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Lack of evidence of hepatitis E virus infection among renal transplant recipients in a disease-endemic area

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SUMMARY. Persistent hepatitis E virus (HEV) infection has been reported among solid-organ transplant recipients in nonendemic areas. Such chronic infections have all been related to genotype 3 HEV, which is prevalent in these areas. Whether persistent infection occurs with genotype 1 HEV, prevalent in areas where the infection is hyperendemic, is unclear. We therefore tested sera from renal transplant recipients receiving immunosuppressive agents in India, where genotype 1 HEV infection is endemic, for alanine aminotransferase levels, and presence of IgM and IgG anti-HEV antibodies and HEV RNA. Of the 205 subjects studied [aged 16–65 (median, 38) years, 182 male], 46 (22.4%) had abnormal ALT levels (>40 IU/mL). IgG anti-HEV was detected in 52 (20.5%) and IgM anti-HEV was detected in 14 (6.8%) subjects, including four who had IgG anti-HEV; antibody positivity had no relation with serum ALT or serum creatinine. All the sera tested were negative for HEV RNA. These findings suggest that chronic infection with genotype 1 HEV is infrequent.

Keywords: genotype, hepatitis E, hepatitis E virus, immunosuppression, organ transplantation.

BACKGROUND

Hepatitis E is a form of viral hepatitis endemic in several developing countries in Asia, the Middle East, Africa and Latin America [1,2]. In recent years, the disease has also been increasingly reported from developed countries. The causative agent, hepatitis E virus (HEV), is a 32–34 nm diameter, enveloped virus with a 7.2-kilobase single-stranded RNA genome. It has at least four genotypes that infect mammals [3]. Of these, genotypes 1 and 2 have a restricted geographic distribution and host range, causing infection among humans residing in or travelling to areas where the disease is hyperendemic. In contrast, genotypes 3 and 4 have been reported from all continents and circulate widely in several mammals, particularly pigs, deer, wild boars and rats, with occasional transmission to humans.

In disease-endemic regions, the disease occurs as outbreaks and sporadic cases, caused by faecal-oral transmission usually through contaminated water, and predominantly affecting young adults [1]. Most of these cases present as self-limited acute hepatitis and some as acute liver failure [4]. Infected pregnant women are at particularly high risk of severe disease and mortality. In contrast, in low-endemicity areas, HEV infection is characterized by sporadic disease mainly in elderly men who often have other concomitant illnesses, rarity of disease outbreaks, zoonotic and/or parenteral transmission and absence of severe disease in pregnant women [2].

In low-endemicity regions, persons who have undergone solid-organ transplants and are receiving immunosuppressive drugs are at increased risk of HEV infection [5]. These persons often develop chronic HEV infection, which can lead to chronic hepatitis, liver fibrosis and cirrhosis. All cases of chronic HEV infection have had infection with genotype 3 HEV.

There are no data on chronic HEV infection among immunosuppressed groups or solid-organ transplant recipients in hyperendemic regions where genotype 1 virus predominates. We therefore looked for evidence and frequency of HEV infection among kidney transplant recipients in India, a disease-endemic area.

METHODS

Persons who had previously received an allogeneic renal transplant and were on follow-up in our Renal Transplant Clinic were enrolled in this study, irrespective of the duration since transplantation, nature and dose of immunosuppressive therapy and presence/absence of liver injury. For each subject, clinical details related to renal transplantation and current immunosuppressive regimen were recorded, and a blood specimen was collected. Serum

Abbreviation: HBV, hepatitis E virus.

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bilirubin level and alanine aminotransferase activity were measured on the same day. Sera, stored at −80 °C, were tested for IgM and IgG anti-HEV antibodies using commercial immunoassays (Genelabs, Singapore). Any specimens testing borderline were retested.

In addition, one serum aliquot (100 μL) from each patient was tested for HEV RNA. In brief, RNA was extracted using QIAamp viral RNA minikit (Qiagen, Valencia, CA, USA) and subjected to one-step reverse transcription and polymerase chain reaction (PCR) with real-time detection of PCR products (Quanti Fast Probe RT PCR kit; Qiagen, Hilden, Germany and ABI 7500 Real-Time PCR System; Applied Biosystems, Carlsbad, CA, USA). The primers used were 5′–GGTGGTTTCTGGGGTGAC–3′ and 5′–AGGGGTTGGTTGGATGAA–3′, respectively. Reverse transcription was done at 50 °C for 10 min, followed by inactivation of RNA at 95 °C for 5 min, and PCR involved 40 cycles of 95 °C for 10 s and 60 °C for 35 s. The rate of synthesis of a 70-bp region in ORF2 of HEV genome was monitored using a fluorescent probe (5′–TGA TTC YCA GCC CTT CGC–3′) in the reaction mixture [6].

Each assay included a relevant synthetic cRNA prepared from a plasmid as positive control and an appropriate negative control. Using a dilution series of this cRNA, the assay was determined to have a detection sensitivity of better than 300 copies of HEV RNA per millilitre of serum. The study also envisaged amplification and sequencing of selected segments of HEV genome to determine the genotype of the infecting virus from any specimens that tested positive for HEV RNA.

Our institution's Ethics Committee approved the study protocol, and each subject provided informed consent. Wilcoxon's rank-sum test was used for inter-group comparisons.

RESULTS AND DISCUSSION

The 205 study subjects were aged 16–65 (median, 38) years and included 182 (89%) males. The time interval between renal transplantation and enrolment in this study varied from 18 days to 16 years (median, 3 years). The majority of subjects had received organs from live-related donors, as is common in India. Median serum creatinine in the study subjects was 1.4 (range, 0.5–5.7) mg/dL; most subjects had normal renal function. Most patients were receiving triple drug immunosuppression, including low-dose prednisolone, mycophenolate and a calcineurin inhibitor (cyclosporin or tacrolimus). Six (3%) and 10 (5%) patients had chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, respectively.

Serum ALT level was abnormal (>40 IU/mL) in 46 (22.4%; including five with HBV infection and three with HCV infection) subjects. Of these, 10 (4.9%; including three with HCV infection) had ALT levels exceeding 80 IU/mL.

Of the 205 sera tested, 52 (20.5%) were positive for IgG anti-HEV and one tested borderline repeatedly. In addition, 14 (6.8%) specimens tested were positive for IgM anti-HEV antibodies (including 4 that were positive for IgG anti-HEV) and three tested borderline repeatedly. The median (range) ALT level was similar in patients with IgM anti-HEV [33 (26–62) IU/L] and those without [29 (11–218) IU/L]; of the 14 persons with IgM anti-HEV, one (7.1%) had abnormal ALT, compared to 13 (6.8%) of the 191 who lacked this antibody. Median serum creatinine levels were similar in persons with and without IgG anti-HEV (1.5 [0.5–3.2] *vs* 1.4 [0.8–5.7] mg/dL).

None of the sera tested were positive for HEV RNA.

Discussion

HEV infection in solid-organ transplant recipients was first reported in a case series of 14 French patients who had liver dysfunction and HEV viremia [7]. Of these, seven progressed to chronic HEV infection. In a retrospective review of 85 cases with HEV infection in recipients of solid-organ transplants (kidney 47, liver 26, kidney-pancreas 6, liver-kidney 2, heart 2, islet cell 1 and lung 1) in 17 centers from France, the Netherlands, Germany, England, Belgium and USA, 56 patients developed chronic hepatitis [5]. The factors associated with chronicity were as follows: liver transplantation, shorter time since transplantation, lower levels of liver enzymes and serum creatinine, low platelet counts and use of tacrolimus-based immunosuppressive therapy (over cyclosporin A). All these patients had infection with genotype 3, the prevalent HEV genotype in the regions where these cases occurred. Persistent genotype 3 HEV infection has also been reported in other conditions associated with immunosuppression, including chemotherapy for haematological or lymphoid neoplasms, HIV infection. Whether genotype 1 HEV, which is much more prevalent globally, also causes chronic infection remains unclear.

In India, HEV infection is highly prevalent, accounting for frequent large outbreaks and a large proportion of cases with sporadic acute hepatitis [1]. Molecular studies show circulation of only genotype 1 HEV among humans, and of genotype 4 HEV among pigs [8], with no evidence to date of animal-to-human transmission of HEV. In particular, genotype 3 HEV infection has not been reported. Given the high rate of exposure to HEV infection in the Indian population, if genotype 1 HEV infection persists among immunosuppressed persons, one would expect at least some renal transplant recipients to have detectable HEV RNA. In contrast, we found absence of detectable HEV RNA in sera obtained from a fairly large unselected group of Indian renal transplant recipients. This indicates that genotype 1 HEV either may be incapable of causing chronic infection or may do so very rarely. Although we had planned to determine the viral genotype in transplant recipients who had infection with HEV, we could not do this because no patient had detectable HEV RNA.

It may be argued that absence of HEV viremia in our patients was limited by the fact that our patients did not have specific symptoms of liver disease and often had normal ALT. However, it is notable that HEV infection in organ transplant recipients in the low-endemicity areas has not been associated with specific symptoms of liver disease. For instance, in the retrospective series referred to above, only one of 85 patients reported jaundice [5]. Thus, this factor cannot explain our negative results.

Further, it is possible that transplant recipients receiving immunosuppressive drugs adopt habits that reduce the risk of exposure to HEV. These factors would be at least partly countered by our use of a very sensitive assay for HEV RNA and a fairly large sample size of our study, increasing the chance of picking up evidence of chronic HEV infection.

The IgM and IgG anti-HEV positivity rates in our study subjects were similar to those reported in the general Indian population. Presence of detectable IgM anti-HEV in some of our patients may need comment. This antibody is generally taken as a marker of recent (within the last 6 months) infection with HEV. However, this assay is known to give false-positive results in some healthy residents of disease-endemic areas. Similarly, the IgG anti-HEV assays are suboptimal and lack sensitivity [9]. Hence, the detection of IgG or IgM anti-HEV antibodies in some of our study subjects may not have much significance.

Information on chronic HEV infection among organ transplant recipients is important because such infection may be preventable using HEV vaccines. Two subunit vaccines have already been shown to have high protective efficacy in human trials. However, their efficacy in immunosuppressed persons has not yet been studied.

In conclusion, the current study failed to find evidence of HEV viremia in a large group of unselected renal transplant recipients in India, where genotype 1 HEV infection is hyperendemic. This suggests that the risk of chronicity with genotype 1 HEV infection among immunosuppressed persons is low.

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CONFLICT OF INTERESTS

None of the authors has any conflict of interest to report.

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