ORIGINAL RESEARCH

Molecular Detection of Hepatitis A & Hepatitis E Viruses by Duplex RTPCR

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Abstract

Introduction: AVH (Acute viral Hepatitis) is also a major health problem in younger children & adults in India. Causative agents of AVH, Hepatitis A (HAV) & Hepatitis E (HEV) viral infections are transmitted by faeco-oral route in developing countries. Several incidences of AVH have been reported in Rajasthan since 2011 Hence, the present study was aimed to study the incidence of HAV & HEV viral infections by Duplex Rt-PCR.

Material & Method- Out of 9685 suspected Jaundice cases attending at MGMCH Jaipur. Total 300 HAV & HEV suspected cases who were having symptoms of jaundice less than 15 days of fever history were enrolled in this study. All these samples were collected from the patients attending Mahatma Gandhi Medical College & Hospital from periods of 2018-2020. These cases were tested for IgMAb for HAV & HEV and HAV & HEV infection by duplex RT-PCR.

Result- Out of 300 viral hepatitis cases 119 cases were found HAV positive with incidence rate 1.22%. Among 300 cases ELISA, HAV IgM 35% (n=105) cases were found positive whereas HEV IgM 67% (n=29), HAV RTPCR 39.67% (n=119) whereas HEV RTPCR 14.67% (n=44) were found positive.

Conclusion: The present study reveals the molecular identification and trends of the HAV & HEV viruses in the study area. The study also reveals about the early diagnosis of the HAV & HEV viruses in order to control the spread of these viruses efficiently.

Keywords: AVH, HAV IgM, HEV IgM, HAV & HEV RTPCR.

INTRODUCTION

Viral hepatitis, caused by hepatitis viruses A through E, is a major public health problem in India.1 Since 1955, several epidemics of hepatitis have been reported. Although hepatitis A virus (HAV) and hepatitis E virus (HEV), both enterically transmitted, are highly endemic in India, HEV has been responsible for most of these epidemics[1].

AVH (Acute viral Hepatitis) is also a major health problem in younger children & adults in India. Causative agents of AVH, Hepatitis A (HAV) & Hepatitis E (HEV) viral infections are transmitted by faeco-oral route in developing countries. a study was conducted in NIV, Pune in 2004-05[2]. Detectionof HAV & HEV viral infections by IgM ELISA miss the early detection that's why we need a diagnostic tool which can detect both HAV & HEV viral infections simultaneously in very acute phase. The data on the use of Duplex RT-PCR for simultaneous detection of Hepatitis A and E virus is scarce in the literature.

Hence, the present study was aimed to study the incidence of HAV & HEV viral infections by Duplex Rt-PCR among the cases attending, Mahatma Gandhi Medical College & Hospital, Jaipur, to compare HAV & HEV Rt-PCR with HAV & HEV IgM ELISA, and to correlate HAV and HEV Duplex Rt-PCR or ELISA positive cases with LFT (liver function tests).

MATERIALS & METHODS

Out of 9685 suspected Jaundice cases attending at MGMCH Jaipur. Total 300 HAV & HEV suspected cases who were having symptoms of jaundice less than 15 days of fever history were enrolled in this study. All these samples were collected from the patients attending MGMCH, Jaipur from periods of 2018-2020. These cases were tested for IgM Ab for HAV & HEV and HAV & HEV infection by duplex RT-PCR.

Inclusion Criteria –

a) Patients having Jaundice like illness for less than 15 days were included in this study.
b) Patients who had Bilirubin level more than normal included in this study.

Exclusion Criteria –

a) Hemolyzed blood specimens were excluded for the study.
b) Patients with the symptoms of Jaundice along with Dengue positive cases of will be excluded.
c) HBV & HCV Positive cases with Jaundice symptoms were not be tested for HAV & HEV.

RNA Extraction: Viral RNA was extracted by using QIAamp Viral RNA mini kit (CAT No. 52906) & by Qiasymphony Automated extraction system[3].

HAV & HEV Detection by Dulpex Real Time PCR

The identification of reference and clinical samples was determined using type specific taqman Real Time PCR primers & Probes.

RESULTS

In the present study total 9685 Jaundice cases were monitored. Out of which 300 viral hepatitis cases were identified. In these 300 viral hepatitis cases 119 cases were found HAV positive hence incidence rate was observed 1.22%. that concluded the burden of disease was 1:81. While 44 cases were found HEV Positive and concluded incidence rate 0.45% with burden of disease was 1:220. Apart from this, 2 cases of mix infection HAV & HEV were also found in the present study.

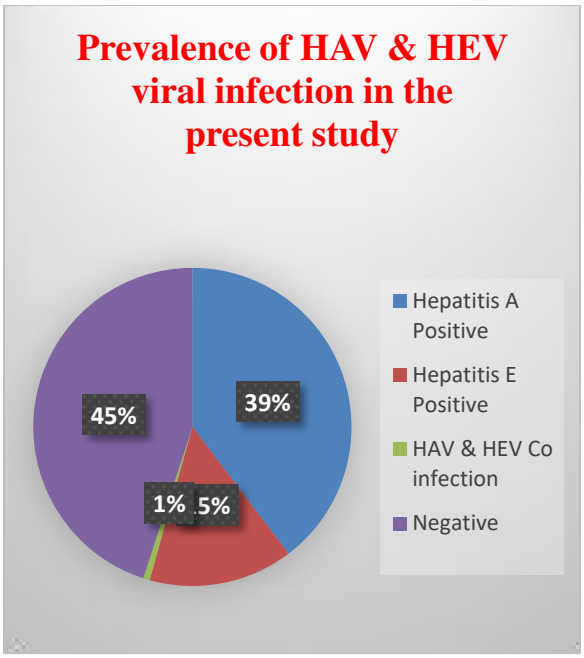

Fig. 1 Prevalence of HAV & HEV in the study area.

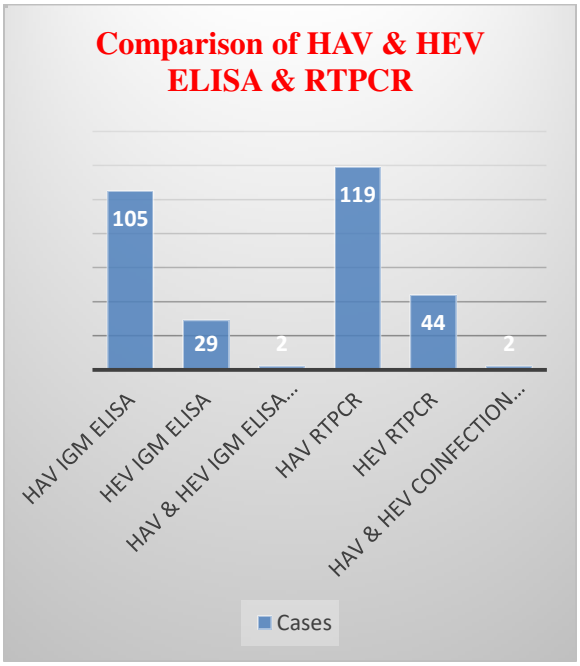

Fig. 2 Comparison of HAV & HEV ELISA & RTPCR

Total 300 HAV & HEV suspected cases who were having sign & symptoms of jaundice less than 15 days of fever history were enrolled in this study. In the present study total HAV were positive 119 (39.6%), HEV 44 (14.6%), Coinfection HAV & HEV 02(0.66% and negative cases were 135 (45.0%) found (Fig.1)

In the present study out of total 300 cases HAV positive Males 80 (26.66%) & Females 39 (13.05)

found. HEV males were 35 (11.66%) while females were 9 (0.03%) positive. One Couple was found positive for HAV & HEV Coinfection. out of 119 cases of HAV, total 100 (84.03%) were belongs to age group of 1-20 yrs. Which strongly represents that HAV mainly cause the disease in Paediatrics age groups, while HEV 21 (47.72%) were belongs to age group of 21-30 yrs. Which strongly represents that HEV mainly cause the disease in adult age groups. Followed by 15 HEV cases (34.09%) in the age group of 11-20 yrs.

Among 300 subjects, it was seen that on ELISA, 35% (n=105) subjects were found positive for hepatitis A virus (HAV), whereas 67% (n=29) subjects were positive for Hepatitis E virus. On Polymerase chain reaction 39.67% (n=119) subjects were found positive for Hepatitis A virus and 14.67% (n=44) subjects for hepatitis E virus.(Fig.2)

DISCUSSION

In the present study total HAV 119 (39.6%), HEV 44 (14.6%), Coinfection HAV & HEV 02(0.66%) were found positive. which is in an agreement with a study from Uttarakhand by Kalita *et al*[4]., 37.6% (HAV 14.7%, HEV 28.0% Combined 5.2%) of the AVH patients had a reactive viral marker along with Mangalore by Joon *et al*. Mangalore by Joon *et al*[5]. as 29.9% (HAV 19.3%, HEV 10.54%, coinfection 11.55%), and Radhakrishnan *et al*. from South India[6]. reported 31.5% (HAV 13.3%, HEV 17.3%, coinfection 0.8%).the study of Sunil R Vaidya et al 2002[7], in his study prevalence was 24.42% (21/86, HAV), 10.98% (9/82, HEV). Prevalence of HAV was significantly higher than HEV (*P*=0.023). During July-Oct months, significantly higher HAV RNA positivity was noted (*P*<0.01) &while Meghna S Palewar et al,2022[8] reported low prevalence in Mumbai HAV 6.70%, HEV 8.5%, and coinfection 0.60%) along with the study by Samaddar *et al*[9]. from Mumbai reporting the AVH (HAV, HEV) infection rate of 18.7% with HAV, HEV, and coinfection rates as 6.96,9.63, and 2.07%, respectively. Gp Capt B Nandi et al[10] conducted a study in Bangalore out of 252 AVH cases16 (6.34%) cases of mixed infection by Hepatitis A + E seen.

In the present study Among 300 subjects showed that RT-PCR test is found to be most sensitive 100% and specific 100% test for HAV & HEV detection followed by HAV IgM ELISA (sensitivity 92.82% and specificity 100%) & HEV IgM ELISA (sensitivity 94.46% and specificity 100%). Out of 300 Acute viral hepatitis cases 105 (35%) were positive by HAV IGM ELISA & 29 (9.67%) were HEV positive by IgM ELISA. While MP Singh et al,2015 A total of 1334 samples were tested, 290 (21.7%) were positive for anti-HAV IgM antibody. Of these, 78 serum samples (< 7 days old) were subjected to PCR and 47.4% (37/78) samples showed the presence of HAV RNA. Children < 15 yr of age accounted for majority (94%) of cases with highest seropositivity during rainy season while Malhotra B et al, 2015[11] Among the 1269 samples tested, 642 (50.59%) were positive for anti-HAV IgM; among the positive samples, 171 patients having a history of less than seven days were tested by PCR, of whom 141 (82.45%) were found to be PCR positive. Salahuddin Mahmud et al,2016[12] at Bangladesh reported in his study that100 children of the age group 1 to 15 years were studied. Out of them, 76 cases (76 %) were positive for Hepatitis A and 14 cases (14 %) for Hepatitis E. Coinfection with Hepatitis A and Hepatitis E were 4 cases (4%). In another study of Bharti Malhotra et al,2020[13] Among 106 symptomatic patients, HEV IgM was positive in 84/106 (79.2%) patients and HEV RNA in 72/106 (67.9%) patients. Among pregnant women, 6/39 (15.4%) were HEV IgM positive and 5/39 (12.8%) for HEV RNA. One (2.5%) pregnant woman died due to hepatitis. While Al Absi2021[14] Qatar HEV-RNA was detected in 23.1% of the samples. HEV-IgM, HEV-IgG, HEV-Total Ab were detected in 8.65%, 32.1%, and 34.2% of all tested samples, respectively. Elevated ALT levels were highly correlated with the HEV-IgM, HEV-RNA. HEV-IgM demonstrated the best second marker for diagnosis of acute HEV after RT-PCR as jugged by the overall performance parameters: specificity (96.2%), sensitivity (71.4%), PPV (83.3%), NPP (92.7%), agreement with RT-PCR (91.0%), while Divya namdeo et. al AIIMS Bhopal, 2022[15] Among the recruited patients, 11 (23.91%) and 15 (32.6%) patients were positive for anti-HEV IgM and RT-PCR, respectively.

CONCLUSION

The present study reveals about the molecular identification and trends of the HAV & HEV viruses in the study area. The study also reveals about the early diagnosis of the HAV & HEV viruses in order to control the spread of these viruses efficiently.

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