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PII: S0168-8278(11)00564-2
DOI: 10.1016/j.jhep.2011.06.021
Reference: JHEPAT 3926

To appear in: Journal of Hepatology

Received Date: 11 January 2011
Revised Date: 2 June 2011
Accepted Date: 7 June 2011


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Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient

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Running title: HEV transmission through an infected liver

Keywords: occult hepatitis E virus infection, liver transplantation, chronic HEV infection, cirrhosis, immunosuppression

Word count: 2245 words

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Summary
Acute hepatitis E virus (HEV) infection is a self-limiting symptomatic or asymptomatic disease. However, as recently observed it can manifest itself as chronic hepatitis in patients receiving solid organ transplants as well as in patients with HIV infection or severe hematologic disorders.

Here we describe the clinical course of a 73-year-old male patient in whom HEV transmission occurred after receiving a HEV-infected liver from a donor with occult HEV infection, whereby the patient had tested negative for HEV RNA and anti-HEV antibodies shortly before explantation.

Anti-HEV IgG, IgM and HEV RNA were detected in the first tested serum sample of the liver recipient obtained 150 days after liver transplantation and remained positive (earlier samples after OLT were not available). Liver cirrhosis developed within 15 months and the patient died of septic shock. Based on phylogenetic analyses of the donor and recipient’s HEV strains we were able to prove that the occult HEV infection was transmitted via the graft.

Introduction
Hepatitis E virus (HEV) belongs to the family of Hepeviridae (1) and is the sole member of the genus Hepevirus. HEV is subdivided into the genotypes 1-4. Sporadic and epidemic outbreaks in developing countries are mainly caused by genotype 1 and 2 strains. The other genotypes are considered as causative agents of sporadic indigenous cases in industrialized countries (2-4). HEV infection is characterized by an acute self-limiting course in immunocompetent patients, but chronic HEV infections have been described in solid-organ transplant patients (5). Recently we reported the first reactivation of a HEV infection in an acute lymphoblastic leukemia patient after allogeneic stem cell transplantation (6). The CD4+ lymphocyte count seemed to be a marker for the successful clearance of HEV by the cellular immune response since the number of this lymphocyte subset was found to be significantly reduced in patients with lasting viremia (7).
Here we report for the first time HEV transmission via an infected donor liver leading to chronic HEV infection and cirrhosis development in a German patient after orthotopic liver transplantation (OLT).

**Case report:**
We describe the case of a 73-year-old male patient with a 30-year history of elevated liver enzymes. A diagnosis of non-alcoholic fatty liver disease-induced cirrhosis was made in 2001 due to hyperlipidemia, type 2 insulin-dependent diabetes and moderate adipositas. Other causes such as chronic viral hepatitis, autoimmune or hereditary liver disease could be excluded.

In August 2008 a multilocal hepatocellular carcinoma (HCC) (3 nodules, maximum size 3 cm) was diagnosed during a routine computed tomography (CT) scan. Hepatitis B or C virus infection was excluded but markers for previous varizella zoster virus, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) infections were detected by standard serological assays prior to OLT. Transplantation was performed without complications in December 2008. To our knowledge, no blood transfusion was performed within this period. The donor was a 65-year-old male patient who had died of a heart attack. In addition to cardiovascular disease, IDDM and hyperlipoproteinemia were reported. At the point of death, ALT values were four times the upper limit of normal (ULN). No information on the levels of liver enzymes in the preceding months/period was available. To our knowledge, no blood transfusion was performed in the three-month period before transplantation. Histological assessment of the liver allograft showed mild fatty liver changes but no signs of chronic hepatitis or fibrotic alterations (Figure 1A).

After OLT the liver enzymes increased temporarily, most likely due to a reperfusion injury effect, but returned to normal levels within a week. The immunosuppressive regimen consisted of prednisolone (Merck, starting 1mg/kg, tapering), tacrolimus (Prograf®, Astellas) and rapamycine (Sirolimus®, Wyeth). Thirty-seven days after transplantation alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase levels (163 UL) became elevated (Figure 2). Liver biopsy was performed in February 2009 and
revealed mild fatty liver degeneration without evidence of acute or chronic hepatitis or an acute rejection episode (not shown). Weekly tests for CMV and EBV DNA remained negative. In May 2009 (150 days after OLT) another liver biopsy was performed due to increasing ALT levels up to >5 times the ULN. At this stage chronic inflammation with portal and interface hepatitis potentially associated with acute rejection could be observed (Figure 1B) and intravenous steroid bolus therapy (methylprednisolone, Urbasone®, Sanofi Aventis, 500 mg/day for 5 days) was started because of a suspected rejection episode. This resulted in a prompt decrease in liver enzymes. Immunosuppressive regimen was continued with tacrolimus and rapamycin. In November 2009 (333 days after OLT) the patient presented with edema of the lower limb. Liver cirrhosis was diagnosed by ultrasound and CT scan showing an irregular liver surface and a hypertrophy of the lobus caudatus (Figure 1D). Liver biopsy (347 days after OLT) revealed advanced (stage 3 to 4) fibrosis (Figure 1C).

In February 2010 liver disease decompensated as indicated by ascites (CRP 8.7 mg/dL (normal <0.5 mg/dL), creatinine 1.4 mg/dL, bilirubin 4.6 mg/dL, AST 154 U/L, ALT 148 U/L, AP 174 U/L, γGT 77 U/L, Hb11 g/dL, WBC 3.6 /nL, TPZ 51%). The patient died in February 2010 from septic shock probably due to a panenterocolitis (as suspected from the CT scan). E. coli bacteria were found in blood and ascites cultures.

**HEV diagnosis, detection of HEV in donor tissue**

In February 2010 the markers for HEV infection, anti-HEV IgM/IgG antibodies, were detected for the first time by recomWell ELISA (based on HEV genotype 1 antigens) and confirmed by recomBlot (based on HEV genotype 1 and 3 antigens), both Mikrogen, Germany, and HEV RNA in serum by RT-PCR [(8)]. Anti-HEV IgM/IgG antibodies and HEV RNA were also detected in a stored serum sample from May 2009 (150 days after OLT). Serum samples from the period between OLT and day 150 were not available and could not be tested for HEV serum markers. However, HEV serology and HEV RNA were negative in samples obtained before OLT in December 2008 suggesting that HEV infection occurred during or after OLT. We also retrospectively examined premortal blood (obtained one day before
Antibody screening and RT-PCR of the donor serum sample were negative but, surprisingly, HEV RNA was detected in high concentrations in liver tissue of the donor. The 95% detection limit of the RT-PCR was determined by probit analysis at approximately 2000 copies/ml serum. Crossbinding and unspecific amplification were not observed for all other herpes and hepatitis virus genomes. The sequence data of a partial fragment of the ORF3 region (161 base pairs) of both serum- and tissue-derived HEV strains from the recipient and the donor belonged to genotype 3 (Figure 3). Both sequences are identical whereas the identity rate between prototypes of GT1 to GT4 is approximately 73% and approximately 83% among different genotype 3 sequences (not shown). By sequencing an additional fragment within ORF1 (9) the HEV subgenotype 3e was identified. Again, the sequence data obtained from serum and liver tissue were concordant.

These data strongly suggest transmission via the transplanted allograft. To validate these results repeated RNA extractions and amplifications were performed and all confirmed our initial results. Possible contaminations of the PCR reactions could be almost certainly excluded because no other HEV RNA positive samples were handled at this time in our laboratory. Additionally the donor-recipient HEV RNA strain showed significant nucleotide divergence as compared to the positive control sample, which was used in all PCR reactions thereby excluding any contamination or sample mix-up.

Discussion:

This clinical course convincingly demonstrates that HEV can persist in liver tissue without serological evidence for HEV infection. A rather low-level HEV carrier state may be responsible for the lack of an anti-HEV response, but scant sensitivity of the current anti-HEV assays also has to be considered as a possible cause. Furthermore, based on phylogenetic analyses of the donor and recipient's HEV strains we were able to prove that the occult HEV infection was transmitted via the graft to an HEV seronegative liver transplant recipient.
From HEV prevalence studies in wild boar (10) it became evident that a significant proportion of viremic animals do not show an anti-HEV antibody response. Thus overall anti-HEV seroprevalence was approximately 25-30% whereas HEV RNA was found to be positive in up to 68% of the animals. Furthermore, HEV RNA was exclusively detected in the bile in a significant proportion of the boars, supporting the existence of an occult HEV carrier state.

Legrand-Abranevel recently reported a high ratio of HEV infections in solid organ transplant recipients. However, none of those patients tested positive for HEV antibodies before OLT suggesting a greater likelihood of de novo infections than HEV reactivations (11).

Immunosuppressive treatment influenced the rapid clinical course of chronic HEV infection. The first therapeutic successes with pegylated interferon and especially ribavirin monotherapy were recently achieved in transplant patients with chronic hepatitis E (12-15). As clearance of the virus depends on the immune status of the patient, reducing the immunosuppressive therapy is generally recommended.

In conclusion, this is the first report of HEV transmission via an infected organ from a donor with occult HEV infection leading to chronic HEV infection in a liver transplant recipient. HEV might persist in the liver tissue and bile of patients without any serologic evidence of infection. These findings may have implications for future HEV screening strategies. Controlled studies are required to establish recommendations for the management of patients with chronic HEV infection.
Figure 1: Histologic assessment of the liver tissue before and after OLT (A-C) and CT scan after OLT (D).

The liver tissue of the donor revealed absence of significant signs of chronic hepatitis but vesicular fatty liver disease was diagnosed (1A). 150 days after OLT chronic inflammation with portal and interface hepatitis was described which was interpreted as an acute rejection (1B, 2nd biopsy). 347 days after OLT persistence of chronic hepatitis was associated with portal and septal bridging signs of fibrosis (1C, 3rd biopsy). CT scan performed 1 year after liver transplantation revealed signs of portal hypertension including ascites, splenomegaly and gastric varices compatible with decompensated liver cirrhosis (1D).
Figure 2: Course of ALT, bilirubin and HEV RNA levels in a liver transplant recipient with chronic HEV infection leading to liver cirrhosis within one year. Immunosuppressive regimen was started immediately after OLT consisting of prednisolone (reduction scheme) plus tacrolimus (4 mg/day) and rapamycine (4 mg/day) (black bars). After steroid tapering, maintenance immunosuppressive regimen with tacrolimus plus rapamycine was administered (gray bars). Intravenous high-dose methylprednisone bolus therapy (500 mg/day for 5 days) which was given for suspected acute rejection episodes are depicted in black-white boxes.

ALT and bilirubin values are given as x-fold the upper limit of normal (ULN) (normal range of ALT 45 IU/L, and bilirubin 1.1 mg/dL). Positive anti-HEV IgG/M results were obtained on days 150 and 420 after OLT but were found to be negative at the time of transplantation.
Figure 3: Phylogenetic tree constructed from patient and prototype HEV sequences.

HEV nucleotide sequences were aligned to construct a phylogenetic tree using the neighbor-joining method. A rooted phylogram is shown, with an avian HEV strain as outlier. The branch length is proportional to evolutionary distance (scale bar). Results of bootstrap analysis (1000 replicates) are indicated at the nodes of the tree. Accession numbers given in brackets indicate prototype strains from Genbank. The HEV_GT3_PC is obtained from a patient with a chronic HEV infection after stem cell transplantation (6) and serves as the positive control in our PCR reactions.


