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HEV infection in two referral centers in Spain; epidemiology and clinical outcomes

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ABSTRACT

Background and objectives: Hepatitis E virus (HEV) is one of the major causes of icteric hepatitis worldwide. In industrialized countries it is considered an emerging disease, as a growing number of autochthonous cases have been reported in recent years. Occasional extrahepatic manifestations have been described in the setting of HEV infection.
Study design: To characterize the epidemiological pattern and clinical outcomes of new cases of HEV infection diagnosed in two referral centers during the period 2011–2013.
Results: During the study period, four cases of self-limited acute hepatitis E after travel to endemic areas were recorded, as well as five cases of HEV infection after solid organ transplantation. Four patients failed to spontaneously clear the virus and received ribavirin monotherapy; all of them had HEV genotype-3. Ribavirin was effective in inhibiting HEV replication, although in one patient a virological relapse occurred after the end of therapy. Finally, we report a case of HEV-genotype-3 related agranulocytosis in an immunocompetent patient, resulting in a fatal outcome; this is the first case reported of its kind.
Conclusion: Diagnosis of HEV infection needs to be taken into consideration in patients with acute or chronic hepatitis in whom other etiologies have been excluded. Although hematological complications related to acute HEV infection are infrequent, these may affect any of the bone marrow series, even after viral clearance.

1. Background

Hepatitis E virus (HEV) is the causal agent of hepatitis E. Currently, it is considered to be the main cause of enterically transmitted hepatitis worldwide [1,2]. In developing countries, HEV genotypes 1–2 are transmitted through water and are associated to epidemic outbreaks, affecting mostly young adults. On the contrary, genotypes 3–4 have been isolated not only in humans but also in animals, supporting the zoonotic nature of the infection responsible for most of the sporadic cases in industrialized countries [3].

HEV infection may cause a wide range of clinical presentations; from subclinical or asymptomatic forms to fulminant liver failure. In the last decade, there has been increasing knowledge of the possibility of chronic HEV infection in immunocompromised patients [4–6]. It is particularly important to test HEV-RNA in these patients in case of persistently abnormal liver tests because serological diagnostic tools may be negative as a result of a lower humoral response [7]. Patients with chronic hepatitis E should be considered for treatment because prolonged viremia has been associated with the development of liver cirrhosis and hepatic failure [8]. Several

Abbreviations: HEV, hepatitis E virus; HEV-RNA, hepatitis E ribonucleic acid; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; ORF2, open reading frame 2; HIV, human immunodeficiency virus; F, female; M, male; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; GGT, gamma-glutamyl transferase; IS, immunosuppression; ALC, alcoholic liver cirrhosis; PBC, primary biliary cirrhosis; RNH, regenerative nodular hyperplasia; PKD, polycystic kidney disease; N, nephroangiosclerosis; CVI, common variable immunodeficiency; DM, diabetes mellitus; Leu, leucocyte total count; Neu, neutrophil count; Plat, platelet count; G-CSF, Granulocyte-Colony stimulating factor.

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extra-hepatic manifestations such as neurological disorders have been described in relation to acute or chronic HEV infection [9].

In Spain, anti-HEV IgG prevalence in the general population is around 0.6–7%, being our region of Catalonia the one with the highest reported prevalence (7%). This finding may be related to a high prevalence of swine farms, which is clearly considered a risk factor for HEV transmission in industrialized countries [10–12]. In 2010, a study performed in our geographical area detected HEV in 30% of urban sewage samples not related to agricultural sources of contamination [13]. Altogether, these findings would suggest a high incidence of HEV-related infections.

2. Objectives

To characterize the epidemiological pattern of HEV infection in two referral centers in Barcelona, with particular focus on recipients of solid organ transplants, and to analyze the clinical outcomes of confirmed HEV infection in our center.

3. Study design

Between 2011–2013, the Microbiology Department in our hospital received 392 samples to assess the presence of HEV infection. Approximately, half of the suspected cases had been attended in the Liver Unit, including those from another referral center. HEV was suspected in cases of acute hepatitis in patients with epidemiological risk factors (i.e., travel to endemic areas). After the first case of HEV infection in a liver transplant recipient in our center (2011), a diagnostic protocol was established: all solid organ transplant-recipients with acute or chronic hepatitis of unknown origin were screened for HEV infection (anti-HEV status and total RNA). Other causes of liver disease such as hepatitis C, B or A, autoimmune hepatitis or alcohol intake were discarded. Biliary complications were excluded by abdominal ultrasound. In cases of liver transplant recipients, a liver biopsy was also included in the diagnostic protocol to exclude rejection. Epidemiological risk factors were investigated in all positive cases. This protocol was approved by our local ethics committee.

Anti-HEV status was determined by an enzyme immunoassay; Recomwell HEV IgM and Reconwell HEV IgG tests by Mikrogen Diagnostik (Floriansbogen 2–4; 82061; Neuried, Germany).

Total RNA was extracted from 140 uL of serum using QIAamp Viral RNA Mini kit (Qiagen), following the manufacturer's instructions. Amplification of all samples was carried out by a standard retrotranscription and nested polymerase chain reaction protocol (PCR), using two sets of primers encompassing a 348-bp fragment within the ORF2 gene [14]. Briefly, reverse transcription was performed at 42 °C for 1 h, with AMV in the presence of RNAsin (Promega). PCR cycling conditions for both rounds were 5 min at 95 °C, 35 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and a final extension of 7 min at 72 °C. Amplified products were identified by electrophoresis in a 1% agarose gel and, if necessary, purified with the GeneJET Gel Extraction Kit (Thermo Scientific, Vilnius, Lithuania). Amplicons were bi-directionally sequenced using the set of primers of the nested PCR and the ABI PRISM BigDye Terminator Ready reaction kit, v3.01 (Applied Biosystems). Sequences were verified using the Sequencher 4.6 program (Gene Codes Corporation) and compared with reference HEV strains obtained from GeneBank. HEV genotype was determined by phylogenetic analysis using neighbor-joining algorithm (PHYLIP v.3.5c). The resulting trees were visualized with TreeView program v.1.6.6 and statistical evaluation of tree branches was obtained after 1000 replicates of bootstrap sampling.

4. Results

From 392 tested samples, isolated anti-HEV IgG was detected in twenty-three patients (6%); suggesting a past contact with HEV.

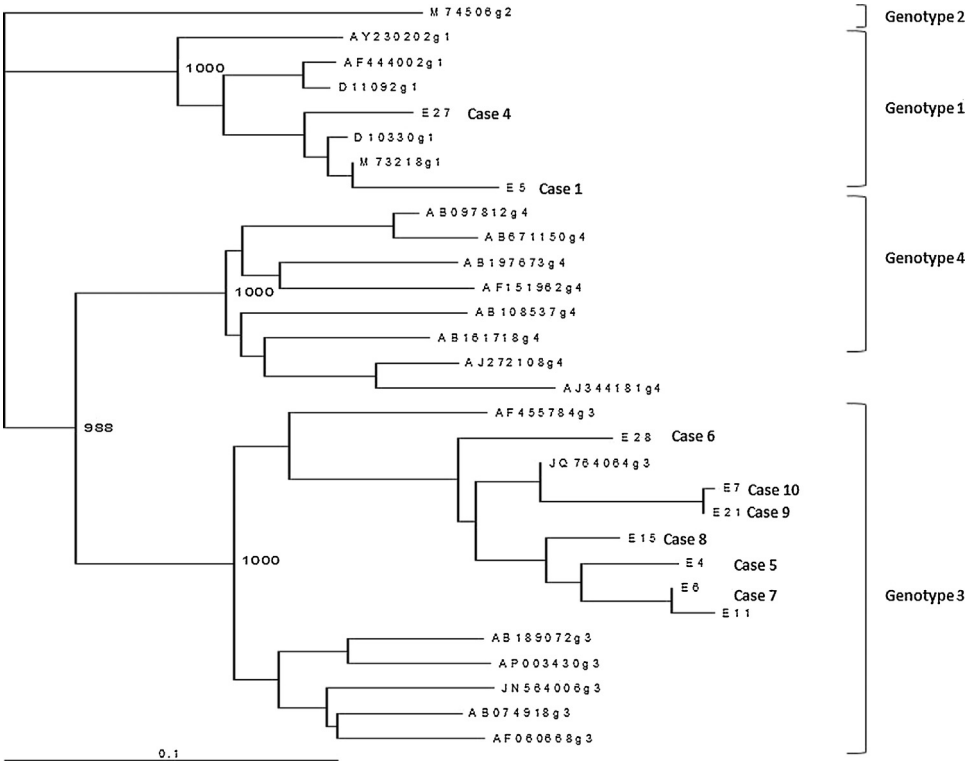

Fig. 1. Phylogenetic tree reconstructed with 7 sequences obtained from amplification of ORF2 region and 21 sequences obtained from genebank from the same viral genome fragment. (E6 and E11 are paired samples of the same patient [case7]).