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***Case Control Study***

**Hepatitis E in haemodialysis and kidney transplant patients in South-east Italy**

Scotto G *et al*. HEV-infection in end-stage renal failure patients

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**Abstract**

**AIM:** We investigate the sero-virological prevalence and clinical features of hepatitis E virus (HEV)-infection in end-stage renal failure patients and in the healthy population.

**METHODS**:HEV infection is a viral disease that can cause sporadic as well as epidemic hepatitis. Previous studies unexpectedly showed a high prevalence of HEV-antibodies in immunosuppressed subjects, including haemodialysis (HD) patients and patients who had undergone kidney transplant. A cohort/case-control study was carried out from January 2012 to August 2013 in two hospitals in Southern Italy (Foggia and S. Giovanni Rotondo, Apulia). The seroprevalence of HEV was determined in 801 subjects, 231 HD-patients, 120 renal transplant recipients, and 450 health individuals. All of the HD-patients and the recipients of renal transplants were attending the Departments of Nephrology and Dialysis at two hospitals located in Southern Italy and were included progressively in this study. Serum samples were tested for HEV antibodies (IgG/IgM); in the case of positivity they were confirmed by a western-blot assay and were also tested for HEV-RNA, and the HEV-genotypes were determined.

**RESULTS**: A total of 30/801 (3.7%) patients were positive for anti-HEV Ig (IgG and/or IgM) and by western-blot. The healthy population presented with a prevalence of 2.7%, HD-patients had a prevalence of 6.0% and transplant recipients had a prevalence of 3.3%. The overall combined HEV-positive prevalence in the two groups, with chronic renal failure, was 5.1%. The rates of exposure to HEV (positivity of HEV-IgG/M in the early samples) were lower in the healthy controls, but the difference among the three groups was not statistically significant (*p >* 0.05). Positivity for anti-HEV/IgM was detected in 4/30 (13.33%) anti-HEV Ig positive individuals, in 2/14 HD-patients, in 1/4 transplant individuals and in 1/12 of the healthy population. The relative risk of being HEV-IgM-positive was significantly higher among transplant recipients compared to the other two groups (OR = 65.4, 95%CI: 7.2-592.7, *p <* 0.001), but the subjects with HEV-IgM positivity were numerically too few to calculate a significant difference. No patient presented with chronic hepatitis from HEV infection alone.

**CONCLUSION**: This study indicated a higher, but not significant, circulation of HEV in haemodialysis patients *vs* the healthy population. Chronic hepatitis due to the HEV-virus wasn’t observed.

**Key words:** hepatitis E virus infection; Prevalence; Immunosuppressed subjects;Haemodialysis patients; Transplant recipients

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**Core tip:** Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. Recently, previous sero-prevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations, and several studies reported an unexpected high prevalence of antibodies against HEV in haemodialysis patients. The purpose of this survey was (1) to compare the rate of HEV infection in renal transplant recipients and patients undergoing chronic haemodialysis to a control population; (2) to determine if these patients have an increased risk for HEV exposure; and (3) to evaluate the stage of liver disease.

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**INTRODUCTION**

Blood-borne viral hepatitis [hepatitis B virus (HBV) and hepatitis c virus (HCV)] infections represent relevant causes of liver disease in end stage renal failure patients on haemodialysis (HD)[1-5]. In recent years, preventive measures and extensive infection control guidelines guided a progressive decrease of HCV and HBV rates in these patients[2-9]. Nevertheless, a proportion of liver illnesses due to non A-B-C hepatitis occurs in these individuals. Recently, previous sero-prevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations[10,11], and several studies reported an unexpected high prevalence of antibodies against HEV in haemodialysis patients[12-15]. The higher prevalence of HEV-IgG in chronic haemodialysis patients could be related to their impaired immunity, with an increased susceptibility to infections and decreased immune responses to antigenic stimuli (*e.g.*, HBV vaccination)[16-18]. Furthermore, they present a reduced response to HBV vaccination. In fact, these patients have an increased risk of contact with nosocomially transmitted agents, and the role of enterically transmitted hepatitis viruses in such cases needs to be defined.

Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. In the recent past, it was believed to be present only in developing countries, where it was associated with epidemic outbreaks through the faecal-oral route from contaminated water supplies, but it is now recognized as a worldwide infection, sometimes related, in developed countries, to an asymptomatic zoonotic infection (also undercook meat products)[19-21] or to parenteral/vertical transmission[22-24]. Furthermore, it has been recently noted that a variable rate of blood donors was positive for HEV-RNA[25-27]. There are scant reports on the prevalence and possible nosocomial transmission of HEV in HD patients. Some authors highlighted high rates of anti-HEV antibodies in their HD patients and hypothesised that there are other routes of transmission besides the faecal-oral route, although the real prevalence of HEV infection through the parenteral route, particularly via haemodialysis, is unknown[28]. Alternatively, other investigators observed low rates of anti-HEV-positivity in their HD populations[15,29,30].

Previous sero-prevalence studies showed anti-HEV/IgG positivity in 6%-16% of renal transplant recipients[31-33]; this variability is often because this virus is not routinely screened for in cases of acute hepatitis in recipients of solid-organ transplants. Recently, HEV infection presented as a chronic infection, sometimes with associated cirrhosis in immunosuppressed individuals. These cases included solid-organ (including kidney) transplant recipients receiving immunosuppressive therapy[33-36], patients with haematological malignancies[37-39] and subjects with HIV infection[40]. It is not known whether HEV can induce chronic hepatitis in subjects with defects of humoral and cellular immunity, such as in patients with end-stage renal failure requiring renal replacement therapy.

To our knowledge, few studies have examined the seroprevalence rate and clinical evolution of HEV infection among HD-patients and in recipients of renal transplants in Italy. The purpose of this survey was (1) to compare the rate of HEV infection in renal transplant recipients and patients undergoing chronic haemodialysis to a control population; (2) to determine if these patients have an increased risk for HEV exposure; and (3) to evaluate the stage of liver disease.

**MATERIALS AND METHODS**

This observational study was carried out from January 2012 to August 2013. The seroprevalence of HEV was determined in 801 subjects (231 HD-patients, 120 renal transplant recipients, and 450 individuals coming from the general population as controls). All of the HD-patients and the recipients of renal transplants were attending the Departments of Nephrology and Dialysis at two hospitals located in Southern Italy (Foggia and S. Giovanni Rotondo, Apulia), and were included progressively in this study. The controls were aged > 18 years and were identified from out-patient populations attending these hospitals for blood tests. Among the control patients, most were healthy, others had a range of acute/chronic general medical conditions, and some (approximately 6%) had a history of liver disease. All of the subjects included in the study were orally informed about the purpose of the study and invited to participate. Each patient gave informed consent. The research was conducted in accordance with the Declaration of Helsinki (as revised in 2008) and according to the local guidelines and laws. Because this was a case-control study, the assent of the local Ethics Committee was not mandatory. At baseline, all study participants were requested to complete a questionnaire to obtain demographic, lifestyle, socio-economic and clinical data to assess their previous exposure to viral hepatitis. These data included sexual orientation, ethnicity and liver function tests; the underlying nephrological diagnosis, previous transplantation (if on chronic haemodialysis), haemodialysis and transplant vintage and previous/current immunosuppressive treatment data were obtained for HD and transplant patients. Routine HD techniques were performed with ¾-hour treatments three times a week. The history of blood transfusion requirements for each patient was evaluated. No patient admitted had a history of intravenous drug abuse. All enrolled subjects also received a full clinical examination and were treated according to their clinical situation. The demographic, clinical and laboratory data of all patients are presented in Table 1.

The samples were investigated for the presence of anti-HEV immunoglobulin (IgG/IgM) using a commercial enzyme immunoassay (EIA) based on recombinant proteins (HEV IgG/IgM; DIA.PRO, Diagnostic BioProbes, Milan, Italy). If repeatedly positive, when sera gave an absorbance greater than the cut-off value, the results were confirmed by a western-blot assay (HEV-Recomblot, Nuclear Laser Medicine, Milan, Italy).

To determine HEV-RNA, a commercially available assay was used (Qiamp viral RNA mini-kit, Qiagen, Chatsworth, CA). After RT-nested PCR, genotyping was performed using restriction endonuclease analysis (a technique in which deoxyribonucleic acid (DNA) fragments obtained from digestion with restriction enzymes are compared to construct a restriction map showing the position of specific sites along a sequence of DNA)[41]. The anti-HEV antibodies, western-blots, determination of HEV-RNA and genotypes assessments were performed using the same assays in a single laboratory (Foggia).

HBV markers were assayed by commercial immunoassay (Abbott-Auszyme Mc, Abbott Laboratories, North Chicago, IL, United States). The presence of antibodies to HCV was determined by the use of a third-generation enzyme-linked-immunoabsorbent assay (HCV-ELISA, Ortho Diagnostic System, Raritan, NJ, United States) and confirmed by a third-generation-recombinant-immunoblot assay (RIBA, Ortho Diagnostic Systems, Raritan, NJ, United States). To determine HBV-DNA and HCV-RNA, a commercially available assay was used (Qiamp viral RNA, Qiagen, Chatsworth, CA). The presence of antibodies to HIV 1+2 was determined by a commercial immunoassay (Ortho Diagnostic Systems, Raritan, NJ, United States). To determine HIV-RNA, a commercially available assay was used (Artus HIV virus 1, Rg RT-PCR kit, Qiagen, Chatsworth, CA, United States).

Serum alanine-amino-transferase (ALT) was quantified by ultraviolet-enzymatic-assay (normal range 0-40 IU/L). Each patient’s hepatic biochemical, epidemiological and virological parameters were recorded; and a serum sample was taken and frozen at -70 °C, prior to being tested for HEV by reverse transcriptase-polymerase chain reaction (RT-PCR), anti-HEV immunoglobulin G (IgG IgM) immunoassays and western blotting.

***Statistical analysis***

The chi-square test was used to compare categorical variables (sex, positivity for anti-HEV IgG/M, western-blot test results for HEV antibodies, HCV antibodies, and HBV markers). When possible, odds ratio and 95% confidence intervals (CIs) were calculated. Continuous variables (age, and ALT levels) were compared by Student’s *t*-test for independent samples and ANOVA. Logistic-regression models were used to account for the confounding effects of patient demographics. *P* values < 0.05 were considered significant. The data were analysed by STATA 10 MP software (Stata Corp., United States) for Mac OS X.

**RESULTS**

A total of 30/801 (3.7%) patients were anti-HEV Ig (IgG and/or IgM) and western-blot positive; almost none of the patients showed any clinical symptom that could be related to acute or chronic hepatitis. The prevalence in dialysis patients was 6.0% (14 patients); in transplant recipients the prevalence was 3.3% (4 individuals) and in the general population, the prevalence was 2.7% (12 subjects). The overall HEV-positive prevalence in the two groups with chronic renal failure combined was 5.1%. The rates of exposure to HEV (positivity of HEV-IgG/M in the early samples) were lower in the healthy controls, but the difference among the three groups was not statistically significant (*p >* 0.05).

Positivity for anti-HEV/IgM was detected in 4/30 (13.33%) anti-HEV Ig positive individuals, in 2/14 HD-patients, in 1/4 transplant individuals and in 1/12 individuals from the healthy population (Table 2).

There was not a statistically significant difference between the rates in HD patients and healthy controls (0.98% *vs* 0.22%, *p >* 0.05). The relative risk to be HEV-IgM-positive was significantly higher in transplant recipients compared to the other two groups (OR = 65.4, 95%CI: 7.2-592.7, *p <* 0.001), but the subjects with HEV-IgM positivity were numerically too few to determine a significant difference. The origin of acute HEV infection (IgM positive and HEV-RNA detecTable) in HD patients, transplant recipients and the healthy population was uncertain.

HEV-RNA determination was positive in all of the IgM-positive patients and in two of the IgG positive patients (1 dialysis and 1 transplant, both HEV/HCV co-infected), who presented with hepatic fibrosis. Among dialysis and transplant patients with acute hepatitis (anti-HEV IgM), one carried genotype 1(an immigrant) and two presented with genotype 3; among the general population population the only anti-HEV IgM patient presented with genotype 3.

There was no significant correlation in both the groups between sex (males 7/14 in HD patients, 2/4 in renal transplants recipients and 8/12 in the healthy population) and HEV-IgG/IgM and western-blot positivity (*p >* 0.05). The mean age in the transplant individuals and in the dialysis patients was not significantly different between subjects who were HEV-positive (the age of transplant subjects 48.5 ± 12.1 years; the age of HD patients 59.0 ± 6.7) *vs* HEV-negative (the age of transplants subjects 48.5 ± 18.9 years; the age of HD patients 62.9 ± 6.3; *p >* 0.05). Instead, the mean age of the healthy population, was significantly higher in HEV-positive subjects (49.8 ± 12.1 years) *vs* HEV-negative (39.7 ± 18.9 years, *p <* 0.05). However, in logistic-regression models adjusted for age, sex and group, the risk of anti-HEV positivity was not significantly higher in HD-patients compared with the other two groups. The only statistically significant association in HD-patients was with age (OR = 11.7, 95%CI: 5.9-23.2, *p <* 0.001). The cohort of patients > 45 years presented with HEV positivity more frequently than the other groups aged 21-45 years. The risk of western-blot positivity was related to age and it was higher in HD patients (OR = 12.3, 95%CI: 5.9-25.5, *p <* 0.001).

The haemodialysis vintage in HD-patients ranged between 4-121 mo (median 79.2 mo) for anti-HEV positive patients and between 1-184 mo (median 79.4 mo) for the anti-HEV negative patients (*p >* 0.05). Among the patients with a functioning transplant, only 2/4 patients who were HEV positive had a prior history of HD treatment (median 21 mo *vs* 17 mo for HEV-negative patients). The length of haemodialysis treatment in these subjects also did not seem to be a significant risk factor for HEV IgG positivity; however, the duration of HD treatment, before renal transplant, in HEV-positive individuals, is lower compared to that of haemodialysis patients.

Five (2.2%) HD-patients and 1/120 (0.83%) transplant recipient presented with HBV infection (hepatitis B surface antigen positive), while 67/231 (29.0%) HD-patients and 29/120 (24.2%) transplant recipients had serological parameters of previous HBV infection (anti-HBc and/or anti-HBs positivity). The individuals who had been immunized with hepatitis B vaccine were not included in this calculation of HBV infection. Co-infection with HBV/HEV was not present in any of the HD and transplant subjects.

Moreover, we observed 18/231 (7.8%) patients with an anti-HCV antibody among HD-patients and 19/120 (15.8%) patients with an anti-HCV antibody among the transplant recipients. Co-infection with HEV/HCV was present in 1/14 (7.1%) of the HD-patients and 1/4 (25%) of the transplant subjects. No co-infection with other hepatitis viruses were present in HEV positive subjects from the general population.

Patients with chronic renal failure and HEV-IgG positivity were for the most part asymptomatic, only 7/18 (38.9%) reported moderate asthenia; whereas jaundice was present in 1/3 (33.3%) IgM-positive patients, and in 2/3 (66.76%) we observed hepato-splenomegaly and distended abdomen.

Among the HD-patients and transplant recipients who were anti-HEV positive, approximately 67% of individuals had normal ALT values. Higher serum levels of ALT were observed in HEV-IgM positive *vs* HEV-IgM negative subjects (178.8 ± 131.1 *vs* 33.7 ± 14.5; *p <* 0.001). Among the HEV-IgG positive subjects, ALT levels were not significantly different among HD-patients (31.59 ± 18.05 UI/ml) *vs* transplant individuals (28.8 ± 13.1) and the general population (54 ± 30 UI/ml), *p >* 0.05.

There is no connection between any stage of chronic hepatitis and only HEV infection in all of the patients groups who presented with chronic renal failure. In fact, the two IgG positive patients (1 dialysis and 1 transplant patient) who presented with chronic active hepatitis with advanced fibrosis were HCV-HEV co-infected, and both patients presented with genotype HEV 3.

**DISCUSSION**

In the past few years, there has been increasing evidence that HEV, with the development of acute/chronic clinical disease (mainly in immune-compromised patients, HD-patients and transplant recipients), may occur in non-endemic areas, where the zoonotic pathway (*porcine zoonosis*) has been found to be the major reason for this infection[20,21,42,43]. There are few data on the prevalence of HEV infection and/or the prevalence of circulating HEV antibodies in Italy or in Southern Italy in end-stage renal failure patients[11,44,45].

In this survey, we studied three cohorts of individuals (haemodialysis patients, kidney transplant recipients and the general population as a control); the overall prevalence of circulating HEV-Ab was 3.7% with appreciable, but not significant, deviations between the general population (2.7%) and HD-patients (6.0%), but not kidney transplant recipients (3.3%), compared to the general population.

There are few studies with conflicting results about HEV epidemiology among HD patients[10,11,14,15]. The different prevalence of HEV infection in the general population[42,43,46], the parameters for the inclusion of patients, the routes of HEV transmission[22,24]and the serological assays used[47,48] could partially explain the different findings. In our research in patients with defects in cellular and humoral immunity, we confirmed HEV-Ig positivity with the western immunoblotting techniques, which validated both the acute-phase and chronic-phase with better sensitivity and specificity[49]. The seroprevalence of anti-HEV/IgG observed in our HD-patients is lower than that reported in other recent studies in the United Kingdom[10], France[46] and Japan[15] and is consistent with the data of the HEV seroprevalence in Greece[13,30], another study in Japan[29], Spain[31] and Saudi Arabia[50]. In all of the studies, there was a higher anti-HEV seroprevalence in HD-patients *vs* controls.

A logistic regression analysis showed that neither length of HD, nor other variables related to HD, such as blood transfusion, were associated with HEV, while a significant link was reported between the presence of antibodies type IgG and older age (> 70 years).

The correlation of HEV/older age could reflect, a cohort phenomenon due to infections acquired some decades ago. Antibodies for HEV/IgG can persist over the long-term, and it may be that water-borne hepatitis outbreaks of unknown aetiology occurred in Apulia earlier in the XX century and became present as sporadic cases owing to the faecal pollution of drinking water with hepatitis E and not A (previously related to the high circulation of HAV in our region, which is approximately 60% of subjects older than 50 years).

HEV is usually associated with an enterically transmitted infection, but the high prevalence of anti-HEV reported in some studies in HD-patients indicated that the faecal-oral route may not be the only route of transmission of HEV and these individuals with a high risk for HBV and HCV could also have been infected by unknown HEV. In fact, experimental transmission of HEV in humans showed a transient phase of viremia preceding the onset of clinical symptoms, and prolonged viremia has been observed in some patients[50]. Therefore, a theoretical possibility of HEV parenteral transmission has been suggested, mainly in endemic areas[50,51]. In our study, only two patients presented with an association between HEV and HCV. Our data are probably different than that of regions with high rates of HEV infection for two reasons: first, in Italy, there is a modest rate of anti-HEV and a low flow of HEV in our community, resulting in a reduced risk of chronic HCV co-infection; second, a relatively small number of patients was tested.

Another interesting and stimulating hypothesis was suggested by Harrison et al, claiming that the use of heparin, derived from porcine small intestine, in HD patients might be one possible cause of HEV infection[52]; HEV has been found in the porcine small intestine after experimental HEV infection in pigs[53]. It is not known whether heparin regularly used in humans is infected with HEV, but this might be a possible route of infection and merits further studies[54].

Acute HEV in HD and transplant patients is infrequent and possibly under-diagnosed. Only 0.9% (2/31) of the HD patients and 1/120 (0.8%) of the transplant recipients, in the present survey were anti-HEV/IgM positive; only one of the patients had any symptoms/signs suspected or diagnosed as acute hepatitis. The clinical events usually associated with acute hepatitis are often less evident in these patients (low grade or subclinical hepatitis); this factor might have contributed to the failure to recognise and document any clinical episodes of hepatitis in our patients. Serum levels of ALT are low in HD patients, and its elevation is usually less pronounced in these individuals, even in the presence of acute hepatitis. In fact, chronic uremic patients present with a reduced immune competence and this situation can be responsible for the attenuated inflammatory reactions in the liver and consequently reduced hepatocyte destruction, and therefore, the AST/ALT levels in HD-patients are usually suppressed[50,55]. The ALT levels in the three patients found to be IgM anti-HEV positive in our study showed only mild abnormalities; the highest ALT levels, with a peak elevation within 1±3 days of the onset of the illness followed by a decline, were less than twice the upper limit of normal level.

We observed that the HD patients had a higher HEV seroprevalence than transplant recipients (6% *vs* 3.3%), suggesting that haemodialysis could represent a risk factor for HEV infection. However, in our study, renal transplant recipients with a prior history of HD had the same rate of HEV infection than that seen in transplants recipients without prior HD treatment. The different seroprevalence between the HD patients and transplant recipients is unexplained, and it is possible that anti-HEV serological assays perform poorly in transplant recipients receiving immunosuppressive treatment, thereby determining a percentage error with false negative results[56]. In the transplant recipients in our survey, the numbers and the doses of immunosuppressive drugs were decreased when they were diagnosed anti-HEV IgM and HEV-RNA positive. In fact, this is the first approach to control HEV infection in these patients[33] because these drugs decrease the synthesis of antibodies and inhibit the cell-cycle progression and differentiation of human B lymphocytes. The humoral immune response is necessary to clear HEV and prevent hepatitis[57,58].

However, in the past few years, many studies have reported a surprisingly high prevalence of chronic HEV hepatitis in immunosuppressed patients, including kidney transplant recipients[34-36], and in our survey at 6 mo of follow-up no patient remained chronically viremic and there is not a correlation between any stage of chronic hepatitis and HEV infection alone in all patients of the two groups with chronic renal failure. The only patients who presented with chronic active hepatitis were HCV-HEV co-infected.

Finally, this study showed a higher circulation of HEV in end-stage renal failure in the district of Foggia *vs* the healthy population, but this high prevalence is mainly related to haemodialysis patients.

There was no relationship between the duration of HD treatment and the risk of acquiring a HEV infection. None of the kidney transplant recipients and HD-patients had evidence of chronic HEV. Although no specific therapy is currently available, unexplained hepatitis in the dialysis setting and in kidney transplant recipients undergoing chronic immunosuppression calls for an evaluation of HEV infection, especially in kidney transplant candidates.

**comments**

***Background***

Hepatitis E, is the main aetiological agent of enteric non-A hepatitis. In the recent past, it was believed to be present only in developing countries, where it was associated with epidemic outbreaks through the faecal-oral route from contaminated water supplies, but it is now recognized as a worldwide infection, sometimes related, in developed countries, to an asymptomatic zoonotic infection (also undercook meat products).

***Research frontiers***

Recently, previous sero-prevalence surveys in developed countries showed variable rates of anti-hepatitis E virus (HEV) positivity in healthy populations, and several studies reported an unexpected high prevalence of antibodies against HEV in haemodialysis patients.

***Innovations and breakthroughs***

There are scant reports on the prevalence and possible nosocomial transmission of HEV in haemodialysis (HD) patients. Some authors highlighted high rates of anti-HEV antibodies in their HD patients and hypothesised that there are other routes of transmission besides the faecal-oral route, although the real prevalence of HEV infection through the parenteral route, particularly via haemodialysis, is unknown. Alternatively, other investigators observed low rates of anti-HEV-positivity in their HD populations.

***Applications***

This study showed a higher circulation of HEV in end-stage renal failure in the district of Foggia *vs* the healthy population, but this high prevalence is mainly related to haemodialysis patients. There was no relationship between the duration of HD treatment and the risk of acquiring a HEV infection. None of the kidney transplant recipients and HD-patients had evidence of chronic HEV. Although no specific therapy is currently available, unexplained hepatitis in the dialysis setting and in kidney transplant recipients undergoing chronic immunosuppression calls for an evaluation of HEV infection, especially in kidney transplant candidates.

***Terminology***

Hepatitis E, a single-stranded RNA virus, is the main aetiological agent of enteric non-A hepatitis. The prevalence of HEV-IgG in chronic haemodialysis patients and transplant recipients could be related to their impaired immunity, with an increased susceptibility to infections and decreased immune responses to antigenic stimuli.

***Peer review***

Scotto *et al* report the results from screening a large number of HD and control patients for prior and current HEV infection. This is of interest because chronic HEV has been shown to infect immunosuppressed populations and hemodialysis patients are in some respects immunocompromised. There has also been relatively high prevalence found in renal transplant patients that come from this population.

**REFERENCES**

1 **Tsianos EV**, Dalekos GN, Elisaf M, Zervou E, Siamopoulos KC. High frequency of antibodies to Hantaan virus and hepatitis C virus in chronic haemodialysis patients. Coincidence or cross-reaction? *J Intern Med* 1993; **234**: 607-610 [PMID: 8258753 DOI: 10.1111/j.1365-2796.1993.tb01021.x]

2 **Fabrizi F**, Lunghi G, Martin P. Hepatitis B virus infection in hemodialysis: recent discoveries. *J Nephrol* 2002; **15**: 463-468 [PMID: 12455711 DOI: 10.1111/j.1525-139x.2007.00311.x]

3 **Fabrizi F**, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. *Hepatology* 2002; **36**: 3-10 [PMID: 12085342 DOI: 10.1053/jhep.2002.34613]

4 **Wong PN**, Fung TT, Mak SK, Lo KY, Tong GM, Wong Y, Loo CK, Lam EK, Wong AK. Hepatitis B virus infection in dialysis patients. *J Gastroenterol Hepatol* 2005; **20**: 1641-1651 [PMID: 16246180 DOI: 10.1111/j.1440-1746.2005.03837.x]

5 **Mina P**, Georgiadou SP, Rizos C, Dalekos GN, Rigopoulou EI. Prevalence of occult hepatitis B virus infection in haemodialysis patients from central Greece. *World J Gastroenterol* 2010; **16**: 225-231 [PMID: 20066742 DOI: 10.3748/wjg.v16.i2.225]

6 **Alter MJ**, Favero MS, Maynard JE. Impact of infection control strategies on the incidence of dialysis-associated hepatitis in the United States. *J Infect Dis* 1986; **153**: 1149-1151 [PMID: 3701120 DOI: 10.1093/infdis/153.6.1149]

7 **Ayoola EA**, Huraib S, Arif M, al-Faleh FZ, al-Rashed R, Ramia S, al-Mofleh IA, Abu-Aisha H. Prevalence and significance of antibodies to hepatitis C virus among Saudi haemodialysis patients. *J Med Virol* 1991; **35**: 155-159 [PMID: 1725179 DOI: 10.1002/jmv.1890350303]

8 **Niu MT**, Alter MJ, Kristensen C, Margolis HS. Outbreak of hemodialysis-associated non-A, non-B hepatitis and correlation with antibody to hepatitis C virus. *Am J Kidney Dis* 1992; **19**: 345-352 [PMID: 1562024 DOI: 10.1016/s0272-6386(12)80452-5]

9 **Okuda K**, Hayashi H, Yokozeki K, Kobayashi S, Kashima T, Irie Y. Acute hepatitis C among renal failure patients on chronic haemodialysis. *J Gastroenterol Hepatol* 1998; **13**: 62-67 [PMID: 9737574 DOI: 10.1111/j.1440-1746.1998.tb00547.x]

10 **Dalton HR**, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis* 2008; **8**: 698-709 [PMID: 18992406 DOI: 10.1016/s1473-3099(08)70255-x]

11 **Scotto G**, Martinelli D, Centra M, Querques M, Vittorio F, Delli Carri P, Tartaglia A, Campanale F, Bulla F, Prato R, Fazio V. Epidemiological and clinical features of HEV infection: a survey in the district of Foggia (Apulia, Southern Italy). *Epidemiol Infect* 2014; **142**: 287-294 [PMID: 23673019 DOI: 10.1017/s0950268813001167]

12 **Dalekos GN**, Zervou E, Elisaf M, Germanos N, Galanakis E, Bourantas K, Siamopoulos KC, Tsianos EV. Antibodies to hepatitis E virus among several populations in Greece: increased prevalence in an hemodialysis unit. *Transfusion* 1998; **38**: 589-595 [PMID: 9661693 DOI: 10.1046/j.1537-2995.1998.38698326339.x]

13 **Stefanidis I**, Zervou EK, Rizos C, Syrganis C, Patsidis E, Kyriakopoulos G, Sdrakas L, Tsianas N, Rigopoulou EI, Liakopoulos V, Dalekos GN. Hepatitis E virus antibodies in hemodialysis patients: an epidemiological survey in central Greece. *Int J Artif Organs* 2004; **27**: 842-847 [PMID: 15560678 DOI: 10.1016/j.jcv.2005.05.007]

14 **Sylvan SP**, Jacobson SH, Christenson B. Prevalence of antibodies to hepatitis E virus among hemodialysis patients in Sweden. *J Med Virol* 1998; **54**: 38-43 [PMID: 9443107 DOI: 10.1002/(sici)1096-9071(199801)54:1<38::aid-jmv6>3.0.co;2-q]

15 **Mitsui T**, Tsukamoto Y, Hirose A, Suzuki S, Yamazaki C, Masuko K, Tsuda F, Endo K, Takahashi M, Okamoto H. Distinct changing profiles of hepatitis A and E virus infection among patients with acute hepatitis, patients on maintenance haemodialysis and healthy individuals in Japan. *J Med Virol* 2006; **78**: 1015-24 [DOI: 10.1002/jmv.20657]

16 **Litjens NH**, Huisman M, van den Dorpel M, Betjes MG. Impaired immune responses and antigen-specific memory CD4+ T cells in hemodialysis patients. *J Am Soc Nephrol* 2008; **19**: 1483-1490 [PMID: 18480314 DOI: 10.1681/asn.2007090971]

17 **Edey M**, Barraclough K, Johnson DW. Review article: Hepatitis B and dialysis. *Nephrology* (Carlton) 2010; **15**: 137-145 [PMID: 20470270 DOI: 10.1111/j.1440-1797.2009.01268.x]

18 **Cohen G**, Haag-Weber M, Hörl WH. Immune dysfunction in uremia. *Kidney Int Suppl* 1997; **62**: S79-S82 [PMID: 9350688 DOI: 10.3390/toxins4110962]

19 **Reuter G**, Fodor D, Forgách P, Kátai A, Szucs G. Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *J Clin Virol* 2009; **44**: 277-281 [PMID: 19217346 DOI: 10.1016/j.jcv.2009.01.008]

20 **Christensen PB**, Engle RE, Hjort C, Homburg KM, Vach W, Georgsen J, Purcell RH. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. *Clin Infect Dis* 2008; **47**: 1026-1031 [PMID: 18781880 DOI: 10.1086/591970]

21 **Meng XJ**, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol* 2002; **40**: 117-122 [PMID: 11773103 DOI: 10.1128/jcm.40.1.117-122.2002]

22 **Mushahwar IK**. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol* 2008; **80**: 646-658 [PMID: 18297720 DOI: 10.1002/jmv.21116]

23 **Somani SK**, Aggarwal R, Naik SR, Srivastava S, Naik S. A serological study of intrafamilial spread from patients with sporadic hepatitis E virus infection. *J Viral Hepat* 2003; **10**: 446-449 [PMID: 14633178 DOI: 10.1046/j.1365-2893.2003.00458.x]

24 **Teshale EH**, Grytdal SP, Howard C, Barry V, Kamili S, Drobeniuc J, Hill VR, Okware S, Hu DJ, Holmberg SD. Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clin Infect Dis* 2010; **50**: 1006-1010 [PMID: 20178415 DOI: 10.1086/651077]

25 **Cleland A**, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, Petrik J. Hepatitis E virus in Scottish blood donors. *Vox Sang* 2013; **105**: 283-289 [PMID: 23763589 DOI: 10.1111/vox.12056]

26 **Mansuy JM**, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V, Rech H, Destruel F, Kamar N, Dalton HR, Izopet J. Hepatitis E virus antibodies in blood donors, France. *Emerg Infect Dis* 2011; **17**: 2309-2312 [PMID: 22172156 DOI: 10.3201/eid1712.110371]

27 **Scotto G**, Giammario A, Centra M, Vittorio F, Martinelli D, Fazio V. Seroprevalence of hepatitis E virus among blood donors in a district of southern Italy. *Blood Transfus* 2012; **10**: 565-566 [PMID: 22682344 DOI: 10.2450/2012.0154-11]

28 **Mitsui T**, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. *J Med Virol* 2004; **74**: 563-572 [PMID: 15484278 DOI: 10.1002/jmv.20215]

29 **Kikuchi K**, Yoshida T, Kimata N, Sato C, Akiba T. Prevalence of hepatitis E virus infection in regular hemodialysis patients. *Ther Apher Dial* 2006; **10**: 193-197 [PMID: 16684223 DOI: 10.1111/j.1744-9987.2006.00363.x]

30 **Psichogiou M**, Vaindirli E, Tzala E, Voudiclari S, Boletis J, Vosnidis G, Moutafis S, Skoutelis G, Hadjiconstantinou V, Troonen H, Hatzakis A. Hepatitis E virus (HEV) infection in haemodialysis patients. The Multicentre Haemodialysis Cohort Study on Viral Hepatitis. *Nephrol Dial Transplant* 1996; **11**: 1093-1095 [PMID: 8671974 DOI: 10.1093/oxfordjournals.ndt.a027461]

31 **Ibarra H**, Riedemann S, Reinhardt G, Ardiles L, Calvo M, Siegel F. Anti-HEV in dialysis and renal transplant patients in an endemic region in Chile. *Clin Nephrol* 1998; **50**: 267-268 [PMID: 9799076 DOI: 10.1016/s0140-6736(94)90315-8]

32 **Buffet C**, Laurent-Puig P, Chandot S, Laurian Y, Charpentier B, Briantais MJ, Dussaix E. A high hepatitis E virus seroprevalence among renal transplantation and haemophilia patient populations. *J Hepatol* 1996; **24**: 122-125 [PMID: 8834035 DOI: 10.1016/s0168-8278(96)80196-6]

33 **Kamar N**, Selves J, Mansuy JM, Ouezzani L, Péron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, Durand D, Vinel JP, Izopet J, Rostaing L. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008; **358**: 811-817 [PMID: 18287603 DOI: 10.1056/nejmoa0706992]

34 **Gérolami R**, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med* 2008; **358**: 859-860 [PMID: 18287615 DOI: 10.1056/nejmc0708687]

35 **Gérolami R**, Moal V, Picard C, Colson P. Hepatitis E virus as an emerging cause of chronic liver disease in organ transplant recipients. *J Hepatol* 2009; **50**: 622-624 [PMID: 19157619 DOI: 10.1016/j.jhep.2008.12.008]

36 **Haagsma EB**, van den Berg AP, Porte RJ, Benne CA, Vennema H, Reimerink JH, Koopmans MP. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2008; **14**: 547-553 [PMID: 18383084 DOI: 10.1002/lt.21480]

37 **Ollier L**, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet JG, Nicand E. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Ann Intern Med* 2009; **150**: 430-431 [PMID: 19293084 DOI: 10.7326/0003-4819-150-6-200903170-00026]

38 **Péron JM**, Mansuy JM, Récher C, Bureau C, Poirson H, Alric L, Izopet J, Vinel JP. Prolonged hepatitis E in an immunocompromised patient. *J Gastroenterol Hepatol* 2006; **21**: 1223-1224 [PMID: 16824086 DOI: 10.1111/j.1440-1746.2006.04209.x]

39 **Tamura A**, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, Mishiro S, Shimizu K, Moriyama M. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatol Res* 2007; **37**: 113-120 [PMID: 17300706 DOI: 10.1111/j.1872-034x.2007.00024.x]

40 **Dalton HR**, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med* 2009; **361**: 1025-1027 [PMID: 19726781 DOI: 10.1056/nejmc0903778]

41 **Gouvea V**, Hoke CH, Innis BL. Genotyping of hepatitis E virus in clinical specimens by restriction endonuclease analysis. *J Virol Methods* 1998; **70**: 71-78 [PMID: 9506814 DOI: 10.1016/s0166-0934(97)00172-9]

42 **Purcell RH**, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 2008; **48**: 494-503 [PMID: 18192058 DOI: 10.1016/j.jhep.2007.12.008]

43 **Aggarwal R**, Naik S. Epidemiology of hepatitis E: current status. *J Gastroenterol Hepatol* 2009; **24**: 1484-1493 [PMID: 19686410 DOI: 10.1111/j.1440-1746.2009.05933.x]

44 **Gessoni G**, Manoni F. Hepatitis E virus infection in north-east Italy: serological study in the open population and groups at risk. *J Viral Hepat* 1996; **3**: 197-202 [PMID: 8871881 DOI: 10.1111/j.1365-2893.1996.tb00095.x]

45 **Fabrizi F**, Lunghi G, Bacchini G, Corti M, Pagano A, Locatelli F. Hepatitis E virus infection in haemodialysis patients: a seroepidemiological survey. *Nephrol Dial Transplant* 1997; **12**: 133-136 [PMID: 9027787 DOI: 10.1093/ndt/12.1.133]

46 **Mansuy JM**, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M, Vischi F, Alric L, Vinel JP, Izopet J. Hepatitis E in the south west of France in individuals who have never visited an endemic area. *J Med Virol* 2004; **74**: 419-424 [PMID: 15368508 DOI: 10.1002/jmv.20206]

47 **Bendall R**, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J Med Virol* 2010; **82**: 799-805 [PMID: 20336757 DOI: 10.1002/jmv.21656]

48 **Drobeniuc J**, Meng J, Reuter G, Greene-Montfort T, Khudyakova N, Dimitrova Z, Kamili S, Teo CG. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis* 2010; **51**: e24-e27 [PMID: 20578874 DOI: 10.1086/654801]

49 **Haqshenas G**, Huang FF, Fenaux M, Guenette DK, Pierson FW, Larsen CT, Shivaprasad HL, Toth TE, Meng XJ. The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and chicken big liver and spleen disease virus. *J Gen Virol* 2002; **83**: 2201-2209 [PMID: 12185274]

50 **Ayoola EA**, Want MA, Gadour MO, Al-Hazmi MH, Hamza MK. Hepatitis E virus infection in haemodialysis patients: a case-control study in Saudi Arabia. *J Med Virol* 2002; **66**: 329-334 [PMID: 11793384 DOI: 10.1002/jmv.2149]

51 **Chauhan A**, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. *Lancet* 1993; **341**: 149-150 [PMID: 8093748 DOI: 10.1016/0140-6736(93)90008-5]

52 **Jaques LB**, Kavanagh LW, Kuo SH. Variation in commercial heparin and its relation to the problem of heparin standardization for clinical use. *Thromb Res* 1973; **3**: 295-306 [DOI: 10.1016/0049-3848(73)90055-8]

53 **Lee YH**, Ha Y, Ahn KK, Chae C. Localisation of swine hepatitis E virus in experimentally infected pigs. *Vet J* 2009; **179**: 417-421 [PMID: 18308595 DOI: 10.1016/j.tvjl.2007.10.028]

54 **Harrison A**, Scobie L, Crossan C, Parry R, Johnston P, Stratton J, Dickinson S, Ellis V, Hunter JG, Prescott OR, Madden R, Lin NX, Henley WE, Bendall RP, Dalton HR. Hepatitis E seroprevalence in recipients of renal transplants or haemodialysis in southwest England: a case-control study. *J Med Virol* 2013; **85**: 266-271 [PMID: 23169048 DOI: 10.1002/jmv.23463]

55 **Fabrizi F**, Lunghi G, Finazzi S, Colucci P, Pagano A, Ponticelli C, Locatelli F. Decreased serum aminotransferase activity in patients with chronic renal failure: impact on the detection of viral hepatitis. *Am J Kidney Dis* 2001; **38**: 1009-1015 [PMID: 11684554 DOI: 10.1053/ajkd.2001.28590]

56 **Legrand-Abravanel F**, Kamar N, Sandres-Saune K, Lhomme S, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerg Infect Dis* 2011; **17**: 30-37 [PMID: 21192851 DOI: 10.3201/eid1701.100527]

57 **Luo H**, Chen H, Daloze P, Chang JY, St-Louis G, Wu J. Inhibition of in vitro immunoglobulin production by rapamycin. *Transplantation* 1992; **53**: 1071-1076 [PMID: 1585470 DOI: 10.1097/00007890-199205000-00019]

58 **Aagaard-Tillery KM**, Jelinek DF. Inhibition of human B lymphocyte cell cycle progression and differentiation by rapamycin. *Cell Immunol* 1994; **156**: 493-507 [PMID: 7517796 DOI: 10.1006/cimm.1994.1193]

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**Table 1 Clinical characteristics**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Transplant patients** | **HD patients** | **General population** |
| **Total** | 120 | 231 | 450 |
| **Sex (**Male:Female) | 82:38 | 126:105 | 178:272 |
| **Median age** | 48 yr | 63 yr | 40 yr |
| **Causes of renal failure** | Chronic glomerulonephritis: 43Nephroangiosclerosis: 21Polycystic kidney disease: 16Diabetic nephropathy: 14Chronic interstitial nephritis: 11Other aetiologies: 15 | Chronic glomerulonephritis: 73Nephroangiosclerosis: 27Polycystic kidney disease: 32Diabetic nephropathy: 44Chronic interstitial nephritis: 26Other aetiologies: 29 |  |
| **Median HD treatment** | 18 mo (range: 1-54 mo)17 mo (range: 1-48 mo) HEV-negative21 mo (range: 3-54 mo) HEV-positive | 79 mo (range: 3-154 mo)78.9 mo (range: 3-149 mo) HEV-negative81 mo (range: 7-154 mo) HEV-positive |  |
| **Immunosuppressive treatment** | All of the patients (120) | Yes: 2 (males) |  |

HEV: hepatitis E virus; HD: haemodialysis.

**Table 2 hepatitis E virus antibodies *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Transplant patients** | **HD patients** | **General population** |
| **Total** | 120 | 231 | 450 |
| **Anti-HEV Ig pos.** | 4 (3.3) | 14 (6) | 12(2.7) |
| **HEV IgM pos.** | 1 | 2 | 1 |
|  **HEV RNA pos.** | 2 | 3 | 1 |
|  |  |  |  |

HEV: hepatitis E virus; HD: haemodialysis.