

Lack of persistent hepatitis E virus infection as a cause for unexplained transaminase elevation in renal transplant recipients in India

Sachin Munjal · Neha Gupta · Raj K. Sharma ·
Amit Gupta · Narain Prasad · Anupama Kaul ·
Dharmendra Bhaduria · Amit Goel · Rakesh Aggarwal

Received: 29 May 2014 / Accepted: 15 September 2014 / Published online: 1 October 2014
© Indian Society of Gastroenterology 2014

Abstract

Background Hepatitis E virus (HEV) infection is highly endemic in India, being the most common cause of acute hepatitis; however, no case of chronic infection has been reported. All the human isolates of HEV from India till date have belonged to genotype 1. In contrast, in non-endemic areas, genotype 3 is the most prevalent, and persistent HEV infection has been reported among solid-organ transplant recipients. Whether persistent infection occurs with genotype 1 HEV is unclear. We therefore looked for evidence of HEV infection among renal transplant recipients with elevated alanine transaminase (ALT).

Methods Renal transplant recipients receiving immunosuppressive therapy were screened for ALT levels, irrespective of time duration since renal transplant. For those with ALT levels equal to or exceeding 50 IU/mL on at least two occasions ≥ 3 weeks apart, serum was tested for HEV RNA using a sensitive real-time reverse transcription polymerase chain reaction assay. For those testing positive, HEV genotyping and follow up for duration of viral persistence were planned.

Results Of the 275 patients studied, 49 (17.8 %, 44 male, median age=39.5 years) had elevated ALT levels (median=62 [range=50–477]IU/L). None of these 49 patients had detectable HEV RNA in the serum using an assay with detection sensitivity of 300 copies of RNA/mL of specimen.

Conclusion Our data indicate that persistent HEV infection is an infrequent cause of ALT elevation in Indian renal transplant

recipients who are receiving immunosuppressive drugs. This suggests that infection with genotype 1 HEV may have either no or low potential to cause persistent infection.

Keywords Immunosuppression · Solid-organ transplantation · Transaminitis · Viral hepatitis

Introduction

Hepatitis E virus (HEV) is a small non-enveloped, single-stranded RNA virus, which is currently classified in genus *Hepevirus* and family *Hepeviridae*. Based on phylogenetic analysis of genomic sequences of various HEV isolates, at least four genotypes (1, 2, 3, and 4) have been identified; some of these genotypes show further division into subgenotypes. Infection with HEV genotypes 1 and 2 is restricted to humans. In comparison, genotypes 3 and 4 HEV appear to primarily infect animals such as pigs, deer, rodents, mongoose, etc. and only infrequently infect humans [1].

Human HEV infection shows two distinct epidemiological and clinical patterns. First and more frequent of these is due to infection with genotypes 1 and 2, and is characterized by disease occurring either as outbreaks or as cases of sporadic hepatitis in areas where sanitation and water quality are sub-optimal, such as Asia, Africa, and Central America. The infection in this form is usually related to fecal-oral transmission, often through contaminated drinking water. The disease more frequently affects young and previously healthy adults and is associated with a high frequency of acute liver failure among pregnant women. The other pattern is observed in developed countries, such as Europe, USA, and developed countries of Asia (e.g., Japan, Taiwan), and is characterized by occurrence of sporadic cases, mostly among elderly persons with other coexisting diseases, who appear to acquire

S. Munjal · N. Gupta · A. Goel · R. Aggarwal (✉)
Department of Gastroenterology, Sanjay Gandhi Postgraduate
Institute of Medical Sciences, Raebareli Road, Lucknow 226 014,
India
e-mail: aggarwal.ra@gmail.com

R. K. Sharma · A. Gupta · N. Prasad · A. Kaul · D. Bhaduria
Department of Nephrology, Sanjay Gandhi Postgraduate Institute of
Medical Sciences, Raebareli Road, Lucknow 226 014, India

genotype 3 or 4 HEV infection from animal sources, either through ingestion of infected meat or close animal contact [1].

Traditionally, HEV infection was believed to cause acute infection, leading to acute hepatitis, with a few patients developing acute liver failure. Patients with acute hepatitis E recover completely in a few weeks with disappearance of the virus from the blood. In recent years, there have been several reports of chronic HEV infection among immunocompromised patient groups [2–5]. Such infection is associated with histological features of chronic hepatitis and may lead to liver cirrhosis [6]. Such chronic infection has, however, been detected only with genotype 3 HEV [7]. An issue that remains unanswered is whether genotype 1 HEV can cause chronic hepatitis E.

In India, human isolates of HEV have all belonged to genotype 1 [8], whereas animal isolates have all been from genotype 4 [9]. If genotype 1 HEV causes chronic infection, one would expect some cases of HEV viremia in immunodeficient patient groups in India, given the high frequency of transmission of the virus belonging to this genotype in India. However, in a previous study of renal transplant recipients in our institution [10], we were unable to find evidence of persistent HEV infection in any of the subjects studied. However, most of those patients had normal serum transaminases. We hypothesized that a study of patients with biochemical evidence of liver injury may be more likely to yield patients with persistent HEV infection. Therefore, in the current study, we looked for evidence of HEV infection in renal transplant recipients receiving immunosuppressive drugs who had elevated serum transaminases.

Methods

Study subjects

Between January 2012 and December 2013, renal transplant recipients attending the Renal Transplant Clinic in the Department of Nephrology at our institution were enrolled in the study, irrespective of the duration since renal transplant, presence of symptoms or signs of liver disease, or nature of immunosuppressive drugs. The patients who had participated in the previous study on detection of HEV infection in renal transplant recipients [10] were specifically excluded.

Each patient provided a written informed consent, and the study was approved by our institution's ethics committee.

Laboratory methods

From each subject, 6 mL of blood was drawn by venipuncture, and serum was separated. One aliquot of serum was used for determination of serum alanine transaminase (ALT) activity on the same day using an automated chemistry analyzer and test kits (Randox), and the remaining aliquots were stored at

–80 °C for future use. Sera from patients who had serum ALT levels exceeding 50 IU/L were tested for HEV RNA. All the stored sera were also tested for HBsAg and anti-HCV. We empirically decided to use an ALT cutoff of >50 IU/L, somewhat higher than the upper limit of reference range (40 IU/L) of our laboratory, to exclude specimens with marginal elevation due to minor laboratory variations.

HEV RNA detection was done using a real-time reverse transcription polymerase reaction assay [11]. In brief, RNA was extracted from a 100- μ L aliquot of serum using QIAamp viral RNA minikit (Qiagen, Valencia, CA, USA) into 30 μ L of water. The RNA (5 μ L) was then subjected to one-step reverse transcription and polymerase chain reaction (PCR) using QuantiFast Probe RT-PCR kit (Qiagen, Hilden, Germany) and an LC480 real-time PCR machine (Roche Applied Science, Indianapolis, IN, USA). In this, a 70-bp region in ORF2 of HEV genome was amplified, using specific forward and reverse primers (5'-GGTGGTTTCTGGGGTGAC-3' and 5'-AGGGGTTGGTTGGATGAA-3', respectively). A fluorescent probe 6FAM-TGATTCTCAGCCCTTCGC-BHQ was used for real-time determination of the rate of synthesis of amplification products. Reverse transcription was done at 95 °C for 10 min, followed by denaturation at 95 °C for 5 min, and PCR for 45 cycles, each of which included heating at 95 °C (denaturation) for 10 s and 60 °C (primer annealing and extension) for 35 s. Each assay included a stool suspension known to contain HEV RNA as positive control and an appropriate negative control. Sensitivity of the assay for detection of HEV RNA was determined separately using a dilution series of synthetic cRNA.

For any specimens that tested positive for HEV RNA on the above assay, it was proposed to amplify and sequence larger segments of ORF1 and ORF2 of the HEV genome to obtain the viral genotype. Also, it was planned to follow up any patients who may be positive to HEV RNA to determine the time to clearance or duration of persistence of HEV viremia.

Results

A total of 298 renal transplant recipients were screened. Of these, 23 were excluded since they had participated in our previous study, and the remaining 275 subjects were enrolled in the current study. Of these 275 subjects, 49 (17.8 %) had ALT levels above 50 IU/L and were analyzed further.

Median age of the 49 patients with elevated ALT levels was 39.5 years (range, 10–69 years), and 44 (90 %) of them were men. Median time since transplantation in these patients was 12 (range 1–132) months. All the patients were receiving three immunosuppressive

drugs, i.e., prednisolone, mycophenolate mofetil, and tacrolimus. Four patients were receiving antitubercular drugs. Median serum levels of ALT and creatinine were 62 (range 50–477) IU/L and 1.2 (range 0.8–3.8) mg/dL, respectively. In all the patients, ALT levels had been or remained elevated for at least 3 weeks. Two of the subjects tested positive for anti-HCV, but none was positive for HBsAg. Both these subjects had HCV infection before renal transplantation.

None of the subjects tested positive for HEV RNA. The HEV RNA assay showed a consistently good detection sensitivity of better than 300 copies per milliliter of solution.

Since no subject was found to have detectable HEV RNA, genotyping and follow up for determination of duration of viremia were not possible.

Discussion

Chronic viral hepatitis is characterized by persistence of virus in a person's body beyond a certain empiric time period, usually 6 months, and is associated with ongoing hepatocyte necrosis and inflammation, often with simultaneous liver regeneration. These changes, over a prolonged period, lead to hepatic fibrosis and architectural changes, culminating in liver cirrhosis and its life-threatening complications. Among hepatotropic viruses, hepatitis B and C viruses are well known to cause persistent infection.

HEV infection was traditionally believed to cause only acute illness without any long-term consequences. During HEV infection, duration of viremia and fecal excretion of virus usually extends up to 45 days [12] and at the maximum till 4 months after the onset of illness [13]. The first report of prolonged HEV viremia, for about 10 months following acute hepatitis, appeared in an immunocompromised patient with anaplastic large cell lymphoma on chemotherapy [14]. Later, the same group extended this observation with a case series of 14 patients with solid-organ transplant who were receiving immunosuppressive drugs and had HEV viremia; of these, 7 went on to develop chronic HEV infection, i.e., infection persisting beyond 6 months [4]. Subsequently, a retrospective review of 85 cases with HEV infection in solid-organ transplant recipients (kidney, 47; liver, 26; kidney-pancreas, 6; liver-kidney, 2; heart, 2; islet cell, 1; and lung, 1) in 17 centers across Europe and USA, 56 were reported to have developed chronic HEV infection [2]. Occurrence of persistent infection was associated with transplantation of the liver (compared to other organs), shorter time since transplantation, lower levels of liver enzymes and serum creatinine, low platelet counts, and use of tacrolimus-based immunosuppressive therapy (over cyclosporin A) [2]. In addition, chronic HEV infection has also been reported in persons with human immunodeficiency virus infection [15], hematological malignancy [16],

etc. Importantly, all cases of chronic HEV infection have been with genotype 3 virus.

Lack of chronic genotype 1 HEV infection in the developed countries could have two alternative explanations, namely (i) non-circulation of genotype 1 virus in those areas or (ii) inability of genotype 1 HEV to cause persistent infection. This issue can be resolved by looking for evidence of chronic hepatitis E in areas where genotype 1 HEV is prevalent, such as India [1]. In India, all the Indian human isolates of HEV have belonged to genotype 1, and those from pigs to genotype 4 [17], with no evidence of animal-to-human transmission; genotype 3 HEV has not been reported either in humans or in animals. Given the high rate of exposure to HEV infection in the Indian population, if genotype 1 HEV infection has the potential to cause persistent infection among immunosuppressed persons, one would expect at least some renal transplant recipients to have detectable HEV RNA. In fact, in Brazil, where genotype 3 HEV infection occurs among humans, a proportion of unselected renal transplant recipients with mild ALT elevation was indeed found to have detectable HEV viremia [18, 19].

In this context, our finding of the lack of detectable HEV RNA in sera obtained from a fairly large group of Indian renal transplant recipients in a previous study [10] suggested that genotype 1 HEV may either be incapable of causing chronic infection or may do so only rarely. However, our previous study was limited by inclusion of an unselected cohort of renal transplant recipients, and this fact could have accounted for the negative study. This prompted us to focus particularly on transplant recipients who have biochemical evidence of liver injury, since the prevalence of HEV infection may be expected to be higher among such patients. Thus, our failure to find HEV infection even in this selected subgroup reaffirms the validity of our previous observation and adds to its robustness.

Our observation also has a clinical relevance. When patients with prior organ transplantation or those belonging to other immunosuppressed groups present to a clinician with liver function test abnormalities, the latter needs to decide on the tests needed. Currently, it is recommended that such patients should undergo tests for detection of HCV RNA in addition to anti-HCV and HBsAg, because of the failure of such patients to mount an antiviral antibody response. The recent discovery of chronic HEV infection has led Indian hepatologists to ask whether a test for HEV RNA should also be done in such patients. Our data indicate that this may not be necessary at present.

In conclusion, our data indicate that (i) genotype 1 HEV, which is highly prevalent in India, is quite unlikely to cause persistent infection, and (ii) HEV infection is unlikely to be the cause of ALT elevation in renal transplant recipients.

Acknowledgments The authors thank Mr. Vishwajeet Yadav and Ms. Pallavi Shukla for technical help.

Conflict of interest SM, NG, RKS, AG, NP, AK, DB, AG, and RA all declare that they have no conflict of interest.

Ethics statement The authors declare that the survey was performed in a manner that conforms to the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning Human and Animal Rights, and that the authors followed the policy concerning informed consent wherever applicable as shown in www.Springer.com.

References

- Aggarwal R, Jameel S. Hepatitis E. *Hepatology*. 2011;54:2218–26.
- Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140:1481–9.
- Bihl F, Negro F. Chronic hepatitis E in the immunosuppressed: a new source of trouble? *J Hepatol*. 2009;50:435–7.
- Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*. 2008;358:811–7.
- Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl*. 2008;14:547–53.
- Neukam K, Barreiro P, Macias J, et al. Chronic hepatitis E in HIV patients: rapid progression to cirrhosis and response to oral ribavirin. *Clin Infect Dis*. 2013;57:465–8.
- Fujiwara S, Yokokawa Y, Morino K, Hayasaka K, Kawabata M, Shimizu T. Chronic hepatitis E: a review of the literature. *J Viral Hepat*. 2014;21:78–89.
- Arankalle VA, Paranjape S, Emerson SU, Purcell RH, Walimbe AM. Phylogenetic analysis of hepatitis E virus isolates from India (1976–1993). *J Gen Virol*. 1999;80(Pt 7):1691–700.
- Arankalle VA, Chobe LP, Walimbe AM, Yergolkar PN, Jacob GP. Swine HEV infection in south India and phylogenetic analysis (1985–1999). *J Med Virol*. 2003;69:391–6.
- Naik A, Gupta N, Goel D, Ippagunta SK, Sharma RK, Aggarwal R. Lack of evidence of hepatitis E virus infection among renal transplant recipients in a disease-endemic area. *J Viral Hepat*. 2013;20:e138–40.
- Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods*. 2006;131:65–71.
- Aggarwal R, Kini D, Sofat S, Naik SR, Krawczynski K. Duration of viraemia and faecal viral excretion in acute hepatitis E. *Lancet*. 2000;356:1081–2.
- Nanda SK, Ansari IH, Acharya SK, Jameel S, Panda SK. Protracted viremia during acute sporadic hepatitis E virus infection. *Gastroenterology*. 1995;108:225–30.
- Peron JM, Mansuy JM, Recher C, et al. Prolonged hepatitis E in an immunocompromised patient. *J Gastroenterol Hepatol*. 2006;21:1223–4.
- Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med*. 2009;361:1025–7.
- Tavitian S, Peron JM, Huynh A, et al. Hepatitis E virus excretion can be prolonged in patients with hematological malignancies. *J Clin Virol*. 2010;49:141–4.
- Arankalle VA, Chobe LP, Joshi MV, Chadha MS, Kundu B, Walimbe AM. Human and swine hepatitis E viruses from Western India belong to different genotypes. *J Hepatol*. 2002;36:417–25.
- Hering T, Passos AM, Perez RM, et al. Past and current hepatitis E virus infection in renal transplant patients. *J Med Virol*. 2014;86:948–53.
- Passos AM, Heringer TP, Medina-Pestana JO, Ferraz ML, Granato CF. First report and molecular characterization of hepatitis E virus infection in renal transplant recipients in Brazil. *J Med Virol*. 2013;85:615–9.