HHS Public Access

Author manuscript

Transpl Infect Dis. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

Transpl Infect Dis. 2015 April ; 17(2): 284–288. doi:10.1111/tid.12366.

Variability of hepatitis E serologic assays in a pediatric liver transplant recipient: challenges to diagnosing hepatitis E virus infection in the United States

P.K. Sue[1], **N. Pisanic**[2], **C.D. Heaney**[2,3], **T. Mixson-Hayden**[4], **S. Kamili**[4], **K. Nelson**[3], **K.B. Schwarz**[5], **M. Forman**[6], **A. Valsamakis**[6], **J. Ticehurst**[3,6], and **W. Karnsakul**[5]

[1]Division of Pediatric Infectious Disease, Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[2]Department of Environmental Health Sciences, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA

[3]Department of Epidemiology, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA

[4]Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, Georgia USA

[5]Division of Pediatric Gastroenterology, Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[6]Division of Medical Microbiology, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Abstract

Hepatitis E virus (HEV) is an emerging cause of viral hepatitis among immunocompromised individuals in developed countries. Yet the diagnosis of HEV infection in the United States remains challenging, because of the variable sensitivity and specificity of currently available tests, and the lack of a U.S. Food and Drug Administration-approved test. We report a case of multiple discordant HEV serology results in a pediatric liver transplant recipient with idiopathic hepatitis, and review the challenges to diagnosis of HEV infection in the U.S.

Keywords

hepatitis E virus; liver transplantation; viral diagnostics

Autochthonous hepatitis E virus (HEV) infection is an emerging cause of viral hepatitis among solid organ transplant (SOT) recipients in industrialized countries (1, 2). In recent

Correspondence to: Wikrom Karnsakul, MD, The Johns Hopkins Hospital, Department of Pediatrics, 600 N. Wolfe Street, Baltimore, MD 21287-8048 USA, Tel: 410-955-8765, Fax: 410-614-1464, wkarnsa1@jhmi.edu.

Author contributions: P.K.S.: Drafted article, data collection, data analysis/interpretation, article concept; N.P.: Laboratory data collection and analysis, critical revision; C.H.: Laboratory and data analysis, critical revision; T. M.-H. and S.K.: Laboratory analysis, critical revision; K.N.: Article concept, critical revision; K.M.S.: Article concept and design, critical revision; M.F.: Laboratory data collection and data analysis; A.V.: Laboratory and data analysis, funding of studies, critical revision; J.T.: Data analysis, critical revision; W.K.: Article concept and design, funding of studies, critical revision.

years, over 200 cases of viral hepatitis caused by HEV infection have been reported among SOT recipients, with the majority of these coming from Western Europe. Among SOT recipients, HEV infection can lead to acute liver failure, cirrhosis, and chronic hepatitis in up to 60% of infected patients (3–5). Yet despite a documented national anti-HEV prevalence of 6–21%, reports of HEV infection among SOT recipients in the United States remain rare, with fewer than 10 cases reported by the U.S. Centers for Disease Control and Prevention (CDC) between 2005 and 2013 (6–9). Barriers to diagnosis include low clinical suspicion, lack of epidemiological risk factors and population prevalence estimates among North American SOT recipients, and paucity of commercially- available, reliable, and accurate testing for HEV markers of infection. The lack of a U.S. Food and Drug Administration (FDA)-approved diagnostic test, and the variability in the performance characteristics of commercially-available serologic assays, make testing for HEV infection especially challenging (10).

We report a case of idiopathic hepatitis in a 4-year-old liver transplant recipient for whom inconsistent testing results for HEV infection from a commercial diagnostic laboratory (CDL) led to unnecessary additional testing and a subsequent delay in care.

Case report

A 4-year-old African-American girl had biliary atresia for which she underwent liver transplantation at 1 year of age. She presented with a 1-week history of acutely elevated liver enzymes, with aspartate aminotransferase (AST) 376 U/L (reference range, 14–35 U/L), alanine aminotransferase (ALT) 408 U/L (reference range, 10–25 U/L), and gamma glutamyl transferase 101 U/L (reference range 5–32 U/L) on day 0. She had a previous history of hepatic vein stricture requiring biliary stent placement 4 months prior to this event, and a known history of low-level intermittent Epstein–Barr virus (EBV) viremia. She was otherwise well, without fever, jaundice, abdominal pain, nausea, or lethargy. Abdominal ultrasound showed no obstruction of the hepatic vein, hepatic artery, or portal vein. Her serum tacrolimus concentration was low at 3.7 μg/L (therapeutic range, 5–20 μg/L), and serologic testing for hepatitis A, B, and C showed no evidence of acute or chronic infection. Baseline plasma EBV viremia was approximately 2000 copies/mL and cytomegalovirus was undetectable in plasma by polymerase chain reaction (PCR).

The patient was admitted for evaluation, and a liver biopsy showed non-specific, patchy lobular hepatitis. Her tacrolimus dose was increased, and she was started on intravenous methylprednisone for presumed rejection. She continued to have rising serum aminotransferases (peak ALT 536 U/L/AST 434 U/L) and, given her immunosuppressed state, HEV infection was considered. Serum was sent for anti-HEV immunoglobulin M (IgM) and immunoglobulin G (IgG) testing to a CDL to which our institution refers such testing, and was reported reactive for both anti-HEV IgM and IgG. While the patient’s transaminases declined from their peak, they remained markedly elevated (ALT >100 U/L), and her findings were thought to be consistent with HEV infection. As a result, her tacrolimus dose was subsequently decreased and her steroid gradually tapered.

The patient had no history of contact with farm animals, ingestion of under cooked meat or solid organ meats, or history of overseas travel. One frequent visitor to the home did recently travel to sub-Saharan Africa, but denied any history of fever, gastrointestinal disturbance, jaundice, or hepatitis.

The patient continued to have ongoing evidence of mildly elevated serum aminotransferases during the course of the following weeks, and repeat HEV serology testing performed at the CDL 1 week later returned reactive for anti-HEV IgM, but non-reactive for anti-HEV IgG. Given the inconsistency of these results, tacrolimus and low-dose prednisone were re-introduced to treat potential rejection, and serial serum and stool samples stored at −20°C in our laboratory were sent to the CDC Division of Viral Hepatitis laboratory for anti-HEV IgM and IgG and HEV RNA testing. None of the samples sent to the CDC tested positive for markers of HEV infection (Table 1). A repeat serum sample collected on day 28 was again sent to the CDL, and was non-reactive for anti-HEV IgM and IgG.

In light of her multiple discordant test results, ongoing hepatitis, and low clinical suspicion at this time for HEV infection, a liver biopsy was again repeated and showed evidence of immune-mediated hepatitis without cytopathic effect, consistent with rejection. The patient was subsequently treated with mycophenolate mofetil, and her tacrolimus dose again increased, with subsequent resolution of her ongoing hepatitis.

Results

Given the inconsistent anti-HEV IgM and IgG results from the CDL over time, Institutional Review Board approval and patient parental consent were obtained, and additional testing performed for HEV markers of infection. An aliquot of the patient's serum sample from day 0, initially reported positive for anti-HEV IgM and IgG by the CDL and subsequently stored in our archives at −20° C, was re-tested at our institution using a commercial research-use enzyme-linked immunosorbent assay (ELISA) (Beijing Wantai Biological Pharmacy Enterprise, Beijing, People's Republic of China), and found to be non-reactive for both anti-HEV IgM and IgG. We also performed real-time one-step reverse-transcription quantitative PCR, using previously described (11, 12) HEV Open Reading Frames 2/3-based primers highly specific to conserved regions of the virus, on this sample and a corresponding stool sample, and detected no HEV RNA. Serum samples from days 14, 28, and 39 were also subsequently tested for HEV IgM and IgG antibody, as well as HEV RNA, by real-time PCR, and found to be uniformly negative. Furthermore, as shown in Table 1, none of the serum or stool samples sent to the CDC tested positive for HEV RNA.

Discussion

Autochthonous infection with HEV is an emerging cause of viral hepatitis in industrialized countries, and has been reported in North and South America, Europe, New Zealand, and Japan (1, 2). Among SOT recipients, HEV infection can lead to acute liver failure, cirrhosis, graft rejection, and to chronic hepatitis in up to 60% of infected patients (3–5). Risk factors associated with autochthonous HEV infection among SOT recipients include the ingestion of insufficiently cooked game, pork, or solid organ meats (13). Chronic HEV infection has

been associated with low lymphocyte count, immunosuppression with calcineurin inhibitors, and thrombocytopenia. Treatment consists of the reduction of immunosuppression, as well as the judicious use of ribavirin or interferon in select individuals (14).

While HEV infection has been well documented among SOT recipients, particularly in Europe, the lack of an FDA-approved diagnostic test makes the diagnosis of HEV particularly challenging in the United States. In the U.S., the diagnosis of HEV is usually made through the use of in–house or commercially-available anti-HEV IgM and IgG test kits. Unfortunately, these serologic assays demonstrate a wide range of variability in independent trials, in terms of both sensitivity and specificity (15–19) (Table 2). HEV PCR assays, while frequently used in the research setting, have also yet to be evaluated by the FDA and are not widely available at this time. In addition, variability in the estimates of anti-HEV seroprevalence in the U.S. over time, a lack of clarity regarding risk factors for HEV infection in the United States, and lack of data on the proportion of HEV infections among SOT recipients in particular, make interpretation of discordant test results even more problematic (8). As a result, testing for HEV markers can often be difficult to interpret and ultimately pose problems in accurate diagnosis of infection. Recently, Yoo et al. (10) reported a case in which an HEV infection was missed in an adult SOT recipient because of the poor sensitivity of a commercial HEV serologic assay.

In our present case, the provisional diagnosis of hepatitis E in the context of a false positive anti-HEV IgM result led to the initial de-escalation of immunosuppression, additional testing, and a delay in optimal therapy. Although our patient's hepatitis ultimately resolved with treatment of her underlying rejection, interestingly, a follow-up serum sample sent to the same CDL on week 8 was again reported reactive for both anti-HEV IgG and IgM. However, given her clinical resolution by this time and consistent negative results in our laboratories and those of the CDC, this was considered to be a false-positive result.

Conclusion

Our case demonstrates the challenges to accurately diagnosing HEV infection among SOT recipients in the United States. In the absence of an FDA-approved diagnostic test, and variable population prevalence estimates, testing for HEV infection should be interpreted cautiously and confirmed through an independent secondary source, such as the CDC, which offers both ELISA testing for HEV antibody as well as stool and serum HEV PCR. Otherwise, inaccurate results may lead to delayed diagnoses or potentially harmful interventions, such as inappropriate changes in immunosuppression, or potentially toxic antiviral therapy. Given the contrast in treatment approaches to infection and graft rejection among SOT recipients, such differences can have serious clinical consequences. While independently published trials may provide some guidance on the performance of commercial HEV serologic assays, in the absence of an FDA-approved test, additional studies will be needed to guide clinical HEV testing and to further characterize the risk of HEV infection among SOT recipients in North America.

Acknowledgments

Support: Support for this study was provided by the National Institutes of Health training grant (T32 AI052071).

References

1. Dalton H, Bendall R. Hepatitis E: an emerging infection in developed countries. Lancet. 2008; 8:697–709.
2. Nelson K, Kmush B, Labrique A. The epidemiology of hepatitis E virus infections in developed countries and among immunocompromised patients. Expert Rev Anti Infect. 2011; 9(3):1133–1148.
3. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ- transplant recipients. N Engl J Med. 2008; 358(8):811–817. [PubMed: 18287603]
4. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. Lancet. 2012; 379:2477–2488. [PubMed: 22549046]
5. Kamar N, Garrosie 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; 140:1481–1489. [PubMed: 21354150]
6. Kuniholm M, Purcell R, McQuillan G, Engle R, Wasley A, Nelson K. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988–1994. J Infect Dis. 2009; 200:48–56. [PubMed: 19473098]
7. Drobeniuc J, Montfort T, Le NT, et al. Laboratory -based surveillance for hepatitis E virus infection, United States, 2005–2012. Emerg Infect Dis. 2013; 19(2):218–222. [PubMed: 23347695]
8. Teshale E, Denniston M, Drobeniuc J, et al. Decline in hepatitis E virus antibody prevalence in the United States From 1988–1994 to 2009–2010. J Infect Dis. 2014 epub ahead of print. 10.1093/infdis/jiu466
9. Te HS, Drobeniuc J, Kamili S, Dong C, Hart J, Sharapov UM. Hepatitis E virus infection in a liver transplant recipient in the United States: a case report. Transplant Proc. 2013; 45(2):810–813. [PubMed: 23498824]
10. Yoo N, Bernstein C, Caldwell C, et al. Hepatitis E virus infection in a liver transplant recipient: delayed diagnosis due to variable performance of serologic assays. Transpl Infect Dis. 2013; 15:E166–E168. [PubMed: 23701647]
11. Jothikumar N, Cromeans TL, Robertson BH, et al. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods. 2006; 131(1):65–71. [PubMed: 16125257]
12. Mokhtari C, Marchadier E, Haim-Boukobza S, et al. Comparison of real-time RT-PCR assays for hepatitis E virus RNA detection. J Clin Virol. 2013; 58:36–40. [PubMed: 23886501]
13. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J Infect Dis. 2010; 202:835–844. [PubMed: 20695798]
14. Kamar N, Izopet J, Tripon S, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med. 2014; 370(12):1111–1120. [PubMed: 24645943]
15. Pas S, Streefkerk R, Pronk M, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. J Clin Virol. 2013; 58:629–634. [PubMed: 24210958]
16. Khudyakov Y, Kamili S. Serological diagnostics of hepatitis E virus infection. Virus Research. 2011; 161:84–92. [PubMed: 21704091]
17. Drobeniuc J, Meng J, Reuter G, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. Clin Infect Dis. 2010; 51(3): 175–186.
18. Ge SX, Zheng YJ, Guo QS, et al. Evaluation of two anti hepatitis E virus kits. Biomed Environmental Sci. 2007; 20:512–515.
19. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG Kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. J Med Virol. 2010; 82:799–805. [PubMed: 20336757]

Table 1

Patient's hepatitis E virus (HEV) test results from 3 laboratories

Sample collection	ALT (U/L)	anti-HEV IgM			anti-HEV IgG			HEV RNA (Lab II)		HEV RNA (Lab III)	
		Lab I	Lab II	Lab III	Lab I	Lab II	Lab III	Serum	Stool	Serum	Stool
Day 0	408	+	−	−	+	−	−	−	−	−	−
Day 7	155	+	NA	NA	−	NA	NA	NA	NA	−	NA
Day 14	52	NA	−	−	NA	−	−	−	NA	−	NA
Day 28	57	−	−	NA	−	−	NA	NA	−	−	−
Week 7–10	44–68	+	−	−	+	−	−	−	NA	−	NA

Polymerase chain reaction assay for HEV RNA performed by in-house assays at Centers for Disease Control and Prevention (CDC) and in the authors' laboratories.

ALT, alanine aminotransferase; IgM, immunoglobulin M; IgG, immunoglobulin G; Lab I, commercial diagnostic laboratory using in-house anti-HEV EIAs; Lab II, CDC hepatitis laboratory – RPC DSI anti-HEV EIAs; Lab III, authors' laboratory – Wantai anti-HEV EIAs; +, positive; −, negative; NA, not available (test not perfomed); EIAs, enzyme immunoassays.

Table 2

Performance characteristics of select commercial anti-hepatitis E virus (HEV) IgM EIAs

Manufacturer (Country of origin)	**GENELABS/MP (Singapore)**	**MIKROGEN (Germany)**	**WANTAI (China)**	**DIA PRO (Italy)**	**RPC DIAGNOSTIC SYSTEMS (Russia)**	**IMMUNO- DIAGNOSTICS (USA)**
ELISA target	ORF2 /ORF3 Recombinant Ag	ORF2 /ORF3 Recombinant Ag	ORF2 Recombinant Ag	ORF2 Recombinant Ag	ORF2/ORF3 Recombinant Ag	ORF2/ORF3 Recombinant Ag
HEV GT of ELISA target	1, 2	1, 2, 3	1, 2	1, 2, 3, 4	1, 2	1, 2
Sensitivity*	72–74%	52–92.2%	75–97%	81–98%	71–98%	82%
Specificity*	84–93%	96.1–99%	99–100%	93%	90–96%	92%

* See references (15–19).

The antigen (Ag) for each assay is characterized according to the HEV genotype (GT) and associated open reading frames (ORF) used in the development of the enzyme-linked immunosorbent assay (ELISA). Note: The assay used by the commercial diagnostic laboratory to test our patient's serum was an ELISA developed in-house that is not listed here.

IgM, immunoglobulin M; EIAs, enzyme immuoassays.