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Prevalence of hepatitis E in liver transplant recipients in Greece

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Short Paper

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Abstract

Hepatitis E virus (HEV) is a well-known cause of acute hepatitis. Immunocompromised subjects, including liver transplant recipients, are considered to be at risk for HEV infection, which occasionally follows a chronic course. The diagnosis of HEV infection in these patients must be based on HEV RNA testing, as serology has variable performance. The aim of this study was to assess the prevalence of HEV infection in liver transplant recipients in Greece by means of HEV RNA testing. Liver transplant recipients followed in the sole transplant centre in Greece were prospectively included. HEV RNA was detected by real-time RT-PCR. Positive samples were further analysed using a nested reverse transcription RT-PCR kit, which amplifies a 137-nucleotide sequence within the ORF2/ORF3 overlapping region to detect the HEV genotype and perform phylogenetic analysis. The mean age of the included patients ($n = 76$) was 54 years. The most common indication for liver transplantation was viral hepatitis (57%). The majority of the patients (75%) received a calcineurin inhibitor as part of their immunosuppressive regimen and had normal liver enzymes. HEV RNA was found positive in only 1/76 (1.3%) patient. Phylogenetic analysis showed that the sequence clustered into the HEV genotype 3 clade. This patient experienced an acute hepatitis flare, which nonetheless did not become chronic. The prevalence of HEV infection in liver transplant recipients in Greece is similar (1.3%) to that reported previously in other countries. Transplant physicians should be aware of this condition and its associated consequences.

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis, causing approximately 50% of acute hepatitis cases in developing countries. According to the World Health Organization (WHO), approximately one-third of the world population has been exposed to HEV [1]. The main source of HEV transmission in developed countries is the consumption of raw or undercooked infected meat or the direct contact with infected animals, whereas cases of blood-borne transmission have also been reported [2].

Immunocompromised subjects, including liver transplant recipients, are considered to be at risk for HEV infection [3]. They can be infected via the faecal–oral route and through administration of blood products and theoretically via the transplant organ itself. Of note, HEV infection in liver transplant recipients may pose diagnostic difficulties as the serological assays have serious limitations in their sensitivity and specificity [4]. In addition, it can have a chronic course leading to cirrhosis.

Prevalence data (ranging from 3% up to 23%) for HEV infection in liver transplant recipients from different countries were summarised in a recent comprehensive review [4]. Various diagnostic methods were used in these studies, including HEV RNA detection, although most of the studies relied on the detection of anti-HEV IgG. Data about HEV infection in Greece are very limited. A study has estimated that the seroprevalence of hepatitis E in the general population in Greece is approximately 7% [5]. However, no information was up to now available regarding its prevalence in liver transplant recipients.

The aim of this cross-sectional study was to assess the prevalence of HEV infection in liver transplant recipients in Greece by prospectively assessing the serum HEV RNA.

We prospectively included data from consecutive liver transplant recipients followed in the liver transplant centre in Thessaloniki, Greece from December 2016 to March 2017. All patients in our centre had received cadaveric transplants and they were evaluated routinely every 3 months. Demographic, laboratory data and information on immunosuppressive regimens for all patients were recorded. The study was approved by our institutional review board.

Complete blood counts and biochemistries were determined by routine laboratory methods. RNA was extracted from patient's serum samples by QIAamp Viral Mini kit (Qiagen, Hilden, Germany) and HEV RNA was detected by real-time RT-PCR (Hepatitise2@

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ceeramTools kit, Applied Biosystems ABI) according to the manufactures' instructions once for each patient. This method detects as few as five genome copies of HEV per reaction. A nested RT-PCR, which amplifies a 137-nucleotide sequence within the ORF2/ORF3 overlapping region, was applied for positive samples to detect the HEV genotype [6]. Since the amplicons of ORF2/3-137 PCR contain variable sequences, phylogenetic analysis was also performed [7]. In addition, follow-up RNA testing was performed for positive samples as clinically indicated.

Data for 76 patients were analysed in this study. The median age of our patients was 54 years and most of them were males ($n = 58$, 76%). The most common indication for liver transplantation was decompensated cirrhosis due to viral hepatitis ($n = 43$, 57%). The median interval from the date of transplantation to the enrolment in our study was 98.5 months (15–341). The majority of the patients (75%) received a calcineurin inhibitor as part of their immunosuppressive regimen and had normal liver enzymes. Positive HEV RNA was detected in only one patient (prevalence 1.3%, 95% CI 0.00–3.90). Phylogenetic analysis for this patient showed that the sequence clustered into the HEV genotype 3 clade (Fig. 1).

This was a 60-year-old male patient who was transplanted due to alcoholic cirrhosis, 7 months prior to HEV detection. The recipient was discharged 40 days after transplantation in good clinical condition and with normal liver enzymes. His immunosuppressive regimen at that time included steroids, cyclosporine and mycophenolate mofetil. However, a sharp increase in his liver enzymes was noted 3 months after his discharge. A liver biopsy indicated acute cellular rejection and at the same time an inflammatory process compatible with viral infection. Notably, serology for hepatitis B virus, hepatitis C virus and cytomegalovirus was negative for an acute infection. Rejection was treated with steroids leading to the gradual decrease of his liver enzymes. However, they remained elevated for the following 2 months. Thus, an enhancement of his immunosuppressive regimen was decided by replacing cyclosporine with tacrolimus. At that time point, HEV RNA was pending and was retrospectively found to be positive when the patient was evaluated in the context of our study. Patient's liver enzyme levels finally returned to normal approximately 4 months after the initial rise without any specific medication for HEV infection. At that point, HEV RNA was not detectable any more.

Discussion

It was not until the last decade that it became evident that HEV infection can affect liver transplant recipients causing a progressive disease that can lead to liver cirrhosis. The first description of such cases was made exactly 10 years ago in a case series of transplant recipients, including three liver recipients. Notably, all three liver transplant recipients in that study had a chronic clinical course [8]. After that initial report, a number of similar cases have been described from all around the world [9–12]. The overall reported prevalence in most studies was low ranging from 1% to 3%. During the recent years, it also became apparent that the diagnosis of HEV infection in transplant recipients must be based on HEV RNA testing as serology has variable performance. As this is a recently acknowledged topic, any added information that is based on the correct methodology will enhance the understanding of this infection and will consequently facilitate its effective management.

In the present study, we report data on HEV infection prevalence in liver transplant recipients in Greece based on HEV RNA detection. We found only one HEV RNA-positive subject in a cohort of 76 liver transplant recipients followed in one centre. As this is the sole transplant centre in Greece, it is conceivable that our results reflect the national prevalence of HEV in liver transplant recipients. The HEV prevalence in Greece (1.3%) seems to be similar to that reported in other countries previously [9–12].

HEV infection can be diagnosed serologically with the detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. Unfortunately, the performance of the relevant assays is variable and can often mislead the physician [13]. One of the most important problems, especially in the setting of immunosuppression, is the lack of sensitivity of IgM antibodies. In

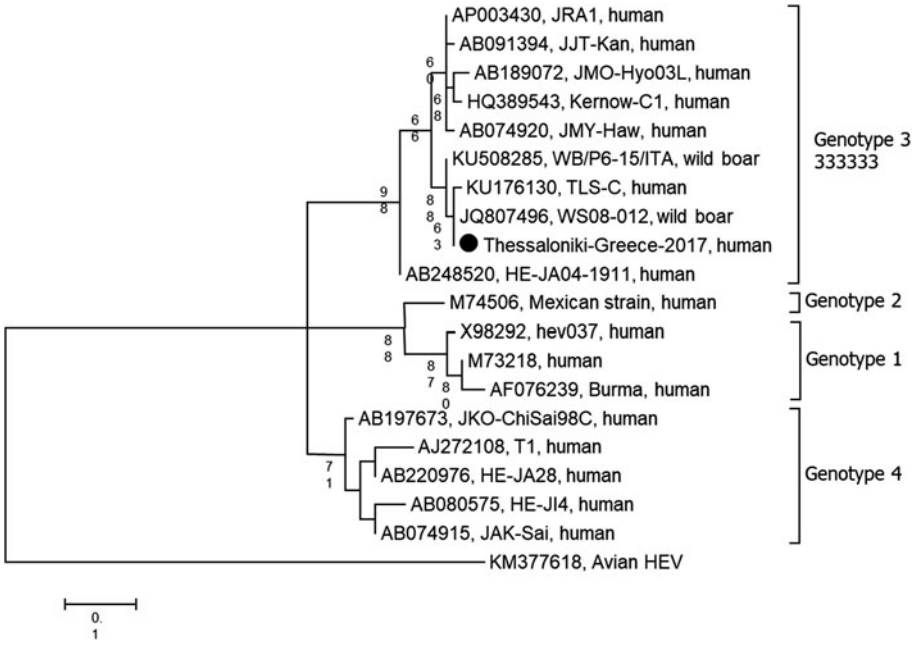

Fig. 1. Phylogenetic tree based on a 97-nt sequence of the ORF e gene constructed by Maximum Likelihood method based on the Tamura–Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

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addition, the IgG antibodies often become positive with a significant delay and furthermore do not remain positive for a long period of time. Currently, the use of commercially available HEV RNA assays is the method of choice for the diagnosis of HEV infection in immunosuppressed patients [4]. However, this still remains an imperfect diagnostic method with substantial variability, as well [14]. In our study, we based the diagnosis of HEV infection only on HEV RNA detection using a real-time RT-PCR kit. The inclusion of serology would have added important information to our findings and certainly constitutes a limitation of our study. However, we elected to use only the most reliable diagnostic method due to the lack of availability of serological tests in our centre and financial issues.

In our study, we identified one patient with positive HEV RNA. Although the available data cannot support conclusively the description of this case as chronic (duration of positive HEV RNA for at least 3 months is warranted), his prolonged clinical course with concurrent elevation of his liver tests suggest that this was a definite case of HEV infection. Notably, this patient had an infection with HEV genotype 3, which is the only genotype that is associated with chronicity among the four (1–4) described genotypes. Genotypes 1 and 2 are responsible for human infections exclusively, while genotypes 3 and 4 can infect humans and other mammals [15]. There is evidence that HEV genotype 3 can be transmitted through the ingestion of undercooked meat from infected animals, thereby highlighting the zoonotic nature of this infection. According to a detailed history, our patient denied eating raw meat. He continuously lived in a semi-rural area with no travel history abroad during the past few months prior to his transplantation. Although the exact mode of transmission in our patient was not evident, it is conceivable that the most probable route was undercooked meat.

In conclusion, this is the first study to report the presence of HEV in liver transplant recipients in Greece using HEV RNA detection. The distribution of HEV seems to be global calling for increased vigilance for its diagnosis. Physicians taking care of liver transplant recipients should be aware of the possibility of HEV infection of the graft since such an infection could potentially become chronic and progress to advanced liver disease.

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References

1. **Khuroo MS** (2016) Hepatitis E: an emerging global disease – from discovery towards control and cure. *Journal of Viral Hepatitis* **23**, 68–79.
2. **Donnelly MC *et al.*** (2017) Review article: hepatitis E – a concise review of virology, epidemiology, clinical presentation and therapy. *Alimentary Pharmacology and Therapeutics* **46**, 126–141.
3. **Marion O *et al.*** (2016) Hepatitis E in transplantation. *Current Infectious Disease Reports* **18**, 8.
4. **Behrendt P *et al.*** (2014) The impact of hepatitis E in the liver transplant setting. *Journal of Hepatology* **61**, 1418–1429.
5. **Politou M *et al.*** (2015) Seroprevalence in hepatitis E in HIV infected patients in Greece. *Journal of Medical Virology* **87**, 1517–1520.
6. **Inoue J *et al.*** (2006) Development and validation of an important RT-PCR assay with universal primers for detection of hepatitis E virus strain with significant sequence divergence. *Journal of Virological Methods* **137**, 325–333.
7. **Di Profio F *et al.*** (2016) Detection and genetic characterization of hepatitis E virus (HEV) genotype 3 subtype c in wild boars in Italy. *Archives of Virology* **161**, 2829–2834.
8. **Kamar N *et al.*** (2008) Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *New England Journal of Medicine* **358**, 811–817.
9. **Pischke S *et al.*** (2010) Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transplantation* **16**, 74–82.
10. **Haagsma EB *et al.*** (2009) Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transplantation* **15**, 1225–1228.
11. **Pas SD *et al.*** (2012) Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerging Infectious Diseases* **18**, 869–872.
12. **Buffaz C *et al.*** (2014) Hepatitis E in liver transplant recipients in the Rhone-Alpes region in France. *European Journal of Clinical Microbiology and Infectious Diseases* **33**, 1037–1043.
13. **Yoo N *et al.*** (2013) Hepatitis E virus infection in a liver transplant recipient: delayed diagnosis due to variable performance of serologic assays. *Transplant Infectious Disease* **15**, E166–E168.
14. **Mokhtari C *et al.*** (2013) Comparison of real-time RT-PCR assays for hepatitis E virus RNA detection. *Journal of Clinical Virology* **58**, 36–40.
15. **Tam AW *et al.*** (1991) Hepatitis E virus (HEV): molecular cloning and sequence of the full-length viral genome. *Virology* **185**, 120–131.

https://doi.org/10.1017/S0950268818001887 Published online by Cambridge University Press