Hepatitis E virus genotype 3 is associated with gallstone-related disease

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ORIGINAL ARTICLE

ABSTRACT
Objective: Hepatitis E virus (HEV) genotype 3 is endemic in Northern Europe and despite a high seroprevalence of anti-HEV IgG antibodies among blood donors (>17%), few clinical cases are notified in Sweden. Low awareness of hepatitis E and its possible symptoms may contribute to this discrepancy. The aim of this study was to investigate the prevalence of acute HEV infection among admitted patients with abdominal pain and elevated liver enzymes.

Materials and methods: During 2016–2017, 148 adult patients with serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > twice normal levels were prospectively enrolled at surgical wards at three Swedish hospitals. Serum samples were analyzed for HEV RNA as well as anti-HEV IgM and IgG, and medical records were reviewed.

Results: Six (6/148, 4.1%) patients were HEV infected confirmed by detectable HEV RNA, but only one of these patients had detectable anti-HEV antibodies. Four of the HEV infected patients were diagnosed with gallstone-related disease: three with biliary pancreatitis and one with biliary colic. The remaining two were diagnosed with bowel obstruction and pancreatic malignancy. Four HEV strains were typed by sequencing to genotype 3.

Conclusions: This study identified acute HEV3 infection in 4% of the patients with elevated liver enzymes admitted to a surgical ward. HEV infection was not the solitary disease leading to hospitalization, instead it was found to be associated with other surgical conditions such as gallstone-related disease including biliary pancreatitis. Additionally, HEV RNA might be the preferential diagnostic tool for detecting ongoing HEV infection.

Introduction

Hepatitis E virus (HEV) is a small nonenveloped RNA-virus forming the Hepeviridae family. Five genotypes, HEV1-4 and HEV7, are known to infect humans, mainly via fecal-oral transmission [1]. Hepatitis E virus genotype 3 (HEV3) is endemic in Sweden, where the anti-HEV IgG seroprevalence is approximately 17% among blood donors [2]. However, in spite of this high seroprevalence, only 42 clinical cases were notified to The Public Health Agency of Sweden in 2017 [3]. Possible reasons for this large discrepancy include scarce awareness of HEV among clinicians, difficulties in accessing diagnostics, and that HEV3 often is associated with sporadic self-limited acute infections, with up to 70% of cases believed to have a mild or asymptomatic infection [4,5].

Clinical symptoms of acute HEV infection are similar to other causes of viral hepatitis, i.e., nausea, fever, malaise, arthralgia, abdominal pain and elevation of liver transaminases. Jaundice is reportedly present in approximately 75% of the symptomatic cases [6]. Additionally, the infection may be associated with extrahepatic manifestations including neurological symptoms, renal injury, hematological disorders, and acute pancreatitis [7].

Since clinical symptoms and laboratory abnormalities in acute HEV infection are nonspecific, they may overlap with several other clinical diagnoses. Patients with an acute HEV infection may present with abdominal pain and elevated liver enzymes, mimicking surgical conditions. Our hypothesis was that patients might be admitted to acute surgical wards due to suspected surgical conditions when the symptoms in fact are caused by an acute HEV infection. The aim of the present study thus was to investigate the prevalence of acute HEV infection in adult patients with elevated liver transaminases admitted to acute surgical wards.

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Materials and methods

Study design

Patients were prospectively enrolled from three different hospitals in the western part of Sweden (Region of Västra Götaland). Patients 18 years or older, who were admitted to an acute surgical ward and showed presence of elevated serum liver transaminases, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) more than twice the upper normal limit, were asked to participate. Written informed consent was obtained before blood sampling for HEV analysis. Enrolling occurred at South Älvsborg Hospital in Borås during March-May 2016, at Sahlgrenska University Hospital in Gothenburg from November 2016 to June 2017, and at Skaraborg Hospital in Skövde during April–September 2017.

Serum samples were collected from all enrolled patients and stored at −20 °C until analyzed. Medical records were reviewed for laboratory parameters, symptoms, surgical procedures and final diagnosis. Patients with either detectable HEV RNA and/or reactive anti-HEV IgM serology were considered to have an acute HEV infection. These patients were subsequently called for a follow-up examination at the local infectious diseases clinic. Two senior consultant surgeons performed a retrospective review of all cases with acute HEV infection, after completion of the study according to a pre-specified protocol.

Ethical considerations

The study conformed to the guidelines of the 1975 Declaration of Helsinki. The ethical committee in Gothenburg, Sweden, approved the study (DNR: 534-16 and 737-12). Written informed consent was obtained from each patient enrolled in the study.

Detection of anti-HEV antibodies and HEV RNA

All serum samples were analyzed a few weeks after sampling for anti-HEV IgG and IgM using the HEV IgM/HEV IgG test (DiaPro, Milan, Italy) according to the manufacturer’s instructions [2] and for HEV RNA by PCR. Samples with OD/cut-off OD ≥1.7 for anti-HEV IgG and OD/cut-off OD ≥1.5 for anti-HEV IgM were considered positive for anti-HEV IgG and IgM, respectively [2]. All serum samples were analyzed for HEV RNA twice in duplicate by RT-qPCR and semi-nested PCR as using the same primers as in the PCR.

Normalized alanine aminotransferase (nALT) and aspartate aminotransferase (nAST)

Normalized ALT was defined as ratio between the measured ALT and the upper limit of normal (men 1.1 µkat/L and women 0.75 µkat/L in the present study). Similarly, normalized AST was defined as ratio between the measured AST and the upper limit of normal (men 0.75 µkat/L and women 0.6 µkat/L). Thus, nALT or nAST values above 1.0 are pathological irrespective of gender or analysis method utilized.

Statistical methods

Mann–Whitney U-test was used to compare laboratory parameters between groups. Statistical analyses were performed using SPSS-version 25 with a p < .05 considered as significant.

Results

A total of 148 patients were prospectively enrolled; 50 from Borås, 48 from Gothenburg, and 50 patients from Skövde. The median age was 60 years (interquartile range IQR: 43–73 years) and 75 (51%) were men. The most common clinical symptoms were abdominal pain (82%), followed by nausea and/or vomiting (58%). Laboratory parameters for all included patients are detailed in Table 1. The most common diagnoses at discharge were gallstone related, e.g., cholecystitis (15%) and biliary pancreatitis (14%) (Table 2). Ninety-six patients (65%) underwent surgical procedures. Acute cholecystectomy (21%) and endoscopic retrograde cholangiopancreatoscopy (ERCP) with sphincterotomy (20%) were the most prevalent acute procedures while 15% of the patients were scheduled for an elective cholecystectomy (Table 3).

Prevalence of anti-HEV IgG and IgM antibodies and HEV RNA

Six of the 148 patients (4.1%) had an acute HEV infection (patients A–E), with HEV RNA detected in serum from all six patients. Only one of these patients (patient E) had a simultaneous positive anti-HEV IgM reactivity. This patient (patient E) additionally had a weak reactive anti-HEV IgG serology, whereas the other five patients (patients A–D, F) lacked anti-HEV antibodies. The HEV strains from four patients could be genotyped by sequencing, all were HEV3 (Table 4). There were 36 patients (24%) with positive anti-HEV IgG serology, of which 18/148 (12%) were weakly reactive for anti-HEV IgG, with an OD/cut-off OD between 1.7 and 3.5. The remaining 112 patients (76%) were negative for anti-HEV IgG.

Table 1. Laboratory parameters in patients with and without acute hepatitis E, the maximum value during admission is reported.

| Laboratory parameters (normal level) | HEV RNA negative | HEV RNA positive |
|-------------------------------------|------------------|-----------------|
| **nALT** (1)                        | 4.7 (2.5–8.5)    | 11.3 (6.1–25.8) |
| **nAST** (1)                        | 3.4 (1.8–6.7)    | 9.5 (1.2–18.3)  |
| **ALP** (0.6–1.8 µkat/L)            | 3.7 (2.2–6.0)    | 3.8 (2.4–11.8)  |
| **Bilirubin** (5–25 µmol/L)         | 44 (18–81)       | 27 (16–88)      |
| **CRP** (<3 mg/L)                   | 69 (19–149)      | 17 (5–29)       |
| **Temperature (°C)**                | 37.7 (37.2–38.3) | 38.0 (37.4–38.3) |

*ALP = alkaline phosphatase.*
Table 2. The final discharge diagnosis according to the medical records.

| Diagnosis                                         | n (%) | HEV clinical cases |
|--------------------------------------------------|-------|--------------------|
| Gallstone-related except biliary pancreatitis     | 66 (45)|                   |
| Cholecystitis                                     | 22    |                    |
| Biliary colic                                     | 13    | 1                  |
| Common bile duct stone without cholangitis        | 21    |                    |
| Common bile duct stone with cholangitis           | 10    |                    |
| Pancreatitis                                      | 28 (19)|                   |
| Biliary                                           | 21    | 3                  |
| Alcohol                                           | 2     |                    |
| Other                                             | 5     |                    |
| Surgical (acute abdomen, trauma, and abdominal pain) | 27 (18)| 1               |
| Gastrointestinal malignancy                       | 18 (12)| 1                |
| Medical (sepsis, mononucleosis)                   | 5 (3) |                    |
| Liver disease                                     | 4 (3) |                    |

Table 3. Surgical procedures performed.

| Intervention | n (%) | HEV clinical cases |
|--------------|-------|--------------------|
| Cholecystectomy acute | 31 (21) | 2 |
| Endoscopic retrograde cholangiopancreatography (ERCP) | 29 (20) | 1 |
| Elective cholecystectomy | 22 (15) | 2 |
| Other surgery             | 14 (9) | 1 |
| No surgery                | 52 (35)|                   |

Table 4. HEV RNA PCR and anti-HEV serology for the patients with acute hepatitis E.

| Patient | A | B | C | D | E | F |
|---------|---|---|---|---|---|---|
| HEV RNA | + | + | + | + | + | + |
| Genotype | HEV3 | HEV3 | – | – | HEV3 | HEV3 |
| Anti-HEV IgM | – | – | – | – | + | – |
| Anti-HEV IgG | – | – | – | – | (+) | – |
| Follow up anti-HEV IgG | Not | Not | Not | Not | Not | Not |
| Follow up tested | tested | tested | tested | tested | tested | tested |

Clinical presentation of the patients with acute HEV

All six patients with acute HEV were diagnosed with a clinically relevant surgical diagnosis. Four of the six HEV infected patients (patients A–D) had a gallstone-related diagnosis. Three of them presented with acute biliary pancreatitis, acute abdominal pain together with an elevated amylase (three times normal level). They all had verified gallstones and underwent cholecystectomy within two weeks. One patient (patient D) had cholecystolithiasis with biliary colic and had an acute cholecystectomy. The remaining two patients with acute HEV infection had differing conditions. Patient E had symptoms consistent with mechanical bowel obstruction and underwent a laparotomy, relieving these symptoms, but liver enzymes remained elevated after surgery. Patient F presented with jaundice without abdominal pain, and an abdominal computed tomography (CT) scan demonstrated a pancreatic malignancy.

The patients with acute HEV infection in the present study had a significantly higher median nALT in comparison to the patients with undetectable HEV RNA (11.3 (IQR 6.1–25.8) vs. 4.7 (IQR 2.5–8.5), respectively; p=.01, Mann–Whitney U-test). When restricting this analysis to patients with gallstone-related diagnoses, nALT was 7.1 (IQR 5.8–20.0, n = 4) vs. 7.2 (IQR 3.7–11.5, n = 83) for patients with and without detectable HEV RNA respectively (p=.47). Similarly, the median C-reactive protein (CRP) among patients with acute HEV infection was significantly lower than among non-infected patients (17 mg/L (IQR 15–29) vs. 69 mg/L (IQR 19–49), respectively; p=.02). Clinical and laboratory data of all HEV cases are detailed in Table 5.

None of the HEV infected patients enrolled were immunosuppressed. Five of the six patients were followed-up regarding recovery and risk factors (one could not be reached). At follow-up examination after 6–14 months, all HEV infected patients had recovered spontaneously from their HEV infections. From two of the six patients we were able to follow-up the serological tests, both patients lacked anti-HEV antibodies one year after the infection (Table 4). Risk factors for HEV were assessed, one patient had eaten game meat (moose) one month prior to admission, and one patient had tasted raw pork while cooking. The remaining patients lacked known risk factors for HEV infection, none had recently received a blood transfusion, and none had a travel history to developing countries.

Discussion

To our knowledge, this is the first prospective study on the association of HEV3 and acute surgical diagnoses. It demonstrated that approximately 4% of patients admitted with elevated liver enzymes and symptoms requiring acute surgical care had an ongoing HEV infection, which is the dominant genotype in high-income countries [13], and pancreatitis. All patients described to date, with HEV associated with pancreatitis, have either...
lived in Southern Asia or recently traveled to HEV1 prevalent regions [14,15]. Only one strain from these patients was genotyped, and identified as HEV1, as expected [14].

The rationale for this study was the large discrepancy between the high seroprevalence of HEV antibodies among blood donors and the low number of clinical cases notified to the Public Health Agency of Sweden [3]. As an active HEV infection can cause unspecific abdominal pain [6] we hypothesized that some of these patients were admitted to a surgical ward without other surgical conditions. Instead, we found that patients with acute HEV infection simultaneously had clinically relevant surgical diagnoses, i.e., 4 out of 87 patients (4.6%) had a combination of gallstone-related disease and concomitant HEV infection. However, it is important to note that the inclusion criteria requiring elevated liver enzymes might result in a selection bias toward finding gallstone-related diseases and that the suggested association between gallstone-related diagnoses and HEV infection in the current observational study does not confirm causality. Patients could have had an asymptomatic HEV infection and simultaneously other confounding conditions.

One limitation of the present study is the lack of a prospective control group. However, when compared with historical controls from previous studies evaluating HEV infection among various patient cohorts [16] we found a considerably higher prevalence of HEV RNA in the current study. This supports the theory that HEV is actually causing symptoms and is not simply an innocent bystander. In a previous study where we evaluated the prevalence of HEV among 204 patients from the same geographical region with chronic hepatitis C virus (HCV) infection and elevated liver transaminases (with 85% having ALT and/or AST in excess of twice the upper normal limit) we only found one patient with asymptomatic HEV infection [16]. The prevalence of detectable HEV RNA in the present study is significantly higher when compared with this historic cohort (6/148 (4.1%) vs. 1/204 (0.5%); \( p = 0.045 \), Fisher’s exact test). Additionally, the prevalence of HEV RNA in serum from patients in the present study was much higher than in sera from healthy Swedish blood donors (4.1% vs. 0.09%; personal communication), also supporting the notion of causality. Confirmation of the HEV RNA positive results by additional sampling of the patients was not possible, due to the time period of several weeks from the first sampling to analysis during which the amount of HEV RNA in serum would have reached undetectable levels. However, the possibility of false-positive HEV RNA results in this study is probably not likely, since all samples were confirmed by repeated HEV PCR tests, and the four samples sequenced all had unique genotype 3 strains.

Among the six patients with detectable HEV RNA, only one had detectable anti-HEV IgM and IgG antibodies. This is a novel and surprising finding, implying that HEV RNA analyses should be included when evaluating for possible HEV infections. Norder et al. [17] have reported a hypothesis aiming at explaining why some patients do not seroconvert despite documented HEV exposure. They noted that HEV RNA positive, but serologically negative, plasma donors have a genomic HEV variant containing a CUG sequence instead of AUG as the start codon for open reading frame ORF3. The subsequent lower expression of the encoded ORF3 protein may alter its impact on innate immune responses as well as type I interferon production, resulting in lower seroconversion rates [17].

All six patients with HEV infection in the present study underwent surgical interventions. After retrospective review by two senior consultant surgeons, it was determined that the surgical management and interventions would not have been altered if the ongoing acute HEV infection had been known during the admissions. Whether the ongoing HEV infection affected the severity of the gallstone-related disease cannot be evaluated in the present study as only hospitalized patients were enrolled. The current findings thus warrant further investigations as the detection of HEV RNA possibly might be useful for prognostication in the setting of gallstone disease.

### Conclusions

HEV 3 infection was found in 4% of patients admitted to surgical wards with abdominal pain and elevated liver enzymes. Among the six cases with HEV infection, four had gallstone-related diagnoses suggesting that HEV might trigger or enhance gallstone-associated disease. Therefore HEV infection should be considered in patients with elevated liver transaminases, even in the presence of surgical conditions.
Current findings also suggest that HEV RNA is the preferential diagnostic tool for the detection of ongoing HEV infection, and should be included in standard HEV testing. More studies are warranted to confirm these results and to further explore the association of acute HEV infection and gallstone-related disease.

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