

PREVALENCE OF HEPATITIS B AND HEPATITIS C INFECTIONS AMONG CRITICALLY ILL PATIENTS ADMITTED TO A TERTIARY CARE HOSPITAL: A COMPARATIVE EVALUATION OF ELISA, CONVENTIONAL PCR, AND TRUENAT MOLECULAR ASSAYS

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ABSTRACT

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections remain major causes of chronic liver disease and liver-related mortality worldwide. Critically ill patients are particularly vulnerable because delayed diagnosis may adversely affect clinical outcomes and infection control practices. Molecular assays may detect additional infections that are missed by conventional serological methods.

Objectives: To determine the prevalence of HBV and HCV infections among critically ill patients admitted to a tertiary care hospital and to compare the diagnostic performance of ELISA, conventional PCR, and Truenat molecular assays.

Materials and Methods: This hospital-based cross-sectional study was conducted among critically ill patients admitted to a tertiary care hospital. A total of 1,317 patients were evaluated for HBV infection and 978 patients for HCV infection. Serological screening was performed using commercially available ELISA kits. For HBV, 593 samples underwent conventional DNA PCR and 724 samples underwent Truenat testing. For HCV, 552 samples underwent conventional RNA PCR and 426 samples underwent Truenat testing. Discordant results between serological and molecular methods were retested using freshly collected blood samples.

Results: Among HBV samples, 443 of 593 (74.7%) tested by conventional PCR and 492 of 724 (68.0%) tested by Truenat were positive. Among HCV samples, 252 of 552 (45.7%) tested by conventional PCR and 203 of 426 (47.7%) tested by Truenat were positive. Molecular assays identified six additional HBV infections and ten additional HCV infections that were not detected by ELISA, whereas ELISA identified three additional HCV cases. Fever (75%) and right upper abdominal pain (60%) were the predominant presenting symptoms. Hepatomegaly was observed in 34% of cases and hepatic encephalopathy developed in approximately 28% of HBV-positive patients.

Conclusion: A substantial burden of HBV and HCV infections was observed among critically ill patients. Molecular assays demonstrated an incremental diagnostic yield over serological methods and identified additional infections that would otherwise have remained undiagnosed. Truenat showed performance comparable to conventional PCR and may serve as a reliable, rapid, and decentralized molecular platform for hepatitis diagnosis in resource-constrained settings.

KEYWORDS: Hepatitis B virus, Hepatitis C virus, ELISA, Polymerase chain reaction, Truenat, Critical care, Molecular diagnosis

INTRODUCTION

Viral hepatitis caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) continues to be a major public health challenge worldwide and remains one of the leading causes of chronic hepatitis, cirrhosis, hepatic decompensation, and hepatocellular carcinoma¹. According to the World Health Organization, approximately 254 million people are living with chronic HBV infection and nearly 50 million people are infected with HCV globally², accounting for more than one million deaths annually due to hepatitis-related complications.³

Critically ill patients represent a particularly vulnerable population because underlying comorbidities, prolonged hospitalization, invasive procedures, blood transfusions, immunosuppression, and multiorgan dysfunction may increase the risk of acquisition and adverse outcomes of viral hepatitis. Early diagnosis is therefore essential not only for patient management and therapeutic intervention but also for prevention of nosocomial transmission and implementation of infection control measures.⁴

Enzyme-linked immunosorbent assay (ELISA) is routinely employed for screening of HBV and HCV infections because of its simplicity, affordability, and suitability for large-scale testing. However, serological assays may fail to identify infections during the window period, occult infections, low-level viremia, and infections in immunocompromised individuals.^{5,6} Consequently, reliance solely on serological methods may lead to underdiagnosis and delayed treatment. Molecular techniques such as polymerase chain reaction (PCR) directly detect viral nucleic acids and are considered highly sensitive and specific diagnostic methods for HBV and HCV infections. Nucleic acid amplification tests can identify infections at an earlier stage and provide valuable information regarding viral replication and infectivity. Recent

advances in molecular diagnostics have resulted in the development of Truenat, a chip-based real-time micro-PCR platform that provides rapid and accurate detection with minimal laboratory infrastructure requirements and shorter turnaround times.^{7,8}

Although several studies have evaluated serological and molecular methods individually, comparative data regarding ELISA, conventional PCR, and Truenat among critically ill patients remain limited, particularly in resource-constrained settings. Therefore, the present study was undertaken to determine the prevalence of HBV and HCV infections among critically ill patients admitted to a tertiary care hospital and to compare the diagnostic performance of ELISA, conventional PCR, and Truenat molecular assays.

MATERIALS AND METHODS

Study design:

based cross-sectional observational study.

Study Setting:

The study was conducted in the Department of Microbiology gsvm medical college kanpur

Study Duration:

25 months (from 2024 to 2026).

Study Population:

All critically ill patients admitted to the tertiary care hospital who were evaluated for hepatitis B and hepatitis C infections during the study period.

Sample Size:

A total of 1,317 patients were investigated for HBV infection and 978 patients were investigated for HCV infection.

Inclusion Criteria:

All critically ill patients admitted to the tertiary care hospital who underwent evaluation for HBV and HCV infection and consented to participate in the study.

Exclusion Criteria:

Duplicate samples, haemolysed or inadequate specimens unsuitable for testing, and patients with incomplete clinical records were excluded.

Sample Collection and Processing:

Three to five millilitres of venous blood was collected aseptically under universal precautions in sterile vacutainers. Serum and plasma were separated by centrifugation and processed according to standard microbiological procedures.

Storage and Transport:

Samples were transported to the laboratory immediately after collection. If immediate processing was not possible, specimens were stored at 2–8°C and processed within 72 hours. For prolonged storage before molecular testing, aliquots were preserved at –20°C or –80°C to maintain nucleic acid integrity.

Serological Testing:

HBsAg and anti-HCV antibodies were detected using commercially available ELISA kits according to the manufacturer's instructions.

Molecular Testing:

HBV DNA detection was performed using conventional PCR in 593 samples and Truenat HBV assay in 724 samples. HCV RNA detection was performed using PCR in 552 samples and Truenat HCV assay in 426 samples according to standard protocols.

Discordant Results:

Samples demonstrating discordance between serological and molecular methods were retested using freshly collected blood specimens to minimize analytical and pre-analytical errors.

Statistical Analysis:

Data were entered into Microsoft Excel and analysed using appropriate statistical software. Results were expressed as frequencies and percentages. Sensitivity, specificity, positive predictive value, and negative predictive value of ELISA were calculated using molecular methods as reference standards. Agreement between methods was assessed using Cohen's kappa coefficient. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Among the 593 HBV samples tested by PCR, 443 (74.7%) were positive, while among the 724 samples tested by Truenat, 492 (68.0%) were positive. For HCV, 252 of 552 samples (45.7%) tested by PCR and 203 of 426 samples (47.7%) tested by Truenat were positive.

TABLE 1. Molecular Positivity Rates of HBV and HCV

Infection	Method	Samples Tested	Positive Cases	Negative Cases	Positivity Rate (%)
HBV	Conventional PCR	593	443	150	74.7
HBV	Truenat	724	492	232	68.0
HCV	Conventional PCR	552	252	300	45.7
HCV	Truenat	426	203	223	47.7

TABLE 2. Total Molecular Positivity

Infection	Total Samples	Total Molecular Positives	Total Molecular Negatives	Overall Positivity (%)
HBV	1317	935	382	71.0
HCV	978	455	523	46.5

TABLE 3. Incremental Diagnostic Yield Beyond ELISA

Infection	Additional Cases by Molecular Assays	Additional Cases by ELISA
HBV	6	0
HCV	10	3

TABLE 4. Clinical Characteristics of Positive Cases

Clinical Feature	Frequency (%)
Fever	75
Right upper abdominal pain	60
Anorexia	28
Vomiting	28
Malaise	25
Hepatomegaly	34
Splenomegaly	5
Hepatic encephalopathy	28

TABLE 5. Molecular Assay Performance Comparison

Parameter	HBV PCR	HBV Truenat	HCV PCR	HCV Truenat
Samples tested	593	724	552	426
Positive cases	443	492	252	203
Negative cases	150	232	300	223
Positivity (%)	74.7	68.0	45.7	47.7

Molecular assays identified six additional HBV infections and ten additional HCV infections that were not detected by ELISA, whereas ELISA identified three additional HCV cases. The predominant presenting symptoms included fever (75%), right upper abdominal pain (60%), anorexia (28%), vomiting (28%), and malaise (25%). Hepatomegaly was observed in 34% of cases and splenomegaly in 5%. Hepatic encephalopathy developed in approximately 28% of HBV-positive patients and represented the most frequent severe complication.

DISCUSSION

The present study demonstrated a considerable burden of hepatitis B and hepatitis C infections among critically ill patients admitted to a tertiary care hospital. The positivity rates observed by both conventional PCR and Truenat indicate that viral hepatitis remains an important clinical problem in the critical care setting.

Molecular assays identified additional HBV and HCV infections that were not detected by ELISA. This finding highlights the limitations of serological methods, particularly during the window period, in occult infections, low-level viremia, and in immunocompromised individuals commonly encountered in intensive care settings. Similar observations have been reported by previous studies demonstrating the superior sensitivity of nucleic acid amplification techniques for detection of viral hepatitis.

Truenat demonstrated positivity rates comparable to conventional PCR, suggesting that this platform may provide reliable molecular diagnosis with shorter turnaround times and minimal infrastructure requirements. Its portability, ease of operation, and rapid reporting make it particularly suitable for decentralized testing facilities and resource-limited healthcare settings.

The predominant clinical manifestations observed in the present study were fever and right upper abdominal pain. Hepatomegaly and hepatic encephalopathy were also frequently encountered. Notably, hepatic encephalopathy occurred

in approximately 28% of HBV-positive patients, indicating a substantial burden of advanced liver disease and emphasizing the importance of early diagnosis and timely therapeutic intervention.

The findings of the present study support the incorporation of molecular assays into routine diagnostic algorithms, particularly in critically ill patients with high clinical suspicion of hepatitis despite negative serological findings.

CONCLUSION

The present study demonstrated a significant burden of hepatitis B and hepatitis C infections among critically ill patients admitted to a tertiary care hospital. Molecular assays provided incremental diagnostic yield over conventional serological methods by identifying additional infections that would otherwise have remained undiagnosed. Truenat exhibited diagnostic performance comparable to conventional PCR and represents a reliable, rapid, and decentralized molecular diagnostic platform. Integration of molecular methods into routine diagnostic strategies may facilitate early diagnosis, improve patient management, strengthen infection control practices, and contribute to global efforts aimed at elimination of viral hepatitis.

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