

## Retrospective Observational Assessment of Seroprevalence of Hepatitis A and E Viruses in Patients of Acute Viral Hepatitis

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Received: 15-03-2022 / Revised: 23-04-2022 / Accepted: 15-05-2022

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Conflict of interest: Nil

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### Abstract

**Aim:** In this study, we have studied the seroprevalence and co-infection of HAV and HEV infections in various age groups. Study on seasonal variation of HAV/HEV infections was also included in the study.

**Methodology:** The present study was a retrospective observational study conducted in Department of microbiology in Anmmc, gaya Bihar for 1 year. All patients presenting to the hospital with features of AVH whose samples were received for serological testing against hepatitis A and hepatitis E in the Microbiology laboratory of the institute during the study period were included in the study. For microbiological confirmation, serological testing was done using enzyme-linked immunosorbent assay (ELISA)-based anti-HAV IgM (DiaPro Diagnostic Bioprobes, Italy) and anti-HEV IgM (DiaPro Diagnostic Bioprobes, Italy) antibody detection tests. Data were collected from the laboratory and hospital records for the study. The categorization into pediatric and adult was as per the hospital protocol for patient enrolment in the pediatrics ( $\leq 12$  years) or medicine ( $> 12$  years) specialities.

**Results:** A total of total 200 serum samples were processed for HAV and HEV IgM. In age group of  $< 12$  years, 23.5% cases; age group of 12-40, 43% cases and age group of  $> 40$  years, 33.5% cases were studied. Among all the samples, 58% samples were of males and 42% samples were of females. Out of 200 samples, 79 samples (39.5%) were positive for HAV and/or HEV. 25 samples were only HAV IgM positive, 43 samples were only HEV IgM positive, and 12 samples were found positive for both HAV and HEV IgM. 121 samples were found negative.

**Conclusion:** The overall prevalence of HAV and HEV infection was found 39.5%. The shift in HAV seroprevalence toward adults, along with an increasing trend of the number of cases reporting to the hospital, warrants the active community-based surveillance to assess the incidence of HAV in adults in this region.

**Keywords:** Seroprevalence, infection, viral, transmission.

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## Introduction

The principal causes of viral hepatitis are hepatitis A, B, C, and E viruses [1–4], among which the hepatitis A virus (HAV) and the hepatitis E virus (HEV) cause self-limiting acute hepatitis. Hepatitis A (HAV) and hepatitis E (HEV) viruses are important public health problems in developing countries such as India [5]. Both these viruses are predominantly enterically transmitted through feco-oral route and cause a spectrum of infection ranging from asymptomatic infection, usually in children, to acute viral hepatitis (AVH) of varying severity in adults [6].

These viruses spread via the fecal-oral route or by ingestion of contaminated water or food [7]. In many developing countries, these infections' prevalence is closely associated with poor hygiene conditions and low socioeconomic status. In recent years, the incidence of HAV and HEV has decreased markedly due to improved health conditions. The risk of infection in youth was more significant than in adults [8]. Also, there was a high risk of vertical transmission of these viruses from the mother to the fetus with subsequent maternal and fetal complications, such as abortion, neonatal death, and premature labor [9, 10]. Thus, the diagnosis of these infections in pregnant women is vital to avoid any adverse outcomes.

Hepatitis A is a vaccine-preventable disease, but the vaccine has not been deployed in India [11] as more than 80% of children by the age of 10 years develop antibodies as a result of natural infection [12] and since the disease is often clinically insignificant in this age group, the use of vaccine is not justified and is still a subject of debate. It is pertinent to note that more than half of the world's population practicing defecation in the open is residing in India [13]. However, there is a noticeable shift in the disease spectrum from children to adults as a result

of improvement in the socio-economic conditions [14]. To add to this, there has been an emphasis on the promotion of increased sanitary infrastructure by India under the Swachh Bharat (Clean India) mission since 2014 [15].

HEV on the other hand is known to cause infection in adult population as compared to children [16], with a greater predilection to cause outbreaks in the community as compared to HAV [17]. It is also documented to cause severe disease in pregnant females leading to increased mortality and pregnancy-related complications [18]. There is evidence in the literature regarding this virus still being a public health menace in industrialized countries as well [19, 20].

There has been no case-based surveillance for these viral diseases in India and the mode of surveillance till date has been outbreak-oriented where the weekly numbers are analyzed for predefined threshold by the Integrated Disease Surveillance Project, depending upon the geographical area [6]. The National Viral Hepatitis Control Program (NVHCP), launched in July 2018, intends to address the public health problem caused by these viruses and it aims to substantially reduce the risk, morbidity, and mortality associated with HAV and HEV by 2030 [21]

In patient with preexisting chronic liver disease, both HAV and HEV infection can worsen the condition. The clinical course of HEV is more problematic than HAV infection, primarily in pregnant females' contract disease in the second and third trimester. Therefore, HAV-HEV co-infection can lead to serious complications and increased mortality because of acute liver failure in patients in children as well as adults [22, 23]. Although clinical diagnosis of co-existence of HAV and HEV viruses as a cause of viral hepatitis is difficult and cannot be differentiated with mono- infection, laboratory diagnosis

either by serology and polymerase chain reaction (PCR) can be a useful tool in the diagnosis of simultaneous presence of both the viruses [24]. In this study, we have studied the seroprevalence and co-infection of HAV and HEV infections in various age groups. Study on seasonal variation of HAV/HEV infections was also included in the study.

### Materials and Methods:

The present study was a retrospective observational study conducted Department of microbiology in Anmmc, gaya Bihar for 1 year.. All patients presenting to the hospital with features of AVH whose samples were received for serological testing against hepatitis A and hepatitis E in the Microbiology laboratory of the institute during the study period were included in the study. Repeat samples were excluded from the study so that each patient is represented by a single sample only.

### Methodology

For microbiological confirmation, serological testing was done using enzyme-linked immunosorbent assay (ELISA)-based anti-HAV IgM (DiaPro Diagnostic Bioprobes, Italy) and anti-HEV IgM (DiaPro Diagnostic Bioprobes, Italy) antibody detection tests. Five milliliters of blood sample were collected in blood

collection vials without additives under strict aseptic precautions and the serum was separated by standard methods. If not possible to put up immediately, the sera were stored at  $-20^{\circ}\text{C}$  till testing (usually up to 1–3 days). Sera exhibiting hemolysis, lipemia, and turbidity were rejected. ELISA testing was performed as per manufacturer instructions supplied as package inserts/kit literature along with the ELISA kits. Positive and negative controls supplied with the kits were run for test validation as per instructions and internal quality control was also set up by testing known positive samples every time ELISA testing was done on patient samples.

Data were collected from the laboratory and hospital records for the study. The categorization into pediatric and adult was as per the hospital protocol for patient enrolment in the pediatrics ( $\leq 12$  years) or medicine ( $>12$  years) specialities.

### Results:

A total of total 200 serum samples were processed for HAV and HEV IgM. In age group of  $< 12$  years, 23.5% cases; age group of 12-40, 43% cases and age group of  $>40$  years, 33.5% cases were studied. Among all the samples, 58% samples were of males and 42% samples were of females.

**Table 1: Demographic details**

Variables		Number	%
Gender	Male	116	58
	Female	84	42
Age (in years)	$<12$	47	23.5
	12-40	86	43
	$>40$	67	33.5

Out of 200 samples, 79 samples (39.5%) were positive for HAV and/or HEV. 25 samples were only HAV IgM positive, 43 samples were only HEV IgM positive, and 12 samples were found positive for both HAV and HEV IgM. 121 samples were found negative.

The overall prevalence of HAV and HEV infection was found 39.5%. The prevalence of HAV infection was found 12.5%, HEV infection 21.5% and HAV-HEV co-infection was 6%.

**Table 2: HAV and/or HEV IgM positive cases**

IgM positive	Number (n=79)	%
Only HAV Positive	25	12.5
Only HEV positive	43	21.5
HAV and HEV both positive	12	6

**Discussion:**

In a study by Murhekar *et al.*, [6] the data from Virus Research and Diagnostic Laboratory (VRDL) network across India from 2014 to 2017 had a HAV: HEV ratio of 1.19 which is consistent with our overall ratio of 1.14 during the study period. However, it should be noted that the ratio of cases of HAV and HEV was unstable throughout our study period with a wide fluctuation ranging from 0.61 to 2.53.

The numbers of HAV cases were more than HEV for most of the study period which is in contrast to previous studies from India where HEV was found to be the dominant agent [25,26]. Joon *et al* [27] reported higher prevalence of HAV (19.31%) as compared to HEV (10.54%) from Karnataka (India). The data from VRDL network laboratory also show higher prevalence of HAV in northern India while the western, central, and eastern part of India have higher prevalence of HEV [6].

With regard to HAV, it is also worthwhile to mention that 40.9% cases in our study were >12 years old. This puts a high proportion of patients at risk of developing jaundice as per the model described by Aggarwal *et al* [28] The rising proportion of adults among hepatitis A patients in this study may have a bearing in the community as well. This is important to investigate further in view of clean India mission 2014 and NVHCP 2018 of India. A seroprevalence of more than 80% in children less than 10 years of age has been described in the literature from India [6]. In the last two decades, there is a

noticeable shift in the seroprevalence with a gradual decrease in seropositivity among children less than 10 years of age [29] In a review of available literature, Agrawal *et al* [30] noted that the shift in seroprevalence towards adolescents and adults was significant in both urban areas as well as populations with high or middle socioeconomic status as compared to rural and lower socioeconomic status, respectively. Arankalle *et al* [31] on the other hand observed that this shift was evident only among higher socio-economic group from urban areas while those belonging to lower socio-economic group from urban areas as well as residents of all socio-economic groups in rural households demonstrated similar, high seroprevalence among children.

HEV is one of the causative agents of enterically transmitted non-A/non-B hepatitis worldwide. It is responsible for the major outbreaks of acute hepatitis in developing countries, especially in tropical and subtropical regions of the world, where outbreaks are usually associated with fecal contamination of drinking water [32]. Although the highest prevalence is found mostly in countries where the disease is endemic, such as India, the geographic prevalence of people with ant-HEV antibodies is worldwide [33]. Given that the sample size used for our study was relatively small, our data might not be an accurate representation of the entire population. However, this study does provide certain important information concerning the prevalence of HAV and HEV based on age, sex, and residency. [34] Furthermore efforts are needed to clarify the prevalence

in the younger generation (i.e. below 10 years) and the modes of transmission involved.

### Conclusion:

The overall prevalence of HAV and HEV infection was found 39.5%. A better coordinated public health effort around this time can help contain the seasonal cases to some extent. The shift in HAV seroprevalence toward adults, along with an increasing trend of the number of cases reporting to the hospital, warrants the active community-based surveillance to assess the incidence of HAV in adults in this region. A long-term, continuous serosurveillance for presence of this virus is important to ascertain the utility of the vaccine for its prevention.

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