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ASSESSING THE ROLE OF RT-PCR (REAL-TIME POLYMERASE CHAIN REACTION) IN DIAGNOSIS OF HEPATITIS E IN INDIAN SUBJECTS

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ABSTRACT

Background: Hepatitis E is a common subvariant seen in subjects affected with hepatitis. However, existing literature data depict a gap in knowledge about appropriate diagnostic tools and the true prevalence of acute hepatitis E infection.

Aim: The present study aimed to assess the prevalence of hepatitis E infection in acute hepatitis subjects in an Indian scenario and to assess the efficacy of prominent diagnostic assays in reaching the diagnosis.

Methods: The study assessed 92 subjects diagnosed with jaundice for <4 weeks with elevated levels of ALT and AST more than 500 U/L. The prevalence of hepatitis E infection in subjects with acute hepatitis was assessed based on several study participants showing a positive reaction in RT-PCR assay and serum anti-hepatitis E virus immunoglobulin M (IgM).

Results: Among 92 study subjects, 32.6% (n=30) and 23.9% (n=22) subjects were positive for RT-PCR and HEV-IgM respectively. Inter-test agreement was poor between the two tests depicting the necessity to perform both tests for accurate diagnosis. A significant difference was seen in RT-PCR negative and positive subjects to illness duration with p=0.007. The mean illness duration was 11.68±5.17 and 8.8±3.52 days respectively in the two groups. The evidence of acute HEV virus infection was 50% with combined RT-PCR and ELISA results.

Conclusions: The present study concludes that HEV is the most common cause of acute hepatitis in adult Indian subjects in the tertiary care center and its diagnosis should be made with the combined use of RT-PCR and ELISA tests.

Keywords: Anti-HEV, ELISA, IgM, hepatitis, hepatitis E, RT-PCR

INTRODUCTION

Hepatitis E virus infection is considered to affect nearly 20 million of the subjects causing them infection and contributing to nearly 3.3 million cases of acute hepatitis E infection every year.

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Following the estimation data of WHO (World Health Organization), the hepatitis virus causes nearly 44,000 deaths and is alone a contributor of 3.3% of all the mortalities attributed to viral hepatitis.[1] Although hepatitis E virus is present globally, the highest prevalence of hepatitis E virus is seen in South and East Asia. The hepatitis E virus presents two epidemiological patterns. In developing countries having poor sanitization and hygiene, the pattern presented is predominant and present as sporadic and outbreak cases after the contamination of drinking water with fecal material. The transmission of HEV genotype 1 is usually seen following the ingestion of a meal that is undercooked. Especially. The animal meat. In such a scenario, the common causative agents seen are HEV3 and HEV 4.[2]

Concerning HEV transmission, India has a hyperendemic presentation. HEV has less contagious ability compared to hepatitis A virus where the Hepatitis A virus shows a household contact rate of 50% to 70%, whereas, the household contact rate of hepatitis E virus is 0.7% to 2%. Also, a higher risk of infection is seen in pregnant females during an epidemic with an infection rate of 12-20%, whereas, in males and non-pregnant females, an infection rate of 1% to 2% is usually seen.[3]

In acute hepatitis cases, biochemical and clinical changes are non-specific, and the assessment of the etiology of the hepatitis E virus needs the use of serological and molecular assays. In various serological assays, IgM in serum is detected after 4 weeks of infection onset and exists till 6 months following disease onset. IgA is detected in the serum after IgM and is detected after 1 month of infection. HEV RNA can also be detected in the serum even in the incubation period, and can be seen in stool and blood for 6 and 4 weeks respectively. Also, in the immunosuppressed subjects, anti-HEV antibodies are usually not detected, and to detect HEV RNA by RT-PCR dhows a higher sensitivity.[4]

The cell-culture assays depict limited application in routine diagnostic procedures, the diagnostic tests for acute HEV infection are usually based on RT-PCR and anti-HEV IgM assay. Conventionally, IgM ELISA (enzyme-linked immunosorbent assay) is the initial diagnostic test for HEV which is usually done additionally with RT-PCR in cases where ELISA is found to be negative, and the subject is clinically suspected of HEV infection. ELISA results are also found to be sub-optimal in subjects that are immunocompromised. As there is no single gold-standard and universally acceptable test to detect HEV the issue of universal guidelines for diagnosis of acute HEV infection.[5]

Considering the existing breach in the knowledge concerning the actual prevalence of HEV infection and the accurate diagnostic tool for assessing acute HEV infection, the present study aimed to assess the prevalence of HEV infection in subjects visiting the Indian healthcare center in India. As the study was done in India which is a developing nation and endemic spot for feco-orally transmitted waterborne pathogens, the present study also aimed to assess the part of HEV in acute hepatitis conditions and to assess the efficacy of prominent diagnostic assays in reaching the diagnosis of acute HEV infection.

MATERIALS AND METHODS

The present prospective clinical study aimed to assess the prevalence of hepatitis E infection in acute hepatitis subjects in Indian scenarios and to assess the efficacy of prominent diagnostic

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assays in reaching the diagnosis. The study was done at Department of General Medicine of the institute.

The study included subjects with a confirmed diagnosis of jaundice for <4 weeks duration with liver enzymes being grossly deranged with ALT (alanine aminotransferase) and AST (aspartate aminotransferase levels of >500 IU/l. The exclusion criteria for the study were subjects who did not give consent for the study and subjects with a history of liver surgery in the past.

After the final inclusion of the study subjects, demographic and clinical data were recorded for all the subjects including their gender, age, laboratory findings conforming to the study, and the presenting signs and symptoms of all the subjects. These data were collected and tabulated in a preformed structured proforma.

For all the included subjects, serum sample was collected following the sterile and aseptic technique. The sample following collection was stored in a 2ml sterile vial and was capped before assessment. The aliquot for HEV RT-PCR was kept at -80^{o}C anti-HEV IgM ELISA (enzyme-linked immune sorbent assay) and was stored at -20^{o}C till further assessment was done. Anti-HEV IgM assay was done using the commercially available kit. The extraction of viral RNA was done from the serum, and the RNA extracted was used for the RT-PCR reaction.

Taqman RT-PCR assay was done to detect the HEV RNA in the serum samples of the study participants. The probe and primer were selected from a highly conserved region of open reading frame-3 from the HEV genome and were used to perform RT-PCR as suggested by Jothikumar et al[6] in 2006. In each run, positive control, extraction control, and no template control were included. All the experiments of RT-PCR were run in duplicate. Samples depicting Ct-values of <40 were taken as positive.

For assessing the prevalence of hepatitis E virus in subjects with acute hepatitis, the proportion of recruited subjects that showed positive reaction to RT-PCR assay and serum anti-HEV IgM. The chi-square test was used for the statistical analysis and positive association of epidemiological, laboratory, clinical, or demographic profiles of study subjects. All the data gathered were analyzed using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA). The data were expressed in mean and standard deviation and frequency and percentage. The significance level was kept at $p<0.05$.

RESULTS

The present prospective clinical study aimed to assess the prevalence of hepatitis E infection in acute hepatitis subjects in Indian scenarios and to assess the efficacy of prominent diagnostic assays in reaching the diagnosis. The study included 92 subjects with a confirmed diagnosis of jaundice for <4 weeks duration with liver enzymes being grossly deranged with ALT (alanine aminotransferase) and AST (aspartate aminotransferase levels of >500 IU/l.

There were 24 males and 22 females in HEV positive group, 30 males and 16 females in HEV negative group, and 54 males and 38 females in total which were non-significant with p=0.34. The mean age of study subjects was 38.96±13.74 years, 39.16±14.76 years in HEV positive, and 38.76±12.86 years in HEV negative subjects which were non-significant with p=0.94. The mean duration of illness was 10.6±4.83 months, 11±4.5 months in HEV-positive subjects, and 10±5.4 in HEV-negative subjects which was non-significant with p=0.6 as shown in Table 1.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

On assessing the liver function, it was seen that globulin levels were 2.44±0.44 in total, 2.42±0.42 in HEV positive, and 2.44±0.44 in HEV negative subjects which were non-significant with p=0.75. Albumin levels were 3.92±0.44, 3.92±0.44 in HEV positive, and 3.86±0.44 in HEV positive subjects which was non-significant with p=0.86. Alkaline phosphate levels were 210.03±132.38 with non-significant difference in HEV positive and HEV negative subjects with p=0.44. The mean unconjugated bilirubin level was 2.34±4.23 which was non-significant in two groups with p=0.77. A similar non-significant difference was seen in HEV positive and HEV negative groups with p=0.57. Hyperbilirubinemia showed a non-significant difference in HEV positive and HEV negative groups with p=0.77. ALT and AST also showed non-significant differences in the HEV positive HEV negative group with p=0.12 and 0.72 respectively (Table 1).

For the evaluation of the prevalence of HEV in study subjects, both HEV RT-PCR and anti-HEV IgM ELISA were undertaken, it was seen that for anti-HEV IgM ELISA, 24 subjects were positive for HEV RT-PCR and 46 subjects were negative for HEV RT-PCR. Also, it was seen that for anti-HEV IgM ELISA positive, 6 subjects were HEV RT-PCR positive and 16 subjects were HEV RT-PCR negative. These results depicted a kappa value of 0.07. In combination, it was seen that HEV had a prevalence of 50% with 46 subjects in the present study as depicted in Table 2.

Concerning the association of RT-PCR and anti-HEV IgM with Ct value and duration illness, it was seen that 6 subjects were RT-PCR and ELISA positive; 24 subjects were ELISA negative and RT-PCR positive, and 8 subjects were ELISA positive and RT-PCR negative as summarized in Table 3.

For the duration of illness, a significant correlation was noted in the duration of illness and HEV diagnosis modality, it was seen that the duration of illness was significantly higher in subjects that were ELISA positive and RT-PCR negative with 14.623±5.01 months compared to ELISA and RT-PCR positive and ELISA negative and RT-PCR positive subjects with mean duration of 11.64±2.75 months and 8.06±5.23 months respectively.

Ct value showed a non-significant correlation with the modality of diagnosing the HEV infection. The mean Ct value was 37.13±3.16 in ELISA negative and RT-PCR positive subjects and was 34.27±4.31 in subjects that were ELISA positive and RT-PCR positive. These results were statistically non-significant with p=0.24 as shown in Table 3.

DISCUSSION

The study included 92 subjects with a confirmed diagnosis of jaundice for <4 weeks duration with liver enzymes being grossly deranged with ALT (alanine aminotransferase) and AST (aspartate aminotransferase levels of >500 IU/l. There were 24 males and 22 females in HEV positive group, 30 males and 16 females in HEV negative group, and 54 males and 38 females in total which were non-significant with p=0.34. The mean age of study subjects was 38.96±13.74 years, 39.16±14.76 years in HEV positive, and 38.76±12.86 years in HEV negative subjects which were non-significant with p=0.94. The mean duration of illness was 10.6±4.83 months, 11±4.5 months in HEV-positive subjects, and 10±5.4 in HEV-negative subjects which was non-significant with p=0.6. These data correlated with studies of Chatterjee S et al[7] in 2019 and Kalita D et al[8] in 2020 where authors assessed subjects with demographic data comparable to the present study.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Concerning the liver function, it was seen that globulin levels were 2.44±0.44 in total, 2.42±0.42 in HEV positive, and 2.44±0.44 in HEV negative subjects which was non-significant with p=0.75. Albumin levels were 3.92±0.44, 3.92±0.44 in HEV positive, and 3.86±0.44 in HEV positive subjects which was non-significant with p=0.86. Alkaline phosphate levels were 210.03±132.38 with non-significant difference in HEV positive and HEV negative subjects with p=0.44. The mean unconjugated bilirubin level was 2.34±4.23 which was non-significant in two groups with p=0.77. A similar non-significant difference was seen in HEV positive and HEV negative groups with p=0.57. Hyperbilirubinemia showed a non-significant difference in HEV positive and HEV negative groups with p=0.77. ALT and AST also showed non-significant differences in the HEV positive HEV negative group with p=0.12 and 0.72 respectively. These data were consistent with the studies of Pathak R et al[9] in 2017 and Kadri SM et al[10] in 2018 where authors evaluated subjects with a liver profile comparable to the subjects of the present study.

It was seen that for the evaluation of the prevalence of HEV in study subjects, both HEV RT-PCR and anti-HEV IgM ELISA were undertaken, it was seen that for anti-HEV IgM ELISA negative, 24 subjects were positive for HEV RT-PCR and 46 subjects were negative for HEV RT-PCR. Also, it was seen that for anti-HEV IgM ELISA positive, 6 subjects were HEV RT-PCR positive and 16 subjects were HEV RT-PCR negative. These results depicted a kappa value of 0.07. In combination, it was seen that HEV had a prevalence of 50% with 46 subjects in the present study. These results were in agreement with the studies of Kamar N et al[11] in 2011 and Narayanan S[12] in 2019 where authors reported comparable results for the prevalence of HEV as in the present study.

The study results showed that concerning the association of RT-PCR and anti-HEV IgM with Ct value and duration illness, it was seen that 6 subjects were RT-PCR and ELISA positive; 24 subjects were ELISA negative and RT-PCR positive, and 8 subjects were ELISA positive and RT-PCR negative. These results correlated with Kamar N et al[13] in 2014 and Echevarria JM et al[14] in 2011 where a similar correlation in RT-PCR and ELISA was reported in their studies.

Concerning the duration of illness, a significant correlation was noted in the duration of illness and HEV diagnosis modality, it was seen that the duration of illness was significantly higher in subjects that were ELISA positive and RT-PCR negative with 14.623±5.01 months compared to ELISA and RT-PCR positive and ELISA negative and RT-PCR positive subjects with mean duration of 11.64±2.75 months and 8.06±5.23 months respectively. These data were in line with Chandra NS et al[15] in 2014 and Tholen ATR et al[16] in 2016 where authors reported a significant correlation between HEV diagnosis modality and disease duration.

The study results showed that the Ct value showed a non-significant correlation with the modality of diagnosing the HEV infection. The mean Ct value was 37.13±3.16 in ELISA negative and RT-PCR positive subjects and was 34.27±4.31 in subjects that were ELISA positive and RT-PCR positive. These results were statistically non-significant with p=0.24. These findings were coordinated with Nandi B et al[17] in 2009 and Jain P et al[18] in 2013 where authors reported for Ct a non-significant correlation with the modality of diagnosing the HEV infection.

CONCLUSIONS

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Considering its limitations, the present study concludes that HEV is the most common cause of acute hepatitis in adult Indian subjects in the tertiary care center and its diagnosis should be made with combined use of RT-PCR and ELISA tests. Further studies with large samples and longer monitoring are needed to reach a definitive conclusion.

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ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

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TABLES

Characteristic	HEV positive (n=46)	HEV negative (n=46)	Total (n=92)	p-value
Gender	24:22	30:16	54:38	0.34
Age (years)	39.16±14.76	38.76±12.86	38.96±13.74	0.94
Illness duration	11±4.5	10±5.4	10.6±4.83	0.6
Globulin	2.42±0.42	2.44±0.44	2.44±0.44	0.75
Albumin	3.92±0.44	3.86±0.44	3.92±0.44	0.86
Alkaline phosphate	195.38±89.68	224.64±165.36	210.03±132.38	0.44
Unconjugated bilirubin	2.4±3.47	2.55±4.97	2.34±4.23	0.77
Conjugated bilirubin	2.62±5.25	1.87±3.62	2.23±4.7	0.57
Hyperbilirubinemia	2.4±3.47	2.51±4.97	2.38±4.23	0.77
ALT	15071.12±437.42	21137.22±614.97	787.24±544.24	0.12
AST	187226.6±727.62	914.87±586.741	1106.71±753.76	0.72

Table 1: Demographic and disease data of study participants

Factors	HEV RT-PCR positive	HEV RT-PCR negative	Kappa value
Anti-HEV IgM ELISA negative	24	46	0.07
Anti-HEV IgM ELISA positive	6	16	

Table 2: HEV RT-PCR versus HEV ELISA

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 12, 2023

Factor	ELISA and RT-PCR positive	Negative ELISA RT-PCR positive	ELISA positive RT-PCR negative	p-value
No. of subjects	6	24	8	
Illness duration (mean)	11.64±2.75	8.06±5.23	14.623±5.01	**0.004**
Ct (Mean ± S. D)	34.27±4.31	37.13±3.16	-	0.24

Table 3: Association of RT-PCR and anti-HEV IgM with Ct value and duration illness