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Quantitative Analysis Of HCV Viral Load In Chronic Hepatitis C Patients In Western Uttar Pradesh

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Abstract

Background: Human hepatitis C virus (HCV) infection is very bad for health in developing countries. Understanding viral load distribution and its association with demographic and clinical factors is essential for effective disease management.
Aim: The present study aimed to assess the distribution of HCV viral load and its association with demographic and clinical risk factors among patients with chronic hepatitis C in Western Uttar Pradesh.
Methods: There were 409 participants in the cross-sectional study was done in a hospital. Structured proforma was used to gather clinical and demographic data. Real-time RT-PCR was used to detect and quantify HCV RNA. The viral load was classified as high (>6×10^1 IU/mL), moderate (2×10^3–6×10^1 IU/mL), and low (<2×10^3 IU/mL). In this study, statistics were done with SPSS version 26.0, and associations were analyzed at with non-parametric and chi-square tests.
Results: Among participants, 57.5% were females and 42.5% were males. The majority belonged to the 20–39 years (38.9%) and 40–59 years (37.4%) age groups. HCV RNA was detectable in 52.4% of cases. No significant association was seen between viral load and gender ($p = 0.91$) or age ($p = 0.92$). However, significant associations were found with alcohol use ($p = 0.023$), smoking ($p = 0.046$), and combined alcohol and smoking exposure ($p = 0.004$), which were linked to higher viral load levels. Comorbidities such as hypertension, diabetes, and HBsAg positivity showed no significant association, while COPD and CKD showed borderline significance.
Conclusion: Alcohol consumption and smoking are significantly associated with higher HCV viral load, while age and gender show no significant effect. Such results indicate the possibility of lifestyle interventions to enhance viral outcomes in patients with HCV.

Keywords: Hepatitis C virus, Viral load, Risk factors, Blood transfusion, Cross-sectional study, RT-PCR.

INTRODUCTION

The hepatitis C virus (HCV) is what causes chronic liver disease around the world, resulting in cirrhosis, [1] hepatocellular carcinoma (HCC) and high mortality rates (approximately 58 million chronic and 1.5 million new infections per year).[2]In India, Seroprevalence varies between 0.5-1.5 which is a significant burden in resource constrained regions.[3]The initial stages tend to be asymptomatic and this delays diagnosis and increases the hepatic complications.[4] India is also the cause of approximately 115,000 deaths because of complications related to hepatitis C. Hepatitis C virus infection has parenteral, sexual and vertical modes of transmission. The hepatitis C infection prevalence in India is not known. In India, the prevalence of HCV is about 6 million. Other risk factors of hepatitis C infections are intravenous drug users, spouses of infected people, gay men, health care workers and frequent recipients of blood and blood products, and patients undergoing hemodialysis and organ transplantation. [5]
The alcohol consumption, tobacco use, obesity, and metabolic syndrome have been conclusively linked to the worsening of the HCV-induced liver damage. Liver disease progression in patients is accelerated by co-infection with other viruses, particularly the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV),[6] positively affects the outcome of treatment and is caused by the age of infection, with old patients having a higher tendency to persistent infection and severe liver disease.[7]Gender impacts the progression to fibrosis and

cirrhosis,[8]with males often exhibiting a more accelerated advancement, possibly because of variations in immune response and hormonal factors.[9]
When someone has a chronic HCV infection, a pre-treatment elevated HCV RNA level predicts diminished Long-lasting reductions in virus (SVR) after treatment with pegylated interferon and ribavirin, whether administered alone or in conjunction with Boceprevir or Telaprevir.[10] Although HCV RNA levels had minimal influence on the treatment efficacy of novel interferon-free direct-acting antiviral (DAA) treatments[11,12]and possibly reduced clinical significance, they continue to provide insights about HCV immune pathogenesis.[10]
In the present study, Comparative assessment of quick hepatitis C antibody test against HCV viral load and evaluate the relationship of determinants affecting transmission and clinical parameters to HCV chronicity.

Material & Methods

Study Design
This hospital-based cross-sectional study, was authorized in March 2025 by the College Research Advisory Committee (CRAC) and the Institutional Ethics Committee (IEC). Following the rules of the Declaration of Helsinki and the guidelines for good clinical practice, the study was done. Each participant had to sign a written consent form before the study could start. This ensured both their privacy protection and their ability to leave at any time.

Study Population and Sampling
Anti-HCV antibody-reactive patients by rapid assay as per using Tri-dot (Antibody) was used according to manufacturer's protocol (J Mitra and Co Pvt. Ltd, India)[13]were enrolled per inclusion/exclusion criteria. Demographic/clinical data were captured via structured forms. Venous blood (4 mL) was collected aseptically into EDTA vacutainers, centrifuged (3000rpm, 10min), and plasma aliquoted/storedat−80°Cunder cold chain (2–8°C) to preserve RNA.

RNA extraction
Viral RNA was extracted from plasma samples of RDT reactive patients with Mag MAX^{TMV} Viral/Pathogen Nucleic Acid Isolation kit(Applied Biosystems, A42352). An aliquot of 200µL of plasma was subjected to Binding bead under highly denaturation condition as provided by the Binding bead mix. Following binding, washing step were conducted with wash buffer and ethanol to remove impurities. The purified RNA was ultimately eluted in 50µLof Elution buffer. The purified nucleic acid was stored at -20℃ until analysis.[14]

HCV RNA Quantification (Viral Load)
HCV RNA amplification was performed with TRUPCR®HCV Viral load kit in accordance with the manufacturer's instructions. Quantification of viral load was carried out on the 3B BlackBio Dx Ltd.(TRUPCR) HCV real-time PCR detection.[15] Real-time PCR assays quantified HCV RNA in IU/mL via standard curves. Categories (low: $<2\times10^3$ IU/mL; moderate: 2×10^3–6×10^5 IU/mL; high: $>6\times10^5$ IU/mL) assessed disease severity, infectivity, and therapy response.

Data Analysis
Clinical parameters (age, gender, risk factors) were correlated with viral load/genotype. Samples processed in duplicate ensured reproducibility.

Statistical Analysis
"Data were entered, cleaned, and analyzed using (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were summarized as frequencies and percentages (%). For comparisons between groups, the independent t-test (for twogroups) andone-way analysis ofvariance (ANOVA) (for more than two groups) were applied for normally distributed variables, whereas non-parametric tests such as the Mann–Whitney U test and Kruskal–Wallis test were used for skewed data. Chi-square test was used to assess associations between categorical variables. Variables with $p < 0.20$ in univariate analysis were included in multivariate analysis."

Results
The study included a total of 409 participants**.** Among them, 235 were females (57.5%)**,** while 174 were males (42.5%)**,** indicating a higher proportion of female participants compared to males in the study population.

Table1: Gender-wise Distribution of Study Participants

Gender	Count (N)	Percentage (%)
Female	235	57.5%
Male	174	42.5%
Total	**409**	**100%**

The study included a total of 409 participants. The majority of participants members of the 20–39 age group made up 38.9% of the group, followed by people in the 40–59 age group on margins (37.4%).Participants aged ≥60 years constituted 21.8%**,** while the 0–19 years age group represented the smallest proportion (2.0%) of the study population.

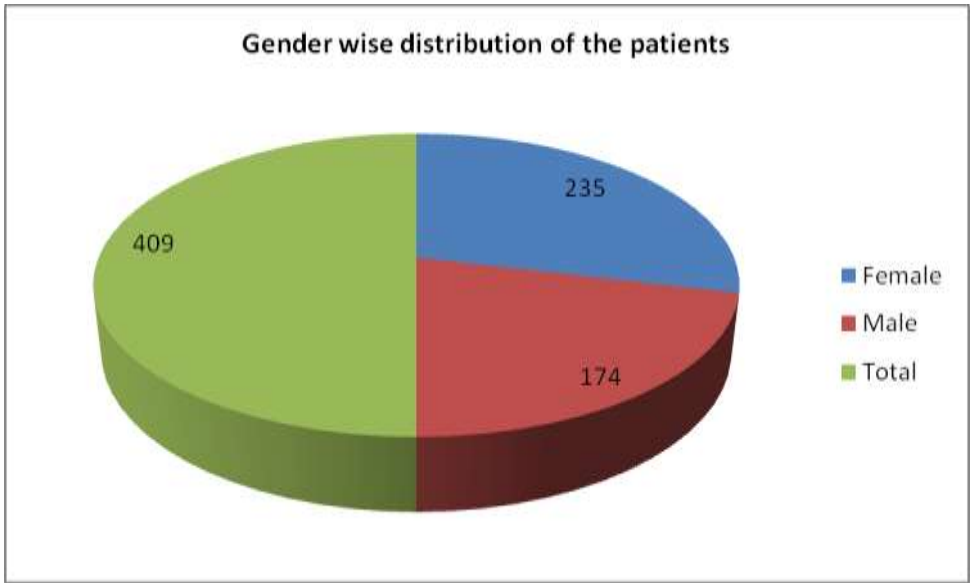

Fig: 1 Gender distribution of the patients.

Table2-Age-wiseDistributionofStudyParticipants

Age Group(Years)	Number of Participants(N)	Percentage (%)
0–19	8	2.0%
20–39	159	38.9%
40–59	153	37.4%
≥60	89	21.8%
Total	**409**	**100%**

Among 409 participants**,** 215(52.4%) were HCV RNA detectable**,** while 194 (47.6%) werenon-detectable**.**

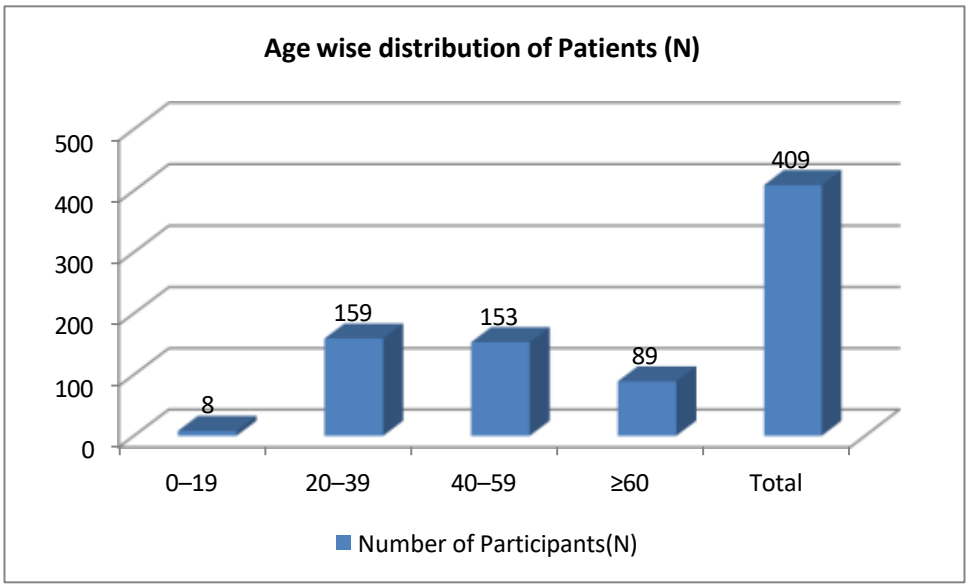

Fig: 2 Graphical represents age wise distribution of the patients Table 3- Viral Load Detection Status

Category	Number of Participants(N)	Percentage (%)
Non-Detectable	194	47.6%
Detectable	215	52.4%
Total	**409**	**100%**

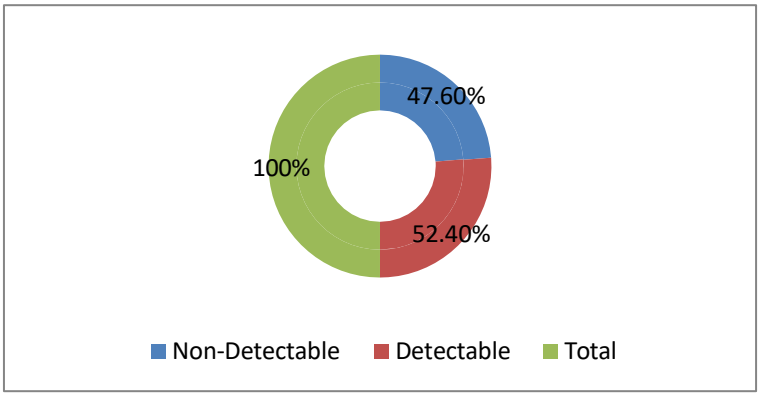

Fig: 4 Viral Load Detection.

Table 4- Association of Viral Load Categories with Gender and Age Groups among HCV-Positive Participants (n = 215)

Variable	Category	Low (<2×10³)	Moderate(2×10³–6×10⁵)	High (>6×10⁵)	Total	χ^2 p-value	KW p-value
Gender	Male	10	57	22	89	**0.91**	
	Female	14	84	28	126		
							0.39
Age Group (Years)	0–19	0	3	1	4		
	20–39	10	62	18	90	**0.92**	
	40–59	9	55	23	87		
	≥60	5	21	8	34		

There was no statistically significant association between gender and the categorized variable levels (low, moderate, high), as indicated by the Chi-square test (p = 0.91). Similarly, no significant difference was observed across age groups according to the Kruskal–Wallis test (p = 0.39). This suggests that the distribution of the variable was comparable between males and females and across different age categories.

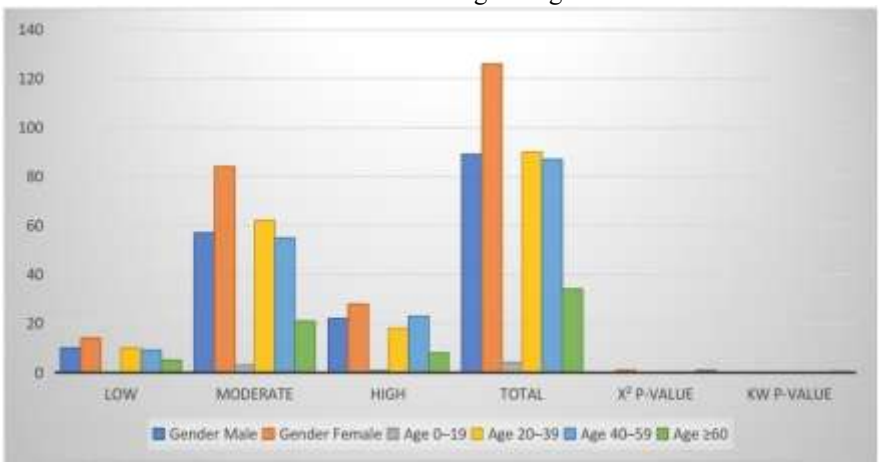

Fig: 5 association of viral load categories with gender and age groups among HCV-positive patients (n = 215)

Table 5: Association of Risk Factors with Viral Load (VL) Categories among Study Patients.

Risk Factor	Low VL n (%)	Moderate VLn(%)	High VLn(%)	Total n(%)	p-value
Alcohol use	8 (11.1)	46 (63.9)	18 (25.0)	72 (100)	0.023
Smoking	10 (10.2)	66 (67.3)	22 (22.4)	98 (100)	0.046
Alcohol+ Smoking	5 (9.6)	32 (61.5)	15 (28.8)	52 (100)	0.004

Blood transfusion	5 (11.9)	29 (69.0)	8 (19.0)	42 (100)	0.234
Hypertension	12 (13.5)	65 (73.0)	12 (13.5)	89 (100)	0.679
Diabetes	5 (13.2)	26 (68.4)	7 (18.4)	38 (100)	0.346
Surgery	3 (14.3)	14 (66.7)	4 (19.0)	21 (100)	0.457
COPD	1 (6.7)	10 (66.7)	4 (26.7)	15 (100)	0.079
CKD	1 (16.7)	3 (50.0)	2 (33.3)	6 (100)	0.057
HBsAg+	1 (12.5)	5 (62.5)	2 (25.0)	8 (100)	0.123
Total	**24 (11.2)**	**141 (65.6)**	**50 (23.3)**	**215 (100)**	-

Alcohol use, smoking, and combined alcohol plus smoking showed a statistically significant association with viral load categories ($p = 0.023$, 0.046, and 0.004, respectively), indicating higher proportions of moderate to high viral load among these participants. Other risk factors including blood transfusion, hypertension, diabetes, surgery, COPD, CKD, and HBsAg positivity did not show significant associations with viral load categories ($p > 0.05$). Overall, moderate viral load was the most common category among participants (65.6%).

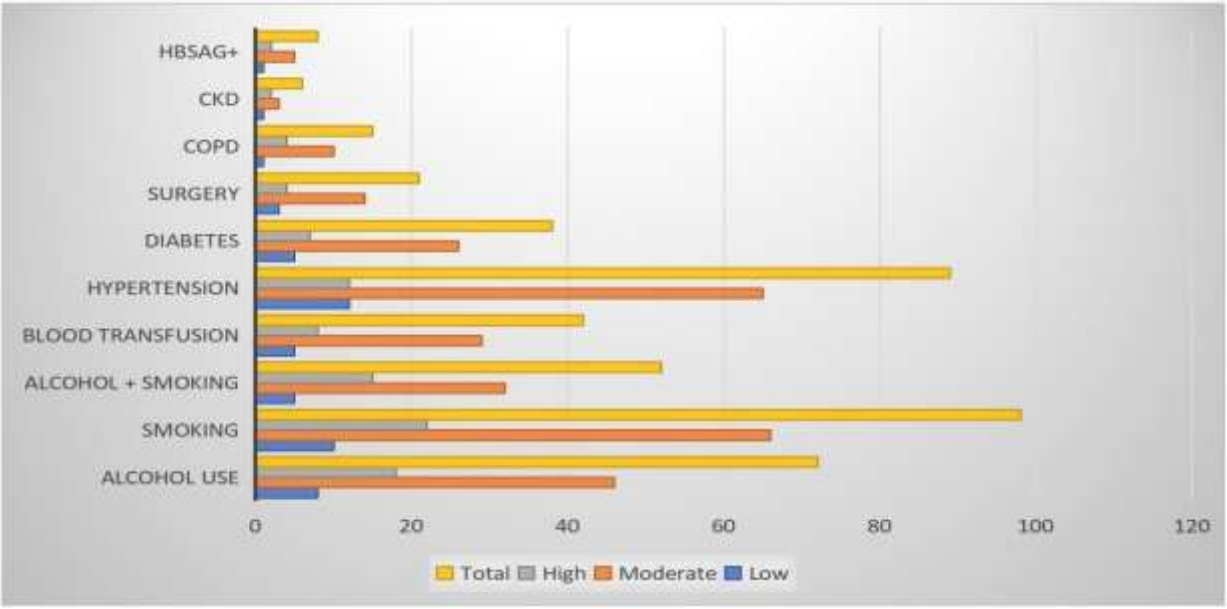

Fig: 6 Graphical represents association of risk factors with viral load (vl) categories among study patients.

Discussion:

The current study included a total of 409 individuals, with more women (57.5%) than men (42.5%) being involved. This female predominance is consistent with findings reported by Khan et al. (2025),[16] also observed a similar higher proportion of females among HCV-infected individuals in a hospital-based cohort. The authors suggested that

this pattern may reflect increased healthcare utilization and screening among females, particularly during antenatal care and routine medical visits, rather than a true biological difference in susceptibility.[16] The present study also showed that The age range of 20 - 39 years (38.9%) comprised the bulk of participants and 40–59 years (37.4%) age groups, indicating that HCV infection is more common in economically productive age groups.
This observation concurs with the report by Singh et al. (2024),[17] who found a similar pattern of age distribution and highlighted that working-age adults were more often diagnosed with it related to cumulative exposure to risk factors associated with healthcare and the community. The results of the present study indicate that 52.4% of the subjects had HCV RNA that could be detected whereas 47.6% could not, indicating a variation in viral replication among the seropositive individuals. A similar finding was obtained by Al-Rashid et al. (2023),[18] who found almost equal rates of detectable and non-detectable viral RNA in their sample population. In terms of viral load distribution, there was no statistically significant correlation between gender and the viral load ($p = 0.91$). The numbers of male and female participants were almost similar in the low, moderate, and high categories of viral load. Chen et al. (2022),[19] have also supported this finding by showing that there were no significant gender-based differences in the level of HCV viral load. Likewise, age group and viral load distribution did not show any significant correlation (2 $p = 0.92$; KW $p = 0.39$). This is consistent with Patel et al. (2021),[20] who found that the levels of viral loads do not always differ among age categories even though there are differences in the duration of the disease. One of the current study's conclusions was that alcohol use and other behavioural risk variables were significantly correlated, smoking and co-exposure with increased levels of viral load. The percentages of high viral load were greater among alcohol users and smokers, and combined exposure had the most significant association ($p = 0.004$). The fact that alcohol consumption increases the severity of HCV replication under oxidative stress conditions supports this observation (Mahmoud et al., 2020)[21].

Additional evidence to this, Ahmed et al. (2022),[22] discovered that smoking plays a major role in hepatic inflammation and viral persistence in chronic HCV infection. Likewise, Li et al. (2023),[23] showed that there is a synergistic effect of alcohol and tobacco use, which has resulted in poorer virological outcomes than each of the two factors alone. Conversely, comorbidities like hypertension, diabetes, history of blood transfusion, surgery, and HBsAg positivity failed to exhibit statistically significant relationships with viral load in the current study. But there were borderline correlations with COPD and CKD. This is substantiated by Garcia et al. (2025), [24] who indicated that systemic comorbidities do not necessarily affect viral replication, but can impact host immune responses.

Also, Zhang et al. (2024),[25] emphasized that CKD can change the immune clearance processes, which could affect viral persistence in HCV-positive people. Equally, Hassan et al. (2023),[26] hypothesized that chronic inflammation conditions in COPD patients can lead to the subtle enhancement of viral activity, but the results are frequently incongruent. Moreover, Patel et al. (2021),[20] stressed that metabolic diseases (diabetes and hypertension) are more closely associated with the progression of liver fibrosis than the level of viral load, which is also consistent with the results of the current study. The current findings were also supported by Wang et al. (2020) [27], who found that there was no significant correlation between metabolic comorbidities and HCV RNA levels in a large cohort study.

In general, the current research proves that demographic variables (age and gender) do not play an important part in the distribution of the HCV viral load, and behavioral variables (alcohol consumption and smoking) have a significant impact on an increase in viral load. These results are in line with various studies carried out in the period of 2020-2025 and highlight the value of lifestyle change in HCV management. Specific alcohol cessation and smoking reduction interventions could enhance the virological outcomes and disease progression among affected people.

Conclusion: The present study demonstrated that alcohol consumption and smoking were significantly associated with higher HCV viral load among chronic hepatitis C patients, whereas demographic factors such as age and gender showed no significant association. Most patients had moderate viral load levels, and detectable HCV RNA was observed in more than half of the participants. These findings emphasize the importance of behavioral risk factor modification,
particularly alcohol cessation and smoking reduction, in improving disease management and clinical outcomes in HCV-infected individuals.

Novelty of the study: This study provides recent regional data on quantitative HCV viral load distribution among chronic hepatitis C patients in Western Uttar Pradesh, an area with limited molecular epidemiological evidence. The

study uniquely evaluates the association of viral load with multiple demographic and behavioral risk factors simultaneously using real-time RT-PCR–based quantification. The findings highlight the significant impact of alcohol consumption and smoking on higher HCV viral load, emphasizing the role of lifestyle-related factors in viral replication and disease progression. Additionally, the study contributes important evidence regarding the lack of significant association between viral load and age or gender in the studied population.

Limitations of the Study

- Hospital-based single-center study with limited generalizability.
- Cross-sectional design cannot establish causality.
- Self-reported behavioral data may introduce bias.
- HCV genotyping and long-term follow-up were not performed.
- Small sample size in some subgroups may affect statistical accuracy.

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