untreated patients correlate well to wonderfully (r = 0.59–0.89) [28–30]. Real-time polymerase chain reaction techniques based on the quick amplification of complementary DNA ends can be used to quantify HBV pgRNA [27,31], and new developments in RNA assay technologies have brought performance closer to WHO guidelines [32]. Serum HBV pgRNA levels before therapy are usually 1-2 logs lower than HBV DNA levels. The serum RNA:DNA ratio reverses after NUC therapy as a result of a fall in HBV RNA levels, which is less pronounced than that of HBV DNA [33]. The relationship between serum HBV pgRNA and cccDNA weakens after therapy. HBV RNA decreases by almost 1.46 log following 48 weeks of NUC treatment [33]. After 48 weeks of treatment, HBV RNA in HBeAg-positive patients treated with PEG-IFNa decreases by 3.07 log from a baseline mean of 7.73 log to 4.66 log [34]. After 48 weeks of PEG-IFNa therapy, a 1.72 log drop is seen in HBeAg-negative individuals, from a mean baseline level of 4.4 log [35]. Despite these results, HBV pgRNA is still mostly used as a research tool because there is currently no widely recognized standard for its clinical application and there haven't been many studies comparing various tests [32, 33].

Translational Products in Treatment Response Evaluation

The use of specialized viral biomarkers, especially those found in the blood, to track viral replication activity is crucial for assessing therapy response in chronic hepatitis B (CHB). These indicators act as stand-ins for continuing viral activities and are now crucial for determining how well treatment plans are working. With different stages of therapeutic response, from on-treatment virological suppression to functional cure—often regarded as the ideal objective in clinical trials—key biomarkers like HBV DNA and HBsAg have been integrated into treatment protocols as endpoints in the management of CHB. Only a small fraction of patients experience functional cure, which is defined as persistent HBsAg seroclearance and undetectable HBV DNA for at least six months. Despite this, it improves clinical outcomes. Because of this, functional cure is a major area of ongoing therapeutic research, with phase 3 trials specifically focusing on the rate of HBsAg loss six months after treatment as a gauge of effectiveness [18]. Due to the difficulties in directly detecting intrahepatic cccDNA, which normally requires liver biopsy, serum viral indicators must be measured. However, cccDNA quantification is still mostly a research area due to the intrusive nature of biopsies, the hazards involved, and the difficulties in achieving uniform results [19,20]. As a result, several blood-based biomarkers that are categorized as translational products and viral nucleic

acids have been found to function as surrogates for cccDNA.

Viral Nucleic Acids

HBV DNA, which is primarily found as enveloped/encapsidated rcDNA, is one of the essential viral nucleic acids that are evaluated in CHB patients. Especially in patients who have not received treatment, this marker shows a moderate to strong connection with intrahepatic cccDNA [21]. Due to the high sensitivity of the nucleic acid amplification methods used for HBV DNA detection, low virus loads can be detected. Serum HBV DNA levels usually drop to undetectable levels in a considerable portion of patients receiving NUC therapy; patients who are HBeAg-negative have the best response [26]. On the other hand, PEG-IFNa therapy results in a reduced amount of HBV DNA, and both HBeAg-positive and HBeAg-negative people had lower rates of undetectability [26]. HBV pgRNA, which is encapsulated in virus-like particles, is another significant viral nucleic acid. In untreated individuals, there is a substantial link between HBV pgRNA levels and cccDNA; however, this association becomes weaker following antiviral medication. Since there are no widely recognized assays for its clinical use, serum HBV pgRNA testing is usually used in research settings. But when HBV RNA levels in the serum are examined, they tend to drop less than HBV DNA throughout treatment, indicating changes in the RNA:DNA ratio [33]. HBV RNA assays have become more useful, although they are still mostly employed in research, and their clinical applicability is still up for debate [32, 33].

Translational Products

HBV-related proteins, including HBsAg, HBcrAg, and HBeAg, are useful indicators for evaluating the course of CHB and the effectiveness of treatment. Especially in individuals who are HBeAg-positive, HBeAg is essential for classifying disease stages and forecasting therapy results. HBeAg's seroconversion or clearance is a frequent treatment outcome, and its presence signifies active viral replication. A percentage of patients in NUC and PEG-IFNa clinical trials achieve HBeAg seroclearance; rates are higher in PEG-IFNa-treated patients than in NUC-treated patients [26]. Predictive risk models for HBeAg seroconversion have included quantitative measures of HBeAg, which can also shed light on viral load and treatment response [36,37]. Another important marker in CHB is HBsAg, which is primarily found as subviral particles. A useful indicator of the existence of viral particles and the possibility of a functional cure is the assessment of HBsAg levels. Quantitative HBsAg assays are employed to track the effectiveness of antiviral therapy and forecast the likelihood of developing serious liver disease. Even while it gets better with longer treatment, the rate of HBsAg seroclearance with NUC therapy is still modest [26,48]. Early HBsAg reductions have been incorporated into treatment algorithms to inform therapeutic decisions, such as stopping treatment in non-responders [26]. Positive clinical results are correlated with the decrease in HBsAg levels during treatment, especially with PEG-IFNa. Lastly, HBcrAg, a protein composite consisting of HBcAg and HBeAg, has shown a strong connection with cccDNA and may be used as a marker to track viral activity. Antiviral therapy causes HBcrAg levels to drop, but its applicability is currently constrained by high detection thresholds in certain patients, particularly those who are HBeAg-negative [57]. Particularly in difficult cases of CHB, newer HBcrAg tests are being developed to increase sensitivity and offer improved insights into therapy dynamics [61].

Potential Application of Viral Markers in Hepatitis B Management

Blood-based biomarkers for Hepatitis B virus (HBV) are vital for assessing treatment eligibility and response, particularly in the context of both established therapies and experimental drugs under investigation.

Determining Treatment Candidacy

Antiviral treatment is not appropriate for all patients with chronic hepatitis B (CHB). The use of serum viral indicators to assess a patient's suitability for treatment is emphasized in clinical guidelines. The most important indicator is serum HBV DNA, and guidelines like those from the Asian Pacific Association for the Study of the Liver (APASL) and the American Association for the Study of Liver Diseases (AASLD) use qualitative HBeAg to help determine the threshold above which treatment is advised. Treatment eligibility is usually indicated by an HBV DNA level of more than 20,000 IU/mL for HBeAg-positive patients or more than 2,000 IU/mL for HBeAg-negative patients, together with increased alanine aminotransferase (ALT) or other risk factors. The threshold for starting treatment is lower for patients with cirrhosis [26,62,63]. Lower serum qHBsAg levels are associated with an inactive carrier state and HBsAg seroclearance, whereas elevated serum HBcrAg levels have been observed to correspond with immunological tolerance in HBeAg-positive patients [64–68]. In both the HBeAg-positive and HBeAg-negative stages of infection, these markers are crucial in determining which individuals need antiviral therapy.

The World Health Organization (WHO) advises HBV DNA testing to assess whether antiviral prophylaxis during pregnancy is necessary in order to avoid mother-to-child transmission [69]. Tenofovir (TDF) medication should be started when the HBV DNA threshold is greater than 200,000 IU/mL. Qualitative HBeAg and qHBsAg results are helpful stand-ins in situations when HBV DNA testing is not possible. When detecting HBV DNA levels exceeding 200,000 IU/mL, HBeAg positive has a sensitivity of 88.2% and

a specificity of 92.6% [70]. Similarly, for detecting patients with HBV DNA levels above 200,000 IU/mL, a serum qHBsAg level larger than 4 log shows high sensitivity (85.1%) and specificity (96.5%) [71,72]. The ability to precisely measure HBV DNA in dried blood spots has been demonstrated using a new Xpert® HBV Viral Load test. With a detection limit of 7.5 IU/mL and an 85.4% concordance with serum viral load, this approach may be especially useful in settings with limited resources where the GeneXpert® system is already being used to test for other pathogens like Mycobacterium tuberculosis and SARS-CoV-2 [73,74]. Furthermore, an HBcrAg point-of-care rapid diagnostic test (RDT) has been created, offering a streamlined method of determining viral load in situations when qHBsAg or HBV DNA readings are unavailable. The RDT-HBcrAg has a detection limit of 4.3 log U/mL and is particularly helpful in clinical settings without molecular laboratory facilities because it is extremely sensitive (90.5–96.6%) and specific (83.2–96.8%) [75].

Dose Adjustment and Regimen Modification

Monitoring viral markers, particularly HBV DNA and qHBsAg, during treatment is crucial. In nucleos(t)ide analogue (NUC) therapy, HBV DNA levels are regularly tracked to detect virological breakthrough, signaling either primary resistance or treatment non-compliance. For patients receiving PEG-IFNa, stopping rules based on qHBsAg levels help determine when therapy should be discontinued [26].

Risk Stratification for Hepatocellular Carcinoma (HCC)

When determining the risk of HCC in individuals with treated CHB, viral biomarkers are also very helpful. There is a correlation between elevated blood qHBsAg levels and a higher risk of developing HCC. For example, compared to individuals with qHBsAg levels below this threshold, those with low viremic HBeAg-negative status (HBV DNA <2,000 IU/mL) and qHBsAg ≥3 log had a hazard ratio of 13.7 [76]. Furthermore, independent of antiviral treatment status, reaching HBsAg seroclearance (functional cure) considerably lowers the risk of HCC, especially in those who do so before the age of 50 [77,78]. Studies like the REVEAL-HBV cohort have shown that serum HBV DNA exhibits a biological gradient, making it a well-established risk factor for HCC [79]. It has been demonstrated that long-term NUC therapy reduces the incidence of HCC [80]. However, alternative viral indicators including serum HBcrAg and pgRNA are being investigated to help with HCC risk classification, as HBV DNA becomes undetectable in many patients during NUC therapy. Significantly higher chances of developing HCC within two years are linked to both detectable serum pgRNA and elevated post-treatment HBcrAg [81–85]. Even in patients whose serum HBV DNA remains undetectable after treatment, these indicators offer important information about the continued risk of HCC.

Other Applications:

Predicting a partial or functional cure in patients with chronic hepatitis B (CHB) is essential for assessing the effectiveness of treatment and deciding whether to stop long-term nucleos(t)ide analog (NUC) therapy. Higher baseline blood HBcrAg levels have been found to be independently linked to nucleoside analog (NA)-induced HBeAg seroconversion in HBeAg-positive persons [64]. On the other hand, it has been demonstrated that a drop in HBcrAg levels at week 12 of pegylated interferon (PEG-IFN) treatment predicts HBeAg seroclearance and a drop in HBV DNA to less than 2,000 IU/mL at 24 weeks after treatment [60]. Before considering stopping long-term NUC therapy in HBeAg-positive patients after extended HBV DNA undetectability, or reaching a state of incomplete cure, it is necessary to achieve HBeAg seroclearance or seroconversion. The success rates and determinants for off-therapy virological management have been the subject of numerous investigations [14,86]. According to these research, both host and viral variables may offer important information about the likelihood of a virological or clinical relapse following the end of long-term NUC treatment. For example, low end-of-therapy (EOT) serum qHBsAg, usually less than 100 IU/mL, has been found to be a reliable indicator of partial cure [87–89]. A subset of patients who are more likely to successfully stop NUC therapy with a lower risk of recurrence can also be identified by low EOT blood HBcrAg levels [90], undetectable EOT serum HBV pgRNA [91], or a combination of both [92,93]. These individuals may achieve a functional cure if they have positive viral biomarker profiles [94,95]. Individuals who are likely to achieve a low serum qHBsAg (<100 IU/mL) or HBsAg seroclearance in the long run can be identified by early evaluation of viral biomarkers, such as serum HBcrAg and pgRNA as early as week 4 of NUC therapy [96]. Early detection can help guide treatment choices by allowing patients to be prioritized for clinical trials and offering insight into who might benefit from longer-term therapy. With a number of promising therapeutic approaches that target different steps in the viral replication cycle, boost the host immune response, or target both pathways at once, the landscape of CHB treatment is rapidly changing in terms of assessing the efficacy and target engagement of novel compounds [97,98]. In phase 3 clinical trials for new treatments for CHB, the functional cure is still the ultimate therapeutic objective, as was previously mentioned [18].

As of right now, a number of novel drugs have shown promising outcomes in attaining long-lasting HBsAg reduction. Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) are two examples of RNA interference-based treatments that have demonstrated great promise in lowering HBsAg levels by more than one log during the course of

treatment [98]. Interestingly, when JNJ-3989, a siRNA, was used in conjunction with NUCs, 97.5% of patients experienced a ≥1 log decrease in HBsAg levels at nadir, with a 38% persistence rate one year after EOT [99]. Similarly, 9–10% of subjects assessed 24 weeks after EOT experienced a functional cure when taking the ASO bepirovirsen [100]. The need for more research into the mechanisms of action of these novel therapies and the use of appropriate viral biomarkers to monitor target engagement and interim responses is highlighted by the fact that, despite the large number of ongoing clinical trials, no compound has yet achieved the benchmark of achieving a functional cure in at least 30% of patients [101]. Core protein allosteric modulators (CpAMs) have shown promise in preventing the encapsidation of viral RNA and the development of functional capsids, which lowers the amount of circulating encapsidated pgRNA. Although there were no notable changes in serum qHBsAg, there were notable decreases in serum HBV DNA and pgRNA in patients receiving vebicorvir (CpAM) at weeks 12 and 24 [102]. Similar to this, patients receiving ABI-H2158 (CpAM) showed signs of pgRNA synthesis suppression as early as day 15, with mean decreases exceeding 2 log from baseline in contrast to the placebo group's negligible changes [103]. In many trials utilizing CpAM, other viral biomarkers, including HBeAg and HBcrAg levels, were assessed [104,105]. Future clinical trials should address the factors predicting durable suppression of various viral biomarkers and the long-term durability of viral kinetic changes induced by these novel therapies. Recent studies involving treatment-naïve cohorts have shown significant declines in HBeAg and HBcrAg levels in response to CpAMs combined with NUC therapy or PEG-IFN [106].

Treatment and Mangement of HBV:

Potential Impact of HBV Treatment on HBV DNA Integration

The intermediate form of hepatitis B virus (HBV) before its integration into the host genome consists of double-stranded linear DNA (dslDNA). This dslDNA is produced through the reverse transcription of HBV RNA [107], and nucleos(t)ide analogs (NAs) are expected to minimize the formation of dslDNA and its subsequent integration into the host DNA. Although research on the effects of antiviral treatment on HBV DNA integration remains limited, existing evidence suggests that NA therapy may play a role in mitigating hepatocarcinogenesis. Analyses of liver biopsies from both treated and untreated patients have indicated that antiviral treatment is associated with reductions in viral load, HBV DNA integrations, and chromosomal translocations. While further investigation is needed to fully understand the influence of antiviral therapy on HBV DNA integration, these preliminary findings provide strong support for the early initiation of treatment in chronic hepatitis B (CHB) patients, particularly with respect to minimizing the risk of hepatocellular carcinoma (HCC).

Potential Impact of Early HBV Treatment on Clinical HBV Parameters

A limited number of studies have assessed the effects of antiviral treatment on virological, serological, and liver-related outcomes in CHB patients who do not meet the current treatment guidelines. However, data on long-term outcomes, including the development of HCC, are currently unavailable, and randomized controlled trials (RCTs) comparing antiviral therapy to no treatment have not been conducted. Nonetheless, a meta-analysis that incorporated two studies of patients ineligible for treatment (IT) found moderate-quality evidence supporting the beneficial effects of antiviral therapy on intermediate outcomes, such as viral suppression and HBeAg seroconversion or loss [108]. Furthermore, a Phase 2 study examining the efficacy of tenofovir disoproxil fumarate (TDF) ± emtricitabine in 126 IT patients revealed that 65% of patients achieved HBV DNA levels <69 IU/mL after 192 weeks of treatment. Additionally, 42% of patients with a moderate aMAP (age, male sex, albumin-bilirubin, and platelets) risk score at baseline transitioned to a lower-risk category, with no cases of HCC reported [109, 110]. However, HBeAg or HBsAg loss was observed in only 4% and 0% of patients, respectively. Based on these results, the authors concluded that routine Nucleos(t)ide analog (NA) treatment for patients with IT CHB is not recommended, a position that aligns with the current treatment guidelines.

A multicenter study evaluating the combination of entecavir (ETV) and peginterferon alfa-2a in 60 children with inactive chronic hepatitis B (IT CHB) revealed that 75% of participants achieved HBV DNA levels ≤1000 IU/mL, with 23% having HBV DNA <20 IU/mL after 48 weeks of treatment. Notably, HBeAg and HBsAg loss was observed in two patients [111]. Similarly, a study involving 28 adult IT patients undergoing ETV + peginterferon alfa-2a treatment demonstrated that 93% of patients achieved HBV DNA ≤1000 IU/mL, and 18% had HBV DNA levels <20 IU/mL after 48 weeks of treatment [112]. In both studies, HBV DNA levels increased following the cessation of treatment [111, 112]. A further analysis of 181 treatment-naïve IT CHB patients, 33% of whom exhibited evident histological liver injury (EHLI) at baseline, reported that 82% and 78% of patients with EHLI experienced histological improvement and fibrosis reversal, respectively, after 72 weeks of ETV therapy. Furthermore, 73% of patients no longer showed evidence of EHLI [113].

Impact of Treatment on 'Gray Zone' Patients

The treatment of 'gray zone' patients, those with HBV DNA >2000 IU/mL, alanine aminotransferase (ALT) levels between 40–80 U/L, and no cirrhosis, was evaluated in the TORCH-B

study, a randomized, double-blind, placebo-controlled trial [114]. Over three years of follow-up, the placebo group exhibited a significantly higher proportion of patients with progression in fibrosis stage compared to the tenofovir disoproxil fumarate (TDF) treatment group (47% vs. 26%; p = 0.013).

Impact of Early HBV Treatment in Patients with HBV/HIV Co-Infection

A distinct cohort of CHB patients who routinely receive early antiviral treatment includes those co-infected with HIV. The administration of antiviral therapy as pre-exposure prophylaxis to high-risk individuals plays a crucial role in HIV control [115]. Many antiretroviral regimens, which typically include a nucleos(t)ide analog (NA) such as TDF or tenofovir alafenamide (TAF), are initiated irrespective of HBV DNA or ALT levels. Thus, examining the impact of early antiviral treatment on hepatocellular carcinoma (HCC) risk in these patients offers valuable insights, albeit with the important consideration that these studies are not randomized controlled trials (RCTs). Among 3,625 HBV/HIV co-infected patients, those receiving NA treatment demonstrated stable HCC incidence, while the incidence increased in patients receiving regimens that did not include an NA [116]. A separate study comparing HBV mono-infected patients (n = 53,974) and HBV/HIV co-infected patients (n = 822) found lower HCC rates among the co-infected group [117]. Similarly, an analysis of claims data indicated that HBV/HIV co-infected patients (n = 7,764) had a lower incidence of HCC compared to HBV mono-infected patients (n = 13,964) [118]. Assuming that HIV co-infection serves as a proxy for early HBV antiviral treatment, these findings suggest that universal antiviral therapy for CHB patients may contribute to a reduced risk of HCC.

Conclusion:

Chronic hepatitis B (CHB) poses a significant global health threat, and although antiviral therapies have greatly improved patient outcomes, challenges persist in eradicating the virus. Pharmacists and laboratories play essential roles in managing this complex disease, particularly through the use of advanced diagnostic tools and biomarkers. Nucleos(t)ide analogues (NUCs) and pegylated interferon alpha (PEG-IFNa) are the mainstays of antiviral therapy. While these treatments effectively suppress viral replication, they do not completely eliminate the virus due to the presence of the stable covalently closed circular DNA (cccDNA) reservoir in hepatocytes. This makes long-term therapy necessary to maintain viral suppression. Laboratory assessments, particularly the measurement of viral biomarkers, are critical in evaluating the efficacy of these therapies. Blood-based viral markers, such as HBV DNA, HBV RNA, and HBsAg, serve as indicators of ongoing viral replication and treatment response. They are used to monitor patients' progress, assess the development of resistance, and determine the potential for a functional cure, defined by the sustained loss of HBsAg and undetectable HBV DNA levels. Although functional cure remains a rare outcome, it is increasingly seen as the ideal treatment goal, and emerging therapies aim to improve the rate of functional cure. Pharmacists are central to the treatment process, not only in dispensing medications but also in counseling patients about the importance of adhering to long-term therapy and helping to adjust treatment regimens based on laboratory results. They also play a pivotal role in educating patients about the chronic nature of CHB and the need for continuous monitoring. Moreover, pharmacists collaborate with healthcare teams to ensure that patients receive individualized care based on the most current diagnostic information. Laboratories, on the other hand, are crucial for providing accurate, timely data on viral markers, which helps clinicians and pharmacists make informed decisions. The use of non-invasive tests for monitoring viral activity, such as serum HBV DNA and HBV pgRNA levels, reduces the need for more invasive procedures like liver biopsies, making patient monitoring more accessible and less burdensome. In conclusion, while significant advancements have been made in CHB treatment, further improvements in both therapeutic strategies and diagnostic techniques are necessary to achieve a functional cure. Pharmacists and laboratories continue to play a vital role in refining the management of CHB, ensuring better patient outcomes, and moving closer to the goal of eliminating the virus from affected individuals.

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