Digestive and Liver Disease 43 (2011) 996–1000

Contents lists available at ScienceDirect

Digestive and Liver Disease

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

Liver, Pancreas and Biliary Tract

Hepatitis E virus infection as a cause of acute hepatitis in Southern Italy

Irene Cacciola[a,*], Federica Messineo[a], Bruno Cacopardo[b], Vito Di Marco[c], Claudio Galli[d], Giovanni Squadrito[a], Cristina Musolino[a], Carlo Saitta[a], Teresa Pollicino[a], Giovanni Raimondo[a]

[a] Unit of Clinical and Molecular Hepatology, Department of Internal Medicine, University Hospital of Messina, Italy
[b] Department of Clinical and Biomolecular Medicine, Hospital Garibaldi Nesima, University of Catania, Italy
[c] Unit Gastroenterology and Hepatology, DiBiMIS, University of Palermo, Italy
[d] Scientific Affairs Abbott diagnostics, Rome, Italy

ARTICLE INFO

Article history:
Received 2 May 2011
Accepted 25 July 2011
Available online 30 August 2011

Keywords:
Acute hepatitis
Epidemiology
Hepatitis E
Serological diagnosis

ABSTRACT

Background: Hepatitis E virus (HEV) is a major cause of acute hepatitis in developing countries, whereas it is not considered a major health problem in Western World.
Aims: To investigate the spread of HEV and its possible role in causing acute hepatitis in Southern Italy.
Methods: Four hundred and thirty patients observed from April to December 2009 were studied and grouped as follows: 55 individuals with acute hepatitis (AH), 33 of whom cryptogenic; 321 individuals with chronic liver diseases (CLD), (278 Italians and 43 immigrants); 54 individuals without liver disease (control-group). Serum samples from all cases were tested for IgG anti-HEV antibodies and those positive to this test as well as all AH cases were also tested both for IgM anti-HEV and HEV RNA.
Results: Two of 33 (6%) cryptogenic AH cases were associated with HEV infection as shown by positive IgM anti-HEV test. Both these patients had not travelled to areas at high HEV endemicity. HEV RNA was not found in any sample tested. IgG anti-HEV antibodies were detected in 5.7% of Italians with CLD and 3.7% of the control-group. No immigrant was found positive for any HEV marker.
Conclusion: Autochthonous HEV infection is present in Southern Italy where it may cause AH.

1. Introduction

1.1. Background

Hepatitis E virus (HEV), the causative agent of hepatitis E, is a single-stranded, positive sense RNA virus of 7.2 kb firstly visualized in 1983 and cloned in 1991 [1,2]. The viral genome consists of short 5′ and 3′ untranslated regions, and three partially overlapping open reading frames (ORF), called ORF-1, ORF-2 and ORF-3 [3,4].

At present, a single HEV serotype and 4 genotypes are recognized. In particular, genotypes 1 and 2 are primarily pathogenic for humans whereas genotypes 3 and 4 have been isolated in primate (including humans) and non-primate (swine, cattle, ovine, rodents, rabbits, etc.) animal species [5–8]. Actually genotype 3 is believed to be a non-primate animal virus that may occasionally cross the species barrier becoming infectious also for humans. Thus, it has been proposed that infection with this genotype may be a zoonosis [9].

Geographic spread and viral transmission are different amongst HEV genotypes [10,11]. Genotypes 1 and 2 are typically faeco-orally transmitted viruses and are endemic in many African, Asian and South-American areas, whereas genotype 3 appears to be common in Western Europe, USA and Japan where its spread may occur also through different routes of transmission including the ingestion of raw or rare cooked meat from infected animals [2,12–17].

In spite of the growing scientific interest in HEV, the real global burden of this infection is not yet well established, since available data are incomplete and often conflicting [18].

From the clinical point of view, HEV is the major cause of acute hepatitis in many developing countries. Recent data suggest that the incidence of acute hepatitis E is increasing also in the most industrialized nations where some cases occur in subjects who have travelled in highly endemic areas, but others develop in non-travellers [15,19,20].

Acute hepatitis E is usually a benign, self-limited disease. However, there is evidence that it may lead to acute liver failure when it occurs in particular conditions, such as pregnancy or pre-existing chronic liver disease [19,21–26]. Moreover, recent studies have highlighted the potential risk of long-lasting persistence of HEV infection accompanied by chronic, progressive liver damage possibly evolving towards cirrhosis in individuals with reduced immune surveillance, such as organ transplant recipients [27–34].

* Corresponding author at: Unit of Clinical and Molecular Hepatology, Department of Internal Medicine, University Hospital of Messina, Via Consolare Valeria, 1, 98124 Messina, Italy. Tel.: +39 0 2212392; fax: +39 0 2213594.
E-mail address: icacciola@unime.it (I. Cacciola).

1590-8658/$36.00
doi:10.1016/j.dld.2011.07.017

Table 1
Demographic characteristics of patients with acute hepatitis.

	HAV	HBV	HCV	EBV	Cryptogenic
Number of patients	1 (1.8%)	10 (18%)	9 (16.4%)	2 (3.64%)	33 (60%)
Mean age (years) ± SD[a]	26	44 ± 15	42 ± 15	20 ± 3	40 ± 13
Males (%)	1 (100%)	3 (30%)	4(44%)	1 (50%)	14 (42%)
Italian origin (%)	0 (0%)	10 (100)	9 (100%)	2 (100%)	32 (97%)

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epsten–Barr virus.
[a] SD, standard deviation.

The aims of this study were to verify the presence of HEV in Southern Italy, to ascertain if it plays any role in cases of acute hepatitis, and to evaluate its diffusion amongst subjects with or without evidence of infection by the major hepatitis viruses (hepatitis A, B and C virus).

2. Patients and methods

2.1. Patients

The 430 patients included in the study belonged to four different groups: 55 patients (12.8%) had acute hepatitis (AH-group); 278 patients (64.6%) were Italians with chronic liver disease (CLD-Ital); 43 patients (10%) were immigrants with chronic liver disease (CLD-Imm); 54 patients (12.6%) were Italians hospitalized for other diseases (control group). Altogether, 385 patients were from two different regions of Southern Italy (305 from Sicily and 80 from Calabria) and 45 were foreigners. Written informed consent was obtained from each patient.

The AH-group included 55 patients (25 males, 30 females; mean age 40.6 ± 14.3) consecutively admitted – from April to December 2009 – to Messina and Palermo University Hospital Liver Units and to the Infectious Disease Division of Catania University Hospital. The diagnosis of AH and its possible aetiology was based on typical clinical, biochemical, serological and virological criteria. Fifty-three patients were from Sicily and 2 were from outside of Italy (1 from Romania, 1 from Morocco). The aetiology of acute hepatitis was: hepatitis A virus (HAV) in one case, hepatitis B virus (HBV) in 10 cases, hepatitis C virus (HCV) in 9 cases, Epstein–Barr virus (EBV) in 2 cases. A diagnosis of cryptogenic or possibly drug-induced acute hepatitis was made in the 33 cases negative for all known causes of hepatitis including alcohol and autoimmune diseases (Table 1).

The CLD-Ital group included 278 patients consecutively admitted to the Messina Liver Unit from June to December 2009. One hundred and fifty six of them had HBV-related (62 cases) or HCV-related (94 cases) chronic liver diseases, whereas 122 had non-viral chronic liver diseases.

The CLD-Imm group included 43 immigrants (mean age 37 ± 16, 26 males, 17 females), with chronic liver disease attending the Messina and Catania units from June to December 2009. Sixteen of them came from Eastern Europe, 8 from South America, 6 from China and 13 from Africa. Eleven of them had CLD related to HBV infection, 9 CLD related to HCV infection, 12 had cryptogenic and 11 alcohol related liver disease.

The control-group included 54 patients hospitalized at the Internal Medicine Unit of the Messina University Hospital and suffering from various diseases (Table 2). Liver disease was carefully excluded in all cases that also tested negative for HBV and HCV serum markers. HBV and HCV markers were detected by using last generation, commercially available assays.

One blood sample was collected from each patient and stored at −80 °C. From AH-group patients admitted to the Messina Liver Unit, additional serum samples were also collected during the follow-up after discharge from hospital.

The study was performed in accordance with the Helsinki Declaration principles and informed consent was obtained from each patient.

2.2. HEV tests

Serum samples from all patients were tested for IgG-class antibodies towards HEV by a commercial enzyme immunoassay (IgG anti-HEV EIA kit, Dia.Pro, Milan, Italy). All serum samples that were IgG anti-HEV positive and all AH-group cases were tested for IgM-class antibodies to HEV by a commercial EIA (IgM anti-HEV kit, Dia.Pro, Milan, Italy). All tests were run in duplicate. The reported sensitivity and specificity of both IgG and IgM assays are >98% and >99.5%, respectively.

All cases that tested positive for IgM and/or IgG anti-HEV and 10 additional cases randomly selected among the IgM/IgG negatives in the AH-group were assayed for serum HEV RNA by previously described techniques [8,35–37] based on two different nested reverse-transcriptase polymerase chain reactions employing primers derived respectively from ORF1 and ORF2 of the HEV genome.

2.3. IgG anti-HAV tests

All samples were tested also for IgG anti-HAV by a commercial EIA (IgG ant-HAV, DiaSorin, Saluggia, Italy). Data regarding HBV and HCV prevalence in the study populations were already available for all cases.

2.4. Statistical analyses

Statistical analysis was carried out using SPSS software (version 13). Differences in frequency were compared by χ^2 test. Mean values were compared by Student's *t* test. *P* values <0.05 were considered significant.

3. Results

None of the 22 AH patients with acute viral hepatitis of known aetiology was found to be positive for any HEV marker.

Conversely, 2 out of the 33 cases (6%) with a previous diagnosis of cryptogenic or drug-induced AH tested positive for both IgM and IgG anti-HEV. These two patients were Sicilian men of 36 and 73 years of age: the first patient was a farmer and the second a clerk living in the city and neither patient had travelled outside of Sicily for at least one year before the onset of hepatitis. One of them was admitted to the Palermo unit after two weeks of treatment at home by the family doctor, whilst the other was admitted to the Messina unit after 12 days of hospitalization in another hospital division. Four serum samples from this latter patient, collected two days after admission to the liver Unit and one, three and six months after discharge from the hospital, respectively, were tested for HEV markers. IgG anti-HEV were detected in all these samples whereas IgM anti-HEV were positive in the first three specimens and negative six months after discharge. In addition, dilution experiments