



DIAGNOSTIC PRECISION OF TRUENAT® TECHNIQUE HBV-DNA VIRAL LOAD AMONG HBSAG POSITIVE PATIENTS AT A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: In India, it is estimated that there are 40 million people suffering from Hepatitis B virus (HBV). A laboratory tool that is useful in managing the disease is the measurement of viral burden. It is still difficult, however, to use different HBV-DNA assays widely because of their high cost and variable diagnostic precision. An evaluation of the diagnostic precision of Truenat®-PCR with HBV-DNA was conducted in this study. **Aim:** This study aims to evaluate the diagnostic accuracy of Truenat®-PCR in detecting HBV-DNA. The second objective aims to determine the sensitivity of Truenat®-PCRs versus rapid HBsAg tests within the same samples and patients. **Materials and Methods:** In this prospective cross-sectional study, 96 serum samples were collected from patients using rapid HBsAg processing. The viral HBV-DNA amplification detection was carried out through by Truenat®-PCR test. **Results & Discussion:** 96 samples were found to be positive by rapid and 71 Truenat®-PCR. 25 samples were negative for HBV-DNA and 71 samples had HBV-DNA levels greater than 100000 copies/ml, which is significantly higher in 71 patients as compared to the patients below 100000 copies/ml. **Conclusion:** HBV infection can be effectively controlled with Truenat®-PCR technique, which is highly sensitive and requires few resources. There was a significant proportion of patients with current viral replication and disease progression based on an evaluation of HBV-DNA levels.

KEYWORDS

Diagnostic Precision, Hepatitis B Virus, Hepatitis B Surface Antigens, HBV Infection

INTRODUCTION

Hepatitis B virus infection is a major public health issue worldwide due to its widespread prevalence and deadly nature. World-wide, the prevalence and mode of transmission of this disease vary greatly. More than 40 million people in India are infected with hepatitis B, accounting for approximately 10–15% of the global HBV carrier population.^[1] Overall, HBV infections remain immoderately high in wage and middle class populations, especially in Asian and African countries. It is estimated that India has the second-highest number of chronic hepatitis infections after China, and the reason for this is that many patients with HBsAg positive are infected. Patients infected prenatally generally remain asymptomatic for most of their lives and are often detected incidentally during routine screening.^[2] Although these patients are asymptomatic and appear healthy on presentation; however, a proportion of them show biochemical and histological abnormalities and may develop symptomatic chronic liver disease or cirrhosis or hepatocellular carcinoma after long-term follow-up.^[3]

Furthermore, recent studies indicate that HBV-DNA levels play a role in the development of these complications. With regard to progression to cirrhosis, the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B virus (REVEAL-HBV) study found that liver cirrhosis incidence increased as a function of HBV-DNA levels, ranging from 5% when viral load was undetectable (300 copies/ml) to 14.9% when viral load was detectable (106 copies/ml). Several studies indicate hepatocellular carcinoma risk is significantly associated with HBV-DNA levels^[4,5,6,7,8,9,10]

The natural course of Chronic Hepatitis B virus infection depends on the interaction between the virus replication and the host immune response^[11]. According to the newer nomenclature of Chronic HBV infection, the two main characteristics of chronicity are infection and hepatitis.^[12] To diagnose hepatitis, a variety of techniques are available, including immunochromatographic tests, reverse passive haemagglutination tests, enzyme-linked immunosorbent assays (ELISAs), enzyme immunoassays (EIAs), chemiluminescent immunoassays (CLIAs), polymerase chain reactions (PCRs) and Truenat®-PCRs. Using Truenat®-PCR, a chip-based technique with rapid turnaround time and no need for biosafety cabinets, staff with minimal laboratory experience can perform the test with good sensitivity and specificity^[14]. In this study, the diagnostic precision of Truenat®-PCR for HBV-DNA will be evaluated. The second objective aims to determine the sensitivity of the Truenat® PCR technique over rapid HBsAg testing in the same patients and samples.^[15]

MATERIALS AND METHODS

This prospective cross-sectional study was conducted at Microbiology Laboratory of Department of Microbiology, Government Medical College Attached R.K. Joshi Hospital Dausa, Rajasthan India under the program run by National Viral Hepatitis Control programme (NVHCP) from September 2025 to March 2026. Inclusion criteria were patient walk-in the OPD and IPD cases with positive HBsAg report from outside or diagnosed positive by HBsAg card test at Microbiology laboratory of the tertiary care hospital and availability of HBV-DNA sample.

Those with chronic hepatitis C, autoimmune hepatitis, jaundice, ascites, alcoholic liver disease, or recent drug-induced hepatitis were excluded. In total, 96 cases were enrolled based on our strict inclusion and exclusion criteria. A total of 3ml of blood sample was taken from all enrolled patients who completed the primary registration process and whose consent was obtained for collecting blood samples (serum & plasma) in vacutainers^[16]. All serological tests were conducted in the hospital's Microbiology laboratory, including liver function tests (LFTs). Truenat® test chips were used to detect viral HBV-DNA on the True lab UNO DX platform^[17]. A patient's age, gender, region of origin, and socioeconomic status were all obtained as part of the demographic information.

Truenat®-PCR technique. In our set-up, TRU-ELAB Uno Dx PCR ANALYSER machine was used for performing HBV, HCV, TB (14). A 0.5mL of the serum/plasma sample was transferred into the lysis buffer bottle using 1 mL transfer pipette. After mixing the mixture, a 1 mL transfer pipette was used to transfer it to the universal cartridge-based sample pre-kit. The cartridge was inserted into the True prep auto V2 extraction machine and the completion of extraction was indicated by a beep sound after 15 minutes. The elute was collected in Elute Collection Tube (ECT), then 6 µL of the purified RNA from the elute collected tube into the micro tube. Allow it to stand for 30-60 seconds. Using the same pipette and barrier tip the clear solution was dispensed into the center of the Truenat® microchip and run was started^[18].

Amplification test and interpretation. Once run completed after 60 minutes, an amplification curves are displayed graphically on the RT-PCR analyzer screen to indicate the progress of the test. Both the target and the Internal Positive Control (IPC) curves will take a sheer, expanding path when the fluorescence will cross the threshold value in case of positive samples. The cycle threshold (Ct) of the specimen will depend on the number of virus copies in the sample. Throughout the test duration, only the IPC curve will take an augmented path in case of negative samples. In case the IPC curve remains horizontal, the test is considered as Invalid. At the end of the test run, the results screen will

display “DETECTED” for positive result or “NOT DETECTED” for negative result. We used factor of 5.26 to convert the result from IU/ml to copies/ml as instruction given in the kit insertion by Molbio Diagnostics Private Limited^[18,19].

RESULT AND DISCUSSION

This study is a laboratory-based descriptive observational study. The study was carried out from September 2025 to March 2026 at the Government Medical College and R.K Joshi Hospital Dausa (Rajasthan) By rapid and 71Truenat®-PCR, 96 samples showed positive results, while 25 were negative. There were 71 patients with high HBV-DNA levels (>100000 copies/ml) compared with those with low levels of HBV-DNA.

Table 1: Distribution of HbsAg Card Positive & Truenat Positive Sample

Total Test	HbsAg Card Positive	Truenat Positive
96(100%)	96(100%)	71(73.95%)

Table 2: Sex Distribution of Study Participants

Total positive sample	Total Male sample positive	Total Female sample positive
71(100%)	41(57.74%)	30(42.25%)

Table 3: Age Distribution of the Study Participants

Age Group	Total test
10-20	4(5.63%)
20-40	42(59.15%)
40-60	20(28.16%)
60-80	5(7.04%)
Total	71(100%)

Table 4 Distribution of Hepatitis B Viral Load Logs

Hepatitis B viral load logs	Total number
1.1x10 – 1.10x10	22(30.98%)
2.1x10 – 2.10 x 10	10(14.08%)
3.1x10 – 3.10 x 10	8(11.26%)
4.1 x 10 – 4.10 x 10	6(8.45%)
5.1x 10 – 5.10 x 10	10(14.08%)
6.1 x 10 – 6.10 x10	8(11.26%)
7.1 x 10 – 7.10 x 10	4(5.63%)
8.1x 10 – 8.10 x 10	2(2.81%)
9.1 x 10 – 9.10 x 10	1(1.40%)
Total	71(100%)

Quantitative real-time PCR is a highly sensitive molecular technique used for the detection and quantification of HBV-DNA viral load. This study provides a cross sectional view of hepatitis B virus infection in Dausa, Rajasthan with a primary objective to identify the precision of Truenat®-PCR technique HBV-DNA viral load among the patients who were incidentally detected HBsAg positive at Government Medical College Dausa attached R.K. Joshi Hospital and also this study assessed the role and assessment of diagnostic precision of Truenat® HBV-DNA over rapid HBsAg immuno- chromatography method^[20,21,22,23,24,25,26]. 96 samples were found to be positive by rapid and 71Truenat®-PCR and 25 were negative for HBV-DNA who had HBV-DNA level (>100000 copies/ml) which is significantly higher in 71 patients as compared to those have below ≤100000 copies/ml.

Among the HBsAg positive subjects enrolled in our study, 46 (64.78%) had an age of at least 40 years, while 25 (35.20%) had an age of over 40 years. The mean age at detection of HBsAg positive status was 35.5 years. A greater proportion of males were detected positive (57.74%) than females (30(42.25%), indicating that men were more likely to become infected than women. In contrast to women, men are more likely to use drugs, engage in risky behavior, apply for jobs and visas, donate blood, and undergo general health screenings at outpatient departments.

Similar findings emerged from a study which showed that of 704 subjects tested positive for HBsAg at medical camps for voluntary testing, 570 (80.96%) were males and 134 (19.03%) were females^[27,28,29,30,31,32,33]. A total of 592 subjects, of which 454 (77%) were males, participated in the future follow-up study. In the study, 456 (77%) participants were aged 40 years or younger, whereas 136 (23%) were above 40 years of age. These findings are consistent with those of Khadaka et al. (2018), who reported a higher prevalence of chronic hepatitis B (CHB) infection among males (59.7%) compared to females (40.3%) in a study of 48 participants.^[34,35,36]

Based on B Nandi et al. (2012), most participants were asymptomatic and were discovered unintentionally during a health check-up or screening at blood donation camps. The majority of their research was conducted by men and their average age was 40 years as compared to women's average age of 34 years.^[37,38] The prevalence of HBV was highest in the 30-39year age bracket, based on a study of 594 HBsAg-positive subjects^[39,40,41].

We tested 96 participants for HBsAg. 25 (26.04%) subjects had no detectable HBV-DNA, while 71 (73.95%) subjects had detectable HBV-DNA. Among them, 32 (45.07%) subjects had viral loads between 2677 and 100000 copies/ml, which corresponds to low to moderate viral loads, whereas 24 (33.8%) subjects had levels above 100000 IU/ml, which corresponds to high viral loads; 15 (21.12) subjects did not have viral loads. One study reported that among 592 HBsAg-positive subjects, 349 (59%) were positive for HBV-DNA^[42].

Although this study demonstrated the diagnostic accuracy and performance of the Truenat®-PCR assay for HBV-DNA viral load estimation, certain limitations should be acknowledged. The accessibility and routine implementation of the Truenat®-PCR assay may be limited by its relatively low sample processing capacity, making it less suitable for high-throughput testing. In addition, the assay is unable to accurately quantify viral loads that fall outside its linear detection range, resulting in values being reported only as above or below the measurable limits. Furthermore, while this study evaluated the correlation between HBV-DNA levels and HBsAg positivity, the findings are insufficient to determine the impact of antiviral therapy across different stages of chronic hepatitis B infection. Longitudinal studies with larger sample sizes and treatment follow-up are needed to assess the clinical utility of Truenat®-PCR for monitoring therapeutic response and disease progression.^[43,44]

CONCLUSION

A large proportion of HBsAg positive patients in India acquire the hepatitis B virus perinatally, remain asymptomatic for a long time, and are diagnosed incidentally. Compared with rapid HBsAg tests, Truenat®-PCR was more sensitive and precise for diagnosing HBV. The technique can also be used for clinicians to make clinical decisions regarding starting antiviral treatment and intervening in the patient's health condition in relation to HBV viral load. In our study, we found that many of these participants harbored ongoing viral replication and liver injury, and were at risk for further disease progression. Using Truenat®-PCR is a highly sensitive and resource-efficient method. The establishment of PCR laboratories in small peripheral laboratories is very convenient for processing fewer samples. Additionally, with this tool, we can identify patients with high HBV-DNA levels who may progress rapidly to the disease.

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Nil

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contribution

Vinita Choudhary contributed to the conceptualization of the study, data curation, formal data analysis, and preparation of the original manuscript draft. She also participated in the review and editing of the manuscript. Ankur Yogi and Ramesh Chand Sharma supervised the study and contributed to the investigation. All authors critically reviewed the manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

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