

**Etiology of Viral hepatitis among acute hepatitis patients at a tertiary
care hospital in North India**

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Abstract

Introduction: Viral hepatitis, a significant public health burden, affects millions of individuals worldwide. HAV, HBV, HCV, and HCV are primary causative agent for viral hepatitis. Large number of outbreaks of HAV and HEV occurs every year. HBV and HCV are primary cause of chronic liver disease. Exploring the etiology and clinic-epidemiological profile of acute viral hepatitis (AVH) becomes crucial for planning the preventive measures to control the diseases.

Methods: This study was conducted at VRDL, Department of Microbiology, JNMC, AMU, Aligarh, Uttar Pradesh, India from January 2018 to October 2022. Patients showing symptoms of hepatitis such as jaundice, fever, malaise, headache, nausea, vomiting, anorexia, diarrhoea, and abdominal pain were included in the study.

Results: Out of 1798 patients presenting with acute viral disease, 21.02% (378/1798) had HAV, 20.96% (377/1798) had HBV, 9.78% (176/1798) had HCV, and 5.45% (98/1798) had HEV. There were 0.5% (09/1798) samples which were positive for both HBV surface antigen and anti-HCV antibodies and 0.3% (06/1798) samples were positive for both anti-HAV IgM and anti-HEV IgM. With the exception of Hepatitis A, which was more common in children, other Viral hepatitis were more common in adults with a male preponderance.

Conclusions: Vaccination, better cleanliness, and appropriate blood screening before blood transfusion are all safety measures that can help to lower the incidence rate of viral hepatitis and stop its spread. Regularly identifying the cause of AVH and keeping track of cases would aid in patient management and help disease control programs make policy decisions.

Keywords: AVH, Viral Hepatitis, HAV, HBV.

Introduction

Viral hepatitis is a global public health problem and a serious health issue in developing countries like India. Due lack of awareness among people, viral hepatitis is spreading at an alarming rate affecting a large population and causing a large number of deaths are every year. Viral hepatitis is caused by any of the four major hepatitis viruses HAV, HBV, HCV and HEV. As per WHO 2017 global hepatitis report there are around 1.34 million deaths occurred due to viral hepatitis. India has a high incidence of HAV-associated hepatitis (10-30%) and acute liver failure (5-15%). Furthermore, HEV is responsible for 10-40% of acute hepatitis and 15-45% of acute liver failure [1]. Infection with either hepatitis B or hepatitis C can last a lifetime, in 2019, the World Health Organization estimates that 1.1 million people died from chronic viral hepatitis and its effects, such as liver cancer and cirrhosis [2]. Hepatitis-A is a RNA virus belongs to family *Picornaviridae* and genus *Hepatovirus*. The majority of children infected with HAV are asymptomatic or mildly symptomatic [3]. A person with hepatitis A often experiences sudden onset and characteristic symptoms that include yellowish discoloration of the eyes, fever, nausea, vomiting, dark urine, and jaundice within a few days to a week [4]. Hepatitis-B virus (HBV) is a DNA virus that belongs to the family Hepadnaviridae; it can cause immune-mediated liver diseases of varied severity and duration and is transmitted by contact with infected blood or body fluids [5]. Hepatitis C virus is a RNA virus of family Flaviviridae and genus *Hepacivirus*; it is commonly transmitted through parental routes and by contact with an infected person through blood or body fluids [6]. Most individuals with HCV infection present with a silent, insidious onset of disease that persists for a long period. This virus has a high risk of worsening chronic liver disease, which may result in chronic hepatitis, cirrhosis, and occasionally hepatocellular carcinoma [7]. Hepatitis-E virus HEV is positive stranded RNA virus belongs to family *Hepeviridae* of genus *hepevirus*, HEV is transmitted mainly through faeco-oral route [8]. There have been several outbreaks of HEV infection in India over the past fifty years. In India, HEV infection is a significant contributor to acute and sub-acute liver failure, compared to women who had other kinds of hepatitis, pregnant women with jaundice and acute viral hepatitis of HEV genesis had greater death rates and worse obstetric and fetal outcomes [7].

In the present study we investigate the prevalence and pattern of acute viral hepatitis in Aligarh region of western Uttar Pradesh, North India.

Materials and methods

Study Population and Sample Collection

This study was conducted at Viral Research and Diagnostic Laboratory (DHR/ICMR), Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh, India from January 2018 to October 2022. Patients showing symptoms of hepatitis such as jaundice, fever, malaise, headache, nausea, vomiting, anorexia, diarrhea, and abdominal pain treated at Jawaharlal Nehru Medical College Hospital were included in the study.

Clinical samples

Venous blood samples about 5 ml in a sterile clot activator vacutainer along with clinical and demographic information were collected from symptomatic patients referred to this laboratory. A total of 1798 patients from all ages and gender were included in the study. The serum was separated by centrifugation and stored at -20°C till further testing.

Ethics statement

Informed consent was taken from all patients or their attendants.

Serological assays for detection of Hepatitis A, B, C, and E virus

Detection of Hepatitis A & E virus: Hepatitis-A and E IgM ELISA kit by Diapro USA, was used for diagnosis of Hepatitis A virus as per manufactures instruction.

Detection of Hepatitis B virus: Hepatitis-B virus surface antigen (HBsAg) ELISA kit by Tulip Diagnostic was used for diagnosis of Hepatitis-B virus.

Detection of Hepatitis C virus: Hepatitis-C virus anti IgG ELISA kit by Tulip Diagnostic was used for diagnosis of Hepatitis-C virus.

All test were performed as per manufacturer's instructions.

Detection of routine clinical indices in blood and serum

Routine blood serum tests, including SGOT or aspartate transaminase (AST), SGPT or alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin total, and bilirubin direct were determined from the blood samples of all patients showing symptoms of viral hepatitis.

Results

1798 suspected patients of viral hepatitis with deranged levels of LFT from all gender and age group were included in the present study. Among them 21.02% (378/1798) were found positive anti-HAV IgM, 20.96% (377/1798) were positive for HBV surface antigen, 9.78% (176/1798) were positive for anti-HCV IgG and 5.45% (98/1798) were positive for anti-HEV IgM. There were 0.5% (09/1798) samples which were positive for both HBV surface antigen and anti-HCV antibodies and 0.3% (06/1798) samples were positive for both anti-HAV IgM and anti-HEV IgM. In the present study we found that males are more prone to viral hepatitis than females (Fig 1).

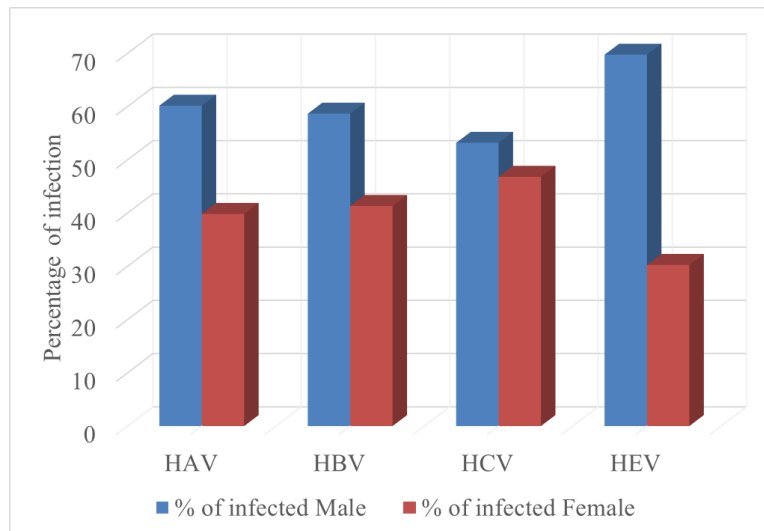


Figure 1: Gender distribution of Acute Viral Hepatitis patients

Out of total positive cases, we found that, HAV infection is found to be highest in the age group of 0-10 years (74.64%), on the other hand HBV infection is more common in the group 21-30 years (34.21%) followed 31-40 years (17.54%), HCV infection is more prevalent in the age group 21-30 years (23.76%) followed 31-40 years (22.77%), and the HEV infection is highest in the age group 11-20 years (38.96%) followed by 21-30 years (31.17%). (Figure 2).

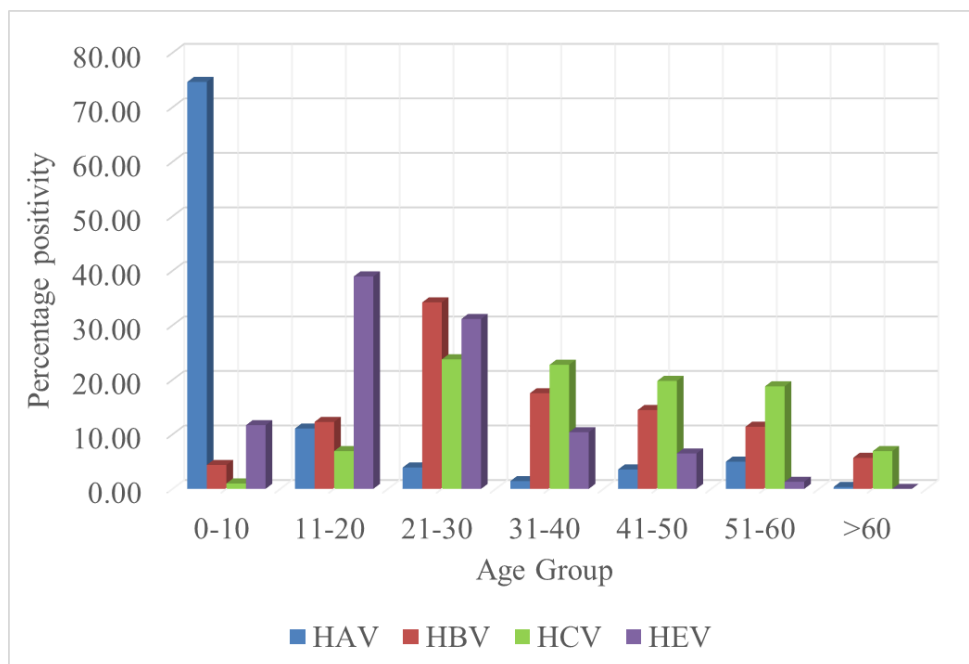


Figure 2: Age distribution of Acute Viral Hepatitis patients

Mean concentration of AST, ALT and ALP was highest in patients suffering from Hepatitis A virus followed by Hepatitis E, on the other hand the mean concentration of Total Bilirubin was highest in patients suffering from Hepatitis E virus followed by patients suffering from Hepatitis B virus (Table 1).

Mean concentration of different liver enzymes in patients suffering different viral hepatitis from 2018 to 2022						
	AST	ALT	ALP	TBI	D	IND
Hepatitis A	1013.5	1209.9	433.6	5.5	4.1	2.1
Hepatitis B	359.60	368.59	188.75	6.98	5.15	2.75
Hepatitis C	229.05	178.03	206.39	2.63	1.73	3.00
Hepatitis E	624.24	643.03	297.40	9.73	5.09	1.97

Table 1: Mean Concentration of Liver Enzymes in Acute Viral Hepatitis Patients

From the study we also found that a higher AST and ALT levels (>1000U/L) were found in 34% and 41.8% of HAV infected patients respectively and only 2.94% and 1.29% of HAV infected patients have normal level (0-50 U/L) of AST and ALT, on the other hand only 5.91% patients have raised level (>1000U/L) of ALP, instead about 46.36% of HAV infected patients have a ALP level in the range of 201-500 U/L, and about 14.09% of HAV infected patients have normal ALP level (Fig 3). A raised level of total bilirubin index (5.1 – 10 mg/dL) is found in about 42.73 % of HAV infected patients.

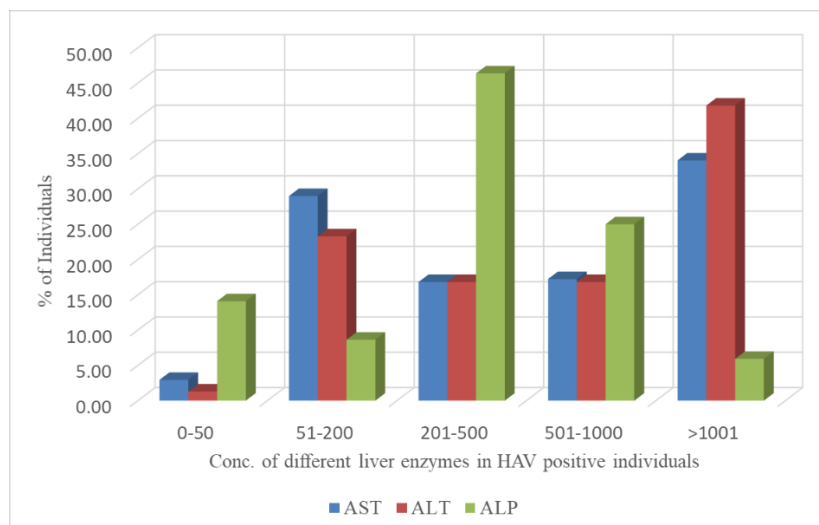


Figure 3: Liver Enzymes level in HAV infected Individuals

In case of HBV infection only 9.93% and 10.98% of infected patients have a higher level (>1000U/L) of AST and ALT respectively, instead 42.55%, 43.29%, and 46.67% of HBV infected patients have AST, ALT and ALP level in the range of 51-200U/L (Fig 4). The total bilirubin index in HBV infected patients was >10 mg/dl in about 22.05% and in the range of 5.1- 10 mg/dL in about 26.77% of HBV infected patients.

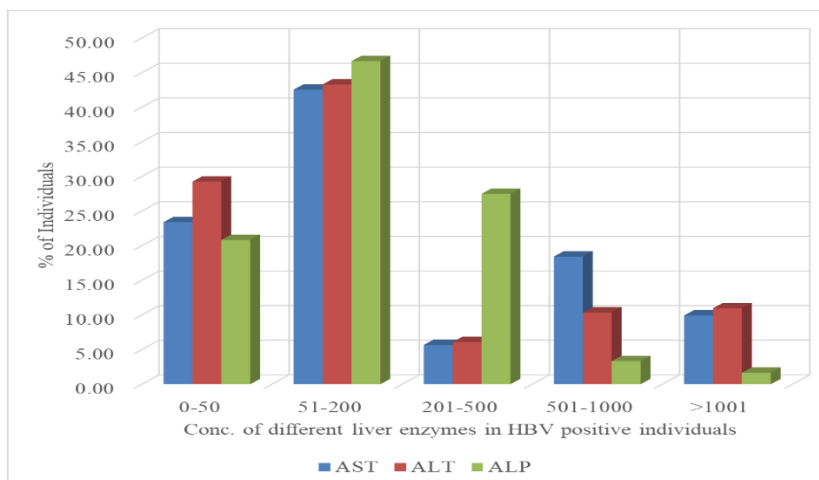


Figure 4: Liver Enzymes level in HBV infected Individuals

In case of HCV infection only 5.56% and 4.44% of infected patients have a higher level (>1000U/L) of AST and ALT respectively, instead about 61.11%, 42.22% and 51.72% infected patients have AST, ALT, and ALP level in the range of 51-200 U/L, 33.33%, 44.44%, and 10.34% of infected patients have normal level (0-50U/L) of AST, ALT, and ALP respectively (Fig 5), on

the other hand only 3.45% of HCV infected patients have higher level (>10 mg/dL) of total bilirubin index, and about 44.83% infected patients have total bilirubin index in the range of 1.1-2 mg/dL.

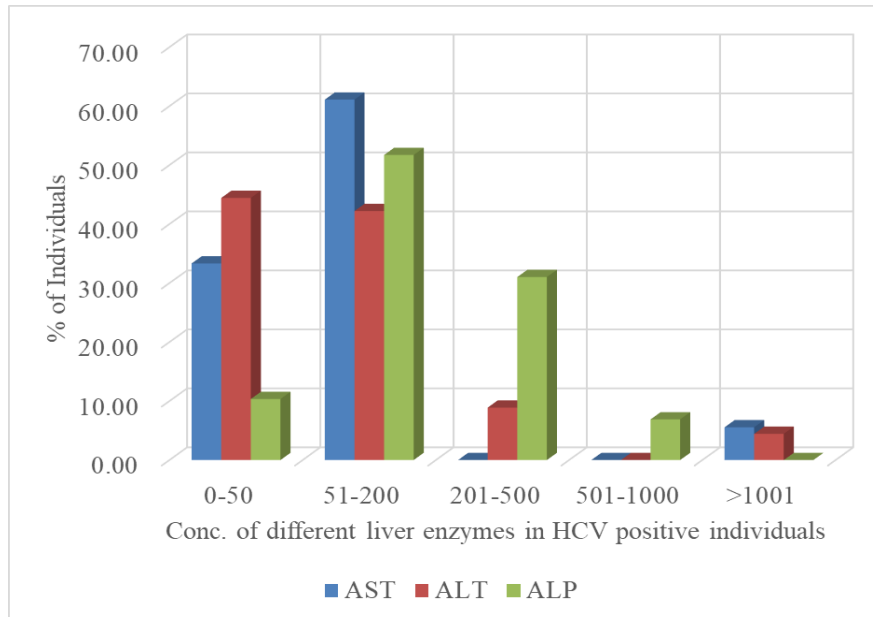


Figure 5: Liver Enzymes level in HCV infected Individuals

In case of HEV infection about 20.34%, 20.63 and 3.92% of infected patients have a higher level (>1000U/L) of AST, ALT and ALP respectively, about 42.37% and 47.62% of infected patients have AST and ALT level in the range of 51-200 U/L (Fig 5). A higher level of total bilirubin index (>10 mg/dL) was found in about 14.29% of infected individuals, majority of infected patients (38.10%) have total bilirubin index in the range of 5.1-10 mg/dL.

Discussion

Viral hepatitis is one of the major health problems in India. Still, due to a lack of awareness among people in the country, viral hepatitis is spreading at a faster rate. Four major viruses are responsible for causing viral hepatitis in India- HAV, HBV, HCV, and HEV [9]. HAV is the primary causative agent of acute viral hepatitis among children. HAV and HEV are waterborne diseases, easily spread through contaminated food and water at unhygienic places like street food [10]. HBV is responsible for causing chronic viral hepatitis, mainly among adults. HBV and HCV are transmitted primarily by an infected individual through the exchange of body fluids like blood; it

is also transmitted through unsafe sexual contact with infected people [11, 12]. A study on the prevalence of HBV and HCV infection is needed to understand its epidemiology and to create strategies to improve public health, which may help in disease prevention and control.

In the present study in the Aligarh region of western Uttar Pradesh, we describe the investigation of viral hepatitis and demonstrate the presence of all major hepatitis viruses in patients with deranged LFT from January 2018 to October 2022. We found that a significant number of individuals have viral hepatitis either by HAV, HBV, HCV, or HEV.

HAV was a predominant etiological agent of viral hepatitis, followed by HBV, HCV, and HEV. This is in concordance with the previous study by Jain *et al.*, 2013 [9], while some other studies by Chandra NS *et al.*, 2014 [13] and PV Barde *et al.*, 2019 [14] reported HEV as the predominant cause of viral hepatitis in India. Hepatitis A infection, a common infection in children but relatively rare in adults, follows the same trend in this study. There were 21.02% of patients had HAV infection; among them, 74.64% HAV infected persons were from the age group of 0-10 years. These results are in support by our previous findings [4], and several other studies by Rajani M. *et al.*, 2016 [15] also reported the same results for HAV prevalence in children. The significant prevalence of HAV in youngsters demonstrates the importance of appropriate sanitation, especially sanitary dietary habits. Parental health education is also required to control these diseases in youngsters. To guard against Hepatitis-A, the WHO, CDC, and Indian Academy of Pediatrics urge regular immunization of all children and susceptible groups. The HBV surface antigen and anti-HCV antibodies were found among 20.96% and 9.78% of individuals, respectively, with the highest share among individuals of age group 21-30 years, 34.21% for HBV and 23.76% for HCV. Similar findings were reported by Jain P. *et al.* 2013 and Singh, K *et al.* 2022 [9, 16]. Chronic HBV infection poses a serious public health concern for our nation since it can cause liver cirrhosis and hepatocellular carcinoma [17]. The higher number of cases of HBV and HCV among adults may be due to unawareness about the infection, e.g., unsafe practices like unsafe sexual contact with a stranger, drug abuse by unsterile syringes, using unsterile blades by barbers, dental treatment by non-qualified persons using unsterile instruments or by blood transfusion at unauthorized places [18, 19, 20]. Hence running awareness programs among people may help in controlling the spread of infection.

On the other hand, in our study, we found that only 5.45% of individuals were infected by HEV, with the highest share among individuals of age group 11-20 years (38.96%) followed by 21-30

years (31.17%) this is in support by the study by Jain P *et al.* 2013 who also found that the HEV infection is prevalent in adults [9]. Over the past five and a half decades, several epidemics of HEV infection have occurred in India. HEV infection is also an important cause of acute and sub-acute liver failure in the country [21]. Pregnant women with jaundice and acute viral hepatitis of HEV etiology showed higher mortality rates and poorer obstetric and fetal outcomes than those with other types of hepatitis [22].

One of the primary reasons for the large number of cases of viral hepatitis is the lack of awareness among people of India about available vaccines for HAV, and HBV, due to which most of the people are not vaccinated against HAV, and HBV [26, 27]. Therefore, to control the spread of viral hepatitis caused by HAV, especially in children and HBV in adults, the health care and government agencies must make some policies like running awareness programs in the community and free immunization to everyone. Unlike HAV and HBV, there is no approved vaccine for HCV and HEV [28, 29], so preventive measures are the only way to survive from getting the infection [23]. As we know, in viral hepatitis liver is the organ that is highly affected laid to an increase in the concentration of different liver enzymes and pigments like AST, ALT, ALP, and bilirubin [24]. In the present study, we found that the mean concentration of AST and ALT was very high (>1000U/L) and ALP (>400U/L) in individuals suffering from HAV infection, followed by HEV, HBV, and HCV infection. Similar findings were reported in previous studies by Ahmad I *et al.* 2020, and Mittal A *et al.* 2016 [4, 25]. In our study, we also reported co-infection of HAV with HEV, which is only 0.3%, and about 0.5% of individuals are co-infected with HBV and HCV.

Regarding HAV and HEV, general hygiene, particularly sanitation, water supply, and food preparation, reflects socioeconomic class and living conditions and significantly impacts its endemicity [4]. Interestingly, we found HBV to be the second most common cause of viral hepatitis in contrast to another study by Irshad M. *et al.*, 2010 [30] in which HBV is the leading cause of viral hepatitis.

There were some limitations of our study, we do not have data for the year 2021 due to covid-19 pandemic, and all data provided here is from 2018, 2019, 2020, and 2022. Also, we did not investigate our samples for HDV and HGV.

Conclusion

From the present study, we conclude that all four viruses (HAV, HBV, HCV, and HEV) actively circulate in the study area, infecting all genders and ages. However, male individuals are more

prone to infection. HAV is prevalent among children and very rare in adults, while HBV, HCV, and HEV are more prevalent among adults. Biochemical analysis shows that liver enzymes were highly elevated during HAV infection, followed by HBV and HEV. However, liver pigment bilirubin was highly elevated in HEV infection, followed by HBV and HAV. Proper sanitation, maintaining good personal hygiene and consuming hygienic food and water, and proper immunization is the only effective way of preventing and controlling the spread of HAV and HEV. The preventive strategy for HBV and HCV infection should include a vigilant screening of blood and blood products and safe practices like using sterile instruments at a barber's shop, dental treatment by a qualified professional, avoiding the reuse of syringes, etc., and safe sex practices and proper immunization against HBV.

List of Abbreviations

VRDL: Viral Research & Diagnostic Laboratory

ELISA: Enzyme linked immunosorbent assay

HAV: Hepatitis-A virus

HBV: Hepatitis-B virus

HCV: Hepatitis-C virus

HEV: Hepatitis-E virus

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Conflict of Interest: Nil

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