Hepatitis E is an autochthonous disease in industrialized countries
Analysis of 23 patients in South-West France over a 13-month period and comparison with hepatitis A

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SUMMARY
Objectives — Hepatitis E virus (HEV) is responsible for acute hepatitis predominantly in developing countries. In Western Europe and in the US, cases of acute HEV infection are uncommon and occur primarily in travelers returning from endemic countries. The aim of this study was to describe patients with acute hepatitis E in South West France and compare them with patients with acute hepatitis A.

Methods — 23 consecutive patients over 13 months were analysed. Acute hepatitis E was diagnosed on the presence of specific serum antibodies or viral RNA detection in serum or stools. Real time PCR products from viraemic patients were sequenced.

Results — All the HEV sequences belonged to genotype 3. Two patients (8%) died during their hospital stay, both suffered from severe underlying disease. Only 3 patients (13%) had travelled outside of Europe, within 3 months of the onset of disease. When compared to 23 patients with acute hepatitis A at the same hospital and during the same time frame, HEV-infected patients were older (54.4 ± 16.6 vs 24.5 ± 16.6, P < 0.05), had lower ALT levels (55.4 X upper normal limit ± 48.6 vs 107.8 X upper normal limit ± 82.8, P < 0.05) and had lower incidence of recent travel outside of Europe (13% in the hepatitis E group vs 60% in the hepatitis A group, P < 0.05).

Conclusions — Hepatitis E can be considered an autochthonous infection in South West France. All strains sequenced were related to genotype III. When compared to hepatitis A, HEV-infected patients were older, had lower ALT levels and had a lower incidence of travel outside of Europe.
Introduction

The hepatitis E virus (HEV) is responsible for acute hepatitis predominantly in developing countries including Central America, Africa, subcontinental India, Asia and the southeast pacific. Infection occurs both sporadically and in large outbreaks. The overall fatality rate ranges from 0.5% to 4% [1]. Pregnant women have a much higher fatality rate reaching 20%, for reasons unknown [2].

HEV is an RNA virus transmitted by the fecal-oral route, often through contaminated water and is therefore often compared to the hepatitis A virus. Both viruses also have similar clinical outcomes resulting in acute, resolving hepatitis. Geographically distinct isolates of HEV have been identified and classified according to four genotypes.

In Western Europe and in the United States of America, cases of acute HEV infection are uncommon and occur primarily in travelers returning from endemic countries. Sporadic cases have been identified in patients who never visited endemic countries and the mode of transmission in these patients remains obscure [3-11]. HEV and HEV-like viruses have been noted in a variety of animals including swine, sheep, cattle, goats and recently deer [12-15]. Zoonosis may therefore be involved in the transmission of HEV, especially in non-endemic areas.

We report here a series of 23 consecutive patients with acute hepatitis E in South West France, most of whom were autochthonous and diagnosed upon finding HEV antibodies in serum and/or viral RNA in serum and/or stool using a real time PCR assay. These patients were compared to 23 patients with acute hepatitis A diagnosed during the same period, in the same hospital.

Patients and methods

Patients

Twenty three consecutive patients were diagnosed with acute hepatitis E from August 2001 to September 2002. All biological samples were collected from patients at the Toulouse University Hospital. Eighteen patients were hospitalized and 5 were seen as outpatients. Hepatitis E was diagnosed based on elevated transaminases and the presence of specific serum antibodies and/or viral RNA detection in serum and/or stools. All sera were negative for hepatitis B virus antigen, antibodies and DNA, hepatitis C virus antibodies and RNA, immunoglobulin-M (IgM) class antibodies to hepatitis A virus, Cytomegalovirus and Epstein Barr Virus. Anti-nuclear, anti smooth muscle and anti LKM-1 antibodies were also negative. Toxic and drug-induced hepatitis was ruled out on medical history. HEV positive patients were compared to 23 consecutive patients with acute hepatitis A observed during the study period in the same hospital. These patients were selected on the basis of positive anti HAV IgM antibodies from the database of the virology department. Patients in whom epidemiological, clinical and biological data were available were considered. Other causes of acute hepatitis were also ruled out.

Clinical and biological features of the patients

Serological testing

Immunoglobulin-G (IgG) class antibodies to HEV were detected with an enzyme immunoassay (Abbott HEV EIA, Laboratoire Abbott, Rungis, France). This EIA uses two recombinant proteins “SG-3” from ORF2 and “8-5” the full length of ORF3 from the Burmese strain (genotype I) expressed in E. coli. Anti-HEV IgM antibodies were not tested as no commercial kits for identifying them were available in France at the time of the study.

ABBREVIATIONS:
- HEV : hepatitis E virus
- HAV : hepatitis A virus
- GGT :gamma-glutamyl transpeptidase
- ALT :alanine transaminase
- AST :aspartate transaminase

Anti-HAV IgM antibody detection was performed with an automated EIA (Axysym HAVAB-M, Laboratoires Abbott, Rungis, France). For both EIAs, data were analyzed according to the manufacturers’ instructions.

HEV genome detection

After extraction, purification of the nucleic acid (High Pure viral Nucleic Acid Kit Roche diagnostics, Meylan, France), and reverse transcription in cDNA (MMLV reverse transcriptase Invitrogen), a real time PCR assay was performed using the TaqMan format for the real time LightCycler (LC) technology. Primer pairing was done from the ORF2 region (viral capsid) to perform the amplification of a 189 base product (primer sense 5’GAGCAGAATGATTCTGGCGGT3’, primer anti-sense 5’GTGTTTGGTACCTCTG3’, fluorogenic probe 5’(6-Fam) GTGCTCTGCAATTGGGACG3’ (Tamra)’).

Real time PCR was carried out in a Light Cycler™ capillary using FastStart™ DNA Master Hybridization probes (Roche Diagnostics, Meylan, France). The reaction, data acquisition and the analysis were done using Light Cycler™ instrument software.

Sequencing of HEV isolates and phylogenetic analysis

Real time PCR products from viraemic patients were sequenced in the sense and anti-sense directions using the fluorescent dye terminator method (Big Dye Terminator cycle sequencing, Applied Biosystems, Paris, France). Electrophoresis and data collection were performed on an Applied Biosystems ABI 3 100 Genetic analyzer. Sequences were aligned using the Sequence Navigator™ programs and compared with other HEV sequences (nucleotide sequences of HEV strains obtained from Burma, BU (M73218), Mexico, ME (M74506), China, CH1 (AJ272108), CH2 (AF005471), India, IN2 (AF082843), IN1 (AF082842), Japan, JP (AB089824), Italy, IT (AF110390), United States, USA1 (AF060668), swine US, sw USA (AF082843), swine Canada, sw CD (AY115488), Spain, SP (AF491001), swine Spain, sw SP (AF491002), Spain sewage water, sw WP (AF491004), swine Netherlands, sw NL (AF336294), Austria, AU (AF279123), France sewage water, sw FR (AF490999)).

Sequences were first aligned with a multiple sequence editor CLUSTAL W version 1.6 [16]. The sequences were gap-stripped and the pairwise matrix was generated with a DNADIST program in the PHYLIP version 3.57c package [17]. Tree topology was inferred by neighbor-joining with a Kimura two-parameter distance matrix (PHYLIP) with a transition/transversion ratio of 2.0 and drawn with TREEVIEW version 1.4 [18]. The bootstrap analysis was performed with CLUSTAL W (1 000 re-samplings) to place approximate confidence limits on individual branches. The numbers at the nodes indicate the frequency with which the node occurred in 1000 bootstrap replicates.

Statistical analysis

Statistical analysis was performed using the SPSS package of statistical programs (SPSS Inc., Chicago, USA). Results were expressed as means ± standard deviations. Student's t-test and chi square test were used for comparing quantitative and qualitative variables respectively. Multivariate analysis used logistic regression.

Results

Clinical and biological features of the patients

Twenty three patients (6 females, none were pregnant) were diagnosed with acute hepatitis E. Their main clinical and biological features are shown in table I. Five patients were diagnosed as outpatients. All but 2 patients were referred with jaundice (91.3%). Fever was present in 11 (47%) patients, diarrhea in 2 (8.7%) and a skin rash in 2. Fever developed 5.3 ± 3.4 days before onset of jaundice (range : 0 to 10 days) and was higher than 39°C in 5 patients (45% of the patients with fever). Six patients (26%) had arthralgia associated to myalgia in 2. One patient was asymptomatic and one had fever alone. This latter patient was an 8 year old child. Asthenia was noted in 12 patients (52%).

Evidence of excessive alcohol consumption (> 40 g/d) was noted in 7 patients.
HEV serological testing, HEV genome detection and phylogenetic analysis

The virological data are shown table II.

Table II. – Virological results for hepatitis E markers. Stools were unavailable for genomic testing in 14 patients.

Résultats des marqueurs viraux pour l’hépatite E. L’analyse de selles n’a pas été possible chez 14 malades.

### Table I. – Baseline characteristics of the patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HEV (n = 23)</th>
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<td>Age (Year)</td>
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<tr>
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<td>12/8*</td>
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* : mean ± standard deviation, ^ : mean percentages ± standard deviation, \ : 3 patients could not be reached and questioned about travel history.

Abbreviations: HEV hepatitis E virus, HAV: hepatitis A virus, N: upper normal limit.

Table II. – Baseline characteristics of the patients.

Caractéristiques clinico-biologiques des malades.

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**HEV contamination**

Seven patients (30%) had travelled outside of France, 3 of these (13%) outside of Europe, within 3 months of the onset of disease. Four patients had visited Spain, 15 days, 21 days, strain (GenBank accession number AF110390). Among the second group, strains were very similar, forming a cluster.

There was no difference in clinical and laboratory presentation between RNA positive and negative patients.

**Course of patients with acute hepatitis E**

Two patients died during their hospital stay, both had a genomic and serological diagnosis. The first patient was a 78-year old man. He had type II diabetes, ischemic cardiomyopathy and no history of alcohol abuse. On admission, ALT activity was 168 times (X) upper normal limit (N), bilirubin 7.9 X N and prothrombin index was 38%. Accelerin was 58%. He died of aspiration pneumonia 21 days after admission. The second patient was a 50-year old man. Clinical examination on admission was remarkable for evidence of cirrhosis including vascular naevi, splenomegaly, palmar erythema, ascites and grade II encephalopathy. He was also found to have a history of alcohol abuse (80 g/day) but no other causes of cirrhosis were revealed. On admission, ALT activity was 35.7 X N, bilirubin 13.8 X N and prothrombin index 16%. Factor V was 25%. He died after 20 days of hospitalisation with terminal hepatic dysfunction and hepatorenal syndrome.

All the other patients survived and recovered without sequelae. ALT activity was normalized within 51 ± 31 days.

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Comparison with patients with acute hepatitis A

Twenty three patients diagnosed with hepatitis A during the same time period at the same hospital were analysed. They were selected on the basis of presence of anti-HAV IgM antibodies. Two patients could not be reached after discharge and history of travel abroad could not be obtained. Patient characteristics are shown in table I. No patient developed encephalopathy and none died. Excess alcohol consumption (> 40 g/d), was noted in 1 patient.

Eight patients (52%) had traveled to North-Africa within 3 months of the onset of disease.

Data from patients with acute hepatitis E were compared to data from patients with acute hepatitis A (table I). Using univariate analysis, HEV-infected patients were older, had lower ALT levels, were of different ethnic origin (100% European for hepatitis E compared to 39% from North Africa for hepatitis A, \( P<0.05 \)) and had lower incidence of recent travel outside of Europe (13% in the hepatitis E group vs 60% in the hepatitis A group, \( P<0.05 \)). Using multivariate analysis only age, ALT levels and recent travel outside of Europe were significantly different between the two populations.

Discussion

Our study showed that hepatitis E can be considered an autochthonous infection in south west France. All strains sequenced were related to genotype 3. When compared to hepatitis A, HEV-infected patients were older, had lower ALT levels and had a lower incidence of travel outside of Europe.

Hepatitis E virus infection is a major cause of epidemic and acute sporadic hepatitis in many areas of Asia, Africa and South or Central America where HEV is considered endemic [1, 19-21]. North America and Europe have traditionally been considered nonendemic areas for HEV [1]. Most HEV infections in these countries are considered to be imported and it is common practice to think of hepatitis E when all other causes of acute hepatitis have been ruled out in patients having visited endemic regions.

The virus is excreted in feces and is transmitted predominantly by the fecal-oral route and therefore often compared to hepatitis A. Clinical illness is similar to other forms of acute viral hepatitis except in pregnant women in whom the disease is particularly severe with a high mortality rate [1, 2].

Our first finding was that acute hepatitis was not rare in our region. While sporadic cases of acute hepatitis E in France [4-7, 9], Europe and the United states have been reported [8, 22-27] this is the largest cohort described over a one year period. The cases were evenly distributed over the year and infections persisted thereafter (data not shown), indicating that this was not an isolated outbreak. More than half of the patients lived in rural communities. Interestingly, the second biggest cohort comes from across the Pyrénées in Barcelona and may therefore be epidemiologically linked [8]. In the study by Bohme et al., 3 different HEV strains were identified in serum from 3 patients, 2 belonged to genotype 3, the predominant genotype found in local urban sewage [8]. Serosurveys have consistently indicated a low 1-5% seroprevalence of antibodies to HEV [5, 23-25].

It is common belief that in industrialized countries, acute hepatitis E occurs mainly in patients that have visited endemic countries. Nevertheless in the present study, only 3 patients (13%) had a history of travel outside Europe within 3 months of jaundice. Two had been to Tunisia and 1 to the Seychelles islands. Because the commonly accepted incubation period of HEV infection is 2 to 9 weeks [31], this indicates that the other 20 cases were of autochthonous origin.

We did not identify cases of acute hepatitis among family members or work colleagues, consistent with the generally believed low probability of intrafamilial (interpersonal) transmission of HEV [20, 32].
Geographical data showed that 16 patients lived in rural communities, 6 in a large city (Toulouse, > 400,000 inhabitants), and 1 in Antibes (South-East France). This latter patient had visited Toulouse the week before his admission and therefore probably contracted the virus in his hometown. No distinct geographical pattern could be found (data not shown). Evidence exists that some animals can be reservoirs for HEV. This may explain sporadic cases in industrialized countries. Anti-HEV antibody has been detected in many animal species including monkeys, pigs, rodents, chickens, dogs, cows, sheep and goats [13, 33-37]. Karetyni et al. [29] found that the anti-HEV antibody prevalence in Iowa field workers was significantly higher (5.7%) than that in normal blood donors (2%). This suggests that human populations with occupational exposures to wild animals may have increased risks of HEV infection. Recently Tei et al. [15] described a case of zoonotic transmission among people who had eaten uncooked deer meat. Some of our patients had household pets including cats, dogs and canaries. One had poultry, but none had contacts with pigs, sheep, cattle or goats. Contaminated water, the usual contaminant source in developing countries, remains a possible source of infection for our cases. However, all our patients are from distinct geographical areas in south west France with distinct water supplies, making this unlikely. Taking all this into account, the source of contamination for our patients remains unknown and is still under investigation.

Two patients (8%) died but none had fulminant hepatic failure. Both had severe underlying disease that decompensated during hospitalization and one had underlying cirrhosis. This further underscores that hepatitis E superinfection in patients with chronic liver disease can be severe [38]. Overall, reported mortality of acute hepatitis E is between 0.5% and 4% except in pregnant women where it reaches 15 to 25% [2, 19, 20, 21]. All other patients normalized transaminase levels within a few months as it is usually observed. However, viral shedding and elevated transaminases may be prolonged in immunocompromised patients. We have recently reported the case of a patient with lymphoma who had persistant elevated liver enzymes and HEV RNA in the serum or the stools over a 10 months period [39].

Genomic testing was available for 18 patients. RNA was detected in serum or feces by real time PCR. The procedure was initially described elsewhere [40]. It is important to look for the virus both in the serum and the feces because most patients had positive titers in only one of the two sites. Since the half-life of HEV is longer in stool, in some patients, the virus may disappear in serum and still be present in the stools. Two patients were positive for RNA but negative for antibodies. One of these is described thoroughly elsewhere [40]. This emphasizes the need to use both serological and genomic testing.

The phylogenetic analysis indicated that all the HEV sequences belonged to genotype 3 (figure 1). Among these sequences, two genetic groups could be identified. The first included the strains from 2 patients which were similar to an Italian strain (GenBank accession number AF103900). Among these two patients, one was living in South East France (Antibes) not far from Italy. He was probably infected in his hometown with an Italian strain before traveling to our region. Other strains from the second group were very similar and seemed to be related to other European strains and specially to Spanish strains [8].

We compared the patients with acute hepatitis E to patients with acute hepatitis A because both viruses are similar in their route of transmission and in causing acute, resolving hepatitis. Moreover, their epidemiological characteristics are similar except for the frequency of autochthonous HAV cases in industrialized countries and the absence of known animal reservoir for this virus. Lastly there is a vaccine only available against HAV.

We found that HEV-infected patients were significantly older. This had already been reported by Su et al. [41] in an Asian population. Also, consistent with the Taiwanese data, HEV patients had lower ALT levels. We found no differences in other serum liver tests, however. We were surprised to find a significant difference in history of travel preceding the onset of the illness in that HEV patients, contrary to HAV patients, had less frequently traveled abroad and in particular to North Africa. This clearly indicates that HEV infection was more frequently local. This work shows that HEV should be sought in unexplained acute hepatitis even in patients who did not travel to endemic countries. HEV genomic detection in both serum and feces should be used systematically in combination with antibody detection. Acute HEV hepatitis can be severe, particularly in patients with concomitant illnesses or chronic liver disease. There are marked differences when comparing hepatitis E and hepatitis A that include an increased age, a lower ALT level and a more frequent autochthonous origin.

ACKNOWLEDGEMENTS. - The authors thank Dr Chris Mascott for his precious help with the manuscript.

REFERENCES


