JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Clinical and virological profile of locally acquired acute hepatitis E in South Bulgaria

Radka Komitova[1], Ani Kevorkyan[2], Elitsa Golkocheva-Markova[3], Mariya Atanasova[4,5], Vanya Rangelova[2], Ralitsa Raycheva[6], Chyidem Ismailova[3], Asya Stoyanova[7], Tencho Tenev[3]

[1] Department of Infectious Diseases, Parasitology and Tropical Medicine, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[2] Department of Epidemiology and Disaster Medicine, Faculty of Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria
[3] National Reference Laboratory "Hepatitis Viruses", Department of Virology, National Center of Infectious and parasitic Diseases, Sofia, Bulgaria
[4] Department of Microbiology and Immunology "Prof. Dr. Elissay Yanev", Faculty of Medicine, Medical University of Plovdiv, Bulgaria
[5] Laboratory of Virology, St George University Hospital, Plovdiv, Bulgaria
[6] Department of Social Medicine and Public Health, Faculty of Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria
[7] National Reference Laboratory of Enteroviruses, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Abstract

Introduction: Acute hepatitis E virus (HEV) infection is recognized as a zoonosis in several European countries. We describe the characteristics and outcomes of locally acquired acute HEV hepatitis.

Methodology: A prospective study was conducted among adult patients with acute HEV hepatitis at the University Hospital in Plovdiv, South Bulgaria between January 2020 and May 2022. An acute HEV infection case was a patient with acute hepatitis and laboratory-confirmed anti-HEV IgM antibodies and/or HEV RNA in serum. Demographic data, clinical manifestations, laboratory test results, and outcomes were recorded.

Results: A total of 46 patients were selected. Median age of 65 years (interquartile range [IQR] 50.8-74.3). 28 (60.87%) were male. 22 (47.83%) had comorbidities such as diabetes (15), liver cirrhosis (3), hepatitis B virus infection (2), and malignancies (2). Of the 46, 18 (39.13%) patients were viremic and, HEV genotype 3 was detected. The median (IQR) serum alanine aminotransferase, aspartate aminotransferase, bilirubin, platelet, and international normalized ratio levels were 992 (495.8-1714.3) U/L, 715 (262.5-1259.3) U/L, 204 (132.3-235.5) µmol/L, 204 (132.3-235.5) $\times 10^9$ L, and 1.0 (0.89-1.19), respectively. Six patients with underlying liver diseases had severe hepatitis. A young patient with osteoarthritis progressed to acute liver failure and died. The persistent HEV infection was ruled out in 2 malignant patients who tested HEV RNA negative three months after discharge.

Conclusions: Acute HEV hepatitis is a diagnosis to consider after excluding other causes of acute viral hepatitis. A diagnostic workup should include timely testing for HEV to identify the most vulnerable to severe consequences.

Key words: hepatitis E virus; chronic liver disease; zoonosis; hepatitis B virus; fulminant hepatitis; Bulgaria.

J Infect Dev Ctries 2024; 18(1):136-144. doi:10.3855/jidc.18341

(Received 10 April 2023 – Accepted 15 July 2023)

Introduction

The hepatitis E virus (HEV) is one of the leading causes of acute hepatitis globally. It causes a significant burden infecting an estimated 20 million in developing countries, resulting in 3.3 million symptomatic infections and 44,000 deaths annually [1]. Recently, HEV has been the most common cause of acute viral hepatitis in many western European countries, thus changing the traditional belief of being only a travel-associated infection [2]. At present, HEV is an RNA virus that comprises of 8 genotypes. HEV-1 through HEV-4 and HEV-7 genotypes are known to infect humans, each with notable distinct characteristics. Routes of transmission vary by genotype and location [3]. HEV-1 and HEV-2 infect only humans, are spread by the faecal-oral route, and cause large waterborne outbreaks in developing countries. The disease mainly affects young adults and, in general, is characterized by

a mild course and is a self-limiting one. Pregnant women are the exception, with a high mortality rate [4].

In industrialized countries, autochthonous (locally acquired) hepatitis E is caused by HEV-3 and HEV-4 (mainly in southeast Asia). It is transmitted zoonotically through the consumption of undercooked pork, contact with wild animals, or blood transfusion [5]. The infection is usually asymptomatic, but there are rare cases of acute symptomatic hepatitis among middle-aged males. In addition, no deaths have been observed during pregnancy. However, HEV-3 causes chronic infection in the immunosuppressed with a rapid evolution to cirrhosis. Moreover, in patients with chronic liver disease (CLD), acute HEV infection, caused by all four genotypes, can be aggravated [6].

HEV infection in Bulgaria has been studied in the animal population (pigs) and seroprevalence in the general human population in the Plovdiv region [7,8]. The HEV genotypic spectrum and phylogenetic analysis have revealed a predominant HEV-3 genotype [9]. In addition, some data on clinical manifestations have been reported but mainly based on serological testing [10]. However, surveillance has been activated following the inclusion of HEV infection in the list of notifiable infectious diseases in 2019. In this study, we describe the characteristics and outcomes of locally acquired acute HEV infection, the diagnosis of which was based on a combination of serological and molecular testing.

Methodology

Study design and population

This prospective single-center study was conducted at the Department of Infectious Diseases, University Hospital Plovdiv, which is a tertiary referral and teaching center in South Bulgaria. Adult patients with acute HEV hepatitis were enrolled at the time of admission to the hospital between January 2020 and May 2022. Detailed demographic, laboratory, and clinical information was prospectively collected by physicians on admission using a standardized data sheet. A structured questionnaire was developed to collect records on food and water intake and contact with animals. Abdominal ultrasonography was performed to evaluate the biliary tract and to detect vascular abnormalities. Abdominal computed tomography (CT) was also performed in some selected patients.

The patients were followed up to the sixth month after discharge to ascertain their vital signs. HEV RNA measurement was repeated in selected cases. Due to the coronavirus disease 2019 (COVID-19) in 2020-2021, the follow-up was performed through telephone interviews.

An acute HEV infection case was defined as a patient with elevated alanine aminotransferase (ALT) ≥ 2.5× upper limit of normal (ULN) [11] and laboratory detection of HEV IgM antibodies and/or HEV RNA in serum. Patients younger than 18 years were excluded. Those with anti-HEV IgG antibodies only, indicating a previous contact with HEV [12], were also excluded. A severe acute hepatitis E case was defined if the patient presented with acute HEV hepatitis associated with coagulopathy (prothrombin time < 50% and/or international normalized ratio, INR > 1.5) [13]. Acute (fulminant) liver failure (ALF) was defined if severe acute hepatitis E was associated with hepatic encephalopathy within 12 weeks after the manifestation of the first symptoms in the absence of pre-existing liver diseases [14]. Cirrhosis was defined by either biopsy-proven histology, liver stiffness measurement or CT/magnetic resonance imaging criteria (irregular liver contour ± portal hypertension or evidence of previous admission with decompensated chronic liver disease [15]. A blood platelet count (PLT) of < 150×10^9/L was defined as thrombocytopenia, and a PLT < 100×10^9/L as severe thrombocytopenia.

A few cases have been partially described in previous publications [16,17].

Serological testing

Anti-HEV IgM and IgG antibodies were determined by enzyme-linked immunosorbent assay (ELISA; Euroimmun, Lübeck, Germany). The samples were considered positive for anti-HEV IgM and anti-HEV IgG at a signal/cutoff ratio of 1.1 or higher according to the manufacturer's instructions. In both IgM and IgG assays, the HEV antigens used in the test kit were a mixture of recombinant target antigens of genotypes HEV-1 and HEV-3. Controls were calibrated against a World Health Organization (WHO) reference serum (WHO hepatitis E antiserum reference reagents, human, 1st IS NIBSC code 95/584).

In certain patients who tested positive for anti-HEV IgM but negative for HEV RNA, anti-HEV IgM was confirmed by other serological assays to exclude possible false positive IgM results. They included an enzyme-linked fluorescence assay (ELFA) with a ready-to-use commercial diagnostic kit for automated hepatitis panel VIDAS (Biomerieux, Paris, France) and RecomWEll Assay (Mikrogen, Neuried, Germany). Serological markers for hepatitis A, B, and C viruses (HAV, HBV, and HCV) were also tested. Antibodies to HAV (anti-HAVIgM), hepatitis B surface antigen

(HBsAg), and antibodies to HCV (anti-HCV) were detected by ELISA (DiaPro, Milan, Italy). In the case of HBsAg positive results, HBV E antigen (HBeAg), antibodies to hepatitis B core antigen (anti-HBcIgM and IgG), and antibodies to HBeAg (anti-HBe) (DiaPro, Mian, Italy) were tested. When clinically indicated, antibodies to HBsAg (anti-HBs) and IgG class antibodies to HAV (anti-HAVIgG) were also tested. All tests were performed in the Virology Laboratory of "St. George" University Hospital, Plovdiv.

Nucleic acid detection and sequencing

All anti-HEV IgM-positive sera were tested for the presence of HEV RNA. Viral RNA was extracted automatically (Exiprep DX16, Bioneer, Daejeon, Republic of Korea) using the ExiPrep Plus Viral DNA/RNA Kit (Bioneer, Daejeon, Republic of Korea). Detection and quantification of HEV RNA were performed with the RealStar HEV RT-PCR kit 2.0 (Altona Diagnostics, Hamburg, Germany) in 25 µL of extracted viral RNA. The kit was calibrated against the first international standard of the WHO for test-based hepatitis E nucleic acid amplification (NAT) techniques (PEI Code 6329/10). The minimum linear limit of quantification of the kit was 10 IU/µL.

HEV RNA-positive samples with a viral load > 150 000 IU/mL were further sequenced on an automated DNA sequencer (Beckman Coulter, Inc., Fullerton, CA, USA), following the Sanger method. The sequences obtained were genotyped online with HEV Genotyping Tool Version 0.1 (RIVM) alignment search tools. further, the identified case sequences were subtyped by phylogenetic analysis using the maximum likelihood method based on the Tamura-Nei model [18] conducted in MEGA7 [19]. The proposed reference sequences for subtypes of the hepatitis E virus were used [20].

In the samples positive for HBsAg, HBV DNA (Cobas HBV, Roche Diagnostics, GmbH, Mannheim, Germany) was quantified. In the anti-HAV-IgM positive samples, the presence of HAV RNA was detected by conventional one-step reverse transcription PCR (RT-PCR, EURx Ltd, Gdansk, Poland).

Molecular testing was performed in the National Reference Laboratory "Hepatitis Viruses" at the National Center of Infectious and Parasitic Disease, Sofia.

All analyses were conducted according to the manufacturer's instructions.

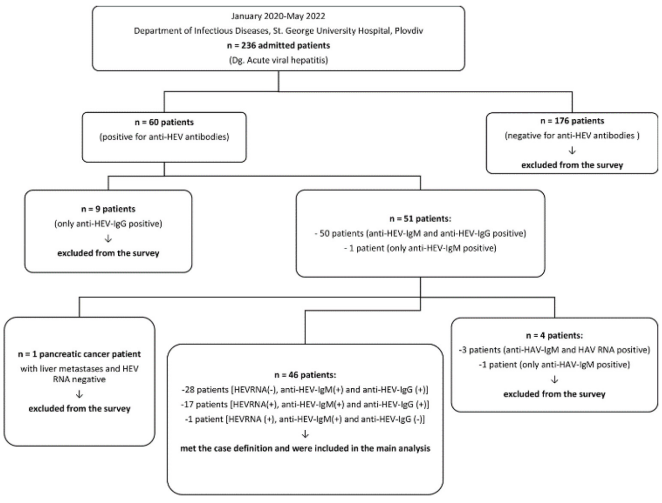
Statistical analysis

Continuous variables were expressed as median (interquartile range, IQR). Categorical variables were expressed as absolute numbers and percentages. Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) v.26 for Windows (IBM Corp. Released 2019. IBM Corp., Armonk, NY, USA). A p value < 0.05 was considered statistically significant.

Ethical statement

The study was approved by the University Ethical Committee of the Medical University of Plovdiv. All patients provided written informed consent.

Figure 1. Flowchart of selection of patients with acute HEV hepatitis.



Dg: diagnosis; HAV: hepatitis A virus; HEV: hepatitis E virus.

Results

A total of 236 adult patients were hospitalized because of acute hepatitis during the study period. Among them, 60 cases were positive for anti-HEV antibodies. Of these 60, nine were excluded from the present study as they did not meet the criteria for acute HEV cases being only anti-HEV IgG positive. The remaining 51 HEV patients were positive for both anti-HEV IgM and IgG (50 cases) and one case was anti-HEV IgM positive but IgG negative. Of these 51 cases, one patient had pancreatic cancer with liver metastases and was excluded. His polymerase chain reaction (PCR) test for HEV RNA was carried out in another hospital and was negative. The lack of active viral replication and malignancy with advancing liver metastases compressing the common bile duct explained his hepatic damage and precluded the possibility of acute HEV infection. Another four cases tested positive for both anti-HAV IgM and anti-HEV IgM and IgG. They were negative for HEV RNA but 3

of them were positive for HAV RNA. These cases were considered ambiguous and were excluded from the study. Nevertheless, being interesting from a clinical point of view, they are described briefly below.

Finally, 46 out of 236 cases (19.4%) met the case definition and therefore, were included in the present study (Figure 1).

Demographic characteristics of the patients

The median age of the patients was 65 years (IQR, 50.8-74.3), and 28 (60.87%) were male. Although most of them were urban (34, 73.91%), more than half stayed in the countryside during the summer and autumn, and engaged in growing vegetables and animal breeding. Thirty-two patients (69.56%) were directly admitted to the hospital, while 14 cases (30.43%) were relocated from other hospitals. In 22 (47.83%) of 46 patients, a comorbidity waspresent, and they received the required medication (Table 1). Two cases presented with the first manifestation of cirrhosis, established by a consultant gastroenterologist. Another patient suffered breast carcinoma and had her cytotoxic chemotherapy stopped. Still, another cancer patient suffered chronic lymphocytic leukemia and did not require immunosuppressive medications. No female patients were pregnant at the time of the study.

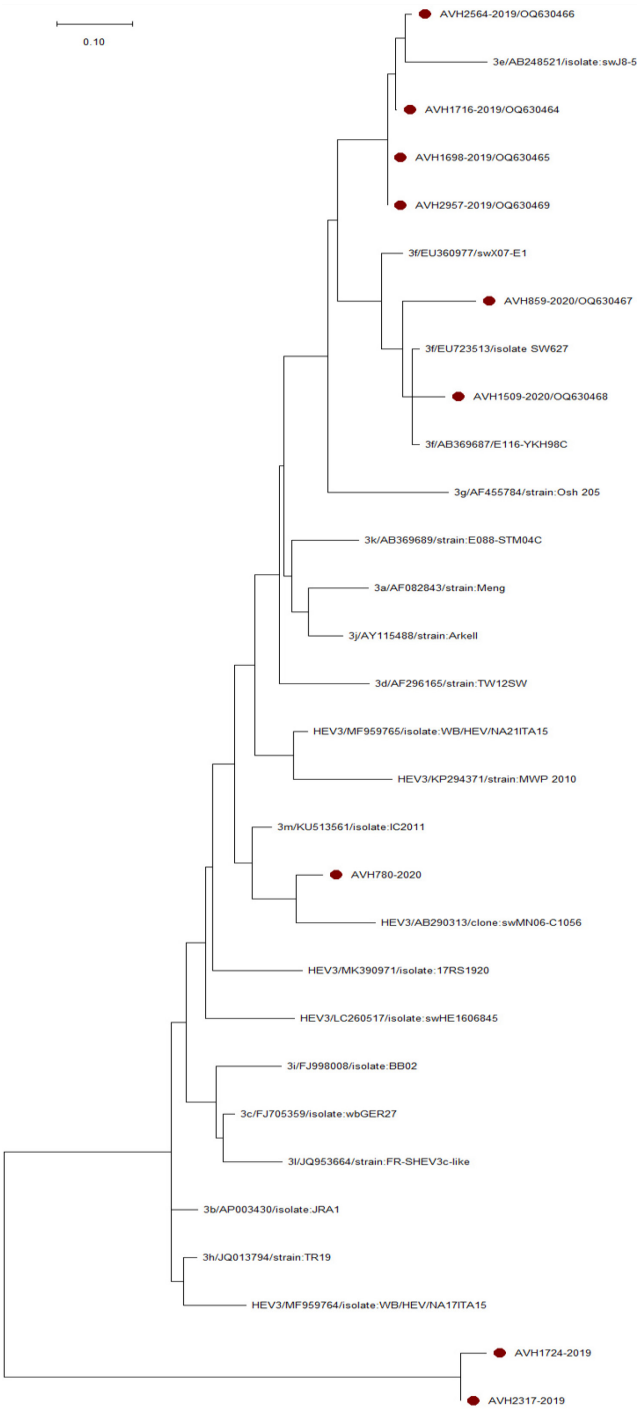
Concerning the eating habits of the participants, none of them was vegan or vegetarian. One case reported consumption of raw shellfish and five homemade sausages containing offal. Most patients (80%, 32 out of 40) reported eating pork, but none reported consumption of undercooked pork or raw meat products.

Table 1. Demographic data of patients.

Variable	n (%)
Age (years) median (IQR)	65 (50.8;74.3)
Gender	
Female	18 (39.13)
Male	28 (60.87)
Residence	
Urban	34 (73.91)
Rural	12 (26.08)
Excessive drinking *	8 (17.39)
Admission	
Direct admission	32 (69.57)
Transferred from another hospital	14 (30.43)
Comorbidities	22 (47.83)
Diabetes	15 (32.61)
Oncological disease	2 (4.34)
Liver cirrhosis	3 (6.52)
Hepatitis B infection	2 (4.34)
Liver steatosis †	25 (62.5)

Data are expressed as the number (%) or the median (IQR). *Excessive alcohol use is defined > 42 g/day for men and > 28 g/ day for women in the recent 12 months [21]. † Examined in 40 patients.

Figure 2. Phylogenetic tree of the HEV sequences isolated from the analyzed cases.



Phylogenetic analysis of the sequenced isolates. Bulgarian sequences are marked with a red dot. The name of the sequence consists of the sequence ID, isolation year, and GenBank accession number, where appropriate. The identified case sequences were subtyped by phylogenetic analysis using the maximum likelihood method based on the Tamura-Nei model [18] analyzed in MEGA7 [19]. The tree with the highest log likelihood is shown. Tree reliability was assessed by setting bootstrap replicates to 500. The proposed reference sequences for subtypes of the hepatitis E virus were used [20]. For each reference sequence, genotype/subtype, accession number, and isolate/strain are reported.

Except for a few cases who occasionally drank water from a well, all consumed tap water. Thirty-five patients had dog or cat as pets.

Virological results

Overall, HEV RNA was detected in the serum of 18 (39.13 %) patients. All the cases were both anti-HEV IgM and anti-HEV IgG positive except one who was only anti-HEV IgM positive. The remaining 28 (60.87%) patients tested negative for HEV RNA but were positive for both anti-HEV IgM and IgG. In eight out of these 28 patients who tested negative for HEV RNA but were positive for anti-HEV IgM, anti-HEV IgM antibodies were confirmed by immunoblot (in three patients who were with cirrhosis, breast carcinoma, and one HBV-HEV co-infection, respectively) and by ELFA (in five patients one of them being the second case with HBV-HEV co-infection).

HEV genotyping was successful in 9 samples, revealing the HEV-3 genotype. HEV-3e was the most common subtype (n = 4), followed by HEV-3f (n = 2). One sample sequence was close to subtype 3m, and two sequences could not be assigned to a recognized subtype (Figure 2).

Clinical characteristics of the patients

All but one of the patients were symptomatic. The only asymptomatic patient was a patient with breast carcinoma diagnosed because of ALT elevation. The chronic lymphocytic leukemia patient manifested a mild course of hepatitis.

Table 2 summarizes the clinical characteristics of the patients. More than half (62.5 %, 25 out of 40 tested) had liver steatosis on abdominal ultrasound check.

HEV hepatitis manifested with a severe course in six patients, especially in five of those with concomitant CLD: two patients with alcoholic cirrhosis, coinfection with HBV in another two patients, and one patient with diabetes. The two patients with HBV co-infection tested positive for HBsAg, anti-HBe, anti-HBc total, and HBVDNA, but were negative for HBeAg. Anti-HBcIgM was positive in one patient and negative in the other. Both were HEV RNA negative, with HEV infection confirmed by ELFA in the latter and RecomWEll Assay (Mikrogen, Neuried,Germany) in the former. The sixth patient was a 42-year-old person with autoimmune thrombocytopenia and osteoarthritis. He progressed to ALF and died. His PCR test for HEV RNA was positive in his archived serum. The latter patient was the only one among the participants who was anti-HEV IgM positive and anti-HEV IgG negative. HAV, HBV, HCV, and autoimmune hepatitis were ruled out.

None of the patients was treated with ribavirin.

Thrombocytopenia was detected in eight patients. One of them was a 79-year-old male who presented with severe isolated thrombocytopenia (15×10^9/L) but was in otherwise good condition without purpura and bleeding. White and red blood counts were in the normal reference range. He had experienced pulmonary thromboembolism 3 months earlier and received rivaroxaban treatment. The consultant hematologist excluded the possibility of acute leukemia and an adverse reaction to rivaroxaban. The patient received 3 units of platelet concentrate and his platelet count gradually increased and reached reference values in 15 days.

The sequencing was successful in two viremic patients discussed earlier. The subtype 3e was detected in one cirrhotic patient and 3f in the lymphocytic leukemia case. HEV infection was mild in both patients, and they recovered uneventfully.

We did not observe other extrahepatic manifestations of the acute infection with HEV.

Table 2. Clinical characteristics of patients.

Variable	n, (%)
HEV testing	
Serologically positive (IgM+)	28 (60.87)
Both PCR and serologically positive	18 (39.13)
Genotyping - HEV-3	9 (50.0[&])
Symptoms*	
Prodromal temperature	6 (14.28)
Diarrhea	5 (11.9)
Abdominal pain	17 (40.47)
Nausea/vomiting	15 (35.71)
Decreased appetite	20 (47.62)
Dark urine	32 (76.19)
Jaundice	27 (64.28)
Pale stools	12 (28.57)
Laboratory results†	
Bilirubin, µmol/L, median, (IQR)	134.5 (44.0;213.3)
ALAT, IU/L, median (IQR)	992.5 (495.8;1714.3)
ASAT, IU/l, median (IQR)	715 (262.5;1259.3)
PLT, 10^9/L, median (IQR)	204 (132.3;235.5)
INR, median, (IQR)	1.0 (0.89;1.19)
Severe hepatitis E	
Yes	6 (13.0)
No	40 (97.0)
Outcome	
Died	1 (2.17)
No change	1 (2.17)
Recovered	44 (95.66)

[&] 9 out of 18 tested; * data available for 42 patients; † data available for 45 patients. Data are expressed as the number (%) or the median (IQR). ALT: alanine aminotransferase; AST: aspartate aminotransferase; INR: international normalized ratio; PLT: platelet count.

Outcome and follow-up

One patient died of ALF, as described above. One of the cirrhotic patients signed a medical release because he refused to complete the treatment. He was discharged without improvement and was lost to follow-up. Thus, 44 patients were discharged from the hospital and their medical condition improved over the course of time. Of these, 26 patients (59.09%) were followed up to the 6th month after hospital discharge. Aminotransferases were checked in nearly all (76.92%, 20 out of 26) and returned to normal. The two patients with malignancies tested HEV RNA negative 3 months after discharge.

Twelve cases diagnosed with acute HEV hepatitis during the COVID-19 pandemic in 2020-2021 were followed by telephone interviews and were doing well. Among them, one patient died 5 months after discharge of HEV-unrelated cause.

In one of the two patients with HBV coinfection who were also anti-HBcIgM positive, HBsAg cleared in 2 months, and anti-HBs developed later, confirming acute HBV infection. In the other case, HBsAg persisted for more than 6 months. He was referred to a gastroenterologist, along with the two cirrhotic patients, for further evaluation and treatment.

The patient tested positive for both anti-HEV IgM and IgG, and anti-HAV IgM but was negative for both HEV and HAV RNA, developed a > 4-fold rise in his anti-HEV IgG levels; thus, he seroconverted.

Ambiguous cases

Four patients tested negative for HEV RNA but at the same time they were positive for both anti-HEV IgM and IgG, and anti-HAV IgM. Three of the patients were also positive for HAV RNA, while in the fourth one HAV RNA was not detected. The latter case later seroconverted to anti-HEV IgG. These four patients were classified as ambiguous cases as to whether they experienced acute HAV infection with false anti-HEV IgM results or HAV-HEV coinfection when HEV RNA had already become negative.

Discussion

In this study, we have identified 46 cases of locally acquired acute HEV hepatitis based on a combination of serology and molecular testing as recommended by the European Association for the Study of the Liver [12]. Additionally, we also used rigorous clinical criteria in this study.

Despite the small sample size, our cohort represented variable clinical manifestations, ranging from asymptomatic infection to severe hepatitis and fulminant hepatitis. This study revealed that HEV hepatitis might be a significant health problem in susceptible adult patients.

HEV infection is usually asymptomatic. As in previous reports, our patients were predominantly male, with a median age of 65 years, and comorbidity was present in almost half of the participants [3,4,6]. Hepatic steatosis was established using ultrasonography in more than 60% of the participants. All these could have been predisposed to the symptomatic manifestations of acute infection with HEV.

According to literature, very few cases present with clinically apparent HEV hepatitis [22]. However, the disease tends to be severe and with a high mortality rate in pregnant women in developing countries. Likewise, it has unfavorable outcomes in those with pre-existing CLD and the immunocompromised in both resource-limited and industrialized countries [23]. The two latter patient categories were represented in our study.

HEV infection is a concern in patients with CLD such as alcoholic liver disease, chronic hepatitis B or C, autoimmune hepatitis, and cirrhosis. As previously described, underlying cirrhosis places patients with acute HEV infection at a high risk of mortality [24].

Due to wide HEV and HBV circulation in southeast Asia, co-infections are common, and hence most studies on the topic are mainly from Asian countries [25]. In these countries, HEV-1 predominates, and patients with decompensated CLD, especially those with cirrhosis, and a superimposed HEV infection, have poorer prognoses than those with liver decompensation from other causes [26].

In Canada and the US, where the prevalence of HBV is low, the role of HEV-3 genotype in liver decompensation is negligible [27]. In the industrialized countries of western and northern Europe, where HEV-3 and HEV-4 predominate and the prevalence of HBV is low, the incidence of HEV is uneven, being significant in Scotland and some areas in southern France. A study in France and the United Kingdom revealed no difference in mortality between CLD patients with and without HEV infection, even among those with liver decompensation [28]. Factors related to the virus might explain the above difference in mortality: HEV-1 is considered more virulent than HEV-3 [26].

All studies investigating mortality in HEV-infected patients with underlying CLD/cirrhosis in Europe and Asia had small sample size. They included only symptomatic HEV-infected patients omitting the prevailing asymptomatic cases. Additionally, they

overestimated the mortality rate and did not accurately represent the clinical outcomes of asymptomatic patients.

Finally, a very recent meta-analysis of 18 studies from 5 countries, mainly in Asia, concerning the prognosis of HEV infection in patients with CLD reported a high prevalence of HEV among these patients. The authors conclude that HEV superinfection could accelerate disease progression in such patients and increase their mortality rates [29].

In one of the two patients with HEV-HBV co-infection that is presented, the acute infection with HEV (HEV RNA negative, anti-HEV-IgM positive by RecomWEll Assay) has led to the first clinical manifestation of liver suffering, followed by an aggressive clinical course. This could be attributed to the unrecognized liver damage due to the underlying and ongoing chronic HBV infection. Despite the severe and prolonged disease course, the patient recovered uneventfully.

Additionally, the acute infection with HEV revealed an underlying CLD in two other cases. These patients revealed their first clinical manifestation of cirrhosis.

The development of ALF in the young patient was intriguing. Negative prognostic factors, such as old age, chronic liver disease, and paracetamol overuse, were not present in this case. We assume that the patient's autoimmune background, recent treatment with steroidal anti-inflammatory drugs, and liver steatosis may have contributed to the fatal outcome. HEV is a rare, recognized cause of acute liver failure. Nevertheless, HEV should be considered when other reasons have been evaluated thoroughly, even if risk factors such as travel to high-risk regions are absent.

CLD patients are the greatest at-risk group for a severe course of acute HEV infection. Immunocompromised patients, especially those with hematological malignancies and transplant recipients, are another at-risk group [30,31]. HEV can persist in almost two-thirds of immunosuppressed patients and lead to cirrhosis after only a few years. However, the burden of HEV infection in other cancer populations is unknown [32]. Reducing immunosuppression and adding ribavirin as the first-line treatment is the regimen for chronically infected patients [12]. Interestingly, the breast carcinoma case in our study had an asymptomatic HEV infection. Her repeated HEV RNA test carried out 3 months after discharge, was negative, thus excluding the possibility of persistent HEV infection. This case highlights the importance of screening immunosuppressed patients with elevated liver enzymes for HEV RNA.

The severe thrombocytopenia in an elderly patient deserves special attention. Very few studies have reported severe thrombocytopenia in autochthonous hepatitis E in Europe [33]. Usually, it is self-limited, but some patients require platelet transfusion, intravenous immunoglobulin, or corticosteroids [34]. According to Liu *et al*. [35], the mortality of HEV infection increases with the severity of thrombocytopenia and age. In contrast, the patient in our study who received a platelet concentrate transfusion had a mild course of hepatitis.

In 18 of the anti-HEV IgM-positive sera (39.13%; 18/46), HEV RNA was detected. One of the possible reasons for the lower number is that viral RNA was examined retrospectively, and it might degrade over time because of the storage and transportation conditions [36]. Additionally, some patients were admitted after nearly a week's stay in other hospitals and hence, HEV RNA testing was not performed early enough during the viremic phase.

Not considering HEV infection as an initial diagnosis suggests that some physicians in Bulgaria might have low awareness of HEV infection in elderly patients, which remain unrecognized. We detected viremia in less than half of them as they came late to our hospital.

HEV-PCR has recently been applied to blood samples of Bulgarian patients by Bruni *et al*. and they detected HEV-3 genotype with 3e, 3f, and 3c as the most frequent subtypes [9]. Our study confirmed the presence of HEV-3 in the country. In the patients discussed earlier who were viremic, the sequencing was successful in two. Subtype 3e was detected in one cirrhotic patient and 3f in the lymphocytic leukemia case. Some recent articles have found a relationship between HEV subtypes and disease severity, reporting a lower risk of hospitalization in adults infected with HEV subtype 3f. [37,38]. The number of HEV subtypes in our study was too small to establish any clinical association. It is to be noted that both patients whose sequencing was successful recovered uneventfully.

The source and route of HEV infection in our patients remain unclear. Although all had consumed pork, none recalled risky food such as undercooked or raw animal meat [39]. A single case reported eating raw shellfish. Apart from shellfish [40], HEV has also been found in fresh fruit and salads [41]. However, the consumption of contaminated imported food cannot be excluded. In agreement with other reports [42], some of the patients in our study were engaged in animal

husbandry and pet care, which put them at risk for HEV infection.

Initially recognized as a zoonosis, HEV infection has emerged as a new threat to blood safety leading to transfusion infections. Moreover, screening for HEV-RNA has been introduced in some European countries [43,44]. We found no cases of blood transfusion, probably due to the small number of patients. Notably, a recent study from Bulgaria reported a high HEV seroprevalence (25.9%) in blood donors [44]. This finding suggests that the prevalence of HEV infection may be higher than expected.

There are several limitations of our study. The study was carried out in a single hospital and hence, the relatively small number of patients enrolled. Second, patients admitted to a tertiary care hospital were with overt hepatitis, and the asymptomatic cases of acute HEV infection that predominate remain undiagnosed. The follow-up was not complete due to the challenges imposed by the COVID-19 pandemic. Nevertheless, our prospective study has recognized HEV as a significant cause of acute viral hepatitis, often misdiagnosed due to a lack of clinical suspicion. Further studies with large numbers of patients from different regions are needed to assess risk factors for disease severity and the possible role of subtypes in clinical presentation.

Conclusions

Acute HEV hepatitis is a diagnosis to consider after other causes of acute viral hepatitis have been excluded. Diagnostic workup should include timely testing for HEV to identify those who are most vulnerable to the severe consequences of this infection.

Acknowledgements

The article has been funded by Scientific Project 02/ 2020, Medical University, Plovdiv, and Project BUL5017 - technical support from the International Atomic Energy Agency (IAEA). We are grateful to Zornica Mladenova for the help with the RecomWEll Assay.

Authors' contributions

Study design and conceptualization: RK, AK, EG-M; data collection: RK, VR; microbiological investigations: EG-M, MV, ChI, AS, TT; data analysis and interpretation: RK, AK, EG; initial draft of the manuscript: RK; critical revision of the article: all authors; final approval of the version submitted for publication: all authors.

References

1. WHO (2023) Hepatitis E. Available: https://www.who.int/news-room/fact-sheets/detail/hepatitis-E. Accessed: 10 March 2023.
2. Aspinall EJ, Couturier E, Faber M, Said B, Ijaz S, Tavoschi L, Takkinen J, Adlhoch C, The Country Experts (2017) Hepatitis E virus infection in Europe: surveillance and descriptive epidemiology of confirmed cases, 2005 to 2015. Euro Surveill 22: 30561. doi: 10.2807/1560-7917.ES.2017.22.26.30561.
3. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, Dalton HR (2017) Hepatitis E virus infection. Nat Rev Dis Primers 3: 17086. doi: 10.1038/nrdp.2017.86.
4. Hoofnagle JH, Nelson KE, Purcell RH (2012) Hepatitis E. N Engl J Med 367: 1237-1244. doi: 10.1056/NEJMra1204512.
5. Doceul V, Bagdassarian E, Demange A, Pavio N. Zoonotic (2016) Hepatitis E virus: classification, animal reservoirs and transmission routes. Viruses 8: 270. doi: 10.3390/v8100270.
6. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, Dalton HR (2012) Hepatitis E. Lancet 379: 2477-2488. doi: 10.1016/S0140-6736(11)61849-7.
7. Tsachev, I, Baymakova M, Dimitrov K, Gospodinova K, Marutsov P, PepovichbR, Kundurzhiev T, Ciccozzi M, Dalton HR (2021) Serological evidence of hepatitis E virus infection in pigs from Northern Bulgaria. Veterinaria Italiana 57: 155-159.
8. Teoharov P, Kevorkyan A, Raycheva R, Golkocheva-Markova E, Trandeva-Bankova D, Andonov A (2014) Data on the prevalence of hepatitis E virus in Bulgaria. Comptes rendus de l'Academie bulgare des Sciences 67: 1427-1432.
9. Bruni R, Villano U, Equestre M, Chionne P, Madonna E, Trandeva-Bankova D, Peleva-Pishmisheva M, Tenev T, Cella E, Ciccozzi M, Pisani G, Golkocheva-Markova E, Ciccaglione AR (2018) Hepatitis E virus genotypes and sub-genotypes causing acute hepatitis, Bulgaria, 2013-2015. PLoS One 13: e0198045. doi: 10.1371/journal.pone.0198045.
10. Pishmisheva M, Shikov P, Golkocheva-Markova E, Kotsev S, Naseva E, Vatev N, Argirova R (2020) Spread of hepatitis E viral infection among hemodialysis patients in Pazardzhik district, Bulgaria. Int J Curr Microbiol App Sci 9: 1086-1092. doi: 10.20546/ijcmas.2020.901.123.
11. Wallace SJ, Swann R, Donnelly M, Kemp L, Guaci J, Murray A, Spoor J, Lin N, Miller M, Dalton HR, Hussaini SH, Gunson R, Simpson K, Stanley A, Fraser A (2020) Mortality and morbidity of locally acquired hepatitis E in the national Scottish cohort: a multicentre retrospective study. Aliment Pharmacol Ther 51: 974-986. doi: 10.1111/apt.15704.
12. European Association for the Study of the Liver (2018) EASL Clinical practice guidelines on hepatitis E virus infection. J Hepatol 68: 1256-1271. doi: 10.1016/j.jhep.2018.03.005.
13. Schiff E R, Maddrey WC, Reddy KR, editors (2017) Schiff's diseases of the liver, 12th edition, New Delhi Wiley-Blackwel 1232 p. doi: 10.1002/9781119251316.
14. European Association for the Study of the Liver (2017) Practical guidelines on the management of acute (fulminant) liver failure. J Hepatol 66: 1047-1081. doi: 10.1016/j.jhep.2016.12.003.
15. de Franchis R; Baveno VI Faculty (2015) Expanding consensus in portal hypertension: report of the Baveno VI consensus workshop: stratifying risk and individualizing care for portal hypertension. J Hepatol 63: 743-752. doi: 10.1016/j.jhep.2015.05.022.
16. Komitova R, Kevorkyan A, Golkocheva-Markova E, Atanasova M, Boykinova O (2021) Acute liver failure associated with hepatitis E infection in a young man with immune thrombocytopenia. J of IMAB 27: 3901-3904. doi: 10.5272/jimab.2021273.3901.
17. Komitova R, Kevorkyan A, Golkocheva-Markova E, Atanasova M, Tenev T (2021) Dual infections with hepatitis E

and hepatitis B virus, General medicine 23: 444-449. [Article in Bulgarian].
18. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512-526.
19. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874. doi: 10.1093/molbev/msw054.
20. Smith DB, Izopet J, Nicot F, Simmonds P, Jameel S, Meng XJ, Norder H, Okamoto H, van der Poel WHM, Reuter G, Purdy MA. (2020) Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). J Gen Virol 101: 692-698. doi: 10.1099/jgv.0.001435.
21. Allen JP, Litten RZ (2003) Recommendations on use of biomarkers in alcoholism treatment trials. Alcohol Clin Exp Res 27: 1667-1670. doi: 10.1097/01.ALC.0000091224.78880.47.
22. Parvez MK (2013) Chronic hepatitis E infection: risks and controls. Intervirology 56: 213-216. doi: 10.1159/000349888.
23. Kamar N, Izopet J, Rostaing L (2013) Hepatitis E virus infection. Curr Opin Gastroenterol 29: 271-278. doi: 10.1097/MOG.0b013e32835ff238.
24. Teshale EH, Hu DJ, Holmberg SD (2010) The two faces of hepatitis E virus. Clin Infect Dis 51: 328-333. doi: 10.1086/653943.
25. Kc S, Mishra AK, Shrestha R (2006) Hepatitis E virus infection in chronic liver disease causes rapid decompensation. JNMA J Nepal Med Assoc 45: 212-215.
26. McGivern DR, Lin HS, Wang J, Benzine T, Janssen HLA, Khalili M, Lisker-Melman M, Fontana RJ, Belle SH, Fried MW (2019) Prevalence and impact of hepatitis E virus infection among persons with chronic hepatitis B living in the US and Canada. Open Forum Infect Dis 6: ofz175. doi: 10.1093/ofid/ofz175.
27. Blasco-Perrin H, Madden RG, Stanley A, Crossan C, Hunter JG, Vine L, Lane K, Devooght-Johnson N, Mclaughlin C, Petrik J, Stableforth B, Hussaini H, Phillips M, Mansuy JM, Forrest E, Izopet J, Blatchford O, Scobie L, Peron JM, Dalton HR (2015) Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study. Aliment Pharmacol Ther 42: 574-581. doi: 10.1111/apt.13309.
28. Qiu LX, Huang Y, Quan JL, Bi ZF, Zhong GH, Wang JY, Huang SJ, Su YY, Wu T, Zhang J, Lu GY, Zhang GM, Xia N (2023) Prognosis of hepatitis E infection in patients with chronic liver disease: a meta-analysis. J Viral Hepat 30: 101-107. doi: 10.1111/jvh.13754.
29. Behrendt P, Steinmann E, Manns MP, Wedemeyer H (2014) The impact of hepatitis E in the liver transplant setting. J Hepatol 61: 1418-1429. doi: 10.1016/j.jhep.2014.08.047.
30. Kamar N, Mallet V, Izopet J (2014) Ribavirin for chronic hepatitis E virus. N Engl J Med 370: 2447-2448. doi: 10.1056/NEJMoa1215246.
31. Chiu CY, Zhang HC, Westin J, Hosing C, Torres HA (2022) Hepatitis E virus infection in cancer patients. Transplant Cell Ther 28: 788.e1-788.e5. doi: 10.1016/j.jtct.2022.08.020.
32. Colson P, Payraudeau E, Leonnet C, De Montigny S, Villeneuve L, Motte A, Tamalet C (2008) Severe thrombocytopenia associated with acute hepatitis E virus infection. J Clin Microbiol 46: 2450-2452. doi: 10.1128/JCM.02295-07.
33. Fourquet E, Mansuy JM, Bureau C, Recher C, Vinel JP, Izopet J, Péron JM (2010) Severe thrombocytopenia associated with acute autochthonous hepatitis E. J Clin Virol 48: 73-74. doi: 10.1016/j.jcv.2010.02.016.
34. Liu L, Xiao D, Yu JH, Shen R, Wang M, Li (2018) Clinical course of sporadic acute hepatitis E in a hepatitis B virus endemic region. Int J Infect Dis 70: 107-114. doi: 10.1016/j.ijid.2018.03.008.
35. Zhang J, Li SW, Wu T, Zhao Q, Ng MH, Xia NS (2012) Hepatitis E virus: neutralizing sites, diagnosis, and protective immunity. Rev Med Virol 22: 339-349. doi: 10.1002/rmv.1719.
36. Abravanel F, Dimeglio C, Castanier M, Péron JM, Kamar N, Lhomme S, Izopet J (2020) Does HEV-3 subtype play a role in the severity of acute hepatitis E? Liver Int 40: 333-337. doi: 10.1111/liv.14329.
37. Subissi L, Peeters M, Lamoral S, Klamer S, Suin V, Van Gucht S (2019) Subtype-specific differences in the risk of hospitalisation among patients infected with hepatitis E virus genotype 3 in Belgium, 2010-2018. Epidemiol Infect 147: e224. doi: 10.1017/S0950268819001122.
38. Meng XJ (2011) From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Res 161: 23-30. doi: 10.1016/j.virusres.2011.01.016.
39. Kmush BL, Nelson KE, Labrique AB (2015) Risk factors for hepatitis E virus infection and disease. Expert Rev Anti-Infect Ther. 13: 41-53. doi: 10.1586/14787210.2015.981158.
40. Terio V, Bottaro M, Pavoni E, Losio MN, Serraino A, Giacometti F, Martella V, Mottola A, Di Pinto A, Tantillo G (2017) Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. Int J Food Microbiol 249: 61-65. doi: 10.1016/j.ijfoodmicro.2017.03.008.
41. Vonesch N, Binazzi A, Bonafede M, Melis P, Ruggieri A, Iavicoli S, Tomao P (2019) Emerging zoonotic viral infections of occupational health importance. Pathog Dis 77: ftz018. doi: 10.1093/femspd/ftz018.
42. Boland F, Martinez A, Pomeroy L, O'Flaherty N (2019) Blood donor screening for hepatitis E virus in the European Union. Transfus Med Hemother 46: 95-103. doi: 10.1159/000499121.
43. Domanović D, Tedder R, Blümel J, Zaaijer H, Gallian P, Niederhauser C, Sauleda Oliveras S, O'Riordan J, Boland F, Harritshøj L, Nascimento MSJ, Ciccaglione AR, Politis C, Adlhoch C, Flan B, Oualikene-Gonin W, Rautmann G, Strengers P, Hewitt P (2017) Hepatitis E and blood donation safety in selected European countries: a shift to screening? Euro Surveill 22: 30514. doi: 10.2807/1560-7917.ES.2017.22.16.30514.
44. Baymakova M, Terzieva K, Popov R, Grancharova E, Kundurzhiev T, Pepovich R, Tsachev I (2021) Seroprevalence of hepatitis E virus infection among blood donors in Bulgaria. Viruses 13: 492. doi: 10.3390/v13030492.

Corresponding author

Vanya Rangelova, MD, PhD.
Department of Epidemiology and Disaster Medicine, Faculty of Public Health,
Medical University of Plovdiv, 4000, 15 A Vasil Aprilov blvd. Plovdiv, Bulgaria
Tel: +359883403683
Fax: +359032200531
Email: vanya.rangelova@mu-plovdiv.bg

Conflict of interests: No conflict of interests is declared.