

TO STUDY SEROPREVALENCE OF VIRAL INFECTION OF HEPATITIS B & C WITH VIRAL LOAD ESTIMATION IN A TERTIARY CARE HOSPITAL OF WESTERN RAJASTHAN

Virology

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ABSTRACT

Background: Hepatitis B (HBV) is the most common hepatitis causing virus accounting for nearly 2 billion infections globally. Similarly, an estimated proportion of 2.8% of the world's population are infected with Hepatitis C (HCV). In India, the National Viral Hepatitis Control Program (NVHCP) was launched by the Ministry of Health and Family Welfare, Government of India in 2018, as an integrated initiative for prevention and treatment of viral hepatitis. **Methods:** A hospital based prospective study was carried out for a period of 3 months (April 2025 to June 2025) in which all patients attending the hospital suspected of having hepatitis were tested for HBV and HCV using Hepatitis Rapid Card Test and Viral load estimation using Chip based Real Time PCR by Truenat molbio according to standard protocols recommended by the manufacturer. **Results:** The study revealed that HBV is more prevalent than HCV with twice the seropositivity and was most common in 11-20 years and 41-50 years. For HCV, age group most affected was between 51-60 and 31-40 years. The results of viral load estimation for HBV DNA detected within the range of assay were found in 54.8%, Below Quantification range were 10.75%, Above Linear range were 7.52% and Not Detected in 26.88% cases. The results of HCV RNA viral load estimation detected within the range of assay were found in 26.31%, Below Quantification range were 15.78%, Above Linear range were 5.26% and Not Detected in 52.63% cases. **Conclusion:** The findings show the need for routine screening, early diagnosis and preventive strategies like HBV vaccination and awareness campaigns. Understanding seroprevalence can further reduce the risk of diseases like cancer in the liver.

KEYWORDS

Hepatitis B, Hepatitis C, NVHCP, Truenat, viral load

INTRODUCTION

Viral hepatitis is liver inflammation due to a viral infection. It may present in acute form as a recent infection with relatively rapid onset, or in chronic form, typically progressing from a long-lasting asymptomatic condition up to a decompensated hepatic disease and hepatocellular carcinoma (HCC).¹

The most common causes of viral hepatitis are the five unrelated hepatotropic viruses' hepatitis A, B, C, D, and E. HBV is a DNA virus while rest all are RNA virus.

Viral hepatitis is a global public health problem, especially in resource poor countries. Complications from HBV or HCV infection are estimated to cause approximately 1.4 million deaths in 2010. About 30% of the disease burden due to viral hepatitis is located in the WHO South-East Asia Region, with estimated 100 million and 30 million people infected with HBV and HCV respectively²

HBV is the only DNA virus among hepatitis viruses. In 1965, it was discovered by Dr Baruch Blumberg in the serum of an Australian Aboriginal person and thus it is also called as Australia antigen. It comes under the family Hepadnaviridae. HBV is the most widespread and most important agent among Hepatitis virus associated with both acute and chronic hepatitis. 1,5 Chronic hepatitis develops in the 15% of adults who are unable to eliminate the virus after an initial infection. Identified methods of transmission include contact with blood, blood transfusion (now rare), unsanitary tattoos, sex (through sexual intercourse or contact with bodily fluids), or mother-to-child by breast feeding; there is minimal evidence of transplacental crossing. However, in about half of cases the source of infection cannot be determined. Blood contact can occur by sharing syringes in intravenous drug use, shaving accessories such as razor blades, or touching wounds on infected persons. Needle-exchange programmes have been created in many countries as a form of prevention.³

Viral hepatitis, caused by hepatitis A to E continues to be a major public

health problem in India. HAV is responsible for 10-30% of acute hepatitis & 5-15% of acute liver failure cases in India. While HEV is responsible for 10-40% of acute hepatitis & 15-45% of acute liver failure in India. Acute HEV mortality is very high in women in late pregnancy, it varies between 15% & 25% in third trimester. Superimposed HEV is responsible for 10-15% of cases of acute or chronic liver failure in India. Acute or chronic liver failure carries a mortality of 50-60% without liver transplantation.

1. Estimation of Seroprevalence of HBV and HCV by Rapid Card Test method.
2. Estimation of Viral Load of HBV & HCV by Real Time PCR test.

AIMS & OBJECTIVES

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MATERIALS & METHODS

A hospital based prospective study was carried out for a period of 3 months (April 2025 to June 2025) in which all patients attending the hospital suspected of having hepatitis (on clinical or laboratory basis) and presented to microbiology lab for testing of HBV & HCV were included. The patients who were affected by HAV and HEV, patients on treatment of hepatitis, patients with known autoimmune hepatitis or drug-induced liver injury or with incomplete clinical or laboratory data were excluded from the study.

The total number of samples included in the study were 3860. Clinical data were obtained using a pre-structured case record form including demographic data (age, gender, residence, occupation), clinical features (jaundice, fatigue, nausea, etc.) and risk factors (blood transfusion, intravenous drug use, surgical history, tattooing, sexual history, water and sanitation conditions)

Serum samples were collected and tested for hepatitis markers. For hepatitis B virus HBsAg Rapid Card Test (Meriscreen Hbs Ag test) and

Viral load estimation using Chip based Real Time PCR by Truenat molbio. For hepatitis C, HCV Rapid Card Test(TREDRO™ HCV Ab) and Viral load estimation using Chip based Real Time PCR by Truenat molbio. All tests were conducted using standard protocols recommended by the manufacturer, and positive samples were confirmed as per the institutional standard operating procedures.

RESULTS

Blood samples from 1973 suspected cases for HBV prevalence were collected amongst which 93 (4.71%) were positive for HBV by Rapid Card Test. (Figure 1)

The maximum seropositivity of HBV is seen in age group 11-20 (6.25%). Second highest is seen in age group 41-50 (5.86%). The minimum seropositivity is seen in 51-60 age group (3.12%). (Table 1)

Table 1 : Age-wise Seroprevalence of HBV

S.No	Age	Total samples	Positive	%
1	<10	0	0	
2	11-20	112	7	6.25%
3	21-30	358	17	4.74%
4	31-40	419	20	4.77%
5	41-50	375	22	5.86%
6	51-60	320	10	3.12%
7	>60	389	17	4.37%
		1973	93	4.71%

The results of HBV DNA viral load are as follows: - HBV DNA Detected within the range of assay were found in 54.8%, Below Quantification range were 10.75%, Above Linear range were 7.52% and Not Detected in 26.88%. (Figure 2,3,4)



Figure 1 : Positive Result of HBV and HCV Rapid Card Test



Figure 2 : Trueprep Auto v2 Universal Cartridge



Figure 3 : Results of Truenat Chip-based RT-PCR

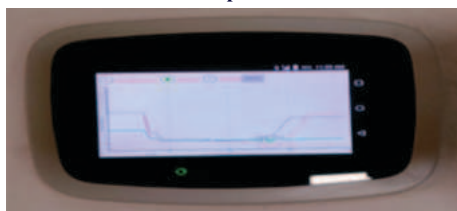


Figure 4: Graphical Results of Truenat Chip-based RT-PCR

Blood samples from 1887 suspected cases for HCV prevalence were collected amongst which 38 (2.01%) were positive for HCV by Rapid

Card Test. (Figure 1)

Maximum seropositivity is seen in age group 51-60 with 3.67% and minimum seropositivity is seen in age group less than 10 and 11-20 with no seroprevalence while age group 31-40 showed second highest seropositivity with percentage of 2.19%. (Table 2)

The results of HCV RNA viral load are as follows: - HCV RNA Detected within the range of assay were found in 26.31%, Below Quantification range were 15.78%, Above Linear range were 5.26% and Not Detected in 52.63%. (Figure 2,3,4)

Table 2 : Age-wise Seroprevalence of HCV

S.No	Age	Total samples	Positive	%
1	<10	0	0	0%
2	11-20	107	0	0%
3	21-30	335	5	1.49%
4	31-40	410	9	2.19%
5	41-50	359	5	1.39%
6	51-60	299	11	3.67%
7	>60	377	8	2.12%
		1887	38	2.01%

DISCUSSION

In study time of 3 months, total 3860 samples were screened. For HBV, 1973 patients were screened and out of which 93 patients were positive. For HCV, 1887 patients were screened and out of which 38 patients were positive. Hence the seropositivity for HBV is 4.71% and that of HCV is 2.01%.

Total 3860 patients were initially screened for hepatitis by the rapid card test. All the positive patients were further screened for confirmation test by viral load estimation.

HBV DNA estimation was done of all the screening positive patients of HBsAg card test samples. It was done under National Viral Hepatitis Control Program and out of 93 positive patients, in 68 patients (73.11%) HBV DNA was detected while in 25 patients (26.88%) HBV DNA was not detected.

HCV RNA estimation was done of all the screening positive patients of Anti-HCV card test samples. It was also done under National Viral Hepatitis Control Program and out of 38 positive patients, in 18 patients (47.36%) HCV RNA was detected while in 20 patients (52.63%) HCV RNA was not detected. In present study, HBV has high prevalence rate of 4.71% than that of HCV which is 2.01%. In Athira Jayaram et al. (2023)⁴ conducted in western India the seroprevalence of the various types of hepatitis markers observed was: HBV: 1.11%; HCV: 0.24%. Similarly, In P Jain et al. (2013)⁵ conducted in North India (in Lucknow, UP) found the seroprevalence of HBV was 16.10% and that of HCV was 11.98%.

And in Michael Owusu et al. (2018)⁶ done in tertiary hospital in Ghana, HBV was the most prevalent virus with seroprevalence of 54.2% and prevalence of HCV was 11.6%. Also, in Birendra Prasad et al. (2018)⁷ done in Kathmandu, Nepal, seroprevalence of HBV was 13.4% and that of was HCV in 11.9% which shows prevalence of HBV is higher than HCV which is in concordance with the present findings.

While in Muchiri et al. (2012)⁸ done in Nairobi, Kenya, the positivity for viral markers were found to be 3% for HBsAg and 5% for HCV RNA which do not match with the current study. In the present study the maximum seropositivity of HBV is seen in age group 11-20 with the percentage of 6.25%. Second highest is seen in age group 41-50 with the percentage of 5.86%. The minimum seropositivity is seen in 51-60 age group with the percentage of 3.12%. The high percentage of seropositivity among young generation of 11-20 age group could be attributed to the fact that the vaccination programs are still not that popular among certain areas and also high-risk rates of sexual contacts and drug use could also be the reason. Athira Jayaram et al³¹ and Manjiyil et al⁹ has documented similar trend in their study.

Some HBsAg-positive samples may show undetectable HBV DNA during viral load estimation by PCR. This can occur when the viral replication is minimal or has ceased, but HBsAg remains in circulation. In some individuals, especially inactive carriers or those on antiviral therapy, the viral load may be below the detection limit of the PCR assay. Additionally, mutations in the HBV genome can affect

viral replication without impacting HBsAg production. Sample handling issues may also contribute to undetectable results. These findings highlight the complex relationship between serological and molecular markers in HBV infection.¹⁰

In the present study, total 1887 blood samples from suspected HCV cases were collected during a period of 3 months from April 2025 to June 2025. In this study seroprevalence of HCV was 2.01% (38/1887).

In Birendra Prasad et al.¹¹, Mohammed Amin Behzadi et al.²⁶, Athira Jayaram et al.³¹ study the seroprevalence of HCV were found to be 1.9%, 0.7% and 0.24% respectively which is in concordance with the present study.

While in the study of P Jain et al.¹², Maasha et al.¹³, Jordi Llaneras et al. the seroprevalence of HCV reported were 11.6%, 13.11% and 17% respectively.

The difference between the seroprevalence rates observed in the present study and those reported by other researchers could be attributed to several factors such as differences in geographical location, study population, sample size, study design, diagnostic methods used, and the time period of data collection. Furthermore, variations in risk behaviour, healthcare infrastructure, and awareness levels among populations may also contribute to these discrepancies.

In the present study the maximum seropositivity is seen in age group 51-60 with 3.67% and minimum seropositivity is seen in age group less than 10 and 11-20 with no seroprevalence while age group 31-40 showed second highest seropositivity with percentage of 2.19%. Athira Jayaram et al.³¹ and Masha et al.³⁰ had reported similar trend in their study. The higher prevalence of HCV in the 51-60 and 31-40 age groups may be due to past exposure before routine blood screening and increased risk behaviours like unsafe injections or tattooing. These age groups may also have delayed diagnosis, contributing to detectable infections during this period.

The seroprevalence of HBV is higher than that of HCV while the exact percentage of prevalence does not match with the other studies possibly due to changes in geographical regions, changes in sample size, study design and many more.

CONCLUSION

This study highlights a considerable prevalence of HBV and HCV in the study population, particularly among males of age group between 31-50 and along among the teenage groups and young adults of age group 11-20 years. The findings show the need for routine screening, early diagnosis and preventive strategies like HBV vaccination and awareness campaigns. Understanding seroprevalence can further reduce the risk of diseases like cancer in the liver.

Limitations of the Study: This study was limited by short duration and limited sample size. Seasonal variation of study could not be studied as this study was done only in summer time for three months. Genome sequencing of viral isolates was not done.

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Conflict of Interest: None declared

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