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Comparative Evaluation of Serological and Molecular Methods for the Diagnosis of Hepatitis C Virus Infection in a Tertiary Care Hospital

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Abstract

Background: Accurate diagnosis of hepatitis C virus (HCV) infection is essential for identifying active infection and preventing progression to chronic liver disease. Serological tests are useful for screening, whereas molecular detection confirms active viraemia.

Methods: A hospital-based observational cross-sectional study was conducted among 220 adults with suspected or confirmed HCV infection and chronic liver disease. Anti-HCV antibodies were detected using the SD BIOLINE HCV Rapid Test and HCV Microlisa ELISA. HCV RNA was detected by real-time RT-PCR using the artus HCV RG RT-PCR Kit on a Rotor-Gene Q system. Statistical analysis was performed using SPSS version 26.0, with $p<0.05$ considered significant.

Results: Of 220 participants, 52 (23.6%) were positive by rapid testing, 60 (27.3%) by ELISA and 66 (30.0%) by RT-PCR. The difference among diagnostic methods was statistically significant ($\chi^2=3.94$, $p=0.047$). Among RT-PCR-positive cases, males predominated (68.2%) and male sex was significantly associated with HCV RNA positivity (OR=1.81, 95% CI: 1.01–3.27; $p=0.041$). Blood transfusion (OR=4.2, $p<0.001$), injectable drug use (OR=5.9, $p=0.002$) and hemodialysis (OR=3.7, $p=0.006$) were significant risk factors. The highest proportion of RT-PCR-positive cases occurred in the 41–50-year age group (36.4%; $p=0.012$).

Conclusion: RT-PCR demonstrated the highest detection of active HCV infection, while rapid testing and ELISA provided complementary serological screening. Molecular confirmation, particularly among high-risk and middle-aged patients, may improve detection of active HCV infection and facilitate timely management.

Keywords: Hepatitis C virus HCV infection Anti-HCV antibody ELISA Rapid diagnostic test RT-PCR HCV RNA Molecular diagnosis Chronic liver disease Viral hepatitis

Introduction

Hepatitis C virus (HCV) infection is a major global health problem and an important cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. HCV is predominantly transmitted through exposure to infected blood. Globally, approximately 47 million people have chronic HCV infection, with nearly 0.9 million new infections annually and 239,000 HCV-related deaths reported in 2024. [1]

HCV infection is frequently asymptomatic, and more than 80% of infected individuals may develop chronic infection, which can progress to hepatic fibrosis, cirrhosis and hepatocellular carcinoma. [2,3] India has considerable variation in HCV prevalence across different populations and geographic regions, emphasizing the importance of reliable laboratory diagnosis. [4]

Detection of anti-HCV antibodies by rapid tests, enzyme immunoassays or chemiluminescence assays is widely used for initial screening. However, antibody positivity indicates exposure and cannot distinguish active from resolved infection. [5] Serological assays generally demonstrate high diagnostic accuracy, with reported sensitivity of approximately 97–98% and specificity of 99–100%.[6,7]

A major limitation of antibody-based diagnosis is the early window period, during which HCV RNA may be detectable before seroconversion. [8] Molecular detection of HCV RNA by nucleic acid amplification testing, particularly RT-PCR, therefore provides direct evidence of active infection and is valuable for confirming viraemia and resolving discrepant serological results. [9] Current diagnostic strategies recommend HCV RNA testing following a reactive anti-HCV antibody result to establish current infection. [10]

Materials and Methods

An observational, cross-sectional investigation was carried out in the Microbiology Department of Index Medical College Hospital and Research Centre, Indore, Madhya Pradesh, spanning 18 months from January 2023 to January 2026. A total of 220 adult participants (≥18 years) presenting to outpatient and inpatient services with clinically suspected or confirmed hepatitis C virus (HCV) infection and chronic liver disease were enrolled. Sample size estimation employed the formula $n = Z^2pq/d^2$, assuming a 15% prevalence and 5% margin of error, yielding 196 subjects; this was augmented to 220 to accommodate a 10% anticipated non-response rate.

Inclusion criteria comprised individuals with chronic HCV infection, chronic hepatitis, cirrhosis, hepatic failure, or hepatocellular carcinoma. Exclusion criteria encompassed acute HCV infection, autoimmune or hereditary liver disorders, drug-induced hepatotoxicity, severe systemic comorbidities, or incomplete clinical/laboratory datasets. Written informed consent was secured from all participants, and institutional ethical clearance was obtained from the Institutional Ethics Committee.

Approximately 5 mL of peripheral venous blood was drawn under aseptic conditions into plain vacutainer tubes. Serum was harvested via centrifugation at 3000 rpm for 10 minutes and aliquoted for storage at 2–8°C for immediate assays or at −20°C for deferred analysis.

Initial screening for anti-HCV antibodies utilized the SD BIOLINE HCV Rapid Test. Specimens yielding reactive results underwent confirmatory testing with the HCV Microlisa ELISA (J. Mitra & Co. Pvt. Ltd., India), following the manufacturer protocol. Absorbance was quantified at 450 nm using a Meril Merilyzer EIAQuant Semi-Automated ELISA Microplate Reader, with requisite controls incorporated in each batch.

For nucleic acid confirmation, 140 µL of serum underwent RNA isolation using the QIAamp Viral RNA Mini Kit (QIAGEN, Germany). Quantitative real-time RT-PCR was executed with the artus HCV RG RT-

PCR Kit (QIAGEN, Germany) on a Rotor-Gene Q Real-Time PCR Cycler. The assay amplified a conserved HCV genomic segment via FAM-labeled hydrolysis probe chemistry. Internal controls, negative controls, and quantitative standards were run concurrently. Viral load was reported in IU/mL, with specimens stratified by viral RNA concentration and cycle threshold (Ct) values. Statistical analyses included the Chi-square test and other relevant parametric/non-parametric tests, with a P-value <0.05 deemed statistically significant.

Result

Among the 220 study participants, the majority were aged 41–50 years (27.7%), followed by 31–40 years (25.5%). Males predominated (62.7%). Chronic hepatitis was the most common clinical diagnosis (43.6%), followed by liver cirrhosis (29.1%). An unknown source of infection was the most frequently reported risk factor (40.0%), followed by blood transfusion (26.4%).

Anti-HCV positivity was 23.6% by rapid testing and 27.3% by ELISA, whereas real-time RT-PCR detected HCV RNA in 30.0% of participants. The difference among the three diagnostic methods was statistically significant ($\chi^2 = 3.94$, $p = 0.047$).

Among the 66 RT-PCR-positive cases, males accounted for 68.2% and females for 31.8%. Male participants had significantly higher odds of HCV RNA positivity than females (OR = 1.81, 95% CI: 1.01–3.27; $p = 0.041$).

Blood transfusion was significantly associated with HCV RNA positivity (OR = 4.2, $p < 0.001$), followed by injectable drug use (OR = 5.9, $p = 0.002$) and hemodialysis (OR = 3.7, $p = 0.006$). Previous surgery was not significantly associated with RT-PCR positivity (OR = 1.8, $p = 0.084$).

Age-wise, the highest proportion of RT-PCR-positive cases was observed in the 41–50-year group (36.4%), followed by 31–40 years (24.2%) and 51–60 years (19.7%). The association between age group and RT-PCR positivity was statistically significant ($\chi^2 = 12.74$, $p = 0.012$).

Table 1. Demographic, Clinical and Epidemiological Characteristics of Study Participants (n = 220)

Variable	Category	Number	Percentage (%)
Age group (years)	18–30	42	19.1
	31–40	56	25.5
	41–50	61	27.7
	51–60	38	17.3
	>60	23	10.4
Gender	Male	138	62.7
	Female	82	37.3
Clinical diagnosis	Chronic hepatitis	96	43.6
	Liver cirrhosis	64	29.1
	Chronic liver disease	36	16.4
	Hepatocellular carcinoma	24	10.9
Risk factors	Blood transfusion	58	26.4
	Hemodialysis	21	9.5
	Previous surgery	37	16.8

	Injectable drug use	16	7.3
	Unknown	88	40.0
Total		**220**	**100.0**

Table 2. Comparative Evaluation of Serological and Molecular Methods and Clinical Profile of RT-PCR-Positive HCV Cases (n = 220)

Parameter	Category	Number	Percentage (%)
Rapid test	Positive	52	23.6
	Negative	168	76.4
ELISA	Positive	60	27.3
	Negative	160	72.7
Real-time RT-PCR	Positive	66	30.0
	Negative	154	70.0
Comparison of diagnostic methods	Rapid Test positive	52	23.6
	ELISA positive	60	27.3
	RT-PCR positive	66	30.0
	χ^2	3.94	—
	p-value	0.047*	—

Table 3. Association between Gender and RT-PCR Positivity

Gender	RT-PCR Positive n (%)	OR (95% CI)	p-value
Male	45 (68.2)	1.81 (1.01–3.27)	0.041*
Female	21 (31.8)		
Total	**66 (100.0)**		

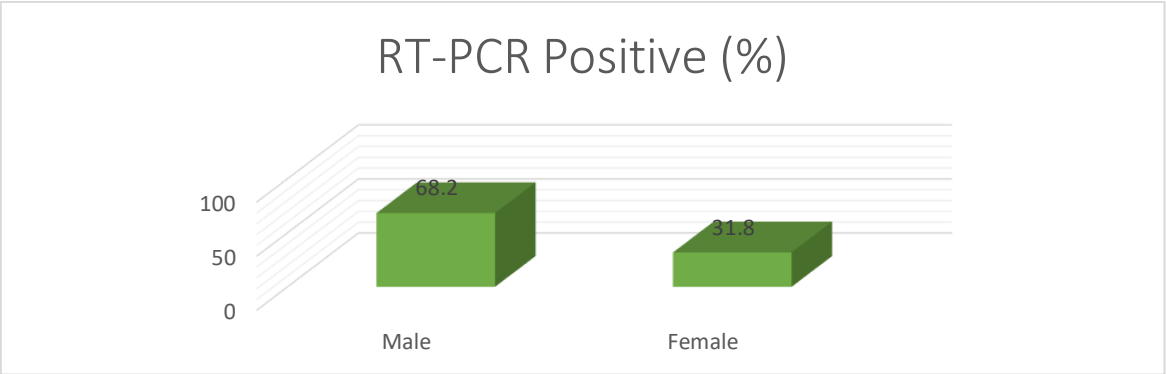

Figure 1. Association between Gender and RT-PCR Positivity

Table 4. Association of Risk Factors with RT-PCR Positivity

Risk Factor	RT-PCR Positive n	OR	p-value
Blood transfusion	28	4.2	<0.001
Hemodialysis	9	3.7	0.006
Previous surgery	11	1.8	0.084

Injectable drug use	8	5.9	0.002

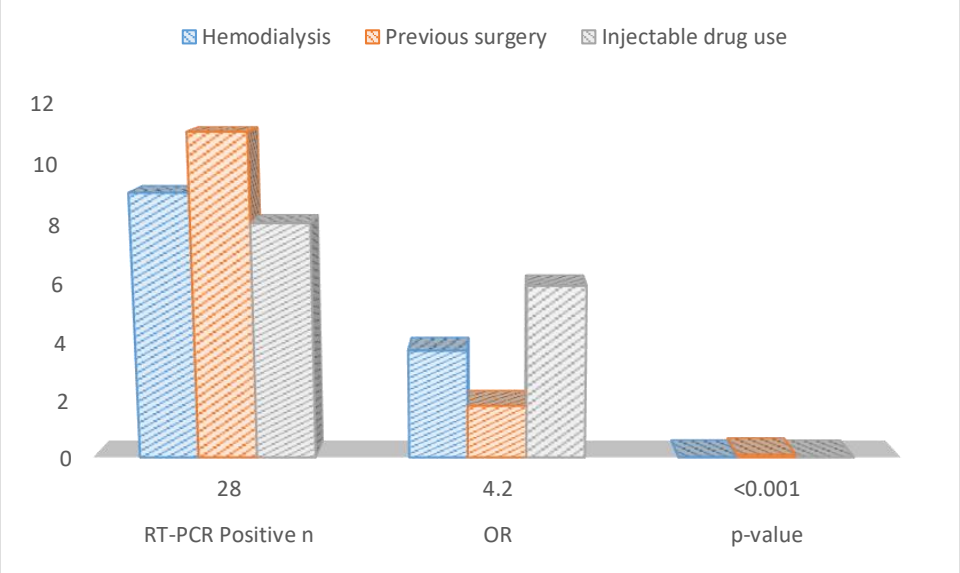

Figure 2. **Association of Risk Factors with RT-PCR Positivity**

Table 5. Age-wise Distribution of RT-PCR-Positive HCV Cases (n = 66)

Age Group (years)	RT-PCR Positive, n (%)
18–30	8 (12.1)
31–40	16 (24.2)
41–50	24 (36.4)
51–60	13 (19.7)
>60	5 (7.6)
Total	**66 (100.0)**

$\chi^2 = 12.74$; $p = 0.012$*

Discussion

The present study evaluated 220 adults with clinically suspected or confirmed HCV infection and chronic liver disease using a sequential diagnostic approach involving rapid anti-HCV testing, ELISA and real-time RT-PCR. The findings demonstrated a progressive increase in positivity from rapid testing (23.6%) to ELISA (27.3%) and molecular testing (30.0%). This pattern supports the importance of combining serological screening with molecular confirmation for identifying active HCV infection.

In the present study, males constituted 62.7% of the study population, and among the 66 RT-PCR-positive patients, 45 (68.2%) were males. Male sex was significantly associated with HCV RNA positivity (OR 1.81, 95% CI 1.01–3.27; p=0.041). A similar male predominance was reported by Prasad et al. (2018) in a tertiary-care hospital study from Mumbai, where males predominated among HCV-infected patients. Importantly, their study also reported that the highest proportion of HCV-infected patients belonged to the 41–50-year age group, which is consistent with the age pattern observed in the present study. [11] A recent tertiary-care study by **Rahman et al. (2026)** also reported male predominance, with males accounting for 66.4% of confirmed HCV cases, further supporting our finding. [12]

Age-wise, the highest proportion of RT-PCR-positive patients in the present study was observed in the 41–50-year age group (36.4%), followed by 31–40 years (24.2%). The association between age and HCV RNA positivity was statistically significant (χ^2=12.74, p=0.012). This finding is comparable with the observations of **Prasad et al. (2018)**, who reported the highest number of HCV-infected patients in the 41–50-year age group in their molecularly confirmed cohort. [11] Similarly, **Chakravarti et al. (2014)** observed that HCV infection in India was more frequently identified among adults with prolonged exposure to healthcare-related and other blood-borne risk factors. [13] The predominance in middle-aged adults may therefore reflect the cumulative effect of past medical exposures and prolonged progression of chronic HCV infection.

The clinical profile of the present study showed that chronic hepatitis was the most common diagnosis (43.6%), followed by cirrhosis (29.1%), chronic liver disease without established cirrhosis (16.4%) and hepatocellular carcinoma (10.9%). Among RT-PCR-positive patients, chronic hepatitis accounted for 47.0%, while cirrhosis and hepatocellular carcinoma together represented 31.8%. These findings are consistent with the established natural history of HCV infection, in which persistent viral infection may progressively result in hepatic fibrosis, cirrhosis and hepatocellular carcinoma. **Poynard et al. (1997)** demonstrated that hepatic fibrosis progresses over time in chronic HCV infection, with the rate of progression varying among individuals. [14] The presence of advanced liver disease among a considerable proportion of molecularly confirmed patients in our study may reflect delayed diagnosis and prolonged untreated infection.

Regarding epidemiological risk factors, blood transfusion was reported by 26.4% of participants and was significantly associated with HCV RNA positivity (OR=4.2, p<0.001). This finding is particularly relevant in the Indian context. **Puri et al. (2014)**, in the INASL consensus report, identified blood transfusion and unsafe therapeutic injections as important routes of HCV transmission in India. [15] Similarly, **Singh et al. (2016)** reported HCV as an important transfusion-transmitted infection among blood donors in Punjab and emphasized the continued importance of sensitive screening strategies for transfusion safety.[16] The relatively high association observed in the present study may partly reflect exposure to transfusion before the widespread implementation of highly sensitive donor-screening strategies.

Hemodialysis was another significant risk factor in the present study, with 9 RT-PCR-positive patients and an odds ratio of 3.7 (p=0.006). This finding is biologically plausible because patients receiving repeated hemodialysis are exposed to frequent vascular access, multiple healthcare contacts and, historically, transfusion-related exposure. **Chaudhary et al. (2019)** reported an HCV prevalence of 18.8% among 250 patients receiving maintenance hemodialysis at five centers in Pune and found a relationship between HCV infection, longer duration of dialysis and greater numbers of blood transfusions. [17] These observations support the importance of continued HCV surveillance and strict infection-control practices in dialysis units.

Injectable drug use showed the strongest association with HCV RNA positivity in the present study (OR=5.9, p=0.002). Although only 16 participants reported this exposure, 8 were RT-PCR positive. This finding agrees with the established role of blood-contaminated injecting equipment in HCV transmission. **Mane et al. (2019)** emphasized the need for expanded HCV screening strategies in India, particularly among populations with increased exposure risk. [18] The high odds ratio observed in our study should nevertheless be interpreted cautiously because of the relatively small number of participants reporting injectable drug use.

Previous surgery was associated with an increased but statistically non-significant risk of HCV RNA positivity (OR=1.8, p=0.084). This finding may indicate a possible relationship with previous healthcare exposure; however, the absence of statistical significance suggests that surgery alone may not be an independent determinant in the present population. Differences between studies may arise from variations in sterilization practices, transfusion history, and timing of exposure and patient characteristics.

An important finding of the present study was the difference in positivity among the three diagnostic approaches. The rapid test detected 52 (23.6%) cases, ELISA detected 60 (27.3%), while RT-PCR detected 66 (30.0%) cases. The difference was statistically significant (χ^2=3.94, p=0.047). Thus, molecular testing identified six additional cases compared with ELISA and 14 additional cases compared with rapid testing. This progressive increase is consistent with the different biological targets of the assays: serological tests detect host antibodies, whereas RT-PCR directly detects HCV RNA.

The higher molecular positivity observed in the present study is supported by the earlier Indian work of Panigrahi et al. (1994) who specifically compared anti-HCV serology with RT-PCR in patients with HCV-associated chronic liver disease and demonstrated the additional diagnostic value of molecular detection. [19] More recently, Sharma et al. (2024) evaluated HCV core antigen, anti-HCV antibody and RT-PCR in an Indian population and highlighted the limitations of antibody testing for detecting acute infection, while RT-PCR remained the molecular reference method for detection of active infection. [20]

The rapid test in the present study correctly identified 49 of 66 RT-PCR-positive cases, while 17 were missed. Among RT-PCR-negative patients, 151 were correctly identified as negative and 3 were false positive. Thus, the rapid assay showed useful screening performance but failed to detect a proportion of molecularly confirmed infections. Jain et al. (2023), in a comparative evaluation of anti-HCV immunochromatographic card tests in India, reported sensitivity values of up to 98% and specificity of 100% for the best-performing rapid assay, while also emphasizing that rapid-test performance can vary between commercially available kits. [21] Differences between the present findings and published rapid-test performance may reflect differences in patient populations, disease stage, viral load, assay characteristics and study design.

ELISA showed better agreement with RT-PCR than the rapid test in the present study. It correctly identified 58 of 66 RT-PCR-positive cases and 152 of 154 RT-PCR-negative cases, with 8 false-negative and 2 false-positive results. The higher positivity observed with ELISA compared with the rapid test is consistent with the established role of laboratory-based immunoassays as more sensitive screening methods. Tang et al. (2017) in a systematic review and meta-analysis of HCV antibody diagnostic tests, reported high overall diagnostic accuracy of antibody-based assays, although performance varied according to the assay and study population. [22] The difference between serological and molecular results may also be explained by the biological window between HCV infection and antibody development, as well as by spontaneous viral clearance. A patient may remain anti-HCV positive after clearance of viraemia, whereas recently infected patients may have detectable HCV RNA before antibody seroconversion. Alter et al. (2003) demonstrated the importance of HCV RNA detection in identifying infection during periods when antibody testing may not yet be informative. [23] Therefore, the higher RT-PCR positivity observed in the present study reinforces the importance of molecular confirmation when the objective is to identify active HCV infection rather than merely previous exposure.

Conclusion

The study demonstrates that serological and molecular methods are complementary: rapid tests enable practical screening, ELISA enhances serological detection, and RT-PCR confirms active HCV viraemia. Significant associations with male sex, middle age, blood transfusion, hemodialysis, and injection drug use underscore the need for targeted screening and molecular confirmation in high-risk groups, while the high burden of chronic hepatitis, cirrhosis, and hepatocellular carcinoma among RT-PCR–positive patients highlights the clinical imperative for early diagnosis and timely antiviral therapy.

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