



Serological and virological profile of viral hepatitis (Hepatitis B and C) in and around Aligarh region

Authors

Parvez Anwar Khan^{1*}, Kanchan Singh², Sarita Mallik²,
Sanjay Sharma¹, Haris Manzoor Khan¹

¹Department of Microbiology, J.N.M.C.H, Aligarh Muslim University, Aligarh, India

²Division of Life Sciences, Department of Biosciences, School of Basic and Applied Sciences, Galgotias University, Uttar Pradesh

*Corresponding Author

Parvez Anwar Khan

Assistant Professor, Department of Microbiology, J.N.M.C.H, Aligarh Muslim University, Aligarh, India

Abstract

Introduction: Viral hepatitis especially hepatitis B and hepatitis C infection is one of the main public health problem affecting million of people. This study was performed in the Department of Microbiology, JNMCH, AMU, Aligarh, to determine the prevalence, serological and virological profile of patients with HBV and/or HCV infection.

Methods: A laboratory based study was carried out on patients with suspected viral hepatitis to find out the clinical profile, spectrum of viral hepatitis infections and the correlation between serology and viral load. Patient blood samples were recruited from the OPDs and Wards from the patients presenting with signs and symptoms of hepatitis. Serum samples were tested for HBsAg, anti HCV antibodies and HBeAg, anti HBeAg by ELISA test. Viral Load of HBV and HCV was done by fully automated Quantitative Real-time PCR.

Results: A total of 3512 samples of HBV and HCV were investigated, out of which 316 (8.99%) patients were found to be positive for viral hepatitis positive. These 316 cases of confirmed viral hepatitis were observed positive during January to May 2022. Occurance of HBsAg positive patients were 194(61.3%), A-HCV were 114(36.0%), HBeAg were 4(1.26%) and A-HBeAg 2(0.63%) and, 8(2.53%) were positive for both HBsAg and A-HCV. Out of 316 positive patients, 150 (47.4%) were male and 166 (52.5%) were female. The majority of male and female patients are found in the age group of 21-40.

Conclusions: Our study provided the information about prevalence of hepatitis B and C, patient's serology and virology profile with viral hepatitis infection and risk transmission. The incidence of HBV and HCV infection were endemic at an intermediate level in the geographical area of Aligarh, Uttar Pradesh. Sufficient measures and intervention are need to be taken by government and public health sector for risk prevention and health education among population to reduce viral infection between adults.

Keywords: HBV hepatitis B virus; HCV hepatitis C virus; HBsAg hepatitis B surface antigen; risk factors.

Introduction

Viral hepatitis especially hepatitis B and C viruses is one of the main public health problem because it

precipitated 1.34 million deaths in year 2015, equivalent to deaths due to tuberculosis and higher than those due to HIV^[1]. In haemodialysis patients,

a hepatitis viral infection elevates the incidence of morbidity and mortality ^[6]. Hepatitis B and C virus belongs to the genus Orthohepadnavirus and hepacivirus, a member of the family hepadnavirus and flavivirus.

Some studies are available on epidemiological details regarding prevalence of hepatitis infections. In one study, the epidemiology of HBV and HCV infection in Morocco was investigated ^[10]. Extensive screening of hepatitis B surface antigen (HBsAg) and anti-HCV antibody was performed by third generation ELISA test. Hepatitis B and C were observed to be parenterally transmitted with respective prevalence of approximately 1.79% and 1.5% of the general population studied ^[10]. Available data on the prevalence of HBV infection was analysed in another study and it indicated an average prevalence of HBsAg seromarkers of 2.6% to 3.2% ^[2].

Health workers are at risk of contracting HBV and HCV infection through mucocutaneous exposure such as (eyes or mouth mucosa or skin), to blood that may be contaminated with blood products or by sharp exposure to contaminated sharp edges (needles, blades, etc.). In the present study, patients with HBV and HCV infection were tested serologically and virologically to determine if they have chronic infection or have experienced exposure to viral hepatitis. To that end, it is necessary to test the serum for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), and antibody for hepatitis B core antigen (anti-HBc) and anti-HCV antibody. The majority of male and female patients were found in the age group of 21-40 years. The youngest patient was 8 years old while the oldest was 84 years old in our study.

Measurement of viral load levels is a very reliable virological indicator for determining active viral replication and, combined with liver enzymes and clinical trials, makes an accurate natural history of hepatitis B, as well as the risk of disease progression among infected patients. Therefore, understanding these symptoms is very important in monitoring the progression of the disease and in the proper

management of patients. We conducted this study to investigate serological and virological HBV and HCV markers distribution among patients in Aligarh region of Uttar Pradesh, India during the period January 2022 to May 2022.

Method and Materials

Laboratory - based study was carried out on patients with suspected viral hepatitis to find out the clinical profile, spectrum of viral hepatitis infections and the correlation between serology and viral load. The patients were recruited from the OPD and wards and, microbiological investigation were performed at Department of Microbiology, JNMCH, AMU, Aligarh. Pre-existing data was also retrospectively collected and tested. The investigation period for present study was from January 2022 to May 2022.

Specimen's collection

Patient's blood (5 ml) was collected in a plain vial tube and centrifuged for 2 to 3 minutes to separate the serum from the blood. Serum sample for molecular testing was stored by freezing at -20 °C until laboratory test was performed.

Serological test

Serological markers such as HBsAg, HBcAg, HBeAg, Anti-HCV antibody, Anti-HBc IgM, were tested with different methods including Rapid test kit (Medsources), ELISA (Qualisa HBsAg), and Roche fully automated immunoassay analyser machine (Cobas e 114) or ECLIA (electrochemiluminescence immunoassay). In samples that were found positive by serology tests, those were further processed for quantitative and qualitative Real-time PCR. The PCR was performed by Hitachi cobas z-4800 or Amplicons onto specific probes after the reaction in Real-time PCR or transcription mediated amplification (TMA) technique.^{[9][7]}

Clinical investigations

To know the detailed clinical history of patients, patient data were evaluated by predesigned performa which contained all suspected case history of the patients. Patients included were from various outpatients department, wards, immunology lab,

ART clinics and VRDL Department of Microbiology Jawaharlal Nehru Medical College and Hospital AMU, Aligarh

Results

In the current study, a total 3512 suspected samples of viral hepatitis were collected and tested for hepatitis positivity by serological tests using serological assay. Out of 3512 total viral hepatitis cases, 316 (8.99%) patients were found hepatitis positive and 3192 (91%) patients were found hepatitis negative (Figure 1).

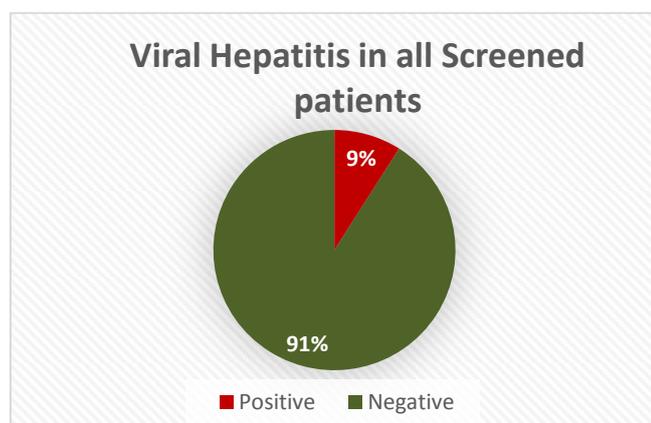


Figure 1: Prevalence of Viral Hepatitis

Distribution of HBV and HCV infection in hepatitis patients

As shown in Table 1, and Figure 2, out of 316 positive cases, the positive patient count of HBsAg was 194 (61.3%), A-HCV was 114 (36.0%), HBeAg was 4 (1.26%) and A-HBc IgM was 2 (0.63%). Further 8 (2.53%) patients were positive for both HBsAg and A-HCV.

Table 1: Details of positive hepatitis cases detecting by using ELISA (total cases = 316)

Type of hepatitis	Number of cases	%age (316)
HBsAg	194	61.39%
Anti-HCV	114	36.0%
HBeAg	4	1.26%
Anti-HBc IgM	2	0.63%
HBsAg+Anti-HCV	8	2.53%

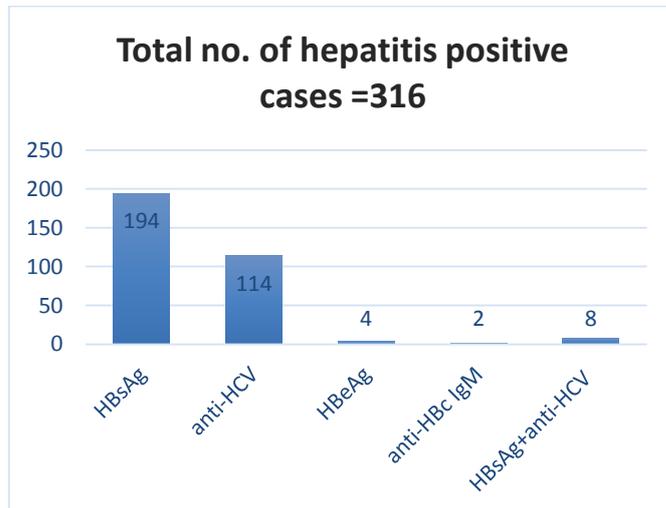


Figure 2: Investigation of various hepatitis cases

Out of 316 positive patients, 150 (47.4%) patients were male and 166 (52.5%) were female positive (Figure 3).

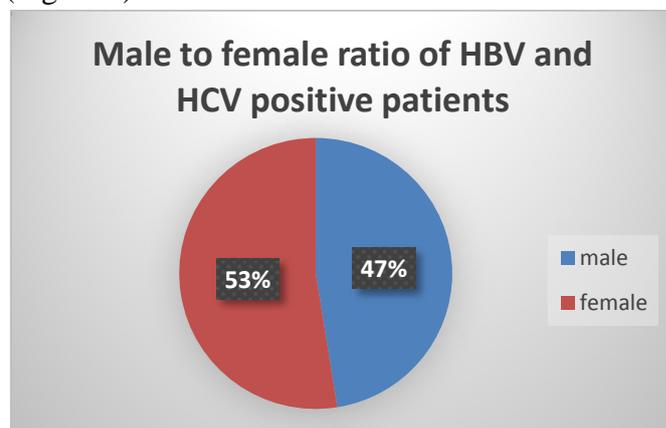


Figure 3: Male to female ratio of HBV and HCV positive patients

Age and sex distribution and demographic profile of patients with suspected viral Hepatitis included in the study

Of the total of 3512 patients with suspected viral hepatitis included in this study, the maximum number of patients (both male and female) was found in the age group of 21-40 years. The youngest patient was 8 year-old while the oldest was of 84 years in our study. Of the total suspected cases, total number of males and females were 1814 (51.6%) and 1698 (48.3%) respectively. The male to female ratio in this study was 1.07:1. The age and sex composition of the study population is shown in Table 2, Figure 4.

Table 2: Age and sex distribution of patients with suspected viral Hepatitis

Age in years	Male	Female	Total (%)
0-20	202	129	331 (9.4%)
21-40	832	1016	1848 (52.6%)
41-60	654	437	1091 (31%)
>60	126	116	242 (6.9%)
Total	1814	1698	3512 (100%)

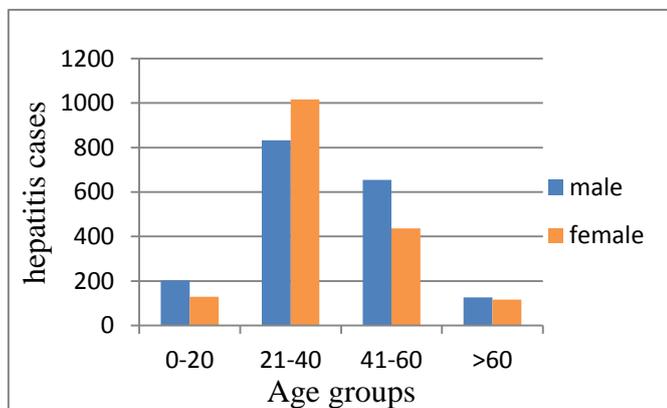


Figure 4: Age and sex distribution of patients with suspected viral Hepatitis

Socio-economic demographic information can be obtained by exploring a population-based demographic and statistical data including marital status, employment, education and housing.

There are different characteristics of Socio-demographic variability in rates of HBV and HCV cases at received in this study (Marital status: married, unmarried; Social background: rural, urban; Occupational: farmers, employed, driver, labourer, business, housewife, army/police, other; Residence: Aligarh, adjacent districts, outside UP state) figure-5. In our study, most 80.6% of the participants were found married. In terms of occupation the majority 37.8% of participants were house wife followed by farmers 15.0% and employed 14.2%. Nearly 34.4% of the patients belonged to Aligarh while 53.6% resided in Adjacent districts and another 11.9% were from outside UP states. All these characteristics and demographic profile of study are as shown in Table 3, Figure 5.

Table 3: Demographic profile of study group

Parameter		Total no. of patients (%)
Marital status	Married	2830 (80.6%)
	Unmarried	682 (19.4%)
Social background	Rural	1885 (53.8%)
	Urban	1627 (46.3%)
Occupation	Farmer	527 (15.0%)
	Employed	499 (14.2%)
	Driver	51 (1.5%)
	Labourer	126 (3.6%)
	Business	785 (22.4%)
	Housewife	1329 (37.8%)
	Army/police	37 (1.0%)
	Others	158 (4.49%)
Residence	Aligarh	1210 (34.4%)
	Adjacent Districts	1884 (53.6%)
	Outside UP state	418 (11.9%)

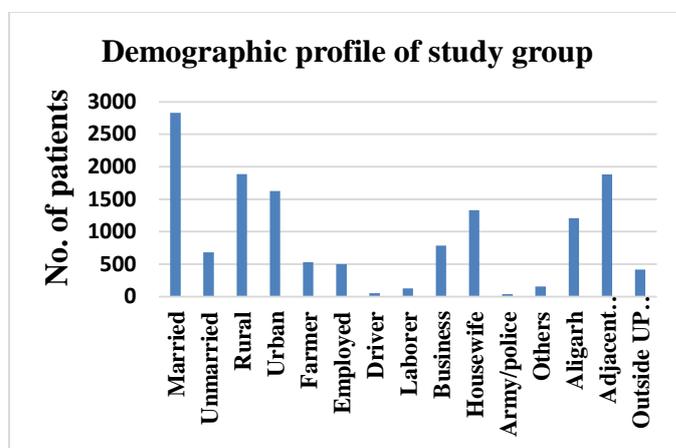


Figure 5: Demographic profile of study group

Serological Profile of patients with Hepatitis B and C Virus infection

According to the OD values obtained in ECLIA, the serological profile of patients with viral hepatitis, higher OD value (mean OD = 2392) was obtained in 194 positive tested samples of HBsAg followed by the 4 HBeAg positive tested samples with mean OD value (509.60), 2 positive tested samples of Anti-HBc-IgM with mean OD value (8.45).

Table 4: Serological Profile of patients with Hepatitis B and C Virus infection

Serological test	Positive	Mean OD in ECLIA
HBsAg	194	2392
Anti-HBc-IgM	02	8.45
HBeAg	04	509.60
HBsAg + Anti-HCV	08	-
Anti-HCV	114	37.81

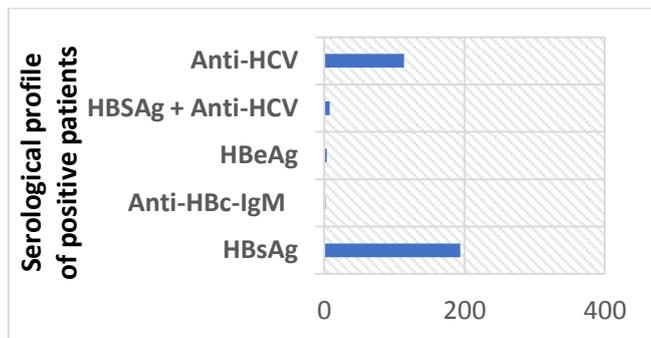


Figure 6: Serological Profile of patients with Hepatitis B and C Virus infection

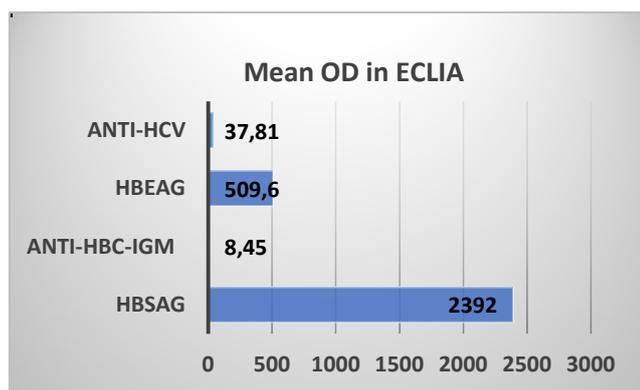


Figure 7: Mean OD in ECLIA in patients with Hepatitis B and C Virus infection

Other 114 positive tested samples of Anti-HCV with mean OD value (37.81) and 8 were positive both HBsAg and Anti-HCV are found with viral hepatitis infection as shown in (Table 4, Figure 6 and 7).

Virological Profile of patients with Hepatitis B and C Virus infection

The viral load testing was done by real-time PCR. The mean viral load of Hepatitis B was 6.13×10^3 and that of hepatitis C was 8.23×10^4 . The viral Load in IU/ml was converted to copies /ml by the formula $1 \text{ IU/ml} = 5.6 \text{ copies/ml}$

Table 5: Hepatitis B and Hepatitis C viral load in patients with viral hepatitis

	No. of Patients	Mean Viral Load (IU/ml)	Mean Viral Load (viral copies/ml)
Hepatitis B	39	6.13×10^3	34.32×10^3
Hepatitis C	13	8.23×10^4	46.08×10^4

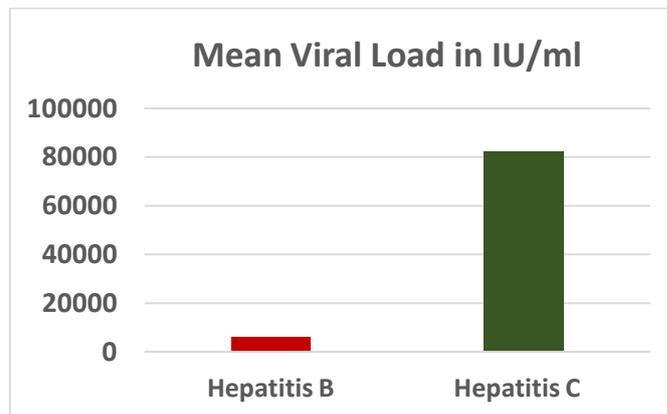


Figure 8: Virological Profile of patients with Hepatitis B and C Virus infection

Discussion

We received a total 3512 samples of HBV and HCV during the study period. Epidemiological studies involving large population study of prevalence of communicable diseases are important to prevent the spread of life threatening diseases such as hepatitis B and hepatitis C and develop measures to improve public health. Some reports are available on distribution of HBV and HCV infection among general population or demographic groups. In comparison to previous studies on the rate of HBsAg and HCV infection in general population of Iran, A study on addict prisoners showed that the HBsAg was 2 times and HCV infection was 70 times more frequent [5]. A recent study in Northern India shows that the average prevalence of HCV and HBV %age positivity of viral hepatitis was 3-4% and 0.09-15% [1]. The overall rate of HBsAg positivity has been reported to range between 2 and 4.7% [8] but in our study on prevalence of HBV and HCV the overall percentage positivity of viral hepatitis was 8.99% (316/3512) was reported. With patient age ranging from 8 years to 84 years, most of the HCV and HBV cases were found in children and young adults. The age distribution signifies the spread of HCV and HBV in young adults and children as older person develop immunity due to production of protective antibodies. The highest prevalence was noted in subjects 21–40 years of age and sex distribution patients with suspected viral hepatitis number of males was 1814 (51.6%) and females was 1698 (48.3%) respectively. Compare to

other study the highest seroprevalence of HBsAg was noted in subjects 31–40 years of age and declined with advancing age^[4].

A recent study involving demographic examination of hepatitis infection patients revealed that majority of the patients were married (70.8%). Among total participants, a higher percentage (39.0%) of participants were government employees while, rest were private workers (27.5%) and unemployed (26.9%)^[2]. Similarly in our study the etiology of viral hepatitis and their associated clinical outcomes differ geographically owing to different social background, marital status, varying occupation and residences. The results showed that (80.6%) of the participants approved the HBV and HCV testing were married. Even though this area is geographically very interior and rural (53.8%). The majority (37.8%) of participants were house wife followed by farmers (15.0%) and employed (14.2%). Nearly (34.4%) of the patients belonged to Aligarh while (53.6%) resided in Adjacent districts and another (11.9%) were from outside UP states as reported by Residence.

In the current study, 316 cases of positive hepatitis infection were observed. Among them, 61.3% were positive for HBsAg, Apart from this, HBsAg A-HCV was positive in 36.0%, HBeAg in 1.26%, A-HBc IgM in 0.63% and 2.53% were positive both HBsAg and Anti-HCV respectively. Co-infection of HBsAg and A-HCV was observed in 2.53% cases by ELISA test. Previous research finding in 183 suspected samples of hepatitis with deranged LFTs, 8.19 % were positive for HBsAg, 2.18 % were positive for anti-HCV, 8.74 % were positive for anti-HEV, and 0.54 % were positive for both anti-HCV IgM and HBsAg antigen by ELISA.^[3]

The patterns of HBV and HCV markers have a defining role to play in characterizing disease phase and requirement for antiviral therapy. In this study, the association of HBsAg and A-HCV was frequent and occurred mostly in the presence of hepatitis B core antibodies of the IgM subclass (HB core IgM). This constellation of markers is well established for the detection of acute or new HBV infection. Patients with detectable hepatitis B surface antigens

are highly viremic with the optical density and often have viral genetic material levels exceeding 823000 IU/mL as also documented in this study.

This study documented a higher proportion viral copies of Hepatitis C compared to the Hepatitis B, but most people with hepatitis B infection had detectable viral load and viral profiles consistent with hepatitis cases. Although our findings are consistent, we report a significantly higher prevalence of HBsAg positive as compared with other serological markers. One reason for these differences may be related to the subjects studied. It is plausible that our subjects had higher viral loads because they were hospital-based patients and thus more symptomatic than the community population in Aligarh. Another important reason may be the different molecular assays used to quantify viral DNA. In support of our study, the standard Roche Amplicor molecular assay used for viral detection and quantification is internationally regulated and associated with a high level of sensitivity and specificity heightening the validity of our finding.

The clustering of HCV and HBV in present study occurred in January to May which is a winter-spring season in this part of India. These are various routes reported for transmission of hepatitis B and hepatitis C viruses: sharing of needles, syringes, and other injection equipment among individuals. Transmission from mother to child occurs when pregnant women pass these infections to their infants. HIV-HCV coinfection in pregnant woman also increases the risk of passing on hepatitis C to the infant. Various other studies in India investigating HCV and HBV outbreaks in the past were able to point towards a common source but no such common source or a single point source could be concluded in our study.

Conclusions

The present investigation revealed the incidence of the HBV infections were endemic at an intermediate level. A low incidence of hepatitis C incidence rate was also observed compared with previous studies. Even though prevalence rate is moderate, there still may be a large reservoir of HCV and HBV-infected

persons in this geographical area of Aligarh, Uttar Pradesh. Hence the risk of developing chronic liver disease linked to viral hepatitis among the population is still high. This added to the fact that affordable antiviral therapy is still a distant reality faced by the vast majority of people in developing countries. Therefore, sufficient measures and interventions need to be taken by government and public health sectors for risk prevention and health education among population to help contain the spread of the blood- born viral infections.

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