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Original Article

Evaluation of ICT and ELISA in Comparison to RT-PCR Assay in the Diagnosis of HCV Infection

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Abstract

Background: *Hepatitis C virus (HCV) infection is a major global health concern, often progressing to chronic liver disease, cirrhosis, and hepatocellular carcinoma if undiagnosed. Early and accurate diagnosis is critical for effective management. This study evaluated the diagnostic performance of the immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA) in comparison to reverse transcription-polymerase chain reaction (RT-PCR) for HCV detection.*

Methods: *A cross-sectional study was conducted among 50 participants, including 24 previously diagnosed HCV cases and 26 clinically suspected cases (History of HCV positive but no document and don't take any treatment), at Chittagong Medical College and Chittagong Veterinary and Animal Science University between January 2019 and December 2019. Serum samples were tested for anti-HCV antibodies using ICT and ELISA. HCV RNA was detected using RT-PCR, considered the gold standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios were calculated to assess the diagnostic performance of ICT and ELISA.*

Results: *The study population had a mean age of 39.16 ± 13.66 years, with a male-to-female ratio of 1.27:1. ICT and ELISA detected anti-HCV in 60% and 52% of cases, respectively. RT-PCR confirmed HCV RNA in 48% of participants. Compared to RT-PCR, both ICT and ELISA showed 91.67% sensitivity. ELISA demonstrated higher specificity (82.62%) than ICT (69.23%). PPV and NPV were 84.62% and 91.67% for ELISA, and 73.33% and 90% for ICT, respectively.*

Conclusion: *Both ICT and ELISA are highly sensitive for anti-HCV detection; however, ELISA provides superior specificity and predictive accuracy. RT-PCR remains essential for confirming active HCV infection. Integrating serological screening with molecular confirmation ensures reliable diagnosis and better clinical management of HCV infection.*

Keywords: *Anti-HCV antibodies, ELISA, HCV diagnosis, Hepatitis C virus, Immunochromatographic test, RT-PCR, Serological assay*

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Introduction

Hepatitis C virus (HCV) infection remains a significant global health challenge due to its capacity to cause chronic liver disease, cirrhosis, and hepatocellular carcinoma if left undiagnosed and untreated.[1] HCV is an enveloped, positive sense single stranded RNA virus belonging to the Flaviviridae family with a global distribution and high potential for chronicity.[2] Despite advances in therapy, the asymptomatic nature of early HCV infection means many cases remain undetected, which contributes to continued transmission and progression to life threatening complications.[3] Accurate diagnosis of HCV infection is therefore a crucial first step in effective disease management, prevention of transmission, and linkage to care. Serological assays that detect antibodies against HCV (anti HCV) are widely used as initial screening tools given their relatively low cost and ease of application. Third generation enzyme linked immunosorbent assays (ELISAs), which detect antibodies to multiple HCV antigens,

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are recommended for reliable anti HCV screening and have shown high sensitivity and specificity across diverse populations.[4,5] Rapid immunochromatographic tests (ICTs) serve as point of care methods that offer quick results without complex infrastructure, making them particularly useful in resource limited settings.[6] However, serological assays have inherent limitations, such as their inability to discriminate between past exposure and current active infection, and they may miss cases during the early "window period" before antibodies are produced.[7] To confirm active HCV infection and determine the presence of ongoing viral replication, nucleic acid amplification tests such as reverse transcription polymerase chain reaction (RT PCR) are essential. RT PCR directly detects HCV RNA, a definitive marker of active infection, and can identify infection during the antibody negative window period.[8] These molecular assays also play a critical role in patient monitoring, assessment of treatment response, and post treatment follow up.[8] Despite the high diagnostic accuracy of RT PCR, its widespread use in clinical practice is often limited by cost, need for specialized equipment, and technical expertise, which may be barriers in low and middle income regions.[9] Several studies have compared the performance of serological assays (ICT and ELISA) with molecular assays. Research in diverse settings has shown that while rapid tests can perform well in antibody detection, their sensitivity and specificity can vary, and they generally require confirmatory testing with ELISA or molecular methods.[6] Evidence also supports that third generation ELISA tests improve anti HCV detection compared to earlier methods and align better with molecular results, although some discordance remains, especially in immunocompromised individuals and during early infection.[5,10] Evaluation of diagnostic performance highlights the complementary nature of combining serological screening followed by molecular confirmation to enhance diagnostic accuracy and clinical decision making.[4,5] Given this context, evaluating the diagnostic accuracy of ICT and ELISA relative to RT PCR remains essential to inform clinical practice and public health strategies, particularly in resource limited settings where the optimal balance of sensitivity, specificity, cost, and feasibility is critical. This study, therefore, compares the diagnostic performance of ICT and ELISA with RT PCR for HCV infection, aiming to provide practical insights for improving diagnostic pathways and clinical outcomes.

Methodology

This cross-sectional study was conducted in the Department of Microbiology, Chittagong Medical College, and Chittagong Veterinary and Animal Science University from January 2019 to December 2019. Ethical approval was obtained from the Ethical Review Committee of Chittagong Medical College. The study included 50 patients, comprising 24 previously diagnosed cases of hepatitis C virus (HCV) infection and 26 clinically suspected cases, recruited from the outpatient department of the Department of Medicine, Chittagong Medical College Hospital, Bangladesh.

Inclusion criteria: Patients were included if they were clinically suspected of hepatitis due to HCV infection (History of HCV positive but no document and don't take any treatment) or had a prior confirmed diagnosis of HCV.

Exclusion criteria: Patients with hepatitis B virus infection, co-infections with HIV or hepatitis delta virus, chronic hepatitis of other causes, connective tissue disorders, or immunocompromised states were excluded.

Study procedure: After informed consent, 5 mL of venous blood was collected under aseptic conditions. Serum was separated and used for anti-HCV detection by ICT and ELISA, while the remaining serum was stored at "80°C for HCV RNA detection by RT-PCR. ICT and ELISA were performed following the manufacturer's protocols. RT-PCR involved viral RNA extraction, reverse transcription, amplification using specific primers, and visualization of PCR products on 1% agarose gel electrophoresis.

Data analysis: Data were analyzed using SPSS version 23. Diagnostic performance of ICT and ELISA was compared with RT-PCR as the reference standard, and results were expressed in terms of sensitivity, specificity, and agreement rates.

Result

The study included 50 patients comprising clinically suspected and previously diagnosed cases of hepatitis C infection, with a mean age of 39.16/ ±/ 13.66 years. The male-to-female ratio was 1.27:1. Age distribution showed 2% of patients were below 20 years, 56% were between 21–40 years, 32% were between 41–60 years, and 10% were above 60 years. Among them, 56% were male, and 44% were female. Screening for anti-HCV antibodies revealed that 60% of cases were positive by ICT, while 52% were positive by ELISA. HCV RNA detection by RT-PCR identified 48% of cases as positive and 52% as negative. A comparative analysis of ICT with RT-PCR revealed that among HCV RNA-positive cases, 91.66% were ICT-positive, while 8.33% were ICT-negative. Among HCV RNA-negative cases, 30.76% were ICT-positive, and 69.23% were ICT-negative. Similarly, ELISA detected 91.66% positive and 8.33% negative cases among HCV RNA-positive patients, whereas 15.38% were false-positive and 84.61% true-negative among HCV RNA-negative cases. Evaluation of

ICT demonstrated a sensitivity of 91.67%, specificity of 69.23%, positive predictive value (PPV) of 73.33%, negative predictive value (NPV) of 90%, positive likelihood ratio (PLR) of 2.98, and negative likelihood ratio (NLR) of 0.12. ELISA showed comparable sensitivity of 91.67%, higher specificity of 82.62%, PPV of 82.62%, NPV of 91.67%, PLR of 5.96, and NLR of 0.10. Overall, both ICT and ELISA demonstrated high sensitivity for anti-HCV detection, with ELISA showing superior specificity and predictive accuracy compared to RT-PCR, which served as the reference standard for HCV RNA detection.

Table 1: Age and sex distribution of study population (N=50)

Age Group	Male	Female	Total
d”20 Years	0 (0%)	1 (2%)	1 (2%)
21–40 Years	18 (36%)	10 (20%)	28 (56%)
41–60 Years	6 (12%)	10 (20%)	16 (32%)
>60 Years	4 (8%)	1 (2%)	5 (10%)

Mean ± SD: 39.16 ± 13.66 years; Male: Female = 1.27:1

Table 2: Detection of anti-HCV antibodies (ICT and ELISA) and HCV RNA (RT-PCR)

Test	Positive	Negative	Total
ICT for HCV	30 (60%)	20 (40%)	50
ELISA for HCV	26 (52%)	24 (48%)	50
HCV RNA (RT-PCR)	24 (48%)	26 (52%)	50

Table 3: Comparison of HCV ICT with HCV RNA (RT-PCR)

HCV ICT	HCV RNA positive	HCV RNA negative	Total
Positive	22 (91.66%)	8 (30.76%)	30
Negative	2 (8.33%)	18 (69.23%)	20
Total	24	26	50

Table 4: Comparison of HCV ELISA with HCV RNA (RT-PCR)

HCV ELISA	HCV RNA positive	HCV RNA negative	Total
Positive	22 (91.66%)	4 (15.38%)	26
Negative	2 (8.33%)	22 (84.61%)	24
Total	24	26	50

Table 5: Evaluation of ICT with respect to RT-PCR as a reference standard

Validity parameter	Value
Sensitivity	91.67%
Specificity	69.23%
Positive predictive value (PPV)	73.33%
Negative predictive value (NPV)	90%
Positive likelihood ratio (PLR)	2.98
Negative likelihood ratio (NLR)	0.12
Accuracy	80%

Table 6: Evaluation of ELISA with respect to RT-PCR as a reference standard

Validity parameter	Value
Sensitivity	91.67%
Specificity	82.62%
Positive predictive value (PPV)	84.62%
Negative predictive value (NPV)	91.67%
Positive likelihood ratio (PLR)	5.96
Negative likelihood ratio (NLR)	0.10
Accuracy	88%

Discussion

The present study evaluated the diagnostic performance of the immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA) compared to reverse transcription-polymerase chain reaction (RT-PCR) for detecting HCV infection. Accurate and timely detection of HCV is critical for initiating antiviral therapy and preventing progression to chronic liver disease, cirrhosis, or hepatocellular carcinoma.[13] In this study, the mean age of the participants was 39.16 ± 13.66 years, with a male predominance (male-to-female ratio 1.27:1), which aligns with previous reports highlighting a higher prevalence of HCV among middle-aged males.[14,15] Most participants were in the 21–40-year age group, reflecting the vulnerability of the economically active population to HCV infection.[16] ICT detected anti-HCV antibodies in 60% of cases, whereas ELISA detected 52%, indicating that both methods are highly sensitive. RT-PCR confirmed HCV RNA in 48% of participants. Sensitivity for both ICT and ELISA was 91.67%, whereas specificity differed—ELISA achieved 82.62%, outperforming ICT (69.23%). These findings are consistent with previous studies showing that while rapid

tests are useful for mass screening, ELISA offers higher specificity and reliability.[17,18] The positive predictive value (PPV) and negative predictive value (NPV) of ELISA were 84.62% and 91.67%, respectively, compared to ICT's 73.33% and 90%, further supporting the superiority of ELISA in distinguishing true positives from false-positive results.[19] Analysis of discordant results revealed that ICT produced more false positives among HCV RNA-negative patients (30.76%) than ELISA (15.38%), emphasizing that rapid immunochromatographic tests, though convenient, may overestimate prevalence if used without confirmatory testing.[20] This finding underscores the importance of integrating serological screening with molecular confirmation to ensure accurate diagnosis, especially in regions with high HCV prevalence. RT-PCR remains the gold standard for HCV diagnosis due to its ability to detect viral RNA even before seroconversion, facilitating early identification of active infections.[21] In this study, combining serological assays with RT-PCR allowed a comprehensive assessment, confirming that ICT and ELISA are reliable screening tools but cannot replace molecular confirmation. Similar conclusions were reported in other comparative studies, which recommended ELISA as the first-line screening assay, followed by RT-PCR to confirm viremia and guide clinical management.[22] From a public health perspective, these findings highlight the need for a tiered diagnostic approach. Rapid ICT can be employed for preliminary screening in resource-limited or community-based settings due to its ease of use and fast turnaround. Positive cases should then be confirmed with ELISA and RT-PCR to reduce false-positive diagnoses and ensure appropriate treatment.[23] Implementing such a strategy can enhance case detection, prevent disease progression, and reduce HCV transmission within communities.

Limitations:

This study was limited by its small sample size and single-center design, which may affect generalizability. Additionally, the study did not include follow-up to assess seroconversion or viral load dynamics, and resource constraints limited the diversity of diagnostic kits tested.

Conclusion

Both ICT and ELISA demonstrated high sensitivity for detecting anti-HCV antibodies, with ELISA showing superior specificity and predictive accuracy. RT-PCR remains the gold standard for confirming active HCV infection. Integrating serological screening with molecular confirmation provides a reliable, cost-effective diagnostic strategy, ensuring early detection, accurate diagnosis, and improved clinical management of HCV infection, particularly in resource-limited settings, thereby supporting efforts to reduce disease progression and transmission.

Recommendation

It is recommended to use ELISA for initial HCV screening due to its higher specificity, followed by RT-PCR confirmation for active infection. Rapid ICT can be used in resource-limited settings, but positive results should always be verified molecularly.

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