Hepatitis E virus genotype 3 infection in a tertiary referral center in the Netherlands: Clinical relevance and impact on patient morbidity

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ABSTRACT

Background: Hepatitis E virus (HEV) genotype 3 infections can have important clinical consequences.

Objectives: To evaluate patients at risk and the effect of treatment strategies, we studied the clinical course and treatment outcome in patients diagnosed with HEV viremia in our hospital.

Study design: Between January 2008 and March 2015 we included all patients with HEV genotype 3 (HEV-3) infections diagnosed by means of quantitative real-time reverse transcription-polymerase chain reaction test (RT-PCR). Clinical data were evaluated retrospectively.

Results: In total 79 patients were included. Forty-nine patients (62%) were male, median age of all patients was 52 years (range 13–79). Sixty-one (77%) patients were immunocompromised. Three patients (3.8%) had only transient viremia, forty-three (54.5%) cleared the infection within six months and twenty-six (32.9%) developed chronic infection. Five patients (6.3%) were lost to follow-up. All patients developing chronic infection were immunocompromised. Overall, thirteen (16%) patients within this cohort died. Three patients had pre-existent liver diseases and died of liver-related causes. Time between diagnosis and death was shorter for patients with pre-existent liver diseases (p < 0.03). Twenty-eight percent of patients on immunosuppressive medication achieved viral clearance after reducing the dose of immunosuppressive therapy. Thirty patients (38.0%) were treated with off-label ribavirin in which 25 (83.3%) a sustained viral response has been documented.

Conclusion: HEV genotype 3 viremia mainly presents in patients with underlying chronic liver diseases or an impaired immune system. Patients with pre-existent liver diseases are at high risk for complications and even death. The off-label use of ribavirin can cure HEV infection.

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1. Background

Chronic HEV-3 infections are increasingly being reported in immunocompromised patients.

2. Objectives

To evaluate patients at risk and the effect of treatment strategies, we studied the clinical course, therapeutic interventions and treatment outcome in all patients diagnosed with HEV-3 viremia in our hospital.

3. Study design

This retrospective cohort-study was conducted at the Erasmus MC, University Medical Center Rotterdam, a tertiary referral and transplant center. We included all patients who tested positive for HEV RNA genotype 3 in serum or blood between January 2008 and March 2015.

Patients were tested prospectively for HEV RNA during yearly routine checkup after transplantation or in case of unexplained elevated liver enzymes.

Patients were included if they tested positive for HEV-3 RNA in serum or blood. Baseline was defined as the time of the first
positive sample preceded by a documented negative PCR sample. Patient’s baseline demographics were obtained, including sex, age, comorbidities, type of medication and biochemistry and virological laboratory results at the time of diagnosis. Patients were immunocompromised based on the following criteria: (1) use of immunosuppressive medication after transplantation (calcineurin-inhibitors and mTOR-inhibitors); (2) use of biologicals (e.g., for rheumatoid arthritis or inflammatory bowel disease); (3) treatment by a course of or continuous chemotherapy during the last three months; (4) presence of haematological malignancy or primary immunodeficiency. The use of glucocorticoids and methotrexate alone was considered as non-immunosuppressive due to their less immunosuppressive action. We evaluated the medical records from recruited patients to explore the clinical course, duration and outcome of the infection, and therapeutic interventions. Causes of death were evaluated by two authors independently.

3.1. Virological parameters

Serum was tested for HEV RNA by means of an internally controlled quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR), as described previously [1]. The RT-PCR has a lower limit of detection (95% hit rate) of 143 IU/ml as determined by the first World Health Organization standard for HEV RNA nucleic acid amplification testing-based assays. When treated with ribavirin, patients were tested for HEV RNA every 4–6 weeks.

3.2. Clinical outcome

In all patients we evaluated the clinical outcome of the infection: transient viremia, acute infection and chronic infection. Mortality due to liver disease or other causes was a secondary outcome parameter. Transient viremia was defined as the presence of HEV RNA in blood without ALT elevation. Acute infection was defined as the presence of HEV viremia for less than six months in combination with an elevation of alanine aminotransferase (ALT) above the upper limit of 44 U/l. If HEV RNA in serum was detectable for at least six months patients were said to have chronic infections. End of infection was stated on the first date RT-PCR was negative. Liver-related mortality was defined as death related to liver failure or liver-related complications. In patients treated with antiviral therapy, an undetectable HEV RNA load at the end of treatment was defined as end of treatment response (ETR) whereas undetectable HEV RNA for at least 3 months post-treatment was defined as sustained viral response (SVR).

3.3. Data analysis

Statistical analysis was performed using SPSS version 21 (IBM, The Netherlands). Within each clinical outcome baseline characteristics of patients were compared. To analyze continuous parameters with a normal distribution, Student’s t test was used. For categorical or dichotomized parameters, proportions between groups were compared using Chi squared test or Fisher’s exact test. A p-value lower than 0.05, was considered statistically significant.

4. Results

A total of 80 patients tested positive for HEV RNA in serum. In all but one patient HEV-3 was detected so a total of 79 patients were included. Thirty-two patients (40.5%) were tested in the context of screening before or after transplantation, 5 (6.3%) were suspected of graft versus host disease (GVHD) and 42 (53.2%) were tested in case of unexplained hepatitis. Forty-nine patients were male (62.0%) and median age was 52 years (13–79). In total 61 patients (77.2%) were immunocompromised. Three patients (3.8%) used low dose prednisone (n = 2) or low dose methotrexate (n = 1). Thirty-eight patients (48.1%) were SOT-recipients of which all but one used immunosuppressive medication, with the following type of SOT: liver (n = 16), heart (n = 13), kidney (n = 5), lung (n = 1), liver and kidney (n = 1), kidney and pancreas (n = 1), heart and lung (n = 1). Fifty percent of SOT-recipients were diagnosed with HEV viremia within 800 days after transplantation (range 7–7304 days). Twelve patients (15.2%) had a history of alloHSCT. The details on immunosuppressive treatment are displayed in Fig. 1. Nineteen patients (24.1%) had pre-existent, non-HEV related active liver disease at time of diagnosis.

4.1. Clinical course of infection

In total, 3 patients (3.8%) had only transient viremia with HEV RNA levels around the lower detection rate of 143 IU/ml. During frequent follow up ALT levels persisted within the normal range. Two patients were heart transplant recipients and treated with immunosuppressive medication. They were infected more than six months after transplantation which made infection by transplantation unlikely given the incubation period of 2–6 weeks for HEV. The other patient was known with alcoholic liver cirrhosis and screened prior to liver transplantation by which transient viremia was detected.

Forty-three patients (54.5%) had signs of acute infection and cleared the infection within six months and 26 patients (32.9%) developed chronic infection. 5 patients (6.3%) were lost to follow up after having signs of acute infection. In these patients we could not determine whether they developed chronic infection or not (Fig. 2).

All patients who developed chronic infection were immunocompromised whereas 65.1% of the patients with an acute infection were immunocompromised (p = 0.001) (Table 1). We found a significant difference in the prevalence of SOT in patients developing chronic infection compared to acute infection: 69.2% vs. 37.2% respectively (p = 0.01). This was linked to the use of immunosuppressive therapy after transplantation (92.3% vs. 39.5%, p < 0.001). Especially heart transplant recipients significantly more often developed chronic infections (p = 0.002). In 2 patients the clinical outcome could not be determined. One patient was screened for HEV after liver transplantation annually. At the time of diagnosis no laboratory findings indicating hepatitis were present. One year later, RT-PCR was repeated and negative. One heart transplant recipient was screened only once and did not have any follow up.

4.2. Deceased patients

Eventually 13 patients died, of which 4 were HEV RNA positive and 8 had cleared the virus at time of death. In 1 patient, the HEV status at the time of death could not be determined.

The four patients who were viremic at the time of death all had haematological malignancies and died of sepsis due to respiratory infections (n = 3) and meningitis (n = 1) (51, 53, 198, and 393 days after HEV infection). None of these patients was treated for the HEV infection.

Eight patients died after clearing the infection. Three of these patients had pre-existent liver diseases with cirrhosis. They died shortly after clearing the infection, i.e., after 4, 17, and 54 days. One died of liver decompensation with multi-organ failure, one of complications due to spontaneous bacterial peritonitis and the third patient died of renal failure. The other five patients who died after clearing the virus were immunocompromised due to haematological malignancies or immunosuppressive therapy.

When comparing time from diagnosis to death between patients with and without pre-existent liver disease, we found a signifi-
Fig. 1. Causes of immunosuppression and use of immunosuppressive treatment.
4.4. Immunocompromised patients not treated

Sixty-one patients were immunocompromised. Of these patients, 27 were not treated. They were immunocompromised due to chemotherapy (n = 8), use of immunosuppressive drugs (n = 16), haematological malignancies (n = 2) and common variable immune deficiency treated with immunoglobulin substitution (n = 1). Two patients had transient viremia, 14 acute infection, 4 chronic infection, 2 were lost to follow up and 5 died while HEV RNA positive. SOT-patients who were not treated did not clear the infection in month 3–6 after diagnosis.

4.5. Treatment by reduction of immunosuppressive drugs

Eight patients were treated solely by reducing their immunosuppressive drugs. Five patients used immunosuppressive drugs after transplantation. In 4 out of these 5 patients the calcineurin-inhibitor was adjusted with 50% dose reduction and in the fifth patient sirolimus was stopped. Prednisone was stopped in 3 out of these 5 patients. The other three patients used immunosuppressive drugs for other indications. In one patient mycophenolic acid for nephrotic syndrome was stopped and dose of cyclosporine was halved. In the second patient adalimumab and methotrexate for
rheumatoid arthritis were temporarily stopped. The third patient, with polycythemia vera, was treated with a temporary stop of hydroxycarbamide. All eight patients successfully cleared the virus, within a median of 207 days (27–1306).

4.6. Ribavirin treatment

In total 30 patients were treated with ribavirin, starting a median of 97 days (0–1825) after the diagnosis of HEV infection (Table 2). Ribavirin was started at the discretion of the treating physician. Thirteen patients started within less than 3 months after diagnosis, 6 within 3–4 months and 11 patients more than six months after time of diagnosis. Median dose administered was 800 mg/day, i.e. 10.4 mg/kg (1.96–25.04) and median duration of treatment was 94 days (10–560). Eighty-seven percent of treated patients were immunocompromised whereas 76% in the non-treatment group were immunocompromised (p=0.036) due to a significantly higher prevalence of SOT and use of immunosuppressive drugs after transplantation. When comparing these groups, other causes for a suppressed immune system were equally distributed between treatment and non-treatment groups. In 10 patients the dose of immunosuppressive therapy was reduced a median of 45 days (10–365) prior to the start of ribavirin treatment. In 11 patients, ribavirin treatment was started concomitantly with reduction of the immunosuppressive therapy.

Eighty-three percent of patients (25/30) achieved SVR. Seven patients had a rapid viral response (<5 weeks from start ribavirin), 10 patients had an early viral response (<13 weeks) and 8 patients had a late viral response (>13 weeks) (Fig. 3). Median time until HEV clearance was 61 days (1–272). Four patients were still being treated at end of follow up (33, 91, 102, and 161 days of treatment). Three of these patients reached negative RT-PCR but had no sufficient follow up yet to ascertain SVR. In one patient treatment was interrupted because of incurable oropharyngeal carcinoma. One patient was treated for only 10 days because of respiratory syncytial virus and had a late viral response after 272 days. This response cannot be assigned to ribavirin with certainty.

Main adverse events were anemia, anorexia and affective disorders. In only one patient this caused a shortening of treatment by three weeks.

Among the 30 patients who were treated with ribavirin, only one patient had recurrence. She initially responded to ribavirin after treatment with 800 mg/day for 90 days. No HEV RNA was detected in serum. After three months, RT-PCR was positive again with rising viral loads. Phylogenetic analysis was performed with concatenated ORF1 and ORF2 sequences, which revealed 9 nucleotide changes in 810 bp hypervariable regions of the HEV genome. After one year, she was retreated successfully with ribavirin 800 mg/day for 120 days.

Table 2
Characteristics of patients treated with ribavirin.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients under observation</th>
<th>Patients who cleared infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, number</td>
<td>19 (63.3%)</td>
<td></td>
</tr>
<tr>
<td>Female, number</td>
<td>11 (36.7%)</td>
<td></td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>26 (86.7%)</td>
<td></td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>19 (63.3%)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>8 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>Heart (including heart + lung)</td>
<td>9 (30%)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>AlloHSCT</td>
<td>4 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive treatment after transplantation</td>
<td>22 (73.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: AlloHSCT: allogeneic stem cell transplantation.

Fig. 3. Time to HEV clearance (negative qualitative RT-PCR) in patients treated with ribavirin. Treatment was started at day 0.
We could not demonstrate a correlation between viral load, AST or ALT levels at start of therapy and time to disappearance of HEV RNA.

5. Discussion

We describe the clinical characteristics and outcome of HEV-3 infections in a tertiary referral hospital based population in the Netherlands and show that HEV can cause significant morbidity and contribute to mortality in patients. The off label use of ribavirin therapy provides viral clearance within a median of two months. To the best of our knowledge this retrospective single center study is the largest series so far to evaluate the clinical course and treatment outcome of HEV infection in Western Europe.

Our study showed a predilection for middle-aged men which is in line with several studies concerning HEV infections in developed countries [2,3].

Our study confirms the results of previous studies, in which chronic HEV infections are solely found in immunocompromised patients, mostly SOT-recipients [4,5]. Several studies revealed that approximately 60% of SOT-recipients develop chronic infections [4,6,7]. In our cohort, 47% of SOT-recipients developed chronic infections. This slight difference can be explained by two reasons. First, six out of 38 patients were successfully treated with ribavirin within six months after diagnosis. Hence, no evolution of the disease to chronicity was awaited. Second, two out of 38 patients had no sufficient follow up so a chronic infection cannot be excluded.

In immunocompetent patients, HEV-3 rarely causes a symptomatic hepatitis but it can result in acute fulminant hepatitis with decomposition in patients with underlying chronic liver disease [8,9]. Although studied among limited numbers, high mortality rates up to 70% in these patients have been reported [8]. In the present study, four immunocompromised patients died while still HEV RNA positive. Moreover, three patients with pre-existent cirrhosis died shortly after clearing the virus due to liver-related causes. The time from diagnosis to death was significantly shorter when compared to patients without underlying liver diseases.

In our study 29 patients had a reduction in dose of immunosuppressive treatment and 8 (27.6%) achieved viral clearance. Eighty-three percent of patients (25/30) were successfully treated with ribavirin for a median of 94 days (10–560) and 10% had a negative RT-PCR but not yet sufficient follow up to determine SVR. Median treatment duration to HEV clearance was two months which demonstrates a rapid response after the start of treatment. Only one patient had recurrence but achieved viral clearance after retreatment with ribavirin. Thirteen patients were treated after being infected with HEV for less than three months. We cannot exclude that (some of) these patients would have had spontaneous clearance without ribavirin treatment. In SOT-recipients it was found by Kamar et al. that if HEV RNA persists for more than three months, no spontaneous clearance will be observed between months 3 and 6 after infection [10]. Based on literature and our finding that SOT-patients did not have spontaneous HEV clearance in month 3–6 we recommend to start ribavirin treatment after three months in SOT-patients to prevent a chronic course with potential adverse events.

Conflicts of interest

None.

Funding

None.

Competing interests

None.

Ethical approval

Obtained from the Institutions Review Board of Erasmus MC, Rotterdam, the Netherlands.

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References