PREVALENCE OF HEPATITIS A AND HEPATITIS E IN WEST BENGAL INDIA- A TERTIARY CARE HOSPITAL BASED STUDY

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ABSTRACT

BACKGROUND

Hepatitis A and E viruses are the most common causes of acute viral hepatitis and account for 10% deaths due to viral hepatitis globally. These viruses are endemic in India, and causes both epidemic and sporadic infections. Though there are several outbreak reports available, data on sporadic infections caused by these two viruses are very few.

METHODS

History of patients were recorded and blood samples collected to investigate possible hepatitis A and E infection. Laboratory assessments for detection of anti-HAV IgG and anti-HEV IgG antibodies was performed using enzyme linked immune-sorbent assay kits.

RESULTS

Total 494 patients were investigated including 159 female and 335 male. Seroprevalence of HAV was found to be 11.2% and that of HEV was 20.05%. Both the infections demonstrated preponderance of male over female. Highest frequencies of HAV were found in age group of 11-20 and that of HEV were in age group 21-30.

Seasonal distribution of both HAV and HEV infections followed a bimodal peak pattern with peaks reported in spring and rainy season.

CONCLUSIONS

Main modes of transmission of both the viruses are through feco-oral route. So by improving community hygiene and administering vaccination (For Hepatitis A) the number of positive cases can be reduced. A community program like "Swatchh Bharat Abhiyan" and a surveillance program by hospitals with multipronged approach of screening, treatment and immunization (against HAV) are expected to reduce incidences of infective viral hepatitis.

KEY WORDS

Hepatitis A, Hepatitis E, Prevalence, Sporadic, Seasonality.

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BACKGROUND

Acute viral hepatitis is a systemic infection affecting the liver predominantly. Almost all cases of acute viral hepatitis are

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caused by one of the five viral agents, HAV, HEV, HBV, HCV and the HBV-associated delta agent or Hepatitis D virus. All human hepatitis viruses are RNA viruses except Hepatitis B which is a partially double stranded DNA virus, but replicates like a retrovirus. Although, these agents can be distinguished by their molecular and antigenic properties, all types of viral hepatitis produce clinically similar illness. These range from asymptomatic and inapparent to fulminant and fatal acute infection common to all types on the one hand and from subclinical persistent infections to rapidly progressive chronic liver disease with cirrhosis, hepato-cellular cancer common in the blood borne types (HBV, HCV, and HDV) on the other hand.^[1]

Hepatitis A (HAV) & Hepatitis E (HEV) viruses are the most common causes of acute viral hepatitis.^[2] Though both the

infections are mostly self-limiting in nature, they are often reported to induce morbidity in patients from developed, developing as well as underdeveloped countries.^[3] Mortality is reported from HAV and HEV infection in risk group e.g. pregnant woman (20%-30%)^[4,5] in the latter and in persons with pre-existing liver diseases.^[6] HAV & HEV together account for 10% of total mortality rate caused by viral hepatitis worldwide.^[7]

HAV is a non-enveloped positive sense single-stranded RNA virus belonging to the family Picornaviridae family with 4 genotypes belonging to one serotype and HEV is positive sense, single stranded RNA virus belonging to the family hepeviridae. HEV has four genotypes of which genotype 1 and 2 infect human.^[8]

Main mode of transmission of both HAV & HEV is through feco-oral route. Other sources of transmission like animal to human transmission by contact with body fluids of infected animal,^[9,10,11] human to human transmission by contact with infected blood products^[12,13,14] and vertical transmission (from mother to fetus) have been reported for HEV.^[15]

The agent of viral hepatitis A is transmitted almost exclusively by the faceo-oral route. It is an outbreak prone disease with an incubation period of around 4 weeks. Person to person spread of HAV is enhanced by poor personal hygiene and overcrowding. Excretion in the stool occurs for only 7-14 day after the onset of the clinical illness and is diagnostic of an acute HAV infection. No carrier state has been identified. Inactivated attenuated vaccine which is safe, immunogenic and effective is available.

The incubation period for HAV ranges from 15-45 days. The prodromal symptoms of acute viral hepatitis are systemic and quite variable. Constitutional symptoms of low grade fever, anorexia, nausea, vomiting, fatigue, malaise, arthralgia, myalgia, headache, photophobia, pharyngitis, cough and coryza may precede onset of jaundice by 1-2 weeks.^[16]

Dark urine and clay colored stools may be noticed by the patient from 1-5 days before the onset of clinical jaundice. With the onset of clinical jaundice, the constitutional prodromal symptoms usually diminish. The liver becomes enlarged and tender, may be associated with right upper quadrant pain and discomfort. During the recovery phase the constitutional symptoms disappear, but some liver abnormalities and abnormal LFT biochemicals persist.^[16]

The early antibody response is predominantly of the IgM class and persists for several (About 3 months) rarely for 6-12 months, followed by IgG. Detection of IgM anti-HAV antibody indicates acute/recent infection whereas the presence of HAV total antibody in the absence of HAV IgM indicates previous infection or immunity. In an infant less than 18 months of age, a positive antibody test result may indicate passive transfer of maternal antibody.

An HAV total antibody test detects both IgG and IgM; when used in combination with the HAV IgM antibody test, it is an effective way to determine current or previous infection and test for immunity before vaccination.

HEV infection is usually an acute self-limiting disease.

Hepatitis E virus (HEV) is a non-enveloped single stranded RNA virus and is a 27- to 34-nm, icosahedral capsid, non-enveloped, HAV-like virus with a 7200-nucleotide, single-strand, positive-sense RNA genome HEV has three open reading frames (ORF) (genes), the largest of which, ORF1, encodes nonstructural proteins involved in virus replication. A

middle-sized gene, ORF2, encodes the nucleocapsid protein—the major structural protein, and the smallest, ORF3, encodes a structural protein whose function remains undetermined. All HEV isolates appear to belong to a single serotype despite genomic heterogeneity of up to 25% and the existence of four genotypes (genotypes 1 to 4 [HEV1, HEV2, HEV3, and HEV4]). Genotypes 1 and 2 appear to be more virulent, whereas genotypes 3 and 4 are more attenuated and account for subclinical infections.

The agent is transmitted almost exclusively by fecal oral route. It is an outbreak prone disease with an incubation period of around 2-10 weeks.

The illness usually begins after the incubation period of 14-70 days as an acute viral syndrome with mild fever, marked loss of appetite, aversion to food, upper abdominal discomfort, nausea and vomiting. Within a few days of onset of these non-specific symptoms, jaundice can appear with the resolution of these non-specific symptoms. Jaundice usually persists for 1-6 weeks and then gradually resolves. In children most HEV infection occurs without any symptom or as a mild illness without jaundice. In contrast, in adults acute hepatitis E may have a prolonged cholestatic phase with significant itching. Acute liver failure may be seen in a small proportion (04.-5%) which is higher in pregnant women normally within a week of onset of symptoms.^[16]

Human infection with HEV has two distinct epidemiological patterns. In areas of poor sanitation and hygiene, genotypes HEV1 and HEV2 are transmitted between humans by the fecal-oral route, usually via contaminated water, is usually a self-limiting illness which last a few weeks and results in frequent sporadic cases and occasional large outbreaks.

In developed countries, genotypes HEV3 and HEV4 are transmitted zoonotically from animal reservoirs, with sporadic cases. Moreover, HEV 3 infection in immune-compromised patients in developed countries causes chronic infection with rapidly progressive cirrhosis (In organ transplant recipients, patients with hematological malignancy requiring chemotherapy, and individuals with HIV). HEV also causes extra-hepatic manifestations, including a number of neurological syndromes and renal injury.

HEV infections can be diagnosed by measuring anti-HEV antibodies, HEV RNA or viral capsid antigen in blood or stool.

HEV infection can be diagnosed either indirectly by detecting serum anti-HEV antibodies or directly by detecting the HEV RNA in blood or other body fluids. Following an incubation period of 2 to 6 weeks, an initial short-lived IgM response is followed by longer-lasting IgG antibodies. The presence of anti-HEV IgM is a marker of acute infection. The presence of anti-HEV IgG alone is a marker of past infection. HEV RNA becomes detectable in early phase of the disease and is undetectable in blood in about 3 weeks after the onset of symptoms but can be detected in feces for another 2 weeks.

India is endemic zone for HAV and HEV infection. Both epidemic and sporadic cases of infection by these viruses had been reported from different parts of India. From 2011-2013, fifty four episodes of HAV and seventy eight episodes of HEV outbreaks have been reported from India.^[17] Most of the reports of HEV outbreak are from Northern, Southern and Western parts of India. Two HEV outbreaks have been reported from West Bengal in 1995 and 2007 respectively.^[18,19]

IDSP data (Obtained from community.data.gov.in website) shows that total number of HAV and HEV outbreak reported from West Bengal in between September 2015 to April 2016 is only one but though numbers of outbreaks are low in this part of the country, sporadic cases occur here all throughout the year. However, systematic studies, investigating these cases are very few in number.

Our tertiary care hospital is a referral hospital of West Bengal, one amongst four viral research and diagnostic labs in West Bengal equipped with state of the art conventional and molecular diagnostic facilities. It receives sample from almost all districts of the state barring a few hilly districts. The present study investigates the sporadic cases of HAV and HEV in relation to age, sex, seasonal prevalence in West Bengal.

METHODS

This hospital based cross sectional study was conducted within a period of July 2017 to June 2018.

Ethics Statement

The study was conducted as a part of a central government funded project "Establishment of a Network of Laboratories for Managing Epidemics and Natural Calamities" which was reviewed & approved by institutional Ethics Committee and funded by ICMR-DHR which contributed to construction of this state of the art viral research and diagnostic laboratory.

Inclusion Criteria

All patients presenting symptoms and signs of viral hepatitis (weakness, lethargy, fatigue, joint pains, jaundice and abdominal pain, elevated liver enzymes and fever) were included in the study. HAV has non-specific constitutional symptoms of low grade fever, anorexia, nausea, vomiting, fatigue, malaise, myalgia, headache, photophobia; pharyngitis, cough and elevated enzyme levels precede the onset of jaundice by 1-2 weeks

Exclusion Criteria

Patients with known history of non-viral causes of hepatitis e.g. obstructive and haemolytic during induced of toxin mediated hepatitis were excluded from the study.

Sample Collection & Processing

After obtaining informed consent from patients, participant's demographic information including age, gender, occupation, locality, symptoms, provisional diagnosis, date of onset of illness etc. were recorded.

Total 494 participants including Outdoor patients & Indoor admitted patients from different departments of our tertiary care hospital were included in the study. They were presenting with symptoms like fever, nausea, vomiting and jaundice. Out of these 494 patients, 23 were advised only HAV screening, 10 were advised only HEV screening. For all other patients both HAV & HEV tests were done.

For each participant, a 5-ml non-fasting venous blood sample was collected aseptically in plastic screw caps vials, and the serum was separated from the blood by centrifugation and transported to laboratory for screening of HAV and HEV.

Laboratory Assessments for detection of anti-HAV IgM and anti-HEV IgM antibodies present in the serum was performed using commercial enzyme linked immune-sorbent assay kits

(DIAPRO Italy), according to the manufacturer's instructions. Results were sent for External Quality Assessment Scheme (EQAS) quarterly to NIV, Pune during the period of study and showed 100% concordance of results.

Statistical Analysis

All parameters were summarized using descriptive statistics. Prevalence of HAV & HEV were analyzed using Fisher's exact test and Chi square test.

RESULTS

Public health impact of viral hepatitis is immensely high worldwide. The burden of viral Hepatitis in India is very large but till few years back surveillance was not well characterized. Only recently, Integrated disease surveillance program (IDSP) program launched by National Center for Disease Control (NCDC) which collects data from district level onwards for effective surveillance of the disease.

Out of total 494 patients screened, 159 were female and 335 were male. Total 56 patients were tested positive for HAV, and 89 patients were tested positive for HEV. (Figure 1)

HAV infections were seen to be prevalent in early stages of life from 0-20 years most prevalent in 11-20 years. HEV infections were found to be prevalent in young adults (21- 30 age group) and almost absent in 0-10 years. The data of age dependent prevalence for both HAV and HEV is presented in Figure 2.

Incidence of both the infections was found higher in rural area (including semi urban areas) as compared to urban area. Data is presented in figure 3.

Month wise assessment based on date of sample collection reveals the positivity rate of HAV varied from 8.8% in December to 27.27% in July. Statistical analysis indicated the difference in month wise incidence pattern was statistically significant. ($\chi^2 = 23.39725$, $p = 0.015528$, $df = 11$)

For HEV, month wise positivity rate varied from 0% in the month of December to 53.84% in the month of March. Incidence of HEV thus varied significantly over months ($\chi^2 = 74.40$, $p = 0.00000000001$, $df = 11$) (Figure 4)

Seasonal distribution of both HAV and HEV infections as shown in Figure 4, followed a bimodal peak pattern with one peak reported in spring and the other in rainy season.

DISCUSSION

The main finding of our study may be put forth as follows: Public health impact of viral hepatitis is immensely high worldwide. The burden of viral Hepatitis in India is very large but till few years back surveillance was not well characterized. Only recently Integrated disease Surveillance program (IDSP) program is launched by National Centre for disease control (NCDC) which collects data from district level for effective surveillance of the disease.

In our study, seroprevalence of HAV was 11.2% while for HEV is 20.05 %. HAV prevalence in previous studies was reported in between 8%- 19%^[20,21] while that of HEV was reported as low as 10.54%, and as high as 78.78%.^[21,22]

In the present study, among all age group, more number of positive cases were reported in males (73.2% and 78.6%) than females (26.7% and 21.3%) for both HAV and HEV infections respectively. Similar observation was made by Manmohan et al. from Tamil Nadu, Antony J and Celine TM et al, from Kerala and Tiwari et al from Delhi India.^[23,24,25] While investigating

the infective causes of viral hepatitis they also showed high male preponderance over female. These observations might be due to more outdoor activities by males for professional and social requirements.

However for HAV, the correlation between genders with disease incidence was not significant statistically. On the other hand a strong correlation (p value 0.01) between gender and disease incidence was observed for HEV infection.

Studies from late nineties reported high (64%-85%) prevalence of HAV infection in children below the age of 10 from both upper and lower socio-economic classes.^[26] In this study the frequency of HAV infection were found to be much lower in this age group. Out of total fifty six positive cases, twelve were reported from this age group (21%). However more number of positive cases (16) was found in age group 11-20 indicating a shift in epidemiology. Similar shifting epidemiology was reported in a recent multicentric seroprevalence study from India where more number of positive cases was found in older children than the younger ones.^[27] This shift in epidemiology might be because of practice of introduction of immunization against HAV antigen over the years. If this practice goes on it might highly impact the severity of the disease.

For HEV, the young adults in age group 20-40 years were found to be predominantly infected than other age group. This data is in accordance with previous studies from other parts of India.^[24,28,29] Another interesting finding in this study is total absence of HEV cases in age group below ten. This finding could not be justified properly as its transmission modality is same as that of HAV. However one report from Pune, India by Arankalle VA et al 1995 described similar finding.^[30] The young adults are in most active phase of life. Travels for occupation are frequent in this period. So they are more exposed to high risk environment and contaminated food than the children below the age of ten. So these might be the reason for more incidence of HEV in young adults.

HAV is very stable and can remain viable for long in the environment. So the infection can occur throughout the year.^[31] HEV though less stable than HAV, can withstand high temperatures and can remain viable for years.^[32] HEV infection in South Asian countries exhibited distinct seasonal pattern with rainy season exhibiting the highest number of cases. However a bimodal pattern of incidence of the disease had been observed in the present study with the highest rate of positivity of HAV infection was observed in the months July followed by March. The highest rate of positivity of HEV infection was observed in the month of February and March followed by July. Similar bimodal distribution of incidence of HAV and HEV had been reported from Punjab, a northern state of India where peaks were observed in February and August.^[30]

Positive cases during the month of February & March might be due to contamination of water distribution line adjacent to sewage collection line. Whereas in rainy season water overflow may contaminate the drinking water sources resulting in infection.

Infective viral hepatitis imposes a major healthcare burden worldwide. To formulate preventive strategies against such infection proper understanding of risk factor associated with the disease is needed. Outbreak investigation reveals consumption of contaminated drinking water is the main cause of both HAV and HEV. Studies from Pakistan, parts of

Nepal and Indonesia had reported increased risk of HEV by exposure to contaminated water.^[33,34,35] A study from Japan also revealed strong association between improper disposals of excreta with both epidemic as well endemic HEV infections. It has been reported that HEV is shredded in patient's excreta after several days of infection.^[36] So disposal of excreta maintaining sanitary guideline could be helpful in bringing down secondary infection

Community sanitation and sewage management might have great role in reducing rates of both HAV and HEV infection worldwide. A study from India reported that individuals using private toilet within the house were less frequently seropositive (33.1%) compared to individuals using an open field for excreta disposal (75%).^[28] Swatchh Bharat Abhiyan" a policy laid down by Government of India in recent years is focusing in these thrust areas. The objectives of this policy are to induce healthy sanitation practices by eliminating open defecation, manual scavenging and proper waste management. Proper implementation of the policy thus might bring down incidences of viral hepatitis in coming years.

It is well know that viral hepatitis causes substantial morbidity and mortality in the developing world, due to both sporadic and epidemic disease caused by two enterically transmitted viruses HAV and HEV. Both viruses are transmitted through feco-oral route and lacks chronic sequelae.

Limitations of the study: This study had some limitations. Firstly, our study was inferred from data recorded from patients. Data quality might vary depending on the individual recording it. We had included only those who attended our tertiary care hospital for treatment in the study. There might be many more cases present in the community who could not reach the facility. So it is likely to underestimate the prevalence data of HAV and HEV in the region

Another limitation of the study is we have used only ELISA to confirm the cases and molecular confirmation by polymerase chain reaction (PCR) was not done.

ELISA has been reported to pose some challenges especially in diagnosing HEV. So inclusion of PCR for laboratory assessment would have confirmed actual rate of infection.

Despite these, the contribution of this research study provides additional data on understudied sporadic cases of HAV and HEV infections and is among very few studies conducted from eastern India. To our knowledge it is the first study to systematically investigate sporadic HAV infection in this part of the country.

CONCLUSIONS

The study has revealed a distinct correlation between factors like age, gender, seasonal pattern on disease incidence. In both cases male preponderance were seen over female in disease prevalence across all age group. The infections were found to occur throughout the year. A community program like "Swatchh Bharat Abhiyan" and a surveillance program by hospitals with multipronged approach of screening, treatment and immunization (against HAV) are expected to reduce incidences of infective viral hepatitis.

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