EXTRAHEPATIC MANIFESTATIONS IN PATIENTS WITH ACUTE HEPATITIS E – PAZARDZHIK, BULGARIA 2014 – 2022

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ABSTRACT

BACKGROUND: Hepatitis E is a global health issue, only partially understood. Bulgarian record started in 2019 and data is not sufficent. AIM: This research aims to analyse extrahepatic manifestation of acute hepatitis E in patients with hepatitis E from Pazardzhik region, between 2014 – 2022. MATERIALS AND METHODS: The analysis includes 247 patients with acute hepatitis E, treated at the Department of Infectious Diseases of Pazardzhik Multiprofile Hospital for Active Treatment, Bulgaria between 2014 – 2022. The methodology includes clinical observation, laboratory tests and medical imaging. The diagnosis was established by serological /ELISA for anti-*HEV* IgM, IgG detection/ and molecular-biological tests /RT-PCR for *HEV* RNA detection/. RESULTS: We observed extrahepatic manifestations in 19% (47/247) of the cases. In 60% (28/47) comorbidities were present, and 9% (4/47) were with underlying acute/chronic coinfection with another hepatotropic virus. Thrombocytopenia was found in 83% (39/47) of the patients; asymptomatic creatine kinase elevation – in 13% (6/47), acute pancreatitis – in 9% (4/47), transitory renal impairent – in 6% (3/47); 2% (1/47) had Guillain-Barré syndrome (GBS), 2% (1/47) – arrhythmia and 13% (6/47) – multiorgan involvement. While 91% (43/47) of the patients recovered, in 9% (4/47) the outcome was fatal. CONCLUSION: Extrahepatic manifestations might prevail, potentially delaying diagnosis *of HEV*-infection. Symptoms associated with comorbidities might also impede the final diagnosis. A diagnostic algorithm is needed to enhance the accurate diagnosis of HEV in patients with dubious symptoms.

Key words: *HEV*; extrahepatic manifestations; thrombocytopenia; CK elevation; acute pancreatitis;

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BACKGROUND

Hepatitis E is a global health issue. In spite of an increasing number of publications from various countries worldwide, there are questions that still remain unanswered.

Hepatitis E is caused by Hepatitis E virus (*HEV*). Mainly 4 *HEV* genotypes (*HEV* 1 – 4) cause disease in humans (1–3). HEV has no envelope, and such "bare" particles are found in gallbladder and faeces. In the bloodstream, *HEV* particles obtain host-derived lipid envelope protecting the virus from the neutralising antibodies. Presumably, the lipid envelope is significant for viral entry in otherwise inaccessible sites as cerebrospinal fluid (CSF). This gives a plausible explanation for the *HEV*-related extrahepatic manifestations (4). The latter might be the only manifestations of hepatitis E, thus complicating and delaying diagnosis. A relation between extrahepatic manifestations and co-infection with other hepatotropic viruses, mainly HBV and HCV was established (5,6). Recently, such have been observed in hepatitis E as well (7).

The first case of a *HEV* infected individual in Bulgaria was reported in 1995 by prof. Pavel Teoharov (8). By 2008 there had been only single case reports from the country (9–11). However, an increasing number of locally acquired hepatitis Ecases have been diagnosed later (12–17).

In 1965, *Austin Bradford Hill* – a British physician and statistician published "*Hill's criteria*" – pivots of the

causation between two events (18). *Pischke et al*. (19) suggested an adapted version of *Hill's criteria* for identifying the extrahepatic manifestations in *HEV* infection, as follows:

1. Strength: the stronger the association, the more likely the causality is.

2. Consistency: an association, repeatedly observed by different observers.

3. Temporality: manifestations observed shortly after or during *HEV* infection.

4. Plausibility and analogy: comparable or similar extrahepatic manifestations and underlying pathophysiological mechanisms have been already established for other viral infections, e.g., HCV infection.

5. Experimental data support a causality.

Considering the above criteria, the association between *HEV* infection and the extrahepatic manifestations is defined as possible, very probable, probable, doubtful and under debate (19). To date, *HEV* infection has been associated with neurological, renal, and haematological disorders, as well as with development of acute pancreatitis and other conditions (4,6,7,18,20–24). Only single publications from Bulgarian authors about the extrahepatic manifestations in *HEV exist*, while their incidence might be higher.

The aim of the present research **was** to analyse the extrahepatic manifestations observed during the acute period of HEV infection in patients from Pazardzhik region diagnosed between January 2014 – December 2022.

MATERIALS AND METHODS

Between January 2014 – December 2022, 247 patients with acute hepatitis E were treated at the Department of Infectious Diseases of Pazardzhik Multiprofile Hospital for Active Treatment. The methodology includes clinical observation, laboratory tests and medical imaging. The diagnosis of all patients was serologically confirmed with specific *anti-HEV IgM* and *IgG* detection (DIA.PRO ELISA Kit, Italy, diagnostic specificity >95% and sensitivity 100%). In 20% of the patients RT-PCR of serum samples for *HEV* RNA detection was also applied (RealStar – *HEV* RT-PCR, Altona Diagnostics, 22767 Hamburg, Germany, Ref.272013). The diagnostic tests were run at the National Reference Laboratory "Hepatitis Viruses" of the National Centre for Infectious and Parasitic Diseases, Sofia, Bulgaria, and the Laboratory of Virology, Acibadem City Clinic – Tokuda Hospital, Sofia, Bulgaria. The results are descriptively analysed, reported as mean (±SD) and numbers /proportions.

RESULTS

Among the 247 patients with acute hepatitis E during the studied period, 69% were males and 31% - females. The mean age *(±SD)* was 57.5 ±11.7 years with no significant difference between the two sexes (57 years for men vs. 57.8 years for women). Extrahepatic manifestations were observed in 19% (47 /247) of the patients (Table 1.). Further on, 59.6% (28/47) of patients with extrahepatic manifestations had comorbidities (Table 2.)

Thrombocytopenia defined as platelets count <150G/l was the most common extrahepatic manifestation observed (83% or 16% of all patients). The lowest platelets count was 4G/l, registered in a female patient with no comorbidities. Clinically manifested bleeding was registered in 13% of thrombocytopenic patients (5 /39) : 2 had haematemesis; 4 - puncture site hematomas; 3 developed skin haemorrhages – petechia and ecchymoses, and 3 patients had both cavitary and skin bleeding. The woman with the lowest Plt count had only petechia on the lower limbs, and was initially admitted to a haematology ward. However, the laboratory tests revealed significant transaminases elevation (ALT>1000IU/l). *HEV* infection was confirmed both serologically (*anti-HEV IgM* and *IgG* in high values) and with *HEV RNA* detection. A month after the acute illness the platelets count was normal and remained within reference range during the follow-up.The patients with haematemesis had alcohol-induced liver cirrhosis.

Creatine kinase (CK) elevation comprised 13% of extrahepatic manifestations (or 2.4 % of all patients). CK elevation was at the expense of the muscle fraction (CK-MM) with no clinical signs. The values normalised within the acute period.

Acute pancreatitis was observed in 9% of patients with extrahepatic manifestations (1.6 % of all). Its

Table 1. Patients with acute *HEV* infection: distribution according to the extrahepatic manifestations.

Extrahepatic manifestation	Number (% related to patients with extrahepatic manifestations, n=47)	Number (% related to the total number of patients, n=247)
Thrombocytopenia	39 (83%)	39 (15.7%)
Creatin kinase /CK/ elevation	6 (13%)	6 (2%)
Acute pancreatitis	4 (9%)	4 (2%)
Transient renal impairment	3 (6%)	3 (1%)
Guillain-Barré syndrome	1 (2%)	1 (0.4%)
Hemophagocytic syndrome	1 (2%)	1 (0.4%)
Cardiovascular disorders: ArrythmiasPulmonary oedema	1 (2%) 1 (2%)	1 (0.4%) 1 (0.4%)
More than one extrahepatic manifestation	6 (13%)	6 (2%)

Table 2. Patients with extrahepatic manifestations – distribution according to the accompanying diseases.

Accompanying diseases	Number (% related to patients with extrahepatic manifestations, n=47)	Number (% related to the total number of patients, n=247)
Alcoholic cirrhosis	9 (19%)	21 (9%)
Diabetes mellitus	7 (15%)	35 (14%)
Cardiovascular diseases	11 (24%)	62 (25%)
Coinfection with another hepatotropic virus	4 (9%)	30 (12%)
Other diseases	10 (21%)	34 (14%)
More than one comorbidity	15 (32%)	78 (32%)

clinical course did not differ pancreatitis with other aetiology. Two patients had liver cirrhosis, one had HAV/HEV coinfection. The disease severity depended on the clinical course of the hepatitis. The outcome of the pancreatitis was favourable in all 4 cases.

Transitory renal impairment – urea and creatinine elevation, were observed in 2% (5 /247) of all patients with hepatitis E. A patient with cirrhosis and concomitant chronic renal failure on haemodialysis, and another one who developed hepato-renal syndrome were excluded from the group. The other three (3/247, 1%) experienced transitory urea and creatinine elevation with no signs of renal injury and values returned to normal within the acute illness. None of them had medical history or sonographic data for underlying renal disorder.

Guillain-Barre Syndrome (GBS) was observed in one patient with alcohol-associated liver cirrhosis. He was initially admitted to a neurology ward due to lower limbs weakness. Electromyographic results showed demyelination and the cerebrospinal fluid analysis revealed proteinorachia 1(.2 g/l). Along with that, elevation of serum bilirubin and transaminases were registered. The diagnosis of acute *HEV* infection was established by specific antibodies detection during the acute illness – *anti-HEV IgM* and *anti-HEV IgG*. The disease ended with recovery 6 – 7 months later

Secondary hemophagocytic syndrome was observed in a female patient with severe acute hepatitis E and liver failure that developed on a damaged terrain: medical history of alcohol abuse, liver steatosis and thyroiditis. In the course of the disease, we registered fever (39ºC), low fibrinogen (1.3g/l) and cholesterol (1.14 mmol/l); elevated ferritin (1482µg/l) and triglycerides (4.2mmol/l); splenomegaly and pancytopenia with low leukocyte and thrombocyte counts and severely affected erythrocytes (Leu 1.9G/l, Plt 54G/l, Hb109g/l). The patient lost over 10

kg. The treatment was complex and continuous: 36 days inpatient period followed by a few months of convalescence. An upper endoscopy, three months after recovery, revealed oesophageal varices grade I.

Other extrahepatic manifestations – **arrhythmia* was observed in a patient who was initially admitted to a cardiology ward with paroxysmal atrial fibrillation. Due to significant transaminase elevation, additional diagnostic tests were performed including serology test for hepatitis E. ** Cardiac asthma/ pulmonary oedema* was observed in a patient with accompanying heart disease. In both patients *HEV* infection was confirmed by the detection of *anti-HEV IgM* and *IgG* antibodies, and ended with recovery.

DISCUSSION

Thrombocytopenia is a common, possibly immune-mediated haematological disorder in hepatitis E (7,19,22,24,28). Low platelets count was the most common extrahepatic manifestation among our patients. Notefully, most of them (87%) had no haemorrhage. Bleeding and its severity might not depend directly on platelets count, since the patient with the lowest (4G/l) presented only with petechia on the lower limbs. Probably, age and comorbidities, the accompanying chronic liver diseases in particular, might be of greater significance. Severe stomach bleeding was observed in two patients, both with underlying liver cirrhosis.

Other HEV-related haematological disorders include hemolytic anemia (29) and aplastic anemia (6,7,24,28,30). The established association between hemophagocytic syndrome and various viruses and bacteria has led to the definition of the so called *reactive secondary hemophagocytic syndrome* (28,31). We did not observe hemolytic or aplastic anemia in any of our patients. However, a woman with comorbidities developed a hemophagocytic syndrome in the course of the acute *HEV*-infection. The diagnosis of secondary hemophagocytic syndrome is clinically-based, the causes are yet to be recognized as well as the risk groups to be identified. Further studies are needed to elucidate the secondary hemophagocytic syndrome. Its spontaneous and malignant evolution requires timely diagnosis for successful management. To date, only sporadic cases of *HEV*-induced hemophagocytic syndrome have been published (31).

Muscle and skeletal disorders are rarely documented and are usually related to acute *HEV* infection (6,28,32,33). We registered CK elevation in few patients without symptoms and clinical signs. Changes were transient and reversible similarly to the observations documented in literature (28,32).

Acute pancreatitis is mainly associated with HEV1; however, other genotypes might cause it as well (6,7,21,27,28). HEV-related pancreatitis is clinically indistinguishable from those with different aetiology. Disease severity depends rather on the course of HEV infection. In some cases, the HEV aetiology of pancreatitis may remain unknown, especially when the patients present without jaundice. Pancreatitis in hepatitis E is more common among males. It is usually benign and treatment results in successful management (21,27). The same was observed among our patients.

** Renal manifestations* – glomerulonephritis in acute hepatitis E has been observed in immunocompetent and immunocompromised patients (7,23,24). Nevertheless, it is mainly related to chronic *HEV* infection. *HEV*-induced glomerulonephritis usually has benign course and favourable outcome (6). Perhaps, some cases remain aetiologically undiagnosed. None of the reported patients developed glomerulonephritis. In those with elevated urea and creatinine the values returned to normal within the acute period without specific treatment. Further studies of renal disorders in acute and chronic *HEV* infection are needed. Such patients should be consulted with nephrologist during the acute illness and the follow-up.

** Neurological manifestations:* The most common peripheral nervous system manifestation of HEV is development of Guillain-Barre Syndrome (GBS) (6,7,24). It is usually observed during or soon after an acute infection , affects predominantly men, and has a benign course that ends with definitive recovery. Neurological amyotrophy (Parsonage-Aldren-Turner syndrome) or brachial neuritis is the other most common neurological complication that affects mainly males as well. Nonetheless, even adequate treatment rarely leads to complete recovery (7).

Central nervous system is less commonly affected with manifestations of meningitis, encephalitis, pseudotumor cerebri, etc. (4,6,7,20,25,26). HEV RNA was isolated from CSF and serum but since the isolates showed differences, the existence of neurotropic HEV strains is still under debate (25,26). Neurologic manifestations are mainly related to HEV3 and HEV4 (4,24–26).

* There are some reports of myocarditis, thyroiditis, and other extrahepatic manifestations in the course of acute *HEV* infection (6,28,34). Cardiac asthma and arrhythmia were reported as initial manifestations of hepatitis E in two patients. Although these were the only cases and their association with *HEV* was not confirmed, they should be kept in mind and thoroughly analysed.

CONCLUSION

According to the adapted criteria of *Pischke et al*. extrahepatic manifestations in *HEV* infection are systematized as follows: *very probable* causality: *Guillain-Barre syndrome*, neurological amyotrophy, acute pancreatitis. *Probable* causality: cryoglobulinemia, haematological disorders. Further data are required to confirm the causal relationship between *HEV* and other extrahepatic manifestations. Therefore, it is important to report every extrahepatic manifestation observed in the course of or soon after an acute hepatitis E as well as in chronic *HEV* infection. Accumulation of more cases would contribute to conclude whether those events are closely related or coincidental, and what is the relative impact of comorbidities, hepatitis severity, patients' age or *HEV* genotype on the development of extrahepatic manifestations.

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