Journal of Clinical Virology 50 (2011) 69–71

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Diagnosis of acute hepatitis E by antibody and molecular testing: A study on 277 suspected cases

J.M. Echevarría*, M. Fogeda, A. Avellón

Service of Diagnostic Microbiology, National Centre of Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

ARTICLE INFO

Article history:
Received 16 June 2010
Received in revised form
16 September 2010
Accepted 23 September 2010

Keywords:
Hepatitis E
Hepatitis E virus
Acute hepatitis
Imported diseases

ABSTRACT

Background: Acute hepatitis due to hepatitis E virus (HEV) infection is both indigenous and imported to Europe. Few studies provide information about the role of HEV as an agent for acute hepatitis in Spain.
Objectives: To investigate the frequency of the HEV infection among patients displaying acute hepatitis of unexplained origin in Spain, comparing the performance of two different diagnostic approaches.
Study design: Specific IgM antibody and HEV RNA tests were used to study samples from 277 patients with acute hepatitis of unknown aetiology received during a six-year period. Samples were sent by 52 hospitals from almost all regions of Spain.
Results: Evidence of acute infection by HEV was obtained for 30 patients in total (10.8%), and 16 cases were unrelated to recent international travel. On samples from 158 patients tested for both anti-HEV IgM and HEV RNA at admission, the yield of IgM antibody testing (11.4%) was higher than the yield of HEV RNA testing (9.5%).
Conclusions: HEV could be responsible in Spain of about 11% of cases of acute hepatitis of unknown origin overall, and of about 8% of cases unrelated to international travel or immigration. India and neighbour countries represent the highest risk for import of epidemic HEV strains into Spain. Both antibody assays and molecular tests are required to optimise the final yield of laboratory diagnosis.

1. Background

Acute hepatitis due to hepatitis E virus (HEV) infection is both indigenous and imported to Europe. Imported cases come mainly from the Indian subcontinent and from the Far East. Indigenous cases have been reported almost all over Europe, from Scandinavia to Spain, and from the United Kingdom to Greece.[1–14]

Eight studies performed in Europe offer data to assess the role of HEV as an agent for acute hepatitis in the region.[2–7,11,14] The diagnostic technology for HEV infection has evolved over the last years, and the information available for some countries (i.e., Italy or Greece) is already out-of-date in regard to it.[1,2] Reports on false reactivity with tests for anti-HEV detection in settings of low endemicity suggest that performance of HEV serology is not yet optimal, and may give rise to mistakes in diagnosis.[15,16]

With such limitations, the participation of HEV in production of acute hepatitis has been assessed for Italy (10.1%),[2] southwest Spain (8.9%),[17] the Netherlands (4.4%),[5] southwest England (9.0–11.7%),[3,6,7] Finland (22.7%)[11] and Hungary (9.6%).[14]

2. Objectives

The objective of the present study was to ascertain the contribution of HEV to acute hepatitis of unexplained origin in Spain. Since use of both serological and molecular tests was planned, the performance of these two diagnostic approaches might also be compared.

3. Study design

In January, 2004, a free service of diagnosis of HEV infection among patients with acute hepatitis of unexplained origin was offered by the National Centre of Microbiology (Majadahonda, Madrid) to all centres from the Spanish National Health System. Anti-HEV IgG and IgM was tested by a recombinant immunoblot test (RIBT) (recomBlot HEV, recombLine HEV, Mikrogen GmbH, Martinsried, Germany) on all samples received for study. HEV RNA was tested at admission by two polymerase chain reaction (PCR) tests[10] on samples received after January, 2008, and retrospectively on a subset of the remaining after selection. Identification of the viral genotype was performed as described elsewhere.[10]

4. Results

From January, 2004 to December, 2009, single serum samples from 277 patients were received from 52 centres from 17

* Corresponding author. Tel.: +34 918223634; fax: +34 915097966.
E-mail address: jmecheva@isciii.es (J.M. Echevarría).

1386-6532/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2010.09.016

Table 1
Results obtained from routine anti-HEV IgM and HEV RNA testing of single serum samples from 158 patients with acute hepatitis of unexplained origin studied from January, 2008 to December, 2009.

Anti-HEV IgM	HEV RNA		Total
	Positive	Negative	
Positive	11	7	18
Indeterminate	1	4	5
Negative	3	132	135
Total	15	143	158

of the 19 regions of Spain. Centres from eight regions placed at the centre (Madrid, Castile-León), the south (Andalusia, Ceuta), the north (Basque Country, Asturias, Galicia) and the east (Valencia) of the country contributed with 88% of patients studied. Twenty-five follow-up samples were received on demand from 16 selected patients. Hepatitis A, B and C virus infections had been ruled-out at origin in all cases. One hundred and fifty-eight patients (57%) were studied in year 2008 or 2009.

Patients ranged in age from 2 to 98 years (mean: 39.6, 45 lacking data), and 50.4% were men. Seventy-nine were immigrants or international travellers who returned to Spain less than one month before the onset of disease. The remaining 198 were born in Spain and had not travelled abroad recently.

Evidence of acute HEV infection was obtained for 30 patients. Twenty-four were studied in years 2008 or 2009. Results obtained from routine anti-HEV IgM and HEV RNA testing of the initial samples from patients studied in that period are summarised in Table 1. Twenty-two patients tested positive for either one marker or both. Anti-HEV IgM yield was 11.4%, and most positive samples tested also positive for HEV RNA (61%). Viral RNA was also found in one sample indeterminate for anti-HEV IgM, and in three samples negative for anti-HEV IgM out of 135 tested (2.2%). Seroconversion for anti-HEV IgG was found in two patients testing negative for anti-HEV IgM and HEV RNA in the index sample.

The HEV genotype responsible for the infection could be identified in 19 patients. Ten strains were from genotype 3, eight from genotype 1, and one from genotype 4. The yield of anti-HEV IgM testing did not display differences in regard to the genotype identified (8/9, 89% for genotype 3 vs. 6/8, 75% for genotype 1).

Sixteen patients with acute HEV infection were born in Spain and did not travel abroad recently. Nine HEV genotype 3 strains were identified among them. The remaining 14 patients were immigrants or international travellers. Eight HEV strains from genotype 1 were found among them, six acquired in the Indian Subcontinent, one in Senegal, and one in Thailand. A single strain from genotype 4 was found in one traveller from Vietnam, and a genotype 3 strain in one patient who spent the vacation in the Dominican Republic.

5. Discussion

In the present study, a diagnosis was established after testing the index sample for anti-HEV IgM and HEV RNA in 28 patients with acute hepatitis E out of 30 found (93%). When both markers were routinely tested on all samples at admission, 50% of HEV-infected patients tested positive for both, but some tested positive for anti-HEV IgM only (32%), or for HEV RNA only (18%). The yield of anti-HEV IgM testing was independent of the viral genotype involved.

Most cases of hepatitis E found among travellers corresponded to patients coming from the Indian subcontinent. Just two cases were found in travellers returning from Latin America. Human HEV genotype 3 infections have not been reported yet from the Caribbean region. Genotype 3 strains infect swine herds in Costa Rica,[18] and genotype 1 strains are responsible for acute hepatitis E in Cuba[19] and Venezuela (Gutiérrez C, personal communication). Importation of HEV genotype 3 infections from the Dominican Republic seems, therefore, unlikely, and the infection found in the present study might have been acquired locally after the travel.

Indigenous hepatitis E was in this series almost as frequent in Spain as the imported disease, and accounted for 8.1% of acute hepatitis unrelated to travel or immigration. With the single exception of Finland,[7] this figure meets data reported before from Spain[13] and from other countries of Europe.[2,3,10,14] The finding was more frequent among patients from the northern regions (Basque Country, Cantabria, Asturias and León) than among the remainder (14.8% vs. 5.1%; $chi^2 = 4.353$, $p < 0.05$). Hepatitis E is also frequent in the neighbouring French region of Midi-Pyrénées,[12] which shares characteristic economic activities, such as livestock farming, with these regions. The virus sources and the transmission routes remain unknown for most cases of hepatitis E acquired in Europe, and livestock farming might perhaps rise the opportunity of infection from sources other than the swine livestock not identified yet.

In conclusion, the yield of anti-HEV IgM testing in diagnosis of acute hepatitis E was higher than the yield of viral RNA detection by PCR, but both approaches were required to optimise the final yield of laboratory diagnosis. Evidence suggesting an influence of the viral genotype in the diagnostic yield of antibody tests was not obtained in the present study. HEV could be responsible in Spain of about 11% of cases of acute hepatitis of unknown origin overall, and of about 8% of cases unrelated to international travel or immigration. India and neighbour countries represent the highest risk for import of epidemic HEV strains into Spain by travellers and immigrants, and Latin American countries seem to involve a much lesser risk, if any.

Conflict of interest

The authors state that no financial or personal relationship exits with other people or organisations that could inappropriately influence their work in regard to the issues of the present report.

Acknowledgments

The authors wish to thank Inés Parera, Lucía Morago, Aránzazu Potente, Mayte Arias and Carmen García Galera for her excellent technical assistance, as well as microbiologists who sent the samples included in the study.

Marta Fogeda enjoys a contract supported by grant 1429/05-17 from the Direction General of Public Health, Ministry of Health and Social Policy. This work was supported by the Instituto de Salud Carlos III, Ministry of Science and Innovation.

References

1. Tassopoulos NC, Krawczynski K, Hatzakis A, Katsoulidou A, Delladetsima I, Koutelou MG, et al. Case report: role of hepatitis E virus in the etiology of community-acquired non-A, non-B hepatitis in Greece. *J Med Virol* 1994;**42**:124–8.
2. Zanetti AR, Schlauder GG, Romanò L, Tanzi E, Fabris P, Dawson GJ, et al. Identification of a novel variant of hepatitis E virus in Italy. *J Med Virol* 1999;**57**:356–60.
3. Dalton HR, Thurairajah PH, Fellows HJ, Hussaini HS, Mitchell J, Bendall R, et al. Autochthonous hepatitis E in southwest England. *J Viral Hepat* 2007;**14**:304–9.
4. Haagsman A, Reuter G, Duizer E, Nagy G, Herremans T, Koopmans M, et al. Seroepidemiology of hepatitis E virus in patients with non-A, non-B, non-C hepatitis in Hungary. *J Med Virol* 2007;**79**:927–30.
5. Herremans M, Vennema H, Bakker J, van der Veer B, Duizer E, Benne CA, et al. Swine-like hepatitis E viruses are a cause of unexplained hepatitis in the Netherlands. *J Viral Hepat* 2007;**14**:140–6.
6. De Silva AN, Muddu AK, Iredale JP, Sheron N, Khakoo SI, Pelosi E. Unexpectedly high incidence of indigenous acute hepatitis E within South Hampshire: time for routine testing? *J Med Virol* 2008;**80**:283–8.
7. Dalton HR, Stableforth W, Hazeldine S, Thurairajah P, Ramnarace R, Warshow U, et al. Autochthonous hepatitis E in Southwest England: a comparison with hepatitis A. *Eur J Clin Microbiol Infect Dis* 2008;**27**:579–85.

8. Lewis HC, Boisson S, Ijaz S, Hewitt K, Ngui SL, Boxall E, et al. Hepatitis E in England and Wales. *Emerg Infect Dis* 2008;**14**:165–7.
9. Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. *J Infect Dis* 2008;**198**:1732–41.
10. Fogeda M, Avellón A, Cilla CG, Echevarría JM. Imported and autochthonous hepatitis E virus strains in Spain. *J Med Virol* 2009;**81**: 1743–9.
11. Kantalaa T, Maunulaa L, von Bonsdorff C-H, Peltomaaa J, Lappalainenb M. Hepatitis E virus in patients with unexplained hepatitis in Finland. *J Clin Virol* 2009;**45**:109–13.
12. Mansuy JM, Abravanel F, Miedouge M, Mengelle C, Merviel C, Dubois M, et al. Acute hepatitis E in south-west France over a five-year period. *J Clin Virol* 2009;**44**:74–7.
13. Norder H, Sundqvist L, Magnusson L, Østergaard Breum S, Löfdahl M, Larsen LE, et al. Endemic hepatitis E in two Nordic countries. *Euro Surveill* 2009;**14**(19):19211.
14. Reuter G, Fodor D, Forgách P, Kátai A, Szucs G. Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *J Clin Virol* 2009;**44**:277–81.
15. Herremans M, Bakker J, Duizer E, Vennema H, Koopmans MP. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 in a setting of low endemicity. *Clin Vaccine Immunol* 2007;**14**:562–8.
16. Fogeda M, de Ory F, Avellón A, Echevarría JM. Differential diagnosis of hepatitis E virus, cytomegalovirus and Epstein-Barr virus infection in patients with suspected hepatitis E. *J Clin Virol* 2009;**45**:259–61.
17. Pérez-Gracia MT, García-Valdivia MS, Galán F, Rodríguez-Iglesias MA. Detection of hepatitis E virus in patient's sera in southern Spain. *Acta Virol* 2004;**48**:197–200.
18. Kase JA, Correa MT, Luna C, Sobsey MD. Isolation, detection and characterization of swine hepatitis E virus from herds in Costa Rica. *Int J Environ Health Res* 2008;**18**:165–76.
19. Montalvo MC, Rodríguez Lay L, Chandra V, Bello M, Sariego S, Gutiérrez A, et al. Hepatitis E virus genotype 1, Cuba. *Emerg Infect Dis* 2008;**14**:1320–2.