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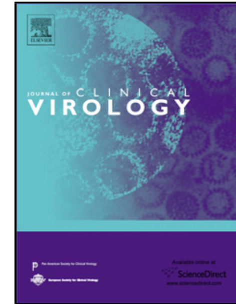

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Clinical burden of Hepatitis E virus infection in a tertiary care center in Flanders, Belgium

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Highlights

- HEV is increasingly recognized as a cause of hepatitis in developed countries.
- Seroprevalence in East and West Flanders, Belgium is 14% in general population.
- All HEV IgM/RNA positive patients were men, aged 41 to 63 years old.
- HEV should be investigated especially in men with elevated liver enzymes.

Abstract

Background: Hepatitis E virus (HEV) infection is increasingly recognized as a cause of hepatitis in developed countries. A high HEV IgG seroprevalence in humans and pigs is reported as well as sporadic clinical cases of autochtonous HEV but there are currently no data available on the clinical burden of HEV in Belgium.

Objectives: The objective of the current study was to evaluate the actual clinical burden of HEV infections in our tertiary care center in Flanders, Belgium

Study design: In the setting of Ghent University Hospital, patients were assessed for the presence of HEV IgG and IgM as well as HEV RNA if no other cause was found for one of the following clinical presentations: a) elevation of liver enzymes in post-liver transplant; b) suspicion of acute or toxic hepatitis; c) unexplainable elevation of liver enzymes; d) cirrhosis with acute-on-chronic exacerbation.

Results: In a period of 39 months (January 2011 – April 2014) 71 patients were enrolled. HEV IgG was found positive in 13 (18,3%) patients; HEV IgM in 6 patients

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(8,5%) and HEV RNA in 4 (5,6%) patients. All HEV IgM/RNA positive patients were male, aged 41 to 63, and classified in the clinical groups a),b) or d). HEV IgG seroprevalence was slightly higher but not significantly different from the seroprevalence in the general population in this region in Belgium previously reported to be 14% (p-value 0.41) by our group.

Conclusions: HEV should be considered as a cause of liver pathology especially in middle-aged men with elevation of liver enzymes.

Keywords

Hepatitis E virus, clinical impact, Belgium

Background

Hepatitis E virus (HEV) is a small, non-enveloped, single stranded RNA virus. Four HEV genotypes exist. HEV genotypes 1 and 2 are typically associated with human epidemic outbreaks in developing countries. They are transmitted between humans by the fecal-oral route. By contrast, HEV genotypes 3 and 4 are transmitted foodborne and zoonotically from animal reservoirs. They usually occur as sporadic cases and are emerging pathogens in the developed world [1-6]. HEV can be complicated by serious complications such as neurologic disorders. Moreover, unlike the other HEV genotypes, HEV genotype 3 can be associated with chronic infection (defined as the presence of HEV RNA in serum or stool ≥6 months) [1-3,6-8]. Immunocompromised persons are at risk [4,8].

Hepatitis E virus (HEV) infection is increasingly recognized as a cause of hepatitis in developed countries [6,9]. A high HEV IgG seroprevalence in humans and pigs is reported in Belgium [10,11]. Van Hoecke et al. observed a seroprevalence rate of 14%

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in humans in East and West Flanders [10]. Also, sporadic cases of autochthonous HEV infection were described [12,13].

Clinical features of a HEV infection are diverse. Most of the infections with HEV are asymptomatic. If symptoms occur, the clinical picture varies from mild disease to (sub)acute or fulminant liver failure. Especially patients with pre-existing liver disease are at risk for serious clinical manifestations and death [7]. Routine laboratory parameters are often nonspecific. Therefore a HEV infection may be indistinguishable from other clinical conditions such as other causes of viral hepatitis, autoimmune hepatitis or drug-induced liver injury [1-9,14].

Objectives

The objective of the current study was to evaluate the clinical burden of HEV infections in the Ghent University Hospital, Belgium, using both serological assays (anti-HEV IgM and IgG) and PCR (HEV RNA).

Study design

In the setting of Ghent University Hospital, clinically suspected patients for HEV infection were assessed for the presence of anti-HEV IgM and IgG as well as HEV RNA. The study was approved by the Ethical Committee of Ghent University Hospital under Belgian Registration number B670201110350. After exclusion of other plausible causes during routine diagnostic workout, patients with the following clinical presentations were included: a) an elevation of liver enzymes post-liver transplant; b) suspicion of acute or toxic hepatitis; c) unexplainable elevation of liver enzymes; d) cirrhosis with acute-on-chronic exacerbation.

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Samples

In total, 71 patients were included in this study during a period of 39 months (January 2011-April 2014). From each included patient, two serum tubes were collected: one for HEV serology and one for HEV PCR. Serum for serology and PCR was stored separately at -20°C and -80°C respectively prior to analysis.

Serology

HEV serology was performed using Wantai anti-HEV IgM and IgG commercial ELISA assays (Wantai Biological Pharmacy, Beijing, China). The assays were executed in accordance with the manufacturer's instructions. Signal to cut-off ratios (s/co) were interpreted as prescribed.

PCR

HEV RNA was determined on all clinical samples using a commercial HEV RT-PCR assay targeting ORF 3 (RealStar® HEV RT-PCR Kit 1.0, Altona Diagnostics GmbH, Hamburg, Germany). The NucliSENS® easyMAG® (bioMerieux, Marcy l'Etoile, France) automated nucleic acid extraction system was used for RNA extraction (bioMérieux, Boxtel, The Netherlands). The Altona assay was performed according to the manufacturer's instructions. PCR assays were performed on the CFX96 real-time PCR detection system (Bio-Rad, Nazareth, Belgium).

Sequencing and phylogenetic analysis

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Sequencing and phylogenetic analysis was performed in the National Reference Centre for Viral Hepatitis (NRC) (WIV-ISP, Brussels, Belgium).

The sequencing was done by an in-house developed method on the ABI3130 automatic capillary sequencer. The primers used are amplifying a fragment of 348bp in the ORF2 region of the HEV genome [16]. The reference set consisted of 18 different know strains of human HEV and the prototype US strain of swine HEV [17].

Sequence analyses, alignments and phylogenetic trees were realized by the statistical programs DNASTAR Lasergene 10 Core Suite (DNASTAR®, Madison, USA) and the MEGA 5.2 software [18].

Statistics

Statistical analysis was carried out using Student t-test in SPSS (IBM® SPSS® Statistics, version 22).

Results

In a period of 39 months (January 2011 until April 2014), 71 patients were enrolled. The studied population consisted of 41 men (58%) and 30 women (42%). The average age was 48years (range 15-75 years). The majority of patients (26 patients, 37%) presented with a clinical suspicion of acute or toxic hepatitis (group b). Besides, there were 21 patients (30%) with an elevation of liver enzymes (group c), 17 post-liver transplant patients (24 %) with an elevation of liver enzymes (group a),4 patients (5,6%) with cirrhosis with acute-on-chronic exacerbation (group d) and 3 patients who belonged to two categories (Table I).

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HEV IgG was found positive in 13 patients (18,3%), HEV IgM in 6 patients (8,5%). HEV RNA was detected in 4 out of 6 IgM positive patients: 3 patients were found HEV RNA positive on the study sample using both our commercial PCR method as well as the commercial PCR used in the NRC; the last patient initially tested negative for HEV RNA in both centers (study sample,29/08/2013), but HEV RNA was detected in the NRC on an earlier sample of the same patient (09/07/2013, with elevation of liver enzymes at that time).

Sequencing revealed two strains belonged to genotype 3, one was subtyped as genotype 3f, the other as genotype 3h. Sequencing of the other two strains failed technically presumably due to a low viral load in the samples. Table II provides a summary of these results.

All four HEV IgM and RNA positive patients were male, aged 41 to 63 years. The patients presented with a varying clinical picture: two of these patients had an acute or toxic hepatitis (group b), one was a cirrhosis patient with an acute-on-chronic exacerbation (group d) and the last was a liver transplant patient with elevation of liver enzymes (group a). Liver function parameters (ALT, AST, gamma-GT and alkaline phosphatase) and bilirubin at the moment of presentation are displayed in table III. All patients showed disturbed liver enzymes. Bilirubin was increased in 2/4 patients. The clinical picture ranged from subclinical (patient 4) over mild/moderate disease with spontaneous recuperation (patient 1 and 3) to an acute and severe hepatitis which necessitated an urgent liver transplantation (patient 2). None of the patients developed a chronic infection.

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HEV IgG seroprevalence in this study, 18,3%, was slightly higher but not significantly different from the seroprevalence in the general population in Belgium reported to be 14% (p-value 0.41) [10].

Discussion

HEV genotype 3 is described as the major cause of sporadic cases of autochthonous HEV in Europe [2,5-8]. Most of the infections with HEV genotype 3 are asymptomatic. If symptoms occur, the clinical picture can vary widely. HEV often presents as an acute viral hepatitis similar to other viral hepatitides. However, some patients exhibit subacute or fulminant acute liver failure [1-9]. HEV can also be complicated by serious complications such as neurologic disorders or chronic hepatitis [1-9]. In our study, two of the HEV IgM and RNA positive patients had a clinical picture of an acute viral hepatitis, one was asymptomatic, and the last suffered an acute hepatic failure which necessitated an urgent liver transplantation. All four patients were male, aged 41 to 63 years. According to the literature, HEV genotype 3 infections most frequently affect middle-aged or elderly persons. Men outnumber women [2-9]. However, these findings may reflect a predisposition of clinically apparent disease rather than a different infection rate depending on age or gender [4,5,7].

HEV IgM was found positive in 6 patients (8,5%). Other studies in Europe report similar numbers (5-15%) [6]. HEV RNA was finally detected in 4 out of 6 HEV IgM positive samples. However the utility of PCR is limited due to the short period of detectable HEV viremia. As has been seen in patient 4, a negative PCR result on a sample positive for HEV IgM cannot completely rule out an acute HEV infection which

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is consistent with the published literature [2,5-9]. On the other hand, commercial HEV ELISA assays also show a wide variety in sensitivity and specificity [1-9]. Thus, a suboptimal specificity, for example due to cross-reactivity, could also explain positive results for HEV IgM.

As expected, sequencing results of the HEV strains found in this study revealed two strains belonged to genotype 3. One was subtyped as genotype 3f, the other as genotype 3h. Genotype 3h consists of animal and human strains and has been isolated in Italy, Uruguay and New Zealand [6,8]. Genotype 3f comprises human, swine and sewage strains isolated from Japan, Thailand, New Caledonia and most European countries [6,8].

Several anti-HEV IgG seroprevalence studies on blood donors or patient groups at risk for HEV infection have been carried out in European countries. These studies show a significant variability among different geographical areas [6]. Belgian data on patients at risk for HEV were lacking. HEV IgG seroprevalence in this study was 18,3% and slightly higher but not significantly different from the seroprevalence in the general population in our region in Belgium reported to be 14% (p-value 0.41) [10]. Differences in patient populations might explain these findings. Van Hoecke et al. [10] used randomly selected, asymptomatic patients presenting at the gynaecological or orthopaedic clinics of the Ghent University Hospital. The average age of the individuals was 45 years (range 17-82 years). The male/female ratio was 1. The patients in our study comprised a population at risk for HEV infection. Moreover, the patients were slightly older (mean age 48 years, range 15-75 years) and there was a small

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preponderance of male (58%), both risk factors for (clinically apparent) HEV infection [4,5,7].

Our findings suggest that serology is useful as a screening tool for HEV infection in a patients at risk for HEV. HEV RNA PCR can be used in HEV IgM positive patients to confirm the diagnosis of an acute HEV infection, as a baseline for patients follow-up and for diagnosis of a chronic HEV infection. We were unable to show an added value of screening all patients with HEV RNA PCR, probably because of the small sample size, heterogeneous composition of studied population and a short period of HEV viremia. The results needs to be confirmed in other specific patient populations.

HEV should be considered as a cause of liver pathology especially in middle-aged men with elevation of liver enzymes. The prevalence of confirmed acute HEV infection was 5,6% in the studied University Hospital population in East and West Flanders. The seroprevalence of HEV IgG was 18,3%. The fact that during >3 years period of the current prospective study only 71 patients were included based on their clinical presentation may reflect the rather low awareness among medical professionals of the importance of HEV infection in liver pathology. This accentuates the importance of future research in this subject in this particular geographical region.

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Authors contribution: L. Cattoir has written the manuscript based on data collection and final analysis of the data; F. Van Hoecke and T. Van Maerken initiated the initial collection of study samples and evaluated the methods to be used in the study; E. Nys and I. Ryckaert performed actual analysis of study samples; M. De Boulle did profound literature search; A. Geerts, X. Verhelst, I. Colle and H. Van Vlierberghe recruited patients and collected clinical information and samples; V. Hutse, V. Suin, M. Wautier and S. Van Gucht performed confirmatory analysis and genotyping; E. Padalko conceptualized and designed the study. All the authors read and approved the final manuscript.

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Table I. Overview of the included patients and study results

Group	Clinical picture	Age Mean (Min-Max)	Gender (M/F)	Number of patients	HEV IgG positive	HEV IgM positive	HEV RNA positive
a	Elevation of liver enzymes in post-liver transplant	54 (27-75)	11/6	17	2	2	1
b	Acute or toxic hepatitis	46 (17-75)	16/10	26	6	2	2
c	Elevation of liver enzymes	45 (15-63)	9/12	21	3	1	0
d	Cirrhosis with acute-on-chronic exacerbation	56 (31-69)	3/1	4	2	1	1
e	Patients belonging to two categories	46 (31-57)	2/1	3	0	0	0
	Total	48 (15-75)	41/30	71	13 (18,3%)	6 (8,5%)	4 (5,6%)

Overview of the included patients and study results

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Table II. Lab results of the HEV IgM positive patients.

Patient	Group	Sample date	HEV IgG	HEV IgM	HEV RNA	HEV genotype	Sample date	ALT (U/L)	AST (U/L)	GGT (U/L)	AP (U/L)	BIL (mg/dL)
1	b	13/04/2011	pos	pos	pos	3f	13/04/2011	5045	2891	445	498	13
2	d	25/07/2013	pos	pos	pos	3h	23/03/2013	1166	2412	281	185	23,5
3	b	10/03/2011	pos	pos	pos	/*	24/02/2011	1452	499	559	253	0,5
4	a	29/08/2013	pos	pos	pos**	/*	09/07/2013	250	215	215	225	0,6

Lab results of the HEV IgM positive patients. Alanine transaminase (ALT), reference range 7-40 U/L for males; Aspartate aminotransferase (AST), reference range 0-37 U/L for males; Gamma-glutamyl transpeptidase (GGT), reference range 0-37 U/L for males; Alkaline phosphatase (AP), reference range 30-120 U/L for males; Total bilirubin (BIL), reference range 0,2-1,3 mg/dL; *: technically failed; **: the original analysis on the study sample (29/08/2013) for HEV RNA was negative while HEV PCR in the NRC was positive on an earlier sample with elevated liver enzymes (09/07/2013).

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