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Acute HEV hepatitis: clinical and laboratory diagnosis

G. MARRONE[1], M. BIOLATO[1], G. MERCURIO[1], M.R. CAPOBIANCHI[1], A.R. GARBUGLIA[2], A. LIGUORI[1], G. VASSALLO[1], A. GASBARRINI[1,3], L. MIELE[1,3], A. GRIECO[1,3]

[1]Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy
[2]"Lazzaro Spallanzani" National Institute for Infectious Diseases, IRCCS, Rome, Italy
[3]Università Cattolica del Sacro Cuore, Rome, Italy

Abstract. – OBJECTIVE: Hepatitis E Virus (HEV) is probably the most common cause of acute hepatitis worldwide. It has been regarded for a long time as a disease limited to developing countries. Recently, the refinement of diagnostic techniques, on the one hand, and migratory flows, on the other hand, have also led to the identification of an increased number of HEV infections in industrialized countries. Four HEV genotypes have been identified across the world, with different epidemiological burdens and a wide range of clinical presentations. Here, we report a case series of acute HEV hepatitis observed in the last three years in our hospital.

PATIENTS AND METHODS: We performed a search for HEV IgM and IgG in all subjects admitted for acute hepatitis without evidence of other possible infectious, toxic or metabolic causes of liver damage. In subjects with HEV IgM positivity, the search for HEV-RNA was performed.

RESULTS: We diagnosed eight acute HEV infections: 2 epidemic and 6 sporadic forms. HEV-RNA was detected in serum in 2 cases.

CONCLUSIONS: HEV infection appears to be a cause of acute hepatitis that we must keep in mind even in developed countries.

Key Words
Hepatitis E virus, Jaundice, Viral hepatitis.

Introduction

For a long time, HEV hepatitis has been considered a peculiar viral infection typical of developing countries, which is transmitted by the oro-faecal route, mainly linked to poor sanitary conditions and similar to HAV infection[1-3]. In developing countries, such as India, Africa and Eastern countries, this infection is endemic, and it is estimated to be responsible for 20-50% of cases of acute hepatitis[2]. Since the 90s, with the sequencing of the HEV genome and the development of serologic diagnostic assays, it has been gradually recognized that such infection is also present in industrialized countries with prevalence rates reaching 21% in the US[4] and with a wider range in Europe (1.3% of blood donors in Italy[5], 52% in France[2], 6.1% in Scotland[6], and 60.9% in Poland[7]). Recently, a German study[8] reported a prevalence of 0.12% HEV RNA positive subjects among a cohort of blood asymptomatic donors; thus, HEV hepatitis is currently considered the most common cause of acute hepatitis in the world[3,9]. It is estimated that HEV causes 20 million new infections per year and 70,000 deaths worldwide[10,11]. In Europe, the real incidence of HEV infection is not fully elucidated, but available data suggest regional differences and a change in the epidemiology of the disease over the time[12]. In general, the incidence of infection seems to have increased in recent years, but this phenomenon can be partly explained with a greater awareness in clinicians of HEV virus circulation and with the improvement and greater availability of diagnostic techniques[13]. Some regional hot-spots have been identified across Europe; in Southwest France, the Netherlands, Scotland, Western Germany, the Czech Republic, Abruzzo in central Italy, and Western/central Poland[9]. The reasons for this epidemiological concentration of HEV cases are not fully elucidated to date.

The epidemiological pattern of HEV infection is divided into four zones: hyper-endemic and endemic zones with a prevalence of genotypes 1 and 2; a distinctive pattern zone, namely, Egypt, where the genotype involved bears a peculiar subtype (subtype 1) and the prevalence is higher among young patients[14]; and the sporadic zone, involving developed countries, where the prevalent genotypes are 3 and 4. Epidemic forms are primarily human

Corresponding Author: Antonio Grieco, MD; e-mail: antonio.grieco@unicatt.it

infections, while genotypes 3 and 4 are more likely zoonotic, in which humans are accidental hosts[15,16]. In developed countries, a well-documented source of infection is the consumption of undercooked infected pig meat, but infected pigs can also be a possible source of environmental contamination for lakes and rivers resulting in contamination of seafood products and vegetables irrigated with infected water[17-19]. The HEV epidemic form is generally a severe disease[3,20-24]. In Europe, where the most frequent agent is genotype 3, the infection is usually clinically unapparent with signs and symptoms of acute infections in less than 5% of cases[13]. Genotype 3-related acute liver failure is rare, although some cases have been reported in Europe, mainly in Germany and France. HEV has also been addressed as a possible cause of acute on chronic liver failure[25,26].

After acute infection, immunocompetent subjects can clear the virus by developing non-sterilizing antibodies, so re-infection can occur even if it is less probable than in non-immune subjects. In immunocompromised subjects, chronicity has been described with potential rapid fibrosis development, leading to cirrhosis[27,28].

In Italy, in real clinical practice, the appearance of acute hepatitis is rarely attributed to HEV infection. Here, we report a series of HEV-induced acute hepatitis cases observed in our hospital.

Patients and Methods

From April 2015 until September 2017 in the Gastroenterology Department of Policlinico Gemelli, Catholic University in Rome (Rome, Italy), all subjects admitted for acute hepatitis were tested for possible cause of infectious hepatitis, including a work-up for HBV, HCV, HAV, EBV, CMV, Herpes, leptospira, and salmonella. Non-infectious causes of acute hepatitis were also investigated including alcoholic, metabolic and toxic ones.

In subjects without the identification of a clear cause of acute hepatitis, the search for HEV antibodies was performed. The diagnostic work-up for HEV infection was performed in collaboration with Spallanzani Hospital in Rome, (Rome, Italy). Anti-HEV IgM and anti-HEV IgG were detected in sera using commercial enzyme-linked immunosorbent assay (ELISA) kits (DIA.PRO, diagnostic bioprobes, Milan, Italy). HEV-RNA test and molecular analysis were performed according to Garbuglia et al[29]. RNA was extracted from 400 µl of plasma, using Qiasynphony (Qiagen, Hilden GmbH, Germany). For HEV detection and genotyping, RNA was subjected to RT-PCR, using primers located in open reading frame 1 (ORF1) of HEV. The sample with nested PCR products was sequenced using 1981 and 1982 primers[29]. A phylogenetic tree was produced based on the best-fit model of nucleotide substitution provided by MEGA6 software[30]. The diagnosis of HEV acute infection was formulated in the presence of HEV IgM positivity. In all subjects with positive IgM, blood and stool samples were tested for HEV-RNA.

Results

We diagnosed eight acute HEV infections: all patients were admitted because of a clinical and laboratory diagnosis of acute hepatitis. In Table I, demographic, clinical patterns of presentation, ethnicity, potential risk factors and outcomes were reported. The pattern of liver damage at presentation was cholestatic in 3 cases and hepatocellular in 5 cases. Clinical presentation was jaundice in 4 cases, flu-like symptoms in 1 case and abdominal symptoms in 3 cases. In 2 cases, the clinical course was complicated by acute renal failure requiring haemodialysis. An underlying chronic liver disease was present in 3 cases including two cases of alcoholic liver cirrhosis (Case 3 and 5) and an autoimmune chronic hepatitis (Case 6). A percutaneous liver biopsy was performed in case 3 and case 6 and revealed acute cholestatic hepatitis. The search for other possible causes of acute liver disease, including infectious, toxic and metabolic causes was negative in all subjects. The search for cryoglobulins was negative in all cases. Table II reports the liver function tests performed during hospitalization.

All the subjects presented a positive HEV IgM and IgG while HEV-RNA was detected in serum in 2 subjects, revealing genotype 1 in the first case (epidemic form) and genotype 3 in the second case (sporadic form). HEV-RNA in stools was absent in all cases. Seven patients had complete clinical and laboratory recovery from the infection with no evidence of chronicity. One subject with a sporadic form of acute HEV died because of sepsis following acute on chronic liver failure and acute renal failure. The median hospital stay was 23.6 days (range 9-47). Interestingly, in four cases, drug-induced liver injury (DILI)

Table I. Demographic of study population.

	Age	Sex	Etnicity	Pattern	Epi-demic/ Sporadic	Hospital stay (days)	ICU stay (days)	Risk factors	HEV geno-type	HEV-RNA serum detection	Out-come
Case 1	72	M	C.	Chol.	Spor.	10	0	absent	n.a.	no	resolved
Case 2	61	M	C.	Hep	Spor.	17	0	present	3	yes	resolved
Case 3	64	M	C.	Chol.	Spor.	47	0	present	n.a.	no	deceased
Case 4	69	F	C.	Chol.	Spor.	13	0	absent	n.a.	no	resolved
Case 5	76	M	C.	Hep	Spor.	45	0	absent	n.a.	no	resolved
Case 6	29	M	C.	Hep	Spor.	9	0	present	n.a.	no	resolved
Case 7	29	M	A.	Hep	Epid.	12	0	absent	n.a.	no	resolved
Case 8	29	M	A.	Hep	Epid.	36	6	present	1	yes	resolved

Legend. ALT: Alanine aminotransferase; INR: International Normalized Ratio; ICU: Intensive Care Unit, C: Caucasian; A: Asiatic; Spor: Sporadic; Epid: Epidemic; Chol: Cholestatic; Hep: Hepatocellular; N.A.: Not Available.

was suspected. At discharge, all patients underwent a follow-up in the outpatient clinic that demonstrated complete resolution of laboratory abnormalities within three months. No patient showed signs of chronic disease.

Discussion

The acute course of HEV is characterized by a wide range of manifestations, ranging from asymptomatic and self-limiting forms to severe forms with possible fatal outcomes[3,31]. Recently, *de novo* autoimmune hepatitis secondary to HEV acute infection has been reported[32]. Acute HEV infection is characterized by fleeting HEV-RNA positivity in stool and serum, with longer IgM serum detection, often for some months after infection[33], while serum IgG may last for years.

Particularly severe forms of acute HEV infections have been described in pregnant women, in the elderly, in subjects with pre-existing liver disease and in immunocompromised subjects[34-37]. In immunocompromised patients, there was also an increased tendency to chronicity and a higher prevalence of HEV genotype 3 infection. Chronic HEV hepatitis, although rare, is characterized by a persistent elevation of serum aminotransferases, with persistent HEV-RNA detection in the serum[27].

In this paper, we report eight cases of severe acute hepatitis secondary to HEV infection, as confirmed by HEV IgM detection in all cases. The only patient who died had pre-existing liver disease. All patients presented with a picture of acute icteric hepatitis requiring hospitalization. HEV-RNA serum positivity was observed only in two cases, an epidemic and a sporadic form, revealing genotypes 1 and 3, respectively. There was no identification of HEV-RNA in stool samples[38].

Two of the observed cases were considered epidemic forms because of recent travel in endemic areas (Pakistan and Bangladesh). In one of these

Table II. Laboratory parameters during the hospitalization.

	ALT (UI/L) at admission	Bilirubin (mg/dl) at admission	INR at admission	Creatinine (mg/dl) at admission	ALT (UI/L) at discharge	Bilirubin (mg/dl) at discharge	INR at discharge	Creatinine (mg/dl) at discharge
Case 1	4335	15.5	2.1	1	14	1.1	1.1	0.77
Case 2	2391	6.9	1.2	0.9	92	7.4	1.12	0.91
Case 3	936	17.6	1	1	22	32	1.79	4.94
Case 4	1047	5.6	1	1	141	1.9	1.03	0.8
Case 5	2215	12.5	1.4	0.9	99	19.8	1.25	0.79
Case 6	1437	17.3	0.9	0.5	850	7.5	1.3	0.81
Case 7	4884	4.8	0.8	0.4	547	4	1.12	0.72
Case 8	1951	2.8	1.8	0.9	249	4.3	1	2.67

Legend. ALT: Alanine aminotransferase; INR: international normalized ratio.

cases, the risk factor for infection was also identified (consumption of undercooked local poultry meat) and HEV genotype 1 was identified. The remaining six cases were considered autochthonous forms with risk factors for HEV infection in 3 cases. HEV genotype 3 was identified in one case. An explanation for the negative serum HEV-RNA in most of the observed subjects probably could be due to the delayed time of search for HEV infection. In fact, in the presence of evident risk factors for HEV infection, such as recent travel to a high-risk country or the consumption of unsafe foods, serological and molecular HEV testing was part of the initial laboratory work-up. In the remaining cases, possible HEV infection was considered only in a subsequent phase of the diagnostic process.

It is well known that the HEV-RNA is detectable in the blood and stool in the early stages of infection, but it is difficult to detect after diagnostic delay due to a lack of clinical suspicion, especially in industrialized countries[14]. Davern et al[39], analysing a cohort of suspected drug-induced liver injury, reported that 5/9 HEV-IgM positive subjects were HEV-RNA negative, estimating that the positivity of HEV-RNA was detectable, on average, in 60% of cases, even if performed at the onset of symptoms.

All patients showed a clinical picture of severe acute icteric hepatitis at admission. In developed countries, the picture of an acute icteric hepatitis in patients lacking evidence of common viral infection, (HAV, HBV, HCV) or metabolic and genetic liver disease, commonly orients the search for xenobiotics or drug-induced liver injury (DILI), while the presence of acute HEV infection is rarely taken into account[40-42]. In our series, a DILI was first considered in 50% of cases. This result is not surprising considering that the presence of prodromal symptoms of acute viral infections, such as flu-like symptoms or abdominal symptoms, can lead to the assumption of antipyretics or antibiotics, which are the most implied class of drugs in DILI[43-45], thus leading to misinterpretation of the clinical picture.

Davern et al[39], on behalf of DILIN, reported that of 50 out of 318 subjects (16%) enrolled for suspected drug-induced liver injury (DILI) were positive for HEV IgG while 9 out of 318 (3%) were also IgM positive. In a retrospective European study, HEV-RNA was identified in 3 out of 157 (2%) cases. Acute HEV hepatitis may be particularly severe and sometimes fatal in immunocompromised and elderly subjects. The hyper-acute form has been described in individuals with pre-existing liver disease, even if asymptomatic[3,35,46]. In our series, a subject with pre-existing, undiagnosed liver cirrhosis died because of sepsis following acute-on-chronic-liver failure. This patient also presented acute renal failure with the need for renal replacement therapy. Another subject in our series presented acute renal failure during the course of acute HEV with the need for haemodialysis and complete normalization of renal function two months later. This subject presented an epidemic form, and genotype 1 was identified.

It is well known that anti-HEV seroprevalence is high in patients undergoing substitutive dialytic treatment[47] with a percentage of 40% HEV-seropositive among kidney transplant candidates in a French cohort[48]. The cause of this association is not fully understood to date. The development of glomerular diseases in a subject with acute HEV infection has been described in the literature in patients infected by GT3[49-51]. The role of HEV infection in the genesis of renal damage is still unclear, although it is possible to hypothesize a direct cytopathic effect by virus replication in the kidney, as suggested by some experimental experiences[52,53], or an immune-mediated mechanism similar to HCV-associated kidney diseases.

Two patients in our series experienced acute kidney injury: in one case bilirubin levels reached dramatically high values (70 mg/dl). Although it is well known that HEV infection may cause glomerulonephritis with or without cryoglobulinemia[50,54], we believe that our patients experienced bilirubin-related kidney damage, rather than a direct virus-related effect because no cryoglobulinemia was found and renal failure recovered with no specific therapy.

The pathogenesis of renal damage in patients with severe hyperbilirubinemia is still not fully elucidated but is probably multifactorial. Romano et al[55] have identified three possible pathogenetic factors for renal damage in the course of prolonged hyperbilirubinemia: haemodynamic changes, obstruction by bile salts, and inflammation.

Despite the severity of clinical onset, we did not observe any significant extrahepatic manifestations, in contrast to reports in the literature, such as aplastic anemia, arthritis or pancreatitis[56,57].

Conclusions

HEV is also a widespread infection in developed countries, such Italy. All patients presenting with a clinical and laboratory picture of acute hepatitis with no evidence of a clear cause of liver damage must be tested for HEV.

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Conflict of Interests

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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