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Seroprevalence Of Hepatitis A And E With Reference To Their Liver Function Tests At Presentation

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Abstract

Background- Acute Viral Hepatitis (AVH) caused by enterically transmitted Hepatitis A (HAV) and Hepatitis E (HEV) can cause infections both in epidemic and sporadic forms. The present study aims to determine the seroprevalence of HAV and HEV along with the demographic characteristics and Liver Function tests (LFT) pattern of the positive cases.

Materials and Methods- It was a retrospective study done at VRDL, Gauhati Medical College over a period of three years. Total of 2612 samples for IgM Anti HAV and 2230 samples for IgM Anti HEV from AVH cases were included. Samples were tested for Anti HAV-IgM and Anti-HEV IgM antibodies using ELISA kits. LFT records were obtained from Department of Biochemistry after taking due approval. Statistical comparisons were performed with SPSS software version 26.

Results- Sero-prevalence of HAV and HEV were found to be 24.69% and 6.27% respectively. HAV and HEV co-infection rate was 0.7%. The 21–30 years age group was found to be most susceptible (36.3%). Prevalence of HAV and HEV was found to be higher in males (65%) as compared to females (35%). Prevalence of HEV in antenatal cases was found to be 37.9%. Majority of the cases (64.4%) showed AST/ALT ratio of <1. Total of 86.3% of the cases had hyperbilirubinemia.

Conclusion- Sero-prevalence of HAV and HEV was found to be highest in the adults. This shift in epidemiology makes adult vaccination even more important than before. Screening of symptomatic pregnant women for HEV is important to reduce the mortality.

Keywords: Hepatitis A, Hepatitis E, Liver Function Tests, Seroprevalence

Introduction

Acute viral hepatitis (AVH) caused by hepatitis E is estimated to cause approximately 44000 deaths in 2015 (accounting for 3.3% of the mortality) and that caused by hepatitis A approximately 7134 deaths in 2016 (accounting for 0.5% of the mortality) as per the WHO estimates[1].

HAV and HEV are both transmitted primarily by the fecal–oral route. India is hyperendemic for HAV and HEV. In spite of improvement in sanitation, socioeconomic conditions and health awareness; these infections continue to take place both in epidemic as well as in sporadic forms in several parts of India[2].

Acute viral hepatitis due to HAV is clinically indistinguishable from other the types and is usually self-limiting and mild when healthy persons are

infected[3]. It is the most common cause of acute hepatitis in pediatric age group (1–3 years). However, due to improvement in socioeconomic conditions, early childhood exposure to the virus has significantly decreased and there has been a gradual shift in the age of acquiring the infection from early childhood to adulthood. In adults, HAV has a more severe course than in children[2].

In the worldwide scenario, HEV is one of the leading causes of hepatitis[4]. HEV affects young to middle aged adults and is uncommon in children younger than 10 years. HEV infection causes high mortality in pregnant women (20–30%) as compared to (0.2–1%) in general population[2].

As limited data on sero-prevalence of HAV and HEV is available from our North Eastern region, the present study was undertaken with the following objectives:

1. To determine the sero-prevalence of Hepatitis A and Hepatitis E infection.
2. To study the demographic characteristics of Hepatitis A and E positive cases.
3. To study the pattern of Liver Function tests (LFT) at presentation in the positive cases.

Materials And Methods: The study was conducted in the Viral Research and Diagnostic Laboratory, Department of Microbiology, Gauhati Medical College and Hospital. A total of 2612 number of samples for IgM Anti HAV and 2230 number of samples for IgM Anti HEV from patients of Acute Viral Hepatitis cases received over a period of three years from January 2017 to December 2019 were included in the study. The study was undertaken after obtaining approval from Institutional Ethics Committee of Gauhati Medical College.

The inclusion criteria included individuals from all age group presenting with the signs and symptoms of Acute Viral Hepatitis.An Acute Viral Hepatitis (AVH) case is defined as a person having an acute illness typically presenting with acute jaundice, dark urine, anorexia, malaise, extreme fatigue and right upper quadrant tenderness[5].

The exclusion criteria included individuals with Acute Viral Hepatitis but rapid card test positive for HBsAg and Anti-HCV.

All the samples were tested for Anti HAV-IgM using Wantai Kits and for Anti-HEV IgM antibodies using kits from MP Diagnostics as recommended by Department of Health Research, Govt of India. The procedures were followed as per the manufacturer's instructions provided within the ELISA kits. The ELISA tests were performed using and the readings were taken spectrophotometrically within it and recorded quantitatively as Optical Density (OD) value. The cut-off value was calculated as mentioned in the manufacturer's instructions and simultaneously OD values were compared and reported as positive or negative. Records of liver function tests were obtained from Department of Biochemistry after taking their due approval.

Statistical comparisons were performed with SPSS software version 26 (SPSS, Inc., Chicago, IL). Data were expressed by frequency or related percent values. Comparative analysis of two groups was done with Mann-Whitney U test for non-normally distributed population. The $P<0.05$ was taken as statistically significant.

Results: A total of 2612 and 2230 number of samples were tested for IgM Anti HAV and IgM Anti HEV respectively. Out of the 2612 samples tested for IgM Anti HAV, 645 samples were positive for IgM Anti HAV, the sero-prevalence of HAV being 24.69%. Out of the 2230 samples tested for IgM Anti HEV, 140 samples were positive, the sero-prevalence of HEV being 6.27%. 34 samples (0.7%) were positive for both Anti HAV and Anti HEV IgM antibodies. The 21–30 years age group was identified as the most susceptible group for HAV and HEV infection (36.3%) followed by the 11-20 years age group (28.2%). The prevalence of both HAV and HEV was found to be higher in males (65%) as compared to females (35%). A total of 29 pregnant women were tested for IgM Anti HEV out of which 11 were found positive for IgM Anti HEV. Thus, the prevalence of HEV in antenatal cases was 37.9%. HAV and HEV positive cases were found throughout the year. No significant seasonal peaks were seen.

Out of the 645 positive cases for IgM Anti HAV, LFT records were available for 532 cases and out of the 140 positive cases for IgM Anti HEV, LFT records were available for 105 cases. The aminotransferases levels were elevated in almost all the cases and elevation in ALT was more than that of

Volume 6, Issue 6; November-December 2023; Page No 113-121

AST. It was seen that majority of the cases (64.4%) showed AST/ALT ratio of <1, and majority of the cases (42.2%) showed moderate elevation of ALT followed by (25.3%) cases which showed severe elevation of ALT. A total of 86.3% of the cases had hyperbilirubinemia and 25.6% of the cases had a bilirubin level of >15 mg/dL. It is seen that there is no statistically significant difference in the mean values of AST, ALT, ALKP parameters in Hepatitis A positive cases according to the gender of the patients. Also in cases of Hepatitis E positive cases, there is no statistically significant difference in the mean values of AST, ALT parameters according to the gender of the patients. However, the mean value of ALKP was significantly higher in females.

Discussion: Many studies from India and abroad have reported a varying prevalence of HAV ranging from 1.7% to 67%[2]. The sero-prevalence of HEV as per various studies reported from different parts of India was found to be from 12-78%[6]. In the current study, the sero-prevalence of HAV and HEV was found to be 24.69% and 6.27% respectively. Thus, in our study a lower prevalence of HEV has been seen. In a similar study done by Khatri et al. at Jodhpur region in India, the sero-prevalence of HAV and HEV was found to be 13.79% and 4.02%, respectively which is similar to the findings of our study[7]. The low sero-prevalence of HEV in our study can be due to various reasons. The test may perform differently in endemic and non-endemic situation as 33-40 % general population has been found to have anti HEV IgG antibodies, so difficulty may occur to distinguish between present and past HEV infection. Moreover, in immunocompromised individuals' antibodies may persist for 6-10 months. Therefore, anti HEV IgM alone may not be informative for diagnosis of acute sporadic HEV infections at times[6].

In the present study, co-infection with HAV and HEV were found in 34 samples (0.7% cases). Prevalence of HAV-HEV co-infection cases varies in different parts of India ranging from 0.8% to 11.5%. The prevalence of HAV HEV co-infection found in the study by Khatri et al. in India was 1.15% cases[7].

In the current study, adults were found to be more susceptible to HAV and HEV infection. The age group of 21–30 years was identified as the most susceptible group (36.3%) followed by the 11-20 years age group (28.2%). Similar findings were observed in the study by Joon et al. where it was found that the prevalence of HAV and HEV infection were highest in the patients with age between 21-25 years[8]. In our study, the prevalence of HAV and HEV in children (<10 years) was found to be 15.9%. Probability of lower HEV infection rates in children may be due to: (1) Anicteric HEV infections, therefore, children can go unnoticed. (2) Subclinical HEV infections in the endemic area make children more immune and adult more vulnerable for HEV infection[9].

In the present study, the prevalence of both HAV and HEV was found to be higher in males (65%) as compared to females (35%). Similar findings have been noted in several other studies across India and other parts of the world[8, 10, 11, 12].This could be because men have increased chances of exposure to contaminated food and drinking water because of outdoor activities.

In the current study, 37.9% of the pregnant women were found positive for IgM Anti HEV. Similar findings were found in a study conducted at All India Institute of Medical Sciences; New Delhi were the prevalence of HEV in pregnant women was found to be 40%[13].

In the present study, HAV and HEV positive cases were found throughout the year. Previous studies have found either no seasonal peaks[14] or peak in summer and monsoon months of the year[15]. Also it has been observed that there is no definite and consistent seasonal pattern on HAV and HEV infection, although evidence points towards spring and summer peak for hepatitis A and E[16].

ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere. AST has cytosolic and mitochondrial forms and is present in tissues of the liver, heart, skeletal muscle, kidneys, brain, pancreas, and lungs, and in white and red blood cells[17]. De Ritis described the AST/ALT ratio as being a useful indicator of the etiology of hepatitis (e.g., acute viral hepatitis). While it is clearly recognized that serum levels of both serum

Volume 6, Issue 6; November-December 2023; Page No 113-121

ALT and AST are elevated several folds in 'acute' viral hepatitis (e.g., hepatitis A and E), De Ritis was the first to describe that the ALT is usually higher than the AST with the AST/ALT ratio usually well below 1.0, and typically in the range 0.5 to 0.7[18]. In the current study, majority of the cases (64.4%) showed AST/ALT ratio of <1.

The highest elevations of AST and ALT occur in severe viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock. Moderately elevated levels of AST and ALT are seen in acute hepatitis, neonatal hepatitis, chronic hepatitis, autoimmune hepatitis, drug induced hepatitis, alcoholic hepatitis and acute biliary tract obstructions[19]. In the present study, it was noted that majority of the cases (42.2%) showed moderate elevation of ALT.

Hyperbilirubinemia is defined as the value of total bilirubin ≥ 2.5 mg/dL[20]. Bilirubin values of 2.5–3.0 mg/dl or greater establish the presence of the icteric phase of hepatitis[21]. Total bilirubin >15 mg/dL or prothrombin time (PT) >3 seconds above the upper reference limit in an individual with viral hepatitis, in the absence of other factors affecting results, indicates severe liver injury and mandates close monitoring for encephalopathy[22]. In the present study it was found that 86.3% of the cases had hyperbilirubinemia and 25.6% of the cases had a bilirubin level of >15 mg/dL.

In the current study it was found that in Hepatitis A sero-positive individuals there was no statistically significant difference in the Mean values of AST, ALT, ALKP parameters according to the gender of the patients. This finding is similar to a study done by Chang ML et al., where Hepatitis A infection had no difference in severity between males and females[23].

In case of Hepatitis E sero-positive individuals also, there was no statistically significant difference in the Mean values of AST, ALT parameters according to the gender of the patients. The mean value of ALKP was however significantly higher in females. There are some data which indicate that HEV has not only hepatocytic but also biliary tropism, and replicates within bile epithelial cells[24-26]. Case studies have been found which have reported associated cholestasis with HEV[27-28].

Conclusion: The sero-prevalence of HAV and HEV was found to be highest in the adults in our study. This shift in the epidemiology makes vaccination of individuals belonging to this group even more important than before. Furthermore, improving sanitation, periodic surveillance of HAV/HEV and universal hepatitis vaccination can together contribute to lowering the burden of hepatitis in the country. Screening of symptomatic pregnant women for HEV is of utmost importance to reduce the mortality associated with the disease.

Limitations Of The Study: One of the limitations of the study is that the HAV and HEV sero-positives patients could not be followed up clinically and hence data on the morbidity and mortality are not available.

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Volume 6, Issue 6; November-December 2023; Page No 113-121

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Volume 6, Issue 6; November-December 2023; Page No 113-121

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FIGURE LEGENDS:

Figure 1: Showing the month-wise distribution of Hepatitis A positive cases

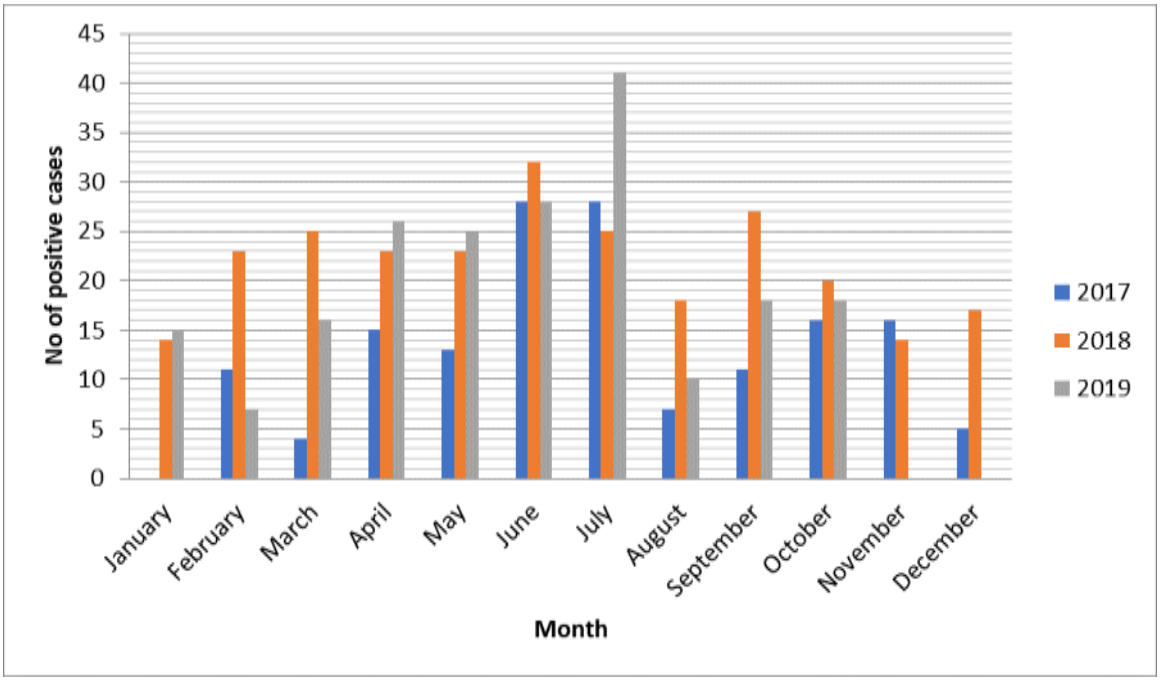
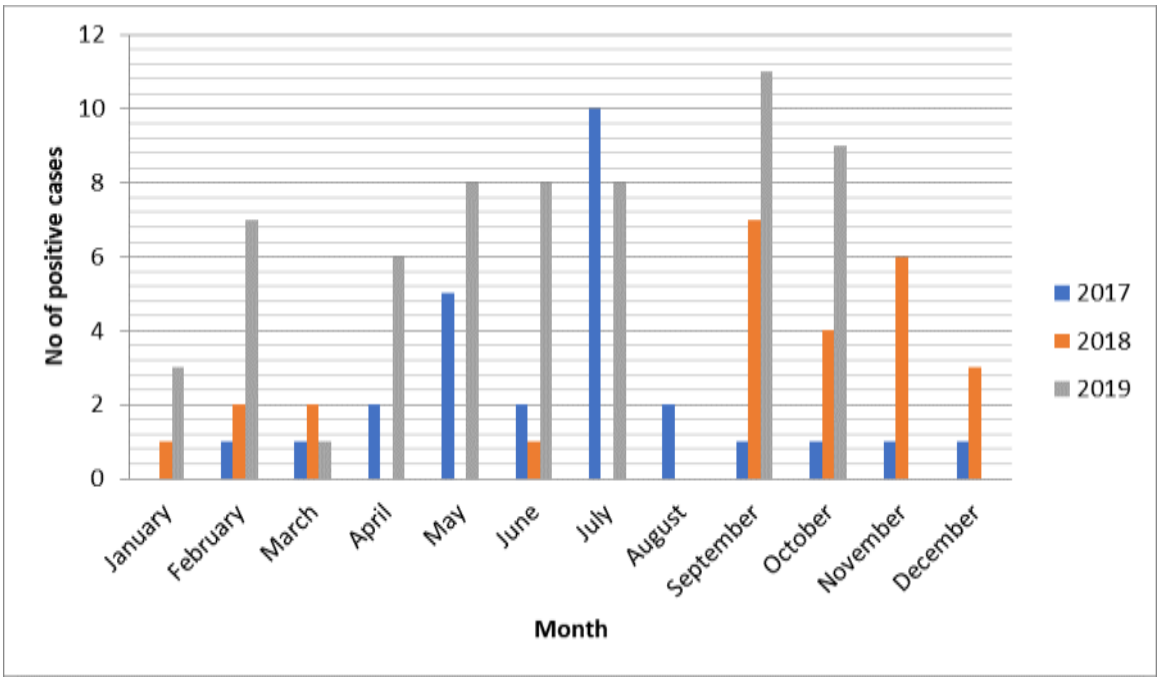


Figure 2: Showing the month-wise distribution of Hepatitis E positive cases:

Volume 6, Issue 6; November-December 2023; Page No 113-121



TABLES:

Table 1: Showing the year wise breakup of the Hepatitis A and Hepatitis E cases.

YEAR	Hepatitis A		Hepatitis E		Total number of samples with Hepatitis A and Hepatitis E Co-infection
	Total number of samples tested for Anti HAV IgM	Total number of samples positive for Anti HAV IgM	Total number of samples tested for Anti HEV IgM	Total number of samples positive for Anti HEV IgM	
2017	814	155(19%)	554	27(4.8%)	7
2018	976	253(25.9%)	877	26(2.9%)	8
2019	822	237(28.8%)	799	87(10.8%)	19
Total	2612	645(24.69%)	2230	140(6.27%)	34

Table 2: Showing the age distribution of the Hepatitis A and Hepatitis E positive cases.

Age Group	2017		2018		2019		Total	
	HAV positives	HEV positives	HAV positives	HEV positives	HAV positives	HEV positives	HAV positives	HEV positives
≤10 years	30	2	38	1	46	8	114	11
11-20 years	55	5	75	7	64	16	194	28

Volume 6, Issue 6; November-December 2023; Page No 113-121

21-30 years	**50**	**10**	**99**	**8**	**85**	**33**	**234**	**51**
31-40 years	**13**	**4**	**26**	**4**	**26**	**17**	**65**	**25**
41-50 years	**2**	**4**	**7**	**4**	**11**	**7**	**20**	**15**
51-60 years	**2**	**2**	**6**	**2**	**2**	**3**	**10**	**7**
>60 years	**3**	**0**	**2**	**0**	**3**	**3**	**8**	**3**
Total	**155**	**27**	**253**	**26**	**237**	**87**	**645**	**140**

Table 3: Showing the sex wise distribution of the Hepatitis A and Hepatitis E positive cases.

Sex	**2017**		**2018**		**2019**		**Total**	
	HAV positives	**HEV positives**	**HAV positives**	**HEV positives**	**HAV positives**	**HEV positives**	**HAV positives**	**HEV positives**
Male	**100**	**18**	**171**	**15**	**155**	**52**	**426**	**85**
Female	**55**	**9**	**82**	**11**	**82**	**35**	**219**	**55**
Total	**155**	**27**	**253**	**26**	**237**	**87**	**645**	**140**

Table 4: Showing the AST/ALT ratio in the Hepatitis A and Hepatitis E positive cases:

AST/ALT ratio	**HAV positives**	**HEV positives**	**Total**
<1	**365**	**45**	**410**
>1	**167**	**60**	**227**

Table 5: Grading ofAlanine aminotransferase (ALT) values[19] in the Hepatitis A and E positives:

Grades of ALT	**Total no of samples**	**HAV**	**HEV**
No elevation	**101**	**76**	**25**
Mild (1-3 times)	**106**	**78**	**28**
Moderate(3-20 times)	**269**	**231**	**38**
Severe(>20 times)	**161**	**147**	**14**

Table 6: Levels of Hyperbilirubinemia[20] in Hepatitis A and E positive cases.

Levels of Total Bilirubin (mg/dL)	**Total no of samples**	**HAV**	**HEV**
<2.5	87	66	21
≥2.5 - <3.0	18	14	4
≥3.0 - <6.0	127	105	22

Volume 6, Issue 6; November-December 2023; Page No 113-121

≥6.0 - <15.0	242	210	32
≥15.0	163	137	26

Table 7: Showing distribution of variables by gender.

Table 7a: Showing distribution of variables by gender for Hepatitis A.

The liver function parameters of the HAV positive patients according to gender are shown in Table 7a.

VARIABLES	**SEX**		**P VALUE**
	FEMALE	**MALE**	
	Mean ±Std Deviation	**Mean ±Std Deviation**	
AST (U/L)	630.52±820.16	727.06±957.24	0.3242
ALT(U/L)	804.35±849.53	977.71±1016.03	0.0724
ALKP(U/L)	275.37±154.74	284.04±504.46	0.05263

Mann Whitney U test (P<0.05 is statistically significant)

Table 7b: Showing distribution of variables by gender for Hepatitis E.

The liver function parameters of the HEV positive patients according to gender are shown in Table 7b.

VARIABLES	**SEX**		**P VALUE**
	FEMALE	**MALE**	
	Mean ±Std Deviation	**Mean ±Std Deviation**	
AST (U/L)	479±560.5	349.7±456.07	0.2037
ALT(U/L)	406.07±540.96	510.89±815.17	0.8683
ALKP(U/L)	319.76±299.81	189.26±143.94	**0.03383**

Mann Whitney U test (P<0.05 is statistically significant)

Volume 6, Issue 6; November-December 2023; Page No 113-121