

Diagnostic Utility of Truenat® for Quantification of HBV DNA and Correlation of ALT Levels with Viral Load among HBsAg-Positive Patients Attending a Tertiary Care Hospital in Uttar Pradesh¹Manoj Kumar, Assistant Professor, Department of Microbiology, GSVM Medical College, Kanpur²Ankita Saha, Senior Resident, Department of Microbiology, S. N. Medical College, Agra³Ambika Bhatiani, Professor, Department of Microbiology, Dr BS Kushwah Institute of Medical Sciences, Kanpur⁴Suraiya K. Ansari, Associate Professor, Department of Microbiology, GSVM Medical College, Kanpur⁵Lubna Khan, Professor, Department of Pathology, GSVM Medical College, Kanpur**Corresponding Author:** Suraiya K. Ansari, Associate Professor, Department of Microbiology, GSVM Medical College, Kanpur**How to citation this article:** Manoj Kumar, Ankita Saha, Ambika Bhatiani, Suraiya K. Ansari, Lubna Khan, “Diagnostic Utility of Truenat® for Quantification of HBV DNA and Correlation of ALT Levels with Viral Load among HBsAg-Positive Patients Attending a Tertiary Care Hospital in Uttar Pradesh”, IJMACR– September– 2026, Volume – 9, Issue – 5, P. No. 613 – 622.**Open Access Article:** © 2026 Manoj Kumar, et al. This is an open access journal and article distributed under the terms of the creative common’s attribution license (<http://creativecommons.org/licenses/by/4.0>). Which allows others to remix, tweak, and build upon the work non - commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.**Type of Publication:** Original Research Article**Conflicts of Interest:** Nil**Abstract**

Background and Objectives: Hepatitis B virus (HBV) infection remains a major public health concern in India, with an estimated 42 million individuals affected. Quantification of HBV-DNA plays a crucial role in disease diagnosis, therapeutic decision-making, and monitoring of treatment response. However, the routine use of molecular assays is often limited by their high cost and differences in diagnostic performance. The present study was undertaken to assess the diagnostic accuracy of the Truenat®-PCR assay and to evaluate the correlation between serum alanine aminotransferase (ALT) levels and HBV-DNA viral load.

Materials and Methods: This prospective cross-sectional study included 760 serum samples obtained from patients who were screened for hepatitis B surface antigen (HBsAg) using a rapid diagnostic test. Liver function tests (LFTs) were performed in other laboratory of GSVM Medical college for all samples, HBV-DNA detection and quantification were carried out using the Truenat®-PCR in department of microbiology.

Results: Out of 760 samples, 610 samples were positive by both the rapid HBsAg test and Truenat®-PCR, while 145 samples were negative for HBV-DNA and 5 samples yielded invalid results. Elevated ALT levels were observed in 78% (127) of patients with HBV-DNA levels >100000 copies/ml, which was significantly higher in

55%(245) patients compared with those having HBV-DNA levels ≤ 100000 copies/ml.

Conclusion: The Truenat®-PCR assay demonstrated high sensitivity and is well suited for use in resource-limited settings for effective HBV diagnosis and management. Assessment of HBV-DNA viral load together with serum ALT levels identified a substantial proportion of patients with active viral replication and an increased likelihood of disease progression.

Keywords: Hepatitis B Virus, Hepatitis B Surface Antigens, HBV Infection, Diagnostic Precision.

Introduction

Viral hepatitis remains one of the major causes of morbidity and mortality worldwide, and infection with the Hepatitis B virus (HBV) continues to be a significant global public health concern. The prevalence of HBV infection and its routes of transmission vary considerably across different geographical regions. It has been estimated that nearly one-fifth of the global population has been exposed to HBV, and India alone accounts for more than 40 million infected individuals, representing approximately 10–15% of the world's HBV carrier population ¹.

The burden of HBV infection remains disproportionately high in low- and middle-income countries, particularly in densely populated regions of Asia and Africa. India ranks second only to China in the number of individuals living with chronic hepatitis B infection. One of the major reasons for this high burden is that a substantial proportion of HBsAg-positive individuals acquire the infection during the perinatal period, remain asymptomatic for prolonged periods, and are often diagnosed incidentally during routine investigations ².

Although many patients appear clinically healthy and do not exhibit symptoms at the time of diagnosis, a

considerable number demonstrate underlying biochemical and histopathological abnormalities. Over time, these individuals may progress to chronic liver disease, liver cirrhosis, or hepatocellular carcinoma (HCC) ³.

Patients with chronic hepatitis B infection are at an increased risk of developing liver cirrhosis and hepatocellular carcinoma ⁴. Emerging evidence has demonstrated a strong association between baseline HBV-DNA levels and the subsequent development of these complications. The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) study reported that the cumulative incidence of liver cirrhosis increased progressively with rising HBV-DNA concentrations, ranging from approximately 5% among individuals with undetectable viral loads (<300 copies/mL) to nearly 36% among those with viral loads of $\geq 10^6$ copies/mL, irrespective of alanine aminotransferase (ALT) levels or hepatitis B e antigen (HBeAg) status ^{5,6}.

Comparable findings have also been observed regarding the development of hepatocellular carcinoma ⁶. The cumulative incidence of HCC ranged from 1.3% among individuals with HBV-DNA levels below 300 copies/mL to approximately 14.9% among those with viral loads of $\geq 10^6$ copies/mL. These observations indicate that elevated HBV-DNA levels are significantly associated with an increased risk of hepatocellular carcinoma ($p < 0.001$) ⁷.

The natural history of chronic HBV infection is largely determined by the complex interaction between viral replication and the host immune response ⁸. Recent nomenclature classifies the different phases of chronic HBV infection based on two principal characteristics: chronic infection and chronic hepatitis ⁹. In this context, both HBV-DNA quantification and serum alanine

aminotransferase (ALT) levels are essential parameters for identifying the phase of disease, determining prognosis, and guiding decisions regarding treatment initiation and long-term monitoring ¹⁰.

Several laboratory techniques are available for the diagnosis of hepatitis B infection, including Immunochromatographic Test (ICT), Reverse Passive Haemagglutination (RPHA), Enzyme-Linked Immunosorbent Assay (ELISA), Enzyme Immunoassay (EIA), Chemiluminescent Immunoassay (CLIA), Polymerase Chain Reaction (PCR), and Truenat®-PCR. Truenat®-PCR is a chip-based, portable molecular diagnostic platform that offers rapid turnaround time, requires minimal biosafety infrastructure, and can be performed by personnel with limited laboratory training while maintaining high sensitivity and specificity ¹¹.

Therefore, the present study was undertaken to evaluate the diagnostic accuracy of the Truenat®-PCR assay and to determine the correlation between serum ALT levels and HBV-DNA viral load among HBsAg-positive patients. Additionally, the study aims to assess the diagnostic performance and sensitivity of Truenat®-PCR in comparison with rapid HBsAg testing using the same patient samples ¹².

Materials And Methods

This prospective cross-sectional study was conducted at the Department of Microbiology, GSVM Medical College, Kanpur, Uttar Pradesh, India, under the National Viral Hepatitis Control Programme (NVHCP) from December 2024 to June 2026. GSVM Medical College this is a tertiary care hospital and serves as a major centre for the diagnosis and management of Hepatitis B, providing free-of-cost services under the NVHCP.

The study included patients attending both the outpatient departments (OPD) and inpatient departments (IPD) who

either had a documented positive hepatitis B surface antigen (HBsAg) report from an outside laboratory or were diagnosed as HBsAg positive by rapid card testing at our virology laboratory of the hospital. Availability of a sample for HBV-DNA testing was mandatory for enrolment in the study. Patients with chronic hepatitis C infection, autoimmune hepatitis, jaundice, ascites, alcoholic liver disease, or a history suggestive of recent drug-induced hepatitis were excluded from the study.

A total of 760 cases were enrolled according to the predefined inclusion and exclusion criteria. All patients who were advised to undergo screening for HBsAg in the outpatient department (OPD) were subjected to the primary registration process. Following the acquisition of informed written consent, approximately 3 mL of venous blood was collected in vacutainers for serum and plasma separation ¹³. All serological investigations, excluding LFT, ALT, AST (conducted in emergency pathology), were performed in the virology laboratory of the hospital. Detection of HBV-DNA was carried out using the Truenat® test chips ¹⁴. Demographic information, including age, gender, region of residence, and socioeconomic status, was recorded for each participant. Patients with elevated HBV viral load and/or raised serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels were considered potential candidates for antiviral therapy. Subjects with detectable HBV-DNA and/or elevated liver enzymes were advised regular follow-up at intervals of 3–6 months.

Truenat ®-PCR Technique: In our laboratory setup, Truenat® test chips were utilized for performing molecular assays for HBV and HCV infections ¹⁴.

Briefly, 0.5 mL of serum/plasma sample was transferred into a lysis buffer bottle using a sterile 1 mL transfer pipette. The lysed sample was subsequently transferred

into the universal cartridge-based sample preparation kit using another sterile transfer pipette. The cartridge was then inserted into the Trueprep Auto V2 extraction device. Completion of nucleic acid extraction was indicated by an audible signal after approximately 15 minutes. The elute obtained was collected in an Elute Collection Tube (ECT), and 6 µL of the purified nucleic acid was transferred into the reaction microtube. After allowing the mixture to stand for 30–60 seconds, the clear solution was dispensed into the centre of the Truenat® microchip using a barrier-tip pipette, and the amplification run was initiated ¹⁵.

Amplification and Interpretation: Upon completion of the run (approximately 60 minutes), amplification curves were displayed graphically on the RT-PCR analyzer screen, indicating the progress and outcome of the test. In positive samples, both the target and Internal Positive Control (IPC) curves exhibited a characteristic sigmoidal amplification pattern, crossing the predetermined threshold fluorescence value. The cycle threshold (Ct) value was inversely proportional to the quantity of viral copies present in the specimen. In negative samples, only the IPC curve demonstrated amplification, whereas the target curve remained absent. If the IPC curve failed to amplify and remained horizontal throughout the run, the test result was considered invalid.

At the completion of the assay, results were automatically displayed as “DETECTED” for positive samples and “NOT DETECTED” for negative samples. Viral load values obtained in IU/mL were converted to copies/mL using a conversion factor of 5.26, as recommended in the manufacturer's kit insert provided by Molbio Diagnostics Private Limited ¹⁵.

Statistical Analysis: All study variables were entered into a master chart and analyzed using Statistical Package

for Social Sciences (SPSS) software version 26.0. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation (SD) for normally distributed data and as median with interquartile range (IQR) for non-normally distributed data. Socioeconomic and demographic characteristics of the participants were assessed using the Modified Kuppaswamy Socioeconomic Scale (Table 1) ¹⁶.

Results

During the study period, a total of 760 samples were collected from patients who fulfilled the inclusion and exclusion criteria. Of these, 320 patients were from Kanpur nagar, Uttar Pradesh, while 440 patients belonged to other districts of Uttar Pradesh. Of 760 HBsAg subjects, 610 (80.3%) were HBV-DNA positive, confirming active viral replication in most cases. Positivity was lowest in children (0–15 years, 45.0%–62.5% female and male respectively) and highest in young adults (16–30 years, 95.8%), then fluctuated gradually with age likely reflecting greater exposure to horizontal transmission routes (unsafe injections, sexual contact) in young adults versus mainly perinatal/early-childhood acquisition in children. Males showed consistently higher HBV-DNA positivity (95.8%) than females (89.5%) across nearly every age group, suggesting greater risk exposure and/or sex-related differences in viral clearance. The 16–30 years group, having both the largest sample size and highest positivity, represents the main burden of active infection in this population and should be prioritized for screening and clinical follow-up. (Table 1)

All 760 samples were initially screened using the rapid HBsAg card test and subsequently confirmed by the Truenat®-PCR technique. Of these, 610 samples were

positive by both rapid HBsAg testing and Truenat®-PCR. However, 145 samples were negative or showed no detectable HBV-DNA on Truenat®-PCR. Only five samples yielded invalid results, possibly due to improper DNA extraction, and fresh samples were requested for repeat testing.

High linearity of HBV-DNA positivity was observed in 10.12% of the total samples, while 3.27% of samples showed HBV-DNA positivity below the linearity threshold. The difference between the rapid HBsAg test and Truenat®-PCR was found to be statistically significant. Among the 760 consecutive patients who tested positive by the rapid HBsAg card test and were included in the study, 610 (80.26%) were confirmed

positive for HBV-DNA by Truenat®-PCR and were considered for baseline biochemical evaluation. (Table-2) Elevated serum ALT levels were observed in 160 participants (26.23%). Among these, 92(15.08%) patients had ALT levels exceeding 100 IU/ml. A significantly higher proportion of patients with elevated ALT levels was observed among those with high HBV-DNA viral loads. Increased ALT levels were present in 78% of HBV-DNA-positive patients with viral loads exceeding 100,000 copies/ml, which was significantly higher compared to patients (n = 447) with HBV-DNA levels of ≤100,000 copies/ml in 55% patients (Table 3).

Table 1: Age- and Sex-wise Distribution of HBsAg-Positive Subjects

Age group (years)	Total screened, (n=760)	Male (n=470)	Male HBV-DNA detected, (n =415)	Female, (n=290)	Female HBV-DNA+, n (%)	Total HBV-DNA+, n (%)
0–15	20	8	5 (62.5)	12	4 (33.3)	9 (45.0)
16–30	286	190	182 (95.8)	96	74 (77.1)	256 (89.5)
31–45	229	135	112 (83.0)	94	62 (66.0)	174 (76.0)
46–60	175	102	88 (86.3)	73	43 (58.9)	131 (74.9)
More than 60	50	35	28 (80.0)	15	12 (80.0)	40 (80.0)
Total	760	470	415 (88.3)	290	195 (67.2)	610 (80.3)

Table 2: HBV Screening and ALT–Viral Load Correlation Study Sample Screening and Confirmation Profile (N = 760)

Parameter	n	%
Total samples screened (rapid HBsAg card test)	760	100.00
Kanpur, Uttar Pradesh	320	42.11
Other districts	440	57.89
Confirmed positive by Truenat®-PCR	610	80.26
Negative	145	19.08
Invalid result (repeat testing requested)	5	0.66

High linearity of HBV-DNA positivity was observed in 10.12% of total samples; 3.27% showed positivity below the linearity threshold. The difference between rapid HBsAg testing and Truenat®-PCR was statistically significant.

Table 3: Distribution of ALT Levels Among PCR-Confirmed Positive Patients (n = 610)

ALT category	n	% of 610
Normal ALT (≤ 45 IU/mL)	450	73.77
Elevated ALT (>45 IU/mL)	160	26.23*
Out of which ALT 46–100 IU/mL	68	11.15
Out of which ALT >100 IU/mL	92	15.08
Total	610	100.00

Table 4: ALT Status by HBV-DNA Viral Load (n = 610)

HBV-DNA viral load	n	Elevated ALT (>45 IU/mL)	Normal ALT (≤ 45 IU/mL)
$\leq 100,000$ copies/mL	447	245 (55%)	201(45)%
$>100,000$ copies/mL	163	127 (78%)	36 (22%)

Discussion

The quantitative real-time polymerase chain reaction (qRT-PCR) technique for the detection and quantification of HBV-DNA viral load is a highly sensitive and specific molecular diagnostic method. The present study provides a cross-sectional overview of hepatitis B virus infection in Kanpur and near districts, Uttar Pradesh. The primary objectives were to evaluate the diagnostic precision of the Truenat® PCR technique and to investigate the relationship between serum alanine aminotransferase (ALT) levels and HBV-DNA viral load among patients who were incidentally detected as HBsAg positive at G.S.V.M Medical College, Kanpur. Additionally, the study assessed the diagnostic performance of Truenat® HBV-DNA testing in comparison with the rapid HBsAg immunochromatographic method ¹⁷.

Males constituted 61.84% (n = 470) of the study population, whereas females accounted for 38.16% (n = 290). These findings indicate a higher prevalence of HBV infection among males compared with females. The greater frequency of HBV infection among men may be attributed to increased exposure to risk factors such as substance use, high-risk behaviours, blood donation

practices, pre-employment and visa-related health screening, and routine health check-ups at outpatient departments.

Similar findings were reported in another study involving 704 individuals who tested positive for HBsAg during voluntary screening camps. Among them, 570 (80.96%) were males and 134 (19.03%) were females ¹⁸. Of the 704 participants, 592 presented for follow-up evaluation, of whom 454 (77%) were males. The study further reported that 456 (77%) participants were aged 40 years or younger, while 136 (23%) were above 40 years of age. Likewise, Khadaka et al. (2018) demonstrated that chronic hepatitis B infection was more prevalent among males (59.7%) than females (40.3%) in their study population (n = 48) ¹⁹.

Similarly, B. Nandi et al. (2012) observed that the majority of participants were asymptomatic and were incidentally diagnosed during blood donation camps, routine health examinations, or screening programmes. Males constituted the majority of cases in their study, with a mean age of 40 years compared with a mean age of 34 years among females ²⁰.

Furthermore, a study conducted among 594 HBsAg-positive individuals reported a male-to-female ratio of 2.9:1, with the highest prevalence of HBV infection observed in the 30–39 years age group ²¹.

Measurement of serum ALT levels in asymptomatic HBsAg-positive individuals is important for assessing ongoing hepatocellular injury. In the present study, elevated ALT levels (>45 IU/mL) were observed in 26.23% (n=160) of subjects, which was lower than that reported by Choudhari G et al. (2019), who observed raised ALT levels in 54% of HBsAg-positive individuals ¹⁸. Similarly, another study involving 317 symptomatic HBsAg-positive patients reported that 65% of subjects had ALT levels greater than 40 IU/mL ²².

Further, all participants underwent quantitative testing for HBV-DNA viral load estimation. HBV-DNA was undetectable in 19.08% (n=145) of subjects, while invalid results were obtained in 0.657% (n=5). The remaining 80.26% of subjects had detectable HBV-DNA levels. Among these, 77.28% (n=447) had viral loads ranging from 2,677 to 100,000 copies/mL, representing low-to-moderate viraemia. Additionally, 2.43% (n=11) had viral loads below the assay linearity range, 26.72% (n=163) had HBV-DNA levels exceeding 100,000 IU/mL, indicating high viraemia, and 8.40% (n=38) had values above the upper linearity limit of the assay. Another study reported detectable HBV-DNA in 85% (n=85) of 100 incidentally diagnosed asymptomatic HBsAg-positive patients ²³. Similarly, one study demonstrated that among 592 HBsAg-positive subjects, 349 (59%) were positive for HBV-DNA ¹⁷. Khadaka et al. (2018) also reported detectable viral load in 90% of 119 asymptomatic HBsAg-positive patients ¹⁹.

The present study further evaluated the relationship between serum ALT levels and HBV-DNA viral load and

demonstrated that HBV-DNA levels were significantly higher among individuals with abnormal ALT levels. Nita et al. (2009) similarly investigated the association between HBV-DNA levels, ALT levels, and HBeAg status and reported that HBV-DNA concentrations were significantly higher in HBeAg-positive individuals and in those with elevated ALT levels ¹.

According to current hepatitis B treatment guidelines, antiviral therapy is generally not recommended for patients with normal ALT levels because they are considered unlikely to have active disease. In our study, approximately 38.95% (n=296) of participants belonged to this category. Since the primary objective of the study was to determine the prevalence of high HBV-DNA viral load and evaluate serum ALT levels among incidentally detected asymptomatic HBsAg-positive individuals, it was anticipated that most patients with elevated ALT levels would represent active disease.

However, our findings demonstrated that a considerable proportion of asymptomatic HBsAg-positive individuals had low ALT levels despite harbouring high HBV-DNA viral loads. Such patients are likely to remain undetected within public healthcare settings where routine HBV-DNA testing is not universally available. Furthermore, previous studies have shown that patients with persistently high HBV-DNA levels are at an increased risk of disease progression and adverse clinical outcomes ²⁴.

Considering the established evidence that elevated HBV-DNA viral load is associated with an increased risk of liver cirrhosis and hepatocellular carcinoma (HCC), we propose that asymptomatic patients with high HBV-DNA levels despite normal ALT values should be regarded as potentially harbouring active infection and as being at increased risk for future complications. Therefore, these

individuals should undergo further evaluation using additional diagnostic modalities and should be monitored more closely through regular follow-up.

The present study had certain limitations. Although it demonstrated the diagnostic utility and performance of the Truenat®-PCR assay for HBV viral load estimation, its practical applicability is limited by its relatively low sample-processing capacity in one times and inability to accurately quantify viral loads that fall below or exceed the assay's linear detection range. Furthermore, although the study explored the correlation between HBV-DNA viral load and serum ALT levels, these findings alone are insufficient to assess the impact of antiviral therapy across different stages of chronic hepatitis B infection. Additionally, the study was conducted at a single tertiary care centre, and the limited number of paediatric participants precluded a comprehensive evaluation of HBV infection patterns in the paediatric population.

Conclusion

In India, a substantial proportion of HBsAg-positive individuals acquire hepatitis B virus (HBV) infection during the perinatal period and remain asymptomatic for prolonged durations, often being diagnosed incidentally during routine screening or evaluation. In the present study, Truenat®-PCR demonstrated superior sensitivity and diagnostic precision compared with rapid HBsAg testing for the detection and assessment of HBV infection. The technique proved valuable in quantifying HBV-DNA viral load, thereby assisting clinicians in making informed decisions regarding initiation of antiviral therapy and appropriate patient management. evaluation of HBsAg-positive participants through measurement of serum HBV-DNA and ALT levels revealed that a considerable proportion had ongoing viral replication and evidence of hepatocellular injury, placing them at increased risk of

progressive liver disease. Truenat®-PCR is a highly sensitive, rapid, and resource-efficient molecular diagnostic platform that can be effectively implemented even in peripheral and resource-limited laboratories processing relatively small numbers of samples.

Furthermore, this molecular approach facilitates the identification of patients with high HBV-DNA levels despite having normal serum ALT values, thereby enabling early recognition of individuals at risk for disease progression and allowing timely therapeutic intervention and long-term monitoring.

Acknowledgements

The authors express their heartfelt gratitude to the faculty member, and technical staff of the Department of Microbiology, GSVM Medical College Kanpur, Uttar Pradesh, India, for their invaluable assistance, guidance, and cooperation throughout the conduct of this research work.

References

1. World Health Organization. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. Geneva: World Health Organization; 2024.
2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol.* 2025;83(2):502-583. doi:10.1016/j.jhep.2025.03.018.
3. You H, Maiwall R, Chen J, Ahn SH, Dokmeci K, Dou X, et al.; APASL Viral Elimination Taskforce. APASL clinical practice guidelines on the management of chronic hepatitis B infection: a 2026 update. *Hepatol Int.* 2026. doi:10.1007/s12072-026-11108-1.

4. Arora A, Sharma P, Dhiman RK, Duseja A, Saraswat V, Mohan VG, et al. Indian National Association for the Study of the Liver Position Statements on Prevention, Diagnosis, and Management of Hepatitis B Virus Infection in India. *J Clin Exp Hepatol*. 2025;15(6):102608. doi:10.1016/j.jceh.2025.102608.
5. Watson AG, Mulay AS, Gill US. Chronic hepatitis B in 2025: diagnosis, treatment and future directions. *Clin Med (Lond)*. 2025;25(6):100527. doi:10.1016/j.clinme.2025.100527.
6. Kumari S, Gautam BK, Singh AK, Gaur V, Kumar A. Diagnostic precision of Truenat® technique and correlation of ALT levels with HBV-DNA viral load among HBsAg positive patients at a tertiary care hospital in Eastern Uttar Pradesh. *Iran J Microbiol*. 2024;16(1):132-138. doi:10.18502/ijm.v16i1.14882.
7. Kumar M, Seema K, Kumar D, Kumar A, Sharma AK, Boipai M, et al. Seroepidemiology of hepatitis B virus (HBV) and relationship to serum transaminase levels in Indian population. [Journal article]. 2024.
8. Gu S, Tao Y, Fan C, Dai Y, Li F, Conklin JL, et al. Impact of hepatitis B virus point-of-care DNA viral load testing compared with laboratory-based standard-of-care approaches on uptake of HBV viral load testing, treatment, and turnaround times: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2024;11(9). doi:10.1093/ofid/ofae483.
9. Lemoine M, EASL collaborators. The 2024 updated WHO guidelines for the prevention and management of chronic hepatitis B: main changes and potential implications for the next major liver society clinical practice guidelines. *J Hepatol*. 2025. doi:10.1016/j.jhep.2024.12.004.
10. Liu M, Zhao T, Zhang J, Bu B, Zhang R, Xia X, et al. Estimating the key outcomes and hepatocellular carcinoma risk in patients in immune-tolerant phase of chronic hepatitis B virus infection: a systematic review and meta-analysis. *Rev Med Virol*. 2024;34(4). doi:10.1002/rmv.2570.
11. Jin YJ, Kim HY, Suh YJ, Lee CH, Yu JH, Kim MN, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using vibration-controlled transient elastography: systematic review and meta-analysis. *Clin Mol Hepatol*. 2024;30(Suppl). doi:10.3350/cmh.2024.0163.
12. Lani L, Stefanini B, Trevisani F. Surveillance for hepatocellular carcinoma in patients with successfully treated viral disease of the liver: a systematic review. *Liver Cancer*. 2024;13(4):376-388. doi:10.1159/000535497.
13. Wang J, Zhu L, Zhang Z, Zhang S, Pan Y, Cao F, et al. Lower HBV DNA level is associated with more severe liver fibrosis in HBeAg-positive chronic hepatitis B with normal alanine transaminase. *Virol J*. 2024;21(1):127. doi:10.1186/s12985-024-02368-0.
14. Zheng D, et al. Circulating metabolites are associated with persistent elevations of ALT in patients with chronic hepatitis B with complete viral suppression. *J Med Virol*. 2024. doi:10.1002/jmv.29723.
15. Hirode G, et al. Limited sustained remission after nucleos(t)ide analog withdrawal: results from a large, global, multiethnic cohort of patients with chronic hepatitis B (RETRACT-B Study). *Am J Gastroenterol*. 2024.
16. Rajalatchumi A, Chinnakali P, Dhodapkar R, Olickal JJ, Subramanian S, Kar SS, et al. Prevalence of hepatitis B infection and its associated factors in rural South India. *Indian J Gastroenterol*. 2025;44(4):517-524. doi:10.1007/s12664-025-01768-6.

17. Swaroop S, Shalimar, Acharya SK. Hepatitis B virus prevalence in India: a wake-up call for action. *Indian J Gastroenterol.* 2025;44(5):585-587. doi:10.1007/s12664-025-01804-5.
18. AGA Institute. AGA Clinical Practice Guideline on the prevention and treatment of hepatitis B virus reactivation in at-risk individuals. *Gastroenterology.* 2025.
19. Wang J, et al. Risk factors for significant histological changes in both HBeAg-positive and negative treatment-naïve chronic hepatitis B with persistently normal alanine aminotransferase level. 2024.
20. Guo L, et al. Efficacy of antiviral therapy in adults with chronic hepatitis B according to baseline hepatitis B virus DNA and alanine aminotransferase concentrations: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2026.
21. Chou R, et al. Natural history of chronic hepatitis B in untreated adults without cirrhosis according to baseline hepatitis B virus DNA and alanine aminotransferase concentrations: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2026.
22. You H, Maiwall R, Chen J, Ahn SH, Dokmeci K, Dou X, et al. APASL clinical practice guidelines on the management of chronic hepatitis B infection: a 2026 update. *Hepatol Int.* 2026. doi:10.1007/s12072-026-11108-1.
23. World Health Organization. Consolidated guidance on hepatitis B and C prevention, testing, treatment, service delivery and monitoring: an implementation handbook for a public health approach. Geneva: World Health Organization; 2026.
24. World Health Organization. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection: updated guidance on HBV DNA testing and treatment eligibility. Geneva: World Health Organization; 2024.