



## Adequate Method of Hepatitis C Virus (HCV) RNA Quantitation and Geographical Distribution of Subjects in Eastern Uttar Pradesh: Findings from a Tertiary Care Hospital

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### Abstract

**Background:** Hepatitis C virus (HCV) have several important features, including worldwide distribution, hepatotropism, modes of transmission, ability to life threatening critical illness and is a growing public health issue worldwide. It's prevalence and mode of transmission varies greatly in different parts of the world. It is estimated that approximately one-fifth of the world population infected with HCV.

**Aim of the Study:** This study was planned to know the prevalence, demographic and geographical distribution of HCV in eastern Uttar Pradesh, India.

**Material and Methods:** An observational study was conducted in the department of Microbiology for 2 years from January 2023 to December 2024. Irrespective of age and sex, a non-duplicate blood samples were taken to detect HCV infection. All samples were tested for rapid HCV antibody test followed by viral load testing by using RT-PCR technique. Results were interpreted according to standard protocol.

**Results:** Out of 1831 patient's samples, 1266 (69.14%) patients were from outpatient department (OPD) and 565 (30.86%) were from inpatient department (IPD). 1053 (57.50%) were male patients and 778 (42.49%) were female patients. In study, most of the patients visited in MTC unit from Gorakhpur division (69.85%) of Uttar Pradesh followed by Basti (13%) division. Among positive cases, only 5.83% of cases has viral load less than 300 IU/ml.

**Conclusion:** Hepatitis virus infection was highest in the age group 19-40 and lowest in the age group above <18 years. Hepatitis C was more in males as compared to females and more in OPD as compared to IPD.

**Keywords:** Hepatitis C Virus; Hepatocellular Carcinoma; Eastern Mediterranean Region; National Viral Hepatitis Control Program; HCV Prevalence; HCV in Eastern Uttar Pradesh

### Introduction

Hepatitis C Virus (HCV) is a major viral pathogen that leads to advanced hepatic fibrosis and cirrhosis and hepatocellular carcinoma [1]. A severity and duration of HCV infection are variable from a small subset of individual's experiences acute hepatitis, while most people remain asymptomatic. The infection can either progress to chronic infection or clear spontaneously depending on the type of infection [2]. Chronic HCV infection is prevalent in the majority of

affected individuals, and in between 5-25% of those with chronic infection develops severe chronic liver conditions such as cirrhosis or Hepatocellular Carcinoma (HCC) over a period of time [3].

Based on 2024 data, the latest global estimates indicate that approximately 50 million people have chronic hepatitis C virus infection, with about 1.0 million new infections occurring per year, resulting in a prevalence of 2% [4]. It was estimated by World Health

organization till 2022 that approximately 242 000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma. The highest prevalence can be found across regions, in the Eastern Mediterranean Region (EMR) around 2.3% which was followed by the European Region (1.5%), the major cause of infection are unsafe use of injections and intravenous drug (IVD) abusers [5,6]. In India, it was estimated that approximately 12–18 million people are thought to be infected with prevalence of HCV infection ranges between 0.5% to 1.5%, with higher rates observed in the north-eastern region, tribal populations, and Punjab, the reported prevalence in this area varies in between 3.2 to 5.2% from the year 2012 and are to be considered as HCV hotspots [7,8].

In other parts of country the prevalence of acute hepatitis C is very low, might be because of the larger number of asymptomatic cases and it is difficult to diagnose unless strongly suspected. Regarding treatment, the WHO has set the goal of eliminating viral hepatitis, as a global public health concern by 2030. In year 2022 a study conducted by Huang P *et al.* approximately 95% of patients received treatment, with a successful Sustained Virological Response (SVR) achieved in 80 to 100% cases [9]. The World Health Organization (WHO) estimated 1.7 million new infections in global hepatitis report 2017 [10]. Direct-acting antiviral agents have proven to be highly effective in reducing the burden of HCV infection by 95%. The “HCV cascade of care” includes understanding the virus’s epidemiology, identifying infected persons, linking them to treatment facilities, and creating surveillance programs for those with severe liver disease [11].

### Aim of the Study

Based on the above background this study was designed to know the prevalence, demographic and geographical distribution of HCV in eastern Uttar Pradesh, India.

### Materials and Methods

- **Study design:** This prospective observational study.
- **Site of study:** The study was conducted at a Viral Diagnostic Research Laboratory, Department of Microbiology, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, Northern India, which serves as a government tertiary care facility with a bed capacity of 1750. The hospital caters to eastern Uttar Pradesh, Bihar and another country Nepal, with an average daily patient’s visit of 3500-4000 out-patients and 350-400 in-patient admissions.

- **Study period:** The study spanned two years, from January 2023 to December 2024.
- **Inclusion criteria:** The study included all consecutive samples from Patients walk-in the OPD and IPD of Model Treatment Centre, Department of Medicine with positive HCV report from outside or diagnosed positive at central laboratory of the tertiary care hospital and samples received in the BSL-3, Department of Microbiology, B.R.D. Medical College, Gorakhpur for HCV vial load testing. The test results were retrieved from the records and subjected to analysis.
- Exclusion criteria were Patient with chronic hepatitis B, Hepatitis B and C co-infection; autoimmune hepatitis and jaundice, ascites, alcoholic liver disease, history of recent drugs hepatitis were excluded.
- All patients were subjected to enrolled in this study was first screened in central pathology of tertiary care hospital by using HCV rapid antibody detection device which was primarily coated with HCV antigen on the nitrocellulose membrane. The tests were performed by following the manufacturer’s instructions [12]. To confirm rapid positive samples and to assess the viral load, a confirmed HCV diagnosis should only be made by using RT-PCR method.
- **NAT: Detection of HCV RNA:** Furthermore, the monitoring of viral load and confirm diagnosis of HCV infection was done by using True lab real time HCV PCR, a chip based Real Time PCR technology. It’s based on Taqman chemistry which used only 6µl of RNA template to obtain the result in respect to minimize the risk of contamination by PCR amplification product. The Truenat HCV assay was able to detect all 6 panel members including genotypes HCV-1A, 2B, 3, 4, 5 and 6. Linear reporting range of the assay is  $3.00 \times 10^2$  IU/ml to  $1.00 \times 10^9$  IU/ml [13].

### Statistical analysis

Data from the manual record (paper and pen) were entered into an Excel sheet for analysis. The statistical analysis was conducted using SPSS software.

Consent was obtained or waived by all participants in this study. Institutional Human Ethics Committee issued approval 177/IHEC/BRD/2024. The study was approved by the Institutional Ethics Committee, Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, India.

### Results

During the study period, 1,45,683 samples were collected from patients who referred for rapid testing of HCV antibody from different departments to central pathology of tertiary care hospital. A total of 1831 rapid positive samples were tested for HCV infection including viral load between 2023 to 2024 and 1371 were tested positive. The true positivity of HCV infection among the rapid HCV Ag positive cases is 74.87% in the study population. The year wise distribution is depicted in Table 1.

**Table 1:** Sero-prevalence of HCV infection in year 2023-2024.

Variables	2023	2024	2023-2024
HCV Positive	627	744	1371
HCV Negative	172	288	460
Total samples tested	799	1032	1831

58.78% (806/1371) of the patients reported positive for HCV infection were male and same trend was observed in year 2024 as presented in Table 2.

**Table 2:** Gender distribution of patients with HCV infection.

Gender	2023	2024	2023-2024
Male	366	440	806
Female	261	304	565
Total	627	744	1371

Approximately, 71% of the HCV infected patients belonged to 19-40 age group followed by 16.34% in age group 41-60 years as shown in Table 3.

**Table 3:** Age wise distribution of patients with HCV infection.

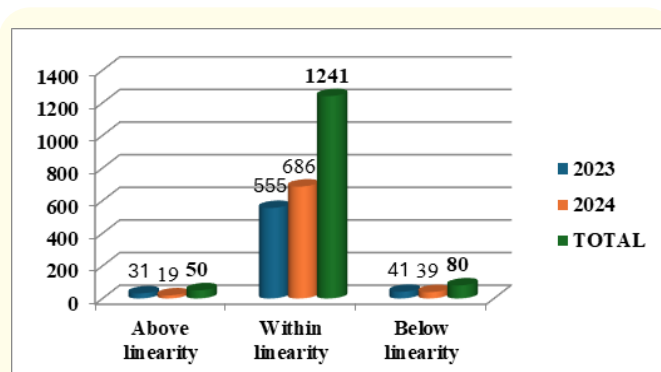
Age (years)	Male (n = 806)		Female (n = 565)		N (%)
	2023	2024	2023	2024	
<18	14	21	05	03	043 (03.14)
19-40	231	351	194	199	975 (71.12)
41-60	81	13	52	78	224 (16.34)
>60	40	55	10	24	129 (09.40)
Total	366	440	261	304	

Among total population study most of the patients visited in MTC unit from Gorakhpur division (69.85%) of Uttar Pradesh followed by Basti (13%) division as illustrated in Table 4. This Eastern U.P hospital covers one more state Bihar and 233 (12.72%) visitors belong to the same. Another country which shares the national boundary with India is Nepal but the total numbers of patient visited during the study period are very few.

**Table 4:** Geographical distribution of subjects enrolled in study (n = 931).

Geographical Area	States	Division	Male	Female	Total
Basti	147	91	238		
Azamgarh	24	13	37		
Ayodhya	04	00	04		
Devipatan	02	14	16		
Varanasi	05	07	12		
Others	04	05	09		
	Bihar		131	102	233
	Nepal		01	02	03

In our study, only 5.83% of cases has viral load less than 300 IU/ml as shown in Figure 1. A low viral load less than  $2 \times 10^6$  RNA copies/ml is considered to be a strong predictor of a sustained response to therapy. Furthermore, patients with a high viral load  $>2 \times 10^6$  RNA copies/ml required longer duration of therapy.



**Figure 1:** HCV patients viral load linearity (n = 1371).

### Discussion

Assessing the prevalence of HCV infection is essential for developing effective strategies for prevention, diagnosis, and control. In this study, HCV sero-prevalence was determined by detecting HCV using RT-PCR. According to hospital protocols, patients were screened for HCV infection prior to surgery, invasive procedures, chemotherapy, dialysis, or if there were suspicions of HCV infection. The study revealed a sero-prevalence rate of 0.94% (1371 out of 145683).

**Table 5:** Comparison sero-prevalence of HCV infection across different region of India.

Study	Place of study	Publisher	Year of study	Study population	Samples tested	HCV sero-prevalence
Retrospective [14]	Mumbai, India	E cancer	2024	Hospital based	71,001	1.17%
Peer reviewed [15]	Jaipur, India,	International Journal of Surgery: Global Health	2023	Survey based	1,64,826	1.0%
Retrospective [16]	Punjab, India	Journal of Advances in Microbiology	2022	Hospital based	122	13.11%
Retrospective [17]	Department at PGIMS, Rohtak India	Adv Res Gastroentero Hepatol	2020	Hospital based	2,13,406	5.18 %
Retrospective [18]	New Delhi, India	Gastroenterol Hepatol Endosc	2018	Hospital based	1,043	NA
Report based [19]	London, UK	Lancet Gastroenterol Hepato	2016	NA	NA	0.96–1.4%
Mini review [20]	Agartala, Tripura, India	Euroasian J Hepato-Gastroenterol	2015	NA	NA	1.0%
Cross-sectional [21]	Mumbai, India	Arq Gastroenterol	2015	Community based	1,833	1%

As summarized in Table 5, the sero-prevalence of HCV infection in India varies from 0.96% to 13.11%. The heterogeneity in sero-prevalence rates may occur due to different study models and sampling bias. When the study is community-based, the selected population may not be a true representative of the community. For example, certain pockets of the community and tribal populations have higher sero-prevalence rates. When the study is hospital-based, there may be patients who are showing symptoms of viral illness and referred to the hospital, leading to overestimation of the sero-prevalence rate.

The test method to detect HCV infection may also affect the result of the study. RT-PCR based tests are considered more sensitive and specific than enzyme immunoassay (ELISA) or immune chromatographic-based tests but require skilled technicians and expensive equipment to run the tests. Tests based on detecting both antigen and viral against HCV are more sensitive, as used in the present study, than tests based on presumptive diagnosis. The performance specifications of the kits to detect HCV may also influence the result of the study. Nevertheless, such variation in HCV prevalence between states implies that the optimum strategies may differ from state to state for screening and treatment for hepatitis C, and their cost-effectiveness.

In the present study, 71% of those who were confirmed positive for HCV infection belonged to the 19-40 year age group, followed by the 41-60 year age group (16.34%). Hence, it is imperative to implement HCV infection screening in this age group along with awareness programs on transmission and prevention of HCV infection.

In 2018, the National Viral Hepatitis Control Program (NVHCP) was launched by the Government of India, with the goal of eliminating Hepatitis C by 2030 [22]. The government is committed to offering free screening, diagnosis, treatment, and counseling services to all individuals, especially those belonging to high-risk groups. The successful implementation of this program is crucial for achieving the elimination of HCV from the country.

### Conclusion

The major mode of transmission of Hepatitis C virus infection varies widely across different regions of the world. In India a large proportion of HCV positive patients acquire hepatitis C 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 HCV antibody for the diagnosis of HCV, this technique also useful to make clinical decisions on starting antiviral therapy for clinician and to intervene the patient health condition in respect to HCV viral load.

Our study showed maximum of such asymptomatic subjects got detected in their third and fourth decade of life and further evaluation of such participants by measuring HCV-RNA viral loads showed that a significant proportion of them harbored ongoing viral replication and liver injury and were at risk for further disease progression. Furthermore, this tool also enables us to find those with high HCV-RNA levels who are at risk of disease progression among patients with asymptomatic status. Proper laboratory diagnosis of HCV infection is the way to access both prevention and treatment.

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. The sero-prevalence of HCV infection in the present study was 0.94%, and over 71% of seropositive individuals belonged to the 19-40-year age group. It is imperative to implement community-wide HCV infection screening programs and awareness campaigns on the transmission and prevention of HCV infection.

The gold standard test for detecting active HCV replication is NAT. HCV NAT is useful in confirming the diagnosis of acute HCV infection, since RNA is detectable as early as one week after exposure and at least 4 to 6 weeks before sero-conversion.

### Conflict of Interest

The authors declare that there is no conflict of interest.

### Ethical Approval

This study was approved by Institutional ethical committee with wide letter no. 177/IHEC/BRD/2025.

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This study was not funded by any agencies/Government/non-government organization.

### Limitations

However, this study has certain limitations. It was conducted within a hospital setting, which restricts the generalizability of the findings to the wider population. This study needs multicentric studies to extrapolate it into common population. Additionally, it also has the limitation due to unidentified genotypic distribution by current strain. Furthermore, study and research require to analyses the current scenario and circulating strain in eastern Uttar Pradesh, India.

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