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ORIGINAL ARTICLE

Diagnostic precision of Truenat® technique and co-relation of ALT levels with HBV-DNA viral load among HBsAg positive patients at a tertiary care hospital in Eastern Uttar Pradesh

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ABSTRACT

Background and Objectives: In India, it is estimated that there are 40 million people suffering from Hepatitis B virus (HBV). Quantification of the viral burden is an important laboratory tool in the management. However, widespread use of different HBV-DNA assays is still affected by the high cost and variable diagnostic precision. The present study was conducted to evaluate the diagnostic precision and co-relation of ALT levels with HBV-DNA by Truenat®-PCR.
Materials and Methods: In this prospective cross-sectional study a total of 567 serums were collected from patients by rapid HBsAg, and processed for liver function tests (LFT). The viral HBV-DNA amplification detection was carried out through by Truenat®-PCR test.
Results: Out of 567 samples, 452 samples were found to be positive by both rapid and Truenat®-PCR and 106 were negative for HBV-DNA followed by 9 invalid. High ALT level found in 73% of positive patients who had HBV-DNA level (>100000 copies/ml) which is significantly higher in 447 patients as compared to those have below ≤100000 copies/ml.
Conclusion: Truenat®-PCR technique is a highly sensitive and can be performed with low resources for effective control of HBV infection. Evaluation of HBV-DNA levels and serum ALT levels showed a significant proportion of patient harbored ongoing viral replication and disease progression.

Keywords: Hepatitis B virus; Hepatitis B surface antigens; HBV infection; Diagnostic precision

INTRODUCTION

Viral hepatitis is one of the leading causes of death and Hepatitis B virus infection is a growing public health issue worldwide. Its prevalence and mode of transmission varies greatly in different parts of the world. It is estimated that approximately one-fifth of the world population and over 40 million hepatitis B infected subjects, accounting for approximately 10-15% of the entire pool of HBV carriers of the world live in India (1). The overall burden of HBV infections remains immoderately high in wage and middle class particularly in Asian and African populated countries. India is second after China in terms of number of chronic hepatitis infection and the reason behind this; a large proportion of HBsAg-positive

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patients in India acquire hepatitis B virus infection prenatally, remain asymptomatic for a long duration and are detected incidentally (2). Although on presentation these patients are asymptomatic and look healthy; a proportion of them show biochemical and histological abnormalities and have been shown to progress to symptomatic chronic liver disease or cirrhosis, or develop hepatocellular carcinoma on long term follow up (3).

Patients with chronic hepatitis B are at an increased risk of developing cirrhosis of liver and hepatocellular carcinoma (HCC) (4). Moreover, there is recent evidence indicating a relationship between the development of these complications and baseline HBV-DNA level. 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 the cumulative incidence of liver cirrhosis increased as a function of HBV-DNA levels, ranging from 5% when viral load was undetectable (<300 copies/ml) to 36% with $\geq 10^6$ copies/ml irrespective of alanine aminotransferase (ALT) or hepatitis B e antigen (HBeAg) status (5, 6). Similar results have been reported with respect to the risk of developing hepatocellular carcinoma. Cumulative incidences were 1.3% with < 300 copies/ml and 14.9% with $\geq 10^6$ copies/ml. HBV-DNA levels were significantly associated with the risk of developing hepatocellular carcinoma ($p < 0.001$) (6, 7).

The natural course of Chronic Hepatitis B virus infection is determined by the interplay between virus replication and the host immune response (8). The newer nomenclature of various phases of natural course of Chronic HBV infection is based on the two main characteristics of chronicity: infection vs hepatitis (9). Thereby the HBV-DNA levels and Alanine aminotransferase (ALT) values have importance in identifying the phase of natural course and subsequently deciding whether the patient requires treatment and/or monitoring (10). There are various techniques, which can be used to diagnosis the hepatitis like Immunochromatographic Test (ICT), Reverse Passive Haemagglutination (RPHA), Enzyme linked ImmunoSorbant assay (ELISA), Enzyme Immunoassay (EIA), Chemiluminescent Immunoassay (CLIA), Polymerase chain reaction (PCR) and Truenat®-PCR. Truenat®-PCR is a chip based technique having rapid turnaround time, does not require biosafety cabinet and staff with minimal laboratory training can perform the test with good sensitivity and specificity (11). Thus this present study aims to evaluate the diagnostic precision and co-relation of ALT levels with HBV-DNA by closed system Truenat®-PCR. Second objective aims to understand the sensitivity of Truenat®-PCR technique over rapid HBsAg within the same sample and patients (12).

MATERIALS AND METHODS

This prospective cross-sectional study was conducted at State Reference Laboratory of Department of Microbiology, BRD Medical Collage, Gorakhpur, Uttar Pradesh, India under the program run by National Viral Hepatitis Control programme (NVHCP) from April 2022 to March 2023. With a bed capacity of around 1300, it is the only tertiary care teaching and referral hospital in the eastern Uttar Pradesh offering free of cost services in the management of Hepatitis B.

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 central laboratory of the tertiary care hospital and availability of HBV-DNA sample.

Exclusion criteria were patient with chronic hepatitis C, autoimmune hepatitis, jaundice, ascites, alcoholic liver disease, history of recent drugs induced hepatitis.

A total of 567 cases were enrolled per our strict inclusion and exclusion criteria. Out of all enrolled patients, those who recommended for screening test for HBsAg in OPD underwent for the primary registration process and their 3ml of blood sample are collected in vacutainer (serum & plasma) after taking proper written consent from them (13). All serological test was performed in central laboratory of hospital including liver function test (LFT). Platform used for the detection of viral HBV-DNA was Truelab UNO DX included Truenat® test chips (14). Demographic information obtained in respect to the patient included age, gender, region of origin and socioeconomic status.

Patient with high HBV viral load and /or high ALT (Alanine aminotransferase) /AST were considered potential candidates for antiviral therapy. Those with detectable DNA and/or high ALT were advised for regular 3-6 months follow-up.

Truenat®-PCR technique. In our set-up, TRU-

ELAB Uno Dx PCR ANALYSER machine was used for performing HBV, HCV, Japanese Encephalitis (JE) and SARS-CoV-2 assays (14).

A 0.5mL of the serum/plasma sample was transferred into the lysis buffer bottle using 1 mL transfer pipette. Then, the mixture was transferred to the universal cartridge based sample pre kit using a 1 mL transfer pipette. The cartridge was inserted into the Trueprep 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 microtube. Allow it to stand for 30-60 seconds. Using the same pipette and barrier tip the clear solution was dispensed into the centre of the Truenat® microchip and run was started (15).

Amplification test and interpretation. Once run completed after 60 minutes, an amplification curves are displayed graphically on the RT-PCR analyser 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 (15).

Statistical analysis. All the required parameters were tabulated in the form of master chart and analyzed using SPSS version: 23.0. The categorical variables were expressed as frequencies and percentages. Continuous variables expressed as means with standard deviation when the distribution was gaussian and medicans with interquartile range when the distribution was non-parametric. The socioeconomic or demographic data were analysed by using modified Kuppuswamy scale (Table 1) (16).

Ethical approval. Consent was obtained or waived by all participants in this study. Institutional Human Ethics Committee issued approval 19/IHEC/BRD/2023. The study was approved by the Institutional Ethics Committee, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, India.

RESULTS

During the study period, 567 samples were collected from patients who fulfilled the study criteria. Of these, 395 were from Gorakhpur division of Uttar Pradesh, 88 were from different division of Uttar Pradesh and 84 were from another state Bihar. Maximum subjects detected HBsAg positive incidentally were between 16-40 years of age. Approximately 2/3rd of the HBsAg-positive subjects were male and 1/3rd were female (Table 1).

Table 1. Ages and sex-wise distribution of HBsAg positive cases (n=567).

Age (years)	Number	Male	#HBV Detected	Female	#HBV Detected
0-15	7	4	2	3	2
16-40	380	207	167	173	136
41-60	127	90	77	37	26
>60	53	39	31	14	11
Total	567	340	277	227	175

Abbreviations: #HBV-Hepatitis B Virus

All 567 samples were processed through HBsAg rapid card and confirmed by Truenat®-PCR technique, but 452 samples were found to be positive on both rapid and Truenat®-PCR. However, 106 samples were negative/not-detected for HBV-DNA on Truenat®-PCR and only 9 samples were found invalid with no result, it happens may be due to improper extraction of DNA and fresh samples asked for the repetition of the test. High linearity of HBV-DNA positive found only in 6.7% of the total samples followed by 1.94% samples found positive for HBV-DNA with below linearity. The difference between the two tests was found to be statistically significant.

The sensitivity of rapid HBsAg with respect to Truenat®-PCR was 79%. Out of 567 consecutive patients who had tested positive on rapid HBsAg card were considered for this study. Out of these, 79.71% (452) were found positive on Truenat® HBV-DNA and considered for baseline biochemical characteristics. In

177 participants (39%), had significantly higher ALT levels, out of which 79 had ALT levels more than 100 IU/ml (Table 2).

Out of 567 consecutive patients who had tested positive on rapid HBsAg card were considered for this study. Out of these, 79.71% (452) were found positive on Truenat® HBV-DNA and considered for baseline biochemical characteristics. In 177 participants (39%), had significantly higher ALT levels, out of which 79 had ALT levels more than 100 IU/ml.

High ALT level found in 73% of positive HBV-DNA patients who had HBV-DNA level (>100000 copies/ml) which is significantly higher in those (447 patients) who had HBV-DNA level below than (≤100000 copies/ml) (Table 3).

Table 2. Distribution of different levels of ALT among HBV positive cases (n=452).

#ALT (IU/ml)	Number	Percentage (%)
Normal(≤45)	275	60.84
Mild (<100)	98	21.68
Abnormal (>45)		
Severe (>100)	79	17.48
Total	452	

Abbreviations: #ALT- Alanine Aminotransferase

Table 3. Distribution of HBV-DNA viral load with levels of different levels ALT among HBsAg cases (n=452).

HBV-DNA Counts (copies/ml)	ALT (IU/ml)		Abnormal (>45)	
	No. of Patients	Normal (≤45)	Mild (≤100)	Severe (>100)
Below linear range	11	6	2	3
≤100000	336	241	82	13
>100000	67	22	5	40
Above linear range	38	6	9	23

DISCUSSION

The quantitative real time PCR technique for the detection of HBV-DNA viral load is a highly sensitive molecular method. This study provides a cross sectional view of hepatitis B virus infection in Eastern Uttar Pradesh, with a primary objective to identify the precision of Truenat®-PCR technique and investigating the relation between ALT level and HBV-DNA viral load among the patients who were incidentally detected HBsAg positive at B.R.D. Medical College Gorakhpur and also this study assessed the role and assessment of diagnostic precision of Truenat® HBV-DNA over rapid HBsAg immunochromatography method (17).

Among the HBsAg positive subjects taken in our study 67.92% (n=307) of the participants had age of ≤ 40 years and 32.08% (145) subjects had age >40 years and the mean age at detection of HBsAg positive status in our study was 35.5 years. Male were more commonly detected positive 61.28% (n=277) than female 38.72% (n=175). That shows men were more likely to develop an infection than women. In contrast to the female population, men are more likely to use drugs, engage in risky behavior, apply for job and visas, volunteer as blood donors and have general health screenings at out patient department (OPD).

In similar finding by a study which showed 704 subjects that tested positive for HBsAg at medical camps for voluntary testing of HBsAg 570 (80.96%) were male and 134 (19.03%) were female (18). Of the 704 subjects 592 subjects came forward for future follow up of which 454 (77%) were males. The study also found that 456 (77%) participants were of the age 40 years or below and 136 (23%) were above 40 years age. Khadaka et al. (2018) in his study showed that chronic hepatitis B (CHB) infection was more common in male gender 59.7% as compared to female 40.3% (n=48) (19).

B Nandi et al. (2012) in their study revealed that majority of the participants were asymptomatic and were unintentionally discovered during blood donation camps health check-up or screening, with the men making up the majority of their research and having mean age of 40 years as compared to women's mean age of 34 years (20).

One study conducted on 594 HBsAg positive subjects revealed a male to female ratio of 2.9:1 and the age bracket of 30-39 years had the highest HBV prevalence (21).

Measurement of serum ALT levels in asymptomatic HBsAg positive subjects is of importance in assessing any ongoing hepatocellular injury. Raised ALT (>45 IU/ml) was seen in 39.15% (n=177) of subjects in our study and this was below than the study conducted by Choudhari G et al. (2019) in which raised ALT was observed in 54% of the HBsAg positive

subjects (18). Another study over 317 symptomatic HBsAg positive subjects showed that 65% subjects had raised ALT greater than 40 IU/ml (22).

Further, the participants underwent quantitative testing for HBV-DNA viral load. HBV-DNA was not detectable in 18.69% (n=106) subjects followed by invalid results in 1.58% (n=9) and the remaining 79.73% subjects had HBV-DNA detectable among them. Among which 74.33% (n=336) subjects had viral load ranging from 2677 to 100000 copies/ml i.e. low to moderate followed by 2.43% (n=11) had values below than linearity and 14.82% (n=67) subjects had HBV-DNA levels >100000 IU/ml i.e. high viral load while in 8.40% (n=38) HBV-DNA values are above the linearity range. Researcher also found HBV-DNA viral load in 85% (n=85) out of 100 incidentally detected asymptomatic HBsAg patients (23). One study revealed that of the 592 HBsAg positive subjects, 349 (59%) patients were found positive for HBV-DNA (17). Khadaka et al. (2018) showed 90% patients had detectable viral load out of 119 asymptomatic patients (19).

This study also assessed the relationship between ALT levels and HBV-DNA levels and showed that HBV- DNA levels are significantly higher in those with abnormal ALT levels. Nita et al. (2009) in their study also assessed the relationship between HBV-DNA and ALT levels and HBeAg status and revealed that HBV-DNA were significantly higher in HBeAg positive individuals and in those with abnormal ALT levels (1).

As per the current guidelines on hepatitis B treatment, no treatment is recommended for patients with normal ALT levels as they are not considered to have active disease but when our subset analysis focused on such individuals approximately 60.84% (n=275) participants fell in this category and as our objective was to assess the prevalence of high viral load in incidentally detected asymptomatic HBsAg positive subjects and to evaluate serum ALT levels in them, since it would be expected that most patients with high ALT levels would have the active disease.

This study also showed that a significant number of patients had this profile of low ALT levels and high HBV-DNA levels in asymptomatic HBsAg positive group and it is probable that such patients would be easily missed within the public healthcare system where HBV-DNA is not routinely tested. Furthermore in another study it was shown that patient with high HBV-DNA levels were more prone to disease progression in future (24).

Considering the evidence that high viral load is associated with increasing probability of liver cirrhosis and HCC, we propose that patients with high HBV-DNA levels and low ALT levels should be kept under suspicion for harboring active infection and to be at elevated risk for complications. Therefore, these patients should be evaluated further with other possible investigational modalities and should be followed-up more closely.

The limitation of the study was that the study demonstrated the diagnostic accuracy of Truenat®-PCR HBV viral load tests, it's performance, but accessibility of Truenat®-PCR need to be considered because of its limitation to process the number of samples in a same time and not able to defined the viral load if the linearity of test either high or below the range of detection. We investigated the co-relation between the HBV-DNA level and ALT levels but this evidence is not enough to assess the effect of an antiviral therapy in patients at different stages. The present study was confined to only one institute, and due to the less number of paediatric group sample; it is very difficult to investigate the rate of infection in the same pool.

CONCLUSION

In India a large proportion of HBsAg positive patients acquire hepatitis B virus infection perinatally and remain asymptomatic for a long duration and are detected incidentally. In our study, Truenat®-PCR was found to be more sensitive and precise than rapid HBsAg test for the diagnosis of HBV. This technique also useful to make clinical decisions on starting antiviral therapy for clinician and to intervene the patient health condition in respect to HBV viral load.

Our study showed evaluation of such participants by measuring HBV-DNA levels and serum ALT levels showed that a significant proportion of them harbored ongoing viral replication and liver injury and were at risk for further disease progression. Truenat®-PCR technique is a highly sensitive and can be performed with low resources. It is very convenient to establish a PCR laboratory in small peripheral laboratories to process less number of sample.

Furthermore, this tool also enables us to find those with high HBV-DNA levels who are at risk of rapid disease progression among patients with normal serum ALT levels.

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