Hepatitis E virus infection in haemodialysis patients: a seroepidemiological survey

F. Fabrizi¹, G. Lunghi², G. Bacchini¹, M. Corti¹, A. Pagano² and F. Locatelli¹

¹Nephrology Department, Hospital, Lecco and ²Institute of Hygiene and Preventive Medicine, University of Milan, Italy

Abstract

Background. Hepatitis E virus (HEV) is the causative agent for enteric non-A, non-B hepatitis. Transmission is via the faecal route but the possibility of transmission by blood has been raised. Data concerning anti-HEV prevalence among chronic haemodialysis (HD) patients are few and give conflicting results.

Methods. We tested for anti-HEV antibody 204 chronic HD patients attending a single dialysis unit. A specific solid-phase enzyme-linked immunoassay (Abbott HEV EIA) was used.

Results. We found six anti-HEV-positive patients, the anti-HEV prevalence was 3% (95% CI 0–6%). The prevalence rates of HBV and HCV infections were 39% (31–45%) and 22% (16–28%) respectively. No anti-HEV-positive patient showed past or current biochemical signs of liver damage. One of six (17%) anti-HEV-positive patients was an immigrant; no risk factor for anti-HEV antibody was identified in the other anti-HEV-positive individuals.

Conclusions. We observed a low anti-HEV prevalence; there was no association between HEV and blood-borne infections (HBV, HCV, and HIV) in our HD patients; most anti-HEV-positive patients we found were probably related to a local infection by HEV. This is one of the first reports concerning seroepidemiology of HEV infection in a large cohort of chronic HD individuals.

Key words: Hepatitis E virus; haemodialysis patients; IgG anti-HEV antibodies

Introduction

Hepatitis E virus (HEV) a non-enveloped, calici-like RNA virus has been shown to be the main aetiological agent of enterically transmitted non-A, non-B hepatitis. HEV has been characterized recently, when part of the viral genome was cloned [1] and serological assays for the detection of antibodies to recombinant expressed HEV antigens have been developed [2]. Only one type of HEV exists, although several strains including those observed from the Mexican and Burmese isolates have been recognized. HEV is largely responsible for water-borne outbreaks [3] and sporadic cases of hepatitis E occurring in many countries worldwide. Large epidemics have been reported in Asia, Africa, and Latin America [4–7]. Reports on the presence of HEV infection in the Mediterranean, Middle East, and areas close to this region have appeared recently [8–14]. HEV usually causes an acute, icteric self-limiting illness with no chronic sequelae. Hepatitis type E generally occurs in young adults and has been documented as having a very high mortality rate (up to 20%) in pregnant women who are infected during the last trimester of pregnancy. HEV is associated frequently with faecal-contaminated drinking water or poor sanitary conditions [15]. Further, vertical transmission of HEV from infected mothers to their children has been recently observed [16]. Also dental treatments were suspected as risk factors for HEV contamination [17]. Some authors [18] observed a high prevalence of anti-HEV antibody in their haemodialysis (HD) patients and hypothesized that the oro–faecal route may not be the only route of transmission of HEV among their patients. Other investigators, in contrast, found few anti-HEV-positive patients in their HD populations [19–21]. Moreover, in most of these reports a small number of patients was studied. These findings prompted the present study.

Subjects and methods

Patients

We studied all patients on chronic haemodialysis treatment at a single dialysis unit in Lecco, northern Italy, in February 1995. Two hundred and four patients were tested. There were 113 males, and 91 females, the mean age was 61.1 years (range 20–89). The median duration of HD treatment was 39 months (range 6–262). Routine HD techniques were performed with 3- or 4-h treatments three times a week. Bicarbonate dialysis was used for all patients. The epidemiol...
ological features of HCV and HBV infections in the same patients have been previously detailed [22–23]. The chronic renal failure of the patients was due to chronic glomerulonephritis (n = 61), nephroangiosclerosis (n = 38), polycystic kidney disease (n = 29), diabetic nephropathy (n = 21), chronic interstitial nephritis (n = 22), and other aetiologies (n = 33). The history of blood transfusion requirement for each patient was evaluated. No patient admitted a history of intravenous drug abuse.

A descriptive analysis of data was made: we calculated mean ± standard deviation and median with respective range of parametric and non-parametric data respectively. Frequency of categorical variables (blood transfusions, raised aminotransferase levels, positivity for viral markers) was assessed.

**Laboratory assays**

The patients were screened for HEV markers using a second-generation enzyme immunoassay (Ortho Diagnostic Systems). Samples repeatedly reactive were confirmed by 4-RIBA (Ortho Diagnostics and Chiron Corporation). The freezing–thawing of specimens was avoided. A single serum sample from each patient was available and was stored at −20°C. All the patients were screened for hepatitis B virus (HBV) markers. Commercially available enzyme immunoassays (Abbott Laboratories, North Chicago, IL) were used for the detection of HBsAg, IgM anti-HBc, IgG anti-HBs. All the patients were screened for antibodies to HIV (anti-HIV) by ELISA assay (Abbott Diagnostics). Serum aspartate and alanine aminotransferase (AST and ALT) levels were assayed by spectrophotometry. The upper normal limits in the AST and ALT were 46 and 40 U/l respectively. A value higher than twice the upper reference level was arbitrarily considered to be significant.

**Anti-HEV IgG detection test (EIA)**

All patients were tested by an enzyme immunoassay (EIA) for specific anti-HEV antibody (Abbott Laboratories, North Chicago, IL). The sample to be tested was diluted 1:441 in a sample diluent containing 2% goat anti-human IgG. The diluted sample was incubated for 1 h at 40°C with the HEV-coated beads. The solid phase was coated with two recombinant antigens expressed in Escherichia coli as fusion proteins (CKS 8-5 and CKS SG-3) that comprise, respectively, all 123 amino acids of ORF-3 protein and 327 amino acids from the carboxyl terminus of ORF-2 protein of an isolated Burmese strain of HEV. After incubation with serum and washing, the bead was further incubated with goat antibody to human IgG (gamma chain specific) conjugated to horseradish peroxidase at 40°C for 1 h. The bead was then washed with water. A substrate solution was added. The enzymic reaction was allowed to proceed for 30 min, at room temperature in the dark. The reaction was stopped by adding 50 μl of 1 NH₂SO₄. The intensity of the colour that was developed as a result of the enzymic catalysis of the substrate was measured at 493 nm. Specimens with absorbance equal to or greater than 9 times the negative control absorbance value were considered to be reactive by the criteria of the test.

**Results**

There were six of 204 chronic haemodialysis patients showing anti-HEV antibody, the anti-HEV prevalence in our population was 3% (95% confidence intervals 0–6%).

All these patients did not show very high reactivity; however, no anti-HEV-positive patient had low titres. In fact, no anti-HEV-positive sample belonged to the positive or negative grey-zone (optical density of the sample/cut-off ratio between 0.7 and 1 and between 0.3 and 0.7 respectively). All anti-HEV-positive patients showed an optical density/cut-off ratio over 1 (range 1.1–3.1).

There were 8/204 (4%) patients with persistent HBV infection (HBsAg positive), and 71/204 (35%) patients showing serological signs of past HBV infection (anti-HBc and/or anti-HBs positive). Seventy-five of 204 (37%) individuals had been immunized with recombinant hepatitis B vaccine and some of them (30/75, 40%) showed detectable anti-HBs antibody at the time of the study, but they were not included in the calculation of previous HBV infection. The prevalence rate of persistent and past HBV infection was 39% (79/204) (95% confidence intervals 31–45%).

We observed 46/204 patients with anti-HCV antibody, the prevalence of anti-HCV positivity was 22% (95% confidence intervals 16–28%).

The descriptive analysis of characteristics of anti-HEV-positive and anti-HEV-negative patient groups is shown in Table 1.

There were 29/204 (14%) individuals with raised AST and ALT levels. Seven of 204 (3%) had current increased aminotransferase values, 22/204 (11%) showed past elevated aminotransferase concentrations. Mean values of increased AST and ALT values were 126.5 ± 56.5 and 165.4 ± 90 U/l respectively. No anti-HEV positive showed past or current biochemical signs of liver disease.

One of six (17%) anti-HEV-positive patients was an immigrant from Albania; there was no history of travel in the other (5/6) anti-HEV-positive individuals. There

<table>
<thead>
<tr>
<th>Table 1. Characteristics of anti-HEV-positive and -negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
</tr>
<tr>
<td>Patients, (n)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Time on HD (months)</td>
</tr>
<tr>
<td>HBV-positive patients</td>
</tr>
<tr>
<td>Anti-HCV-positive patients</td>
</tr>
<tr>
<td>Anti-HIV-positive patients</td>
</tr>
<tr>
<td>Transfused patients</td>
</tr>
<tr>
<td>Patients with raised aminotransferase levels</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages; HD, haemodialysis.
Hepatitis E virus in haemodialysis

were no immigrants in the group of anti-HEV-negative patients.

Discussion

We observed a prevalence of anti-HEV antibody of 3% in chronic HD patients attending a single dialysis unit in northern Italy. Studies concerning HEV epidemiology among chronic HD patients are few and give conflicting results. Differences of HEV prevalence in the general population at regional level, the criteria for inclusion of patients, and the routes of HEV transmission could partially explain the diverse results found.

HEV is usually associated with the faeco-oral route, and blood is not considered an important cause of HEV transmission as the virus does not produce a chronic carrier state. However, experimental transmission of HEV [24] to man showed a transient phase of viraemia preceding the onset of clinical symptoms, and prolonged viraemia has been observed in some patients [25,26]. Therefore a theoretical possibility of HEV transmission in endemic areas via a parenteral route has been suggested. Such a possibility is supported by the observation that anti-HEV antibody is more frequent in transfusion recipients than in the same number of non-transfused controls [27]. However, studies performed in haemophiliacs [28,29] showed a very low prevalence of HEV infection. Our results suggest that HD patients, individuals at risk for parenteral exposure, show a low prevalence of HEV infection. Moreover, we did not find an association between HEV and other blood-borne infections. Both a low prevalence of HEV in the general population and a poor efficiency of HEV spread among HD patients could explain these findings. One of the six anti-HEV-positive patients was a young male immigrant from Albania. To date we have no results concerning the epidemiology of HEV infection in that country. However, a different HEV prevalence rate in the general population of that country could explain the positivity we observed. We did not find a history of travel in the other anti-HEV-positive patients; on the other hand, travelling among HD patients is uncommon due to the need of repeated HD sessions in the week and anti-HEV positivity in such patients is most probably the result of local infection by HEV. Several possibilities may be considered: HEV could exist in an unknown animal reservoir; HEV infection might occur through contaminated food imported from HEV-endemic areas; an avirulent HEV strain might be present in Europe, normally causing only subclinical infections. Moreover, anti-HEV IgG antibody can persist for a long time [30], and anti-HEV positivity might be related to past waterborne outbreaks, previously reported as HAV hepatitis.

A low circulation of HEV in Italy exists [31], as suggested by a very recent survey. The anti-HEV prevalence in European blood donors is 1.3%. However, different prevalence rates have been found by other authors. A high prevalence of anti-HEV antibody (14%) has been observed among a healthy population in the Naples area [32]. Moreover, in that report a striking association between HEV and HCV infection was found. Such an observation is in contrast with our findings. A north–south gradient in HEV prevalence in Europe might be possible. A multicentric study regarding HEV prevalence among chronic HD patients attending dialysis units of northern and southern Italy is in progress at our Department.

HEV infection, as detected by anti-HEV antibody, was associated with no risk factor in most patients, and HEV seems to play no role in the development of liver disease in HD patients. However, our conclusions must be tempered by the small number of patients found to be positive for anti-HEV antibody; we made a descriptive analysis of our findings in order to avoid statistical type 2 errors.

In conclusion, this cross-sectional study showed a low prevalence of anti-HEV antibody in our HD patients; we did not find association between HEV and blood-borne viruses; most anti-HEV-positive patients we found are probably the result of local infection by HEV. At present hepatitis type E is not a major health problem in industrialized countries; however, there is an increasing number of travellers to endemic regions for HEV and a high flux of immigrants from these areas.

A careful surveillance in the general population is required. A periodical anti-HEV screening in risk groups for viral infections such as HD patients is recommended.

References

9. Balayan MS. Hepatitis E virus infection in Europe, a regional

Received for publication: 14.5.96
Accepted in revised form: 19.9.96