ORIGINAL ARTICLE

# Interferon-λ4 gene polymorphisms, circulating interferon λ3, and clinical variables in hemodialysis patients exposed to hepatitis E virus

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**KEY WORDS**

circulating interferon λ3, hemodialysis, hepatitis E virus, high-flux dialysis, *IFNL4* polymorphisms

**ABSTRACT**

**INTRODUCTION** Factors associated with hepatitis E virus (HEV) infection are rarely recognized in patients on renal replacement therapy (RRT), and the results of studies are inconsistent.

**OBJECTIVES** We aimed to search for determinants of HEV seroprevalence among polymorphisms of the interferon-λ4 gene (*IFNL4*) associated with seroclearance of hepatotropic viruses (*IFNL4* rs12979860, rs8099917 near *IFNL4*), circulating interferon λ3 (IFN-λ3), and clinical variables of patients treated with hemodialysis (HD) in a HEV-endemic region.

**PATIENTS AND METHODS** The study was carried out in 90 HD patients. HEV open reading frame 2 antigen (HEV Ag), immunoglobulin M and G antibodies to HEV (anti-HEV IgM and anti-HEV IgG, respectively) and IFN-λ3 were tested, and *IFNL4* polymorphic variants (rs8099917, rs12979860) were genotyped. Survival analysis was conducted concerning anti-HEV IgG positivity.

**RESULTS** In the study group, there were 37.8% anti-HEV IgG-positive subjects. None was HEV Ag or anti-HEV IgM positive. HD modalities utilizing high-flux dialyzers (adjusted odds ratio [OR], 3.586; 95% confidence interval [CI], 1.142–11.263; $P = 0.03$) as well as major homozygosity in rs8099917 (adjusted OR, 4.933; 95% CI, 1.516–16.054; $P = 0.008$) and rs12979860 (adjusted OR, 3.537; 95% CI, 1.136–11.014, $P = 0.03$), but not circulating IFN-λ3 levels, were positive determinants of anti-HEV IgG positivity. Liver enzyme activities and C-reactive protein levels tested as response variables to HEV exposure, as well as survival probability, were not different between patients stratified by anti-HEV IgG positivity.

**CONCLUSIONS** Among HD patients, *IFNL4* polymorphisms and treatment with high-flux HD are explanatory variables for isolated anti-HEV IgG positivity indicating spontaneous HEV resolution.

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**INTRODUCTION** Acute hepatitis E virus (HEV) infection is prevalent worldwide.[1] HEV can also cause chronic infection in solid-organ transplant recipients, human immunodeficiency virus (HIV)-positive individuals with low CD4 counts, subjects with hematological malignancies,[2] and potentially in individuals with an immune system compromised by severe diseases, like end-stage renal disease (ESRD). In ESRD patients treated with hemodialysis (HD), a frequency of immunoglobulin (Ig) G-class antibodies to HEV antigen (anti-HEV IgG) positivity indicated an exposition to HEV infection in a wide range of 6.3% to 28.3%.[3] As serology kits used for determination of anti-HEV IgG differ significantly in the detection of these antibodies,[4] it has to be noted that

differences in the distribution of anti-HEV IgG results shown above were obtained using reagents of the same manufacturer.

Chronic HEV infection has been associated with elevated expression of interferon (IFN)-stimulated genes (ISGs).[5] Retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and IFN regulatory factor 1 are the critical anti-HEV IFN-stimulated genes.[6] A sustained IFN response in human hepatoma cells and primary human hepatocytes persistently infected by HEV was dependent on both RIG-I and MDA5. The Janus kinase/signal transducer and activator of transcription (JAK/STAT) cascade was activated in HEV-infected cells.[5] HEV has a RNA genome and encodes 3 open reading frames (ORFs), of which ORF3 encodes a protein which enhances IFN-β production.[7]

In 2003, IFN-λs, coded by genes positioned on chromosome 19, were discovered by 2 independent groups.[8,9] Among IFN-λs, interleukin (IL)-28B (currently designated IFN-λ3) was attributed to the gene located on chromosome 19, tentatively referred to as IL-28B gene (*IL28B*). In 2009, the polymorphic variants rs8099917[10] and rs12979860,[11] both located near *IL28B*, were strongly associated with treatment-induced clearance of hepatitis C virus (HCV). In more recent studies, *IL28B* is referred to as *IFNL3*, and the polymorphic variants rs12979860 and rs8099917 are shown as *IFNL3* rs12979860[12] and *IFNL3* rs8099917.[13] In 2013, Prokunina-Olsson et al[14] discovered *IFNL4*, located upstream of *IFNL3*, and rs12979860 and rs8099917 are now attributed to this gene or near this gene location, respectively.[15] In this study, we use terms *IFNL4* rs12979860 for the rs12979860 variant lying in the intron 1 of *IFNL4*, and *IFNL4* rs8099917 for the rs8099917 variant located upstream of *IFNL4*, also if we cite publications using the previous names.

Spontaneous HCV clearance was associated with *IFNL4* polymorphic variants and serum IFN-λ3 concentrations.[16] HCV spontaneous resolution[16] and self-limited infection with hepatitis B virus (HBV)[17] were related to higher circulating IFN-λ3 levels. Patients with acute hepatitis E demonstrated higher circulating IFN-λ3 than healthy volunteers.[13] In HD patients, similar associations of HCV/HBV outcomes with IFN-λ3 were observed.[18,19] To our knowledge, there have been no reports concerning HEV seromarkers, *IFNL4*, and circulating IFN-λ3 levels in HD patients.

Our study aimed to look for determinants of HEV seroprevalence in HD patients. We have investigated whether there is a coincidence of anti-HEV IgG positivity with a distribution of *IFNL4* polymorphic variants, circulating IFN-λ3 concentrations, and routine demographic, clinical, and laboratory variables.

## PATIENTS AND METHODS

**Patients** HD patients (n = 90) were recruited for the study in the Greater Poland Voivodship, which is endemic to HEV.[20,21] The majority of patients (n = 50) were dialyzed in 2 dialysis facilities. However, to increase the number of subjects showing features not frequently occurring in HD patients but possibly involved in the prevalence of HEV, we enrolled selective patients (n = 40, infected with HBV and HCV, nonresponders to HBV vaccination) from other 18 dialysis centers located in the same region.

The inclusion criteria were as follows: 1) age over 18 years; 2) updated results of HBV surface antigen (HBsAg), antibodies to HBV core antigen, antibodies to surface antigen of hepatitis B virus, antibodies to HCV, and HCV RNA; 3) established status of a responder (anti-HBs titer ≥10 IU/l) or a nonresponder (anti-HBs titer <10 IU/l) to HBV; and 4) stable clinical condition for at least 2 months before enrollment.

The exclusion criteria included corticosteroid or immunosuppressive therapy for at least 2 months before enrollment; cachectic conditions causing decreases in serum protein levels; and antiviral treatment against HBV, HCV, HIV, and cytomegalovirus before or on enrollment.

All patients were dialyzed 3 times a week using low-flux (LF) dialyzers for LF-HD or high-flux (HF) dialyzers for HF-HD and online hemodiafiltration (HDF). Dialysis sessions lasted approximately 4 hours. Evaluations of dialysis water were performed regularly, including physicochemical examination, endotoxin content, and bacterial contamination. Dialyzers were not reused in any facility.

Patient data and blood samples were obtained since the beginning of January 2014 to the end of December 2016. Patients were followed until June 22, 2017. During this period, 34 patients (37.8%) died, and 8 (8.9%) discontinued HD due to renal transplantation.

**Laboratory methods** Fasting blood samples were collected before the midweek dialysis session.

HEV ORF2 antigen (Ag), anti-HEV IgM, and anti-HEV IgG were determined by enzyme-linked immunosorbent assays (ELISAs), using commercial kits (Wantai Biological Pharmacy Enterprise Co., Beijing, China). The results were considered positive when the ratio of the optical density value to the cutoff value was 1.1 or higher.

The IFN-λ3 concentration was determined with an ELISA kit (Human Interleukin 28B ELISA Kit, Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China). The sensitivity of the IFN-λ3 ELISA was 0.685 pg/ml. The intra-assay coefficient of variation (CV) was less than 10%, and the interassay CV was less than 12%.

Other laboratory parameters were determined using standard methods.

**Genotyping** Genomic locations of rs8099917 (T>G) and rs12 979 860 (C>T) about the IFNL3/IFNL4 positions are shown in Supplementary material, *Figure S1*. Both tested

biallelic *IFNL4* variants have 3 possible genotypes (rs12979860: CC, CT, and TT; rs8099917: TT, GT, and GG). These genotypes form 3 common haplotypes: CT, TG, and TT.

Both single nucleotide variants (SNVs) were genotyped using high-resolution melting curve analysis as previously described.[22] For quality control, 20 of the randomly chosen samples were regenotyped using the same genotyping method; the concordance rate was 100%.

The tested polymorphisms were in concordance with the Hardy–Weinberg equilibrium.

**Statistical analysis** The results are shown as a percentage for categorical variables. We used the Shapiro–Wilk test to determine whether the underlying distributions of continuous variables were normal. Median and range (minimum–maximum) were presented for continuous variables without normal distribution. If the distribution was normal, mean (SD) was used.

Departure from the Hardy–Weinberg equilibrium was determined by the $\chi^2$ analysis (df = 1, $P$ >0.01 for equilibrium).

The Mann–Whitney test or *t* test was used to compare continuous variables. The Pearson $\chi^2$ test or Fisher exact test was applied for the comparison of dichotomous variables. The Cochran–Armitage test for trend was used to show the significance of the trend in the distribution of genotype frequencies.

A logistic regression analysis was used to show the determinants of anti-HEV IgG positivity. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to show the strength of the association. Results were adjusted for sex, age, living in a rural area, renal replacement therapy (RRT) duration, and a history of renal transplantation, as appropriate.

A survival analysis was conducted using the Kaplan–Meier method with the log-rank test. A Cox proportional hazard model was applied in a multivariate analysis assessing the contribution of clinical measures to mortality.

A $P$ value of less than 0.05 was considered significant. However, $P$ values of less than 0.05 obtained for genetic associations were deemed to be significant if they remained significant after a Bonferroni correction. Proper information is given in the legends to tables presenting genetic associations.

The statistical analyses were performed using Statistica version 12 (Stat Soft, Inc., Tulsa, Oklahoma, United States) and R software, version 3.4.0.

Pair-wise linkage disequilibrium (LD) between tested SNVs was computed as both D' and $r^2$ using the genotype data from the tested sample and the Haploview 4.2 software (http://www.broad.mit.edu/mpg/haploview/). D' reflects the recombinational history, whereas $r^2$ summarizes both recombinational and mutational history.[23] The values for both parameters range between 0 and 1. Current research strongly favors the use of $r^2$ as a pairwise measure of LD in the context of association studies.[24] There is a simple inverse relationship between $r^2$ and the sample size required to detect an association between susceptibility loci and SNVs.[25] An $r^2$ of 1.0 is known as perfect LD. Therefore, in our study, thje D' measure is used to model recombination rates and $r^2$—to model association power.

Haplotypes were estimated using the Haploview 4.2 software. Haplotypes were statistically analyzed if their incidence in the examined group was over 1%. Statistical significance was assessed using the 1000-fold permutation test.

## RESULTS

**Baseline data** Characteristics of HD patients are presented in Table S1 in Supplementary material. All patients were Caucasians, tested negative for HIV, and were not vaccinated against HEV (in Poland the vaccine against HEV is not available). Patients received standard care; erythropoietin-stimulating agents were used in those who required them, and blood transfusions were avoided as much as possible.

**Seroprevalence of hepatitis E virus, hepatitis B virus, and hepatitis C virus** Of the 90 HD patients, 34 (37.8%) were positive for anti-HEV IgG. None of the patients were positive for HEV Ag or anti-HEV IgM.

The prevalence of HBV/HCV markers among HEV seropositive and seronegative patients are presented in TABLE 1. There were 26 patients (28.9%) exposed only to HEV; 4 patients (4.4%) showed HEV coinfection with HBV; 2 (2.2%), with HCV; and 2 (2.2%), with HCV and HBV. Among anti-HEV IgG–positive subjects, there were 8 individuals (23.5%) with HBV and HCV coinfection, whereas anti-HEV IgG–negative patients included 13 patients positive for HBV and HCV ($P$ = 0.97).

**Characteristics of patients positive for anti–hepatitis E virus immunoglobulin G** Data of HD patients categorized by anti-HEV IgG are shown in TABLE 1. Anti-HEV IgG–positive subjects revealed a higher frequency of treatment with HF dialyzers (HF-HD/HDF) and longer RRT duration.

Among patients currently treated with HF-HD/HDF, there were 4 subjects not treated exclusively with HF dialyzers, because they started HD utilizing LF-HD. If we compared HEV seroprevalence in patients using only HF-HD/HDF with HEV seroprevalence of subjects utilizing only LF-HD, the prevalence of HEV remained significantly higher in the group treated with HF-HD/HDF (TABLE 1).

When the duration of RRT modalities was compared between HEV seropositive and seronegative subgroups, no significant results were shown. However, longer total time on HD was observed in HEV seropositive subjects (borderline significance, $P$ <0.1), which was related mainly to longer lifespan on LF-HD (TABLE 1).

**TABLE 1** Characteristics of patients positive for anti–hepatitis E virus immunoglobulin G and those negative for tested hepatitis E virus seromarkers among all hemodialysis patients (n = 90)

| Data | Anti-HEV IgG–positive patients (n = 34) | HEV-negative patients (n = 56) | *P* value |
|---|---|---|---|
| **Clinical data** | | | |
| Male sex, n (%) | 19 (55.9) | 30 (53.6) | 0.83[a] |
| Age, y, median (range) | 66.0 (28.4–86.2) | 67.1 (29.5–90.5) | 0.47[b] |
| Living in the rural area, n (%) | 18 (52.9) | 21 (37.5) | 0.15[a] |
| History of symptomatic hepatitis, n (%)[e] | 3 (8.8) | 5 (8.9) | 1.00[c] |
| History of parathyroidectomy, n (%) | 2 (5.9) | 4 (7.1) | 1.00[c] |
| Body mass index, kg/m$^2$, mean (SD) | 26.5 (4.4) | 26.0 (4.7) | 0.62[d] |
| **Causes of ESRD, n (%)** | | | |
| Diabetic nephropathy | 10 (29.4) | 13 (23.2) | 0.51[a] |
| Hypertensive nephropathy | 6 (17.6) | 11 (19.6) | 0.82[a] |
| Chronic glomerulonephritis | 9 (26.5) | 12 (21.4) | 0.58[a] |
| Chronic tubulointerstitial nephritis | 4 (11.8) | 5 (8.9) | 0.73[c] |
| **Type of RRT, n (%)** | | | |
| PD as the first modality of RRT | 1 (2.9) | 3 (5.4) | 1.00[c] |
| Current HF-HD/HDF, n = 43, 47.8% of all | 21 (61.8) | 22 (39.3) | 0.04[a] |
| HD onset as LF-HD, n = 4, 9.3% of all | 2 (5.9) | 2 (3.6) | 0.63[c] |
| Only HF-HD/HDF, n = 39, 90.7% of all | 19 (55.9) | 20 (35.7) | 0.06[a] |
| Only HF-HD/HDF, n = 39, 45.3% of 86 treated exclusively with HF or LF dialyzers | 19 (59.4) | 20 (37.0) | 0.04[a] |
| Current LF-HD, n = 47, 52.2% of all | 13 (38.2) | 34 (60.7) | 0.04[a] |
| History of renal transplantation, n = 10,% of all | 6 (17.6) | 4 (7.1) | 0.17[c] |
| **Duration of RRT, median (range)** | | | |
| Total RRT duration (n = 90), y | 7 (0.3–24.1) | 4.7 (0.8–30.4) | 0.04[b] |
| PD duration before HD (n = 4), y | 3.4 | 4.5 (0.2–5) | 1.00[b] |
| Total time on HD (n = 90), y | 6.7 (0.3–13.1) | 4.6 (0.8–30.2) | 0.08[b] |
| Living on LF-HD only (n = 47), y | 7.5 (1.1–11.7) | 4.2 (0.8–30.2) | 0.09[b] |
| Living on HF-HD/HDF only (n = 39), y | 5.1 (0.3–10.8) | 4.8 (1.3–7.5) | 0.47[b] |
| Living on both LF-HD and HF-HD/HDF (n = 4), y | 11 (8.9–13.1) | 9 (7.2–10.8) | 0.70[b] |
| Timespan with functional renal graft (n = 10), y | 6 (0.003–17.1) | 6.4 (0.1–12) | 0.92[b] |
| **Laboratory data** | | | |
| Anti-HBs titer, IU/l, median (range) | 17.8 (0–1000) | 101.7 (0–1000) | 0.99[b] |
| Anti-HBs titer <10 IU/l, n (%) | 14 (41.2) | 21 (37.5) | 0.73[a] |
| HBsAg positivity, n (%) | 2 (5.9) | 6 (10.7) | 0.71[c] |
| Anti-HBc positivity, n (%) | 6 (17.6) | 9 (16.1) | 0.85[a] |
| Anti-HCV positivity, n (%) | 4 (11.8) | 10 (17.9) | 0.44[a] |
| HCV RNA positivity, n (%) | 3 (8.8) | 4 (7.1) | 1.00[c] |
| IFN-λ3, pg/ml, median (range) | 71.2 (10–232.7) | 90.6 (9–228.8) | 0.84[b] |
| ALT, IU/l, median (range) | l13.5 (1–50) | 17 (3–69) | 0.63[b] |
| AST, IU/l, median (range) | 16 (6–46) | 15 (6–65) | 0.66[b] |
| GGT, IU/l, median (range) | 26 (8–513) | 32 (5–208) | 0.52[b] |
| ALP, IU/l, median (range) | 113.5 (45.7–443) | 98.5 (41–803.8) | 0.82[b] |
| Urea, mg/dl, median (range) | 114.9 (48.6–213) | 104.8 (48.0–192) | 0.86[b] |
| C-reactive protein, mg/l, median (range) | 6.7 (0.7–142) | 5.2 (0.1–104) | 0.74[b] |
| Albumin, g/dl, median (range) | 3.8 (2.8–4.8) | 3.8 (2.0–4.8) | 0.74[b] |

**a** Pearson $\chi^2$ test; **b** Mann–Whitney test; **c** Fisher exact test; **d** *t* test; **e** previous symptomatic hepatitis related to HBV or HCV infection

SI conversion factors: to convert ALT, ALP, AST, and GGT to μkat/l, multiply by 0.0167; albumin to g/l, by 10; C-reactive protein to nmol/l, by 9.524; and urea to mmol/l, by 0.1665.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc, antibodies to core antigen of hepatitis B virus; anti-HBs, antibodies to surface antigen of hepatitis B virus; anti-HCV, antibodies to hepatitis C virus; anti-HEV IgG, immunoglobulin G antibodies to hepatitis E virus; AST, aspartate aminotransferase; ESRD, end-stage renal disease; GGT, γ-glutamyl transferase; HBsAg, surface antigen of hepatitis B virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HD, hemodialysis; HDF, hemodiafiltration; HF-HD, high-flux hemodialysis; HEV, hepatitis E virus; IFN-λ3, interferon λ3; LF-HD, low-flux hemodialysis; PD, peritoneal dialysis; RNA, ribonucleic acid; RRT, renal replacement therapy

**TABLE 2** Interferon-λ4 gene polymorphisms and anti–hepatitis E virus immunoglobulin G positivity in hemodialysis patients not infected with hepatitis B virus or hepatitis C virus

| Genotypes, MAF | Anti-HEV IgG–positive patients (n, frequency) (n = 26) | HEV-negative patients (n, frequency) (n = 43) | OR (95% CI), *P* value[a] |
|---|---|---|---|
| *IFNL4* rs12979860 ($P_{trend}$[b] = 0.07, $P_{genotype}$[a] = 0.12) | | | |
| CC | 15 (57.7) | 14 (32.6) | Reference |
| CT | 7 (26.9) | 18 (41.9) | 0.363 (0.116–1.131), 0.08 |
| TT | 4 (15.4) | 11 (25.6) | 0.339 (0.087–1.318), 0.11[c] |
| CT + TT vs CC | 11 (42.3) | 29 (67.4) | 0.354 (0.129–0.968), 0.04[d] |
| TT vs CC + CT | 4 (15.4) | 11 (25.6) | 0.529 (0.149–1.877), 0.32 |
| MAF | (0.29) | (0.47) | 0.466 (0.224–0.972), 0.04[c] |
| *IFNL4* rs8099917 ($P_{trend}$[b] = 0.02, $P_{genotype}$[a] = 0.02) | | | |
| TT | 17 (65.4) | 14 (32.6) | Reference |
| GT | 8 (30.8) | 27 (62.8) | 0.244 (0.085–0.704), 0.008 |
| GG | 1 (3.8) | 2 (4.7) | 0.412 (0.034–5.029), 0.59[e] |
| GG + GT vs TT | 9 (34.6) | 29 (67.4) | 0.256 (0.091–0.716), 0.008[f] |
| GG vs GT + TT | 1 (3.8) | 2 (4.7) | 0.820 (0.071–9.516), 1.00[e] |
| MAF | (0.19) | (0.36) | 0.422 (0.186–0.957), 0.04 |

**a** Pearson $\chi^2$ test is used unless otherwise indicated. Noncorrected *P* values are shown. A significant *P* value after the Bonferroni correction is <0.004.

**b** Cochran–Armitage test for trend

**c** Significance after the Bonferroni correction might be displayed with the total sample size of 122 persons.

**d** Significance after the Bonferroni correction might be shown with the total sample size of 236 persons.

**e** Fisher exact test

**f** Significance after the Bonferroni correction might be displayed with the total sample size of 135 persons.

Abbreviations: CI, confidence interval; *IFNL4*, interferon-λ4 gene; MAF, minor allele frequency; OR, odds ratio; SNV, single nucleotide variant; others, see TABLE 1

Infections with HBV and HCV could influence the prevalence of some clinical features such as hepatitis episodes and liver enzyme activities, and therefore mask their correlations with anti-HEV IgG. For this reason, also anti-HEV IgG–positive HD patients not infected with HBV or HCV were compared with HD subjects not showing any viral infection-related seromarkers (Supplementary material, *Table S2*). Again, anti-HEV IgG–positive subjects showed longer RRT duration (*P* = 0.04) and higher frequency of treatment with HF-HD/HDF (*P* = 0.04).

**Circulating interferon-λ3 levels** There were no differences in circulating IFN-λ3 levels between anti-HEV IgG–positive and anti-HEV IgG–negative subjects (TABLE 1; Supplementary material, *Table S2*).

**Interferon-λ4 gene and anti–hepatitis E virus immunoglobulin G positivity** As in the case of clinical data, we analyzed all subjects together and, separately, the group without HBV/HCV seromarkers, because patients infected with HBV and HCV may be more susceptible to HEV, independently of *IFNL4* SNVs.[26]

The major homozygosity of both *IFNL4* variants was positively associated with anti-HEV IgG in the dominant inheritance model. However, after the Bonferroni correction, the results were not significant (TABLE 2; Supplementary material, *Table S3*).

Both rs8099917 and rs12979860 SNVs showed a moderate linkage disequilibrium: D' = 1.000, $r^2$ = 0.514 for the entire HD group (Supplementary material, *Figure S1*); D' = 1.000, $r^2$ = 0.638 for the HBV and HCV–negative HD group. Differences in haplotype frequencies were not significant between anti-HEV IgG–positive and anti-HEV IgG–negative subjects (TABLE 3; Supplementary material, *Table S4*). However, in HD patients not exposed to HBV and HCV, a tendency for a higher prevalence of CT rs12979860_rs8099917 (both favorable alleles) and a lower prevalence of TG rs12979860_rs8099917 (both unfavorable alleles) compared with all other haplotypes pooled together was observed in the anti-HEV IgG–positive group (TABLE 3).

**Correlates of anti–hepatitis E virus immunoglobulin G positivity** Treatment with HF-HD/HDF and major homozygosity in both *IFNL4* variants, adjusted for clinical variables, were positive correlates for the anti-HEV IgG occurrence (TABLE 4), also among HBV and HCV–negative patients (TABLE 5).

**TABLE 3** Interferon-λ4 gene rs12979860 (C>T) and interferon-λ4 gene rs8099917 (T>G) haplotype frequencies in hemodialysis patients not infected with hepatitis B virus or hepatitis C virus and stratified by anti–hepatitis E virus immunoglobulin G positivity

| Haplotype | Frequency | Case, control frequencies | $\chi^2$ | *P* value | Corrected *P* value[a] | OR (95% CI)[b], *P* value | OR (95% CI)[c], *P* value |
|---|---|---|---|---|---|---|---|
| CT | 0.601 | 0.712, 0.535 | 4.219 | 0.04 | 0.08 | Reference | 2.145 (1.029–4.472), 0.04 |
| TG | 0.297 | 0.192, 0.360 | 4.388 | 0.04 | 0.07 | 0.401 (0.174–0.924), 0.03 | 0.422 (0.186–0.958), 0.04 |
| TT | 0.101 | 0.096, 0.105 | 0.026 | 0.87 | 1.00 | 0.691 (0.213–2.239), 0.54 | 0.910 (0.288–2.881), 0.87 |

a *P* value was calculated using permutation test and a total of 1000 permutations.

b The most common haplotype was used as the reference.

c All other haplotypes pooled together were used as the reference.

Abbreviations: see TABLE 2

**TABLE 4** Explanatory and response correlates of anti–hepatitis E virus immunoglobulin G positivity among hemodialysis patients

| Parameter | Unadjusted | Adjusted[a] |
|---|---|---|
| | OR (95% CI), *P* value | OR (95% CI), *P* value |
| Male sex | 1.098 (0.466–2.586), 0.83 | 1.142 (0.470–2.275), 0.77 |
| Age (per 10 years) | 0.899 (0.673–1.201), 0.47 | 0.978 (0.701–1.365), 0.90 |
| RRT duration (per 1 year) | 1.033 (0.962–1.110), 0.37 | 1.015 (0.934–1.104), 0.72 |
| Diabetic nephropathy | 1.378 (0.526–3.613), 0.51 | 1.821 (0.635–5.222), 0.26 |
| Hypertensive nephropathy | 0.877 (0.292–2.636), 0.82 | 0.911 (0.284–2.921), 0.88 |
| Chronic glomerulonephritis | 1.320 (0.489–3.567), 0.58 | 1.231 (0.398–3.806), 0.72 |
| Chronic tubulointerstitial nephritis | 1.360 (0.339–5.460), 0.67 | 1.376 (0.328–5.779), 0.66 |
| History of renal transplantation | 2.786 (0.725–10.703), 0.14 | 3.268 (0.440–24.254), 0.25 |
| Living in the rural area | 1.875 (0.790–4.448), 0.15 | 2.018 (0.832–4.897), 0.12 |
| HF-HD/HDF | 2.947 (1.040–5.990), 0.04 | 4.425 (1.541–12.711), 0.006 |
| PD as the first modality of RRT | 0.535 (0.053–5.364), 0.60 | 0.505 (0.042–6.093), 0.59 |
| *IFNL4* rs8099917 TT genotype | 2.497 (1.052–6.111), 0.04 | 2.582 (1.019–6.542), 0.046 |
| *IFNL4* rs12979860 CC genotype | 2.294 (0.950–5.537), 0.07 | 2.677 (1.001–7.160), 0.050 |
| HBsAg positivity | 0.521 (0.099–2.741), 0.44 | 0.246 (0.029–2.057), 0.20 |
| Anti-HBc positivity | 1.119 (0.360–3.478), 0.85 | 0.839 (0.196–3.597), 0.81 |
| Anti-HCV positivity | 0.613 (0.176–2.135), 0.44 | 0.332 (0.064–1.719), 0.19 |
| Anti-HBs titer (per 100 IU/l) | 0.994 (0.890–1.112), 0.92 | 0.989 (0.877–1.115), 0.86 |
| HCV RNA positivity | 1.258 (0.264–5.997), 0.77 | 1.084 (0.190–6.176), 0.93 |
| IFN-λ3 (per 1 pg/ml) | 1.001 (0.995–1.007), 0.77 | 1.001 (0.994–1.008), 0.80 |
| ALT (per 1 IU/l) | 0.991 (0.955–1.028), 0.63 | 0.986 (0.948–1.026), 0.49 |
| AST (per 1 IU/l) | 1.003 (0.958–1.050), 0.90 | 1.003 (0.956–1.053), 0.90 |
| GGT (per 1 IU/l) | 1.004 (0.997–1.010), 0.26 | 1.004 (0.998–1.011), 0.20 |
| ALP (per 1 IU/l) | 1.001 (0.996–1.005), 0.77 | 1.000 (0.995–1.005), 0.86 |
| C-reactive protein (per 1 mg/l) | 1.008 (0.985–1.030), 0.52 | 1.005 (0.981–1.030), 0.66 |

a Adjusted for sex, age, RRT duration, living in the rural area, and history of renal transplantation

Abbreviations: see TABLES 1 and 2

Liver enzyme activities and C-reactive protein, tested as response variables to HEV infection, did not differ between patients stratified by anti-HEV IgG positivity (TABLE 1; Supplementary material, *Table S2*) and were nonsignificant in logistic regression analyses (TABLES 4 and 5).

**Survival and anti–hepatitis E virus immunoglobulin G positivity** The median follow-up duration equaled 2.78 (range, 0.05–3.47) years. The mortality rate was 37.8%. Among 34 deceased patients, there were 11 anti-HEV IgG-positive subjects (32.4%), whereas among 56 survivors, there were 23 anti-HEV IgG–positive patients (41.1%) ($P = 0.41$). Survival curves are shown in FIGURE 1. Adjustment for clinical variables did not reveal an association between anti-HEV IgG and survival ($P = 0.79$).

**TABLE 5** Explanatory and response correlates of anti–hepatitis E virus immunoglobulin G among hemodialysis patients negative for hepatitis B virus or hepatitis C virus seromarkers

| Parameter | Unadjusted | Adjusted[a] |
|---|---|---|
| | OR (95% CI), *P* value | OR (95% CI), *P* value |
| Male sex | 1.302 (0.488–3.473), 0.60 | 1.226 (0.424–3.550), 0.71 |
| Age (per 10 years) | 0.920 (0.660–1.282), 0.62 | 1.059 (0.711–1.577), 0.78 |
| RRT duration (per 1 year) | 1.170 (0.986–1.387), 0.07 | 1.192 (0.998–1.423), 0.05 |
| Diabetic nephropathy | 1.293 (0.440–3.801), 0.64 | 2.128 (0.612–7.401), 0.24 |
| Hypertensive nephropathy | 1.313 (0.398–4.326), 0.66 | 1.394 (0.384–5.059), 0.61 |
| Chronic glomerulonephritis | 1.313 (0.398–4.326), 0.66 | 0.929 (0.224–3.857), 0.92 |
| Chronic tubulointerstitial nephritis | 0.991 (0.216–4.542), 0.99 | 0.806 (0.164–3.963), 0.79 |
| Living in the rural area | 1.894 (0.706–5.077), 0.20 | 2.652 (0.885–7.945), 0.08 |
| HF-HD/HDF | 2.842 (1.017–7.942), 0.046 | 4.412 (1.316–14.793), 0.02 |
| PD as the first modality of RRT | 1.680 (0.101–28.065), 0.72 | 2.253 (0.105–48.428), 0.60 |
| *IFNL4* rs8 099 917 TT genotype | 3.913 (1.398–10.953), 0.009 | 4.826 (1.452–16.036), 0.01 |
| *IFNL4* rs12 979 860 CC genotype | 2.825 (1.033–7.725), 0.04 | 3.289 (1.033–10.470), 0.04 |
| Anti-HBs titer (per 100 IU/l) | 0.995 (0.880–1.124), 0.93 | 0.985 (0.860–1.127), 0.82 |
| IFN-λ3 (per 1 pg/ml) | 1.002 (0.995–1.010), 0.59 | 1.000 (0.991–1.008), 0.93 |
| ALT (per 1 IU/l) | 0.963 (0.905–1.024), 0.23 | 0.959 (0.896–1.026), 0.23 |
| AST (per 1 IU/l) | 0.983 (0.910–1.061), 0.66 | 0.976 (0.897–1.062), 0.57 |
| GGT (per 1 IU/l) | 1.004 (0.997–1.012), 0.23 | 1.005 (0.997–1.013), 0.22 |
| ALP (per 1 IU/l) | 1.004 (0.996–1.012), 0.32 | 1.006 (0.997–1.015), 0.18 |
| C-reactive protein (per 1 mg/l) | 1.009 (0.986–1.033), 0.45 | 0.992 (0.964–1.021), 0.60 |

a Adjusted for sex, age, RRT duration, living in the rural area, and history of renal transplantation

Abbreviations: see TABLES 1 and 2

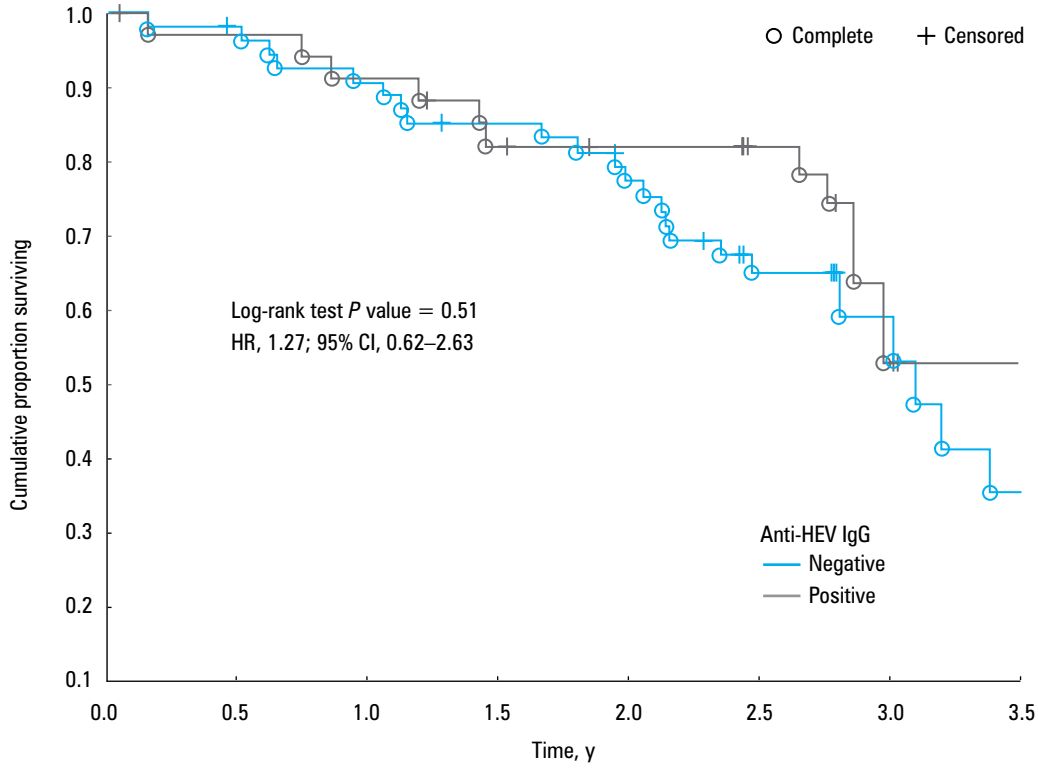


**FIGURE 1** Survival of hemodialysis patients stratified by anti–hepatitis E virus immunoglobulin G positivity (anti-HEV IgG)

**DISCUSSION** We revealed anti-HEV IgG in 37.8% of HD patients living in the HEV-endemic area. All patients were negative for anti-HEV IgM and HEV Ag. Such a pattern of HEV seromarkers suggests the previous exposure to HEV.[27] Determination of HEV RNA, which is the gold standard for diagnosis of ongoing HEV infection,[27] was not done in our study, but approximately 65% of patients with acute HEV infection and 100% of those with chronic HEV infection also show positive results for the anti-HEV Ag-specific ELISA,[28] which was used in our study.

Anti-HEV IgM antibodies are typically observed during about 6 months since HEV infection.[29] Therefore, considering stable clinical status of the examined patients and available results of their HEV seromarkers, we diagnosed past exposure to HEV in all cases.

Available data, including our present results, on factors associated with anti-HEV IgG in HD patients, sex, older age, and HD duration, are inconsistent.[30-34] According to Mitsui et al,[30] 89.7% of HD patients are HEV-infected before the HD initiation. They suggested that anti-HEV IgG positivity may persist in HD patients for years as in the general population.[35] In our study, anti-HEV IgG–positive patients had longer RRT duration compared with anti-HEV IgG–negative ones, but this association was not significant in the logistic regression analysis.

Anti-HEV IgG positivity did not correlate with a history of renal transplantation in our HD subjects, similarly as demonstrated by Psichogiou et al.[31] The proportions of patients with HBV/HCV infections were comparable between HEV seropositive and seronegative HD groups, what is in concordance with the previous studies.[30-33,36] Transmission of HEV with the blood seems to be of less importance in HD patients in the era of standard administration of erythropoietin stimulating agents.

Higher prevalence of anti-HEV IgG seropositive patients on more effective HD modalities (HF-HD/HDF) than on lower efficiency HD therapy (LF-HD) is unclear. Similar timespan on HF-HD/HDF in HEV seropositive and HEV seronegative patients seems to exclude an influence of this factor on the demonstrated association. A few explanations might be discussed. Firstly, an impact of HF-HD on HEV seroprevalence may be evaluated in the context of the increased HEV exposure during this dialysis modality. Secondly, more efficient immune defense mechanisms may be suspected during HF-HD than LF-HD.

Concerning the increased HEV exposure, Sampietro et al[37] indicated a potential risk of HCV cross-infection by dialysis fluids in patients on HF-HD with dialysate back filtration due to suspected microruptures of dialyzer capillaries with subsequent leakage of infected blood. However, no HCV extravasation to spent dialysate was found in further studies,[38] and transmission of HCV via the HD circuit was practically excluded.[39] Additionally, the blood leakage could be associated with HEV infection only when HEV-viremic patients are persistently dialyzed in HD facilities like HCV-infected patients are. Fortunately, HEV RNA is rarely detected among HD patients.[30] Therefore, HEV infection through dialysis circuit seems to be impossible. Pyrogenic reactions in the absence of septicemia, attributed to bacteria and endotoxins in dialysate, were reported by 21% of HD centers and associated with the use of HF-HD.[40] HEV infection is waterborne.[41] Viral contamination of dialysate cannot be excluded if bacterial contaminations are reported.[42] Among developed countries, HEV presence was found in around 30% of sewage samples in Barcelona and Valencia.[43] Although a proper water supply in dialysis facilities is a priority, reverse osmosis applied for water treatment can reject not 100% but 90% to 99% of contaminants, including bacteria, endotoxins, viruses, salts, particles, and dissolved organic substrates.[44] An opposite argument against the concept of HEV infection through dialysate is no reports on HEV outbreaks in dialysis facilities located in the endemic areas.

Concerning less altered immunocompetence, HF membranes offer better removal of middle-molecular-weight uremic toxins, which positively affects responsiveness to HBV vaccination and results in developing higher titers of protective antibodies.[45] As probably not all HEV exposures cause a generation of anti-HEV IgG in immunocompromised patients,[46] better detoxification of ESRD patients may increase the number of patients developing anti-HEV IgG after HEV exposure compared with a respective number in patients treated with LF-HD, independently of the route of HEV infection. Additionally, better removal of uremic toxins may improve immunocompetence and lead to more prolonged maintenance of anti-HEV IgG, also in cases with HEV exposure before the initiation of RRT. A longitudinal, prospective study with regular determination of HEV RNA and HEV seromarkers could confirm this hypothesis.

Finally, it cannot be excluded that an undefined factor (or factors) noncasually associated with effective dialysis modalities is responsible for anti-HEV IgG seropositivity.

In our study, there were no HD subjects with chronic HEV infection. Therefore, no direct evidence was gathered for an association between circulating IFN-λ3 levels and spontaneous resolution of HEV infection. In our earlier studies, HD individuals showing persistent HCV infection, subjects not developing anti-HBs after HBV infection or vaccination, or patients presenting combined HBsAg and HCV RNA positivity demonstrated lower circulating IFN-λ3 levels compared with patients who had favorable outcomes in terms of the above infections and with those never exposed to HB and HCV.[19,22] HD groups with favorable outcomes, as well as noninfected individuals, did not differ in circulating IFN-λ3 levels.[19] The currently studied HD patients with isolated HEV IgG positivity were considered as those with resolved HEV infection. Their plasma IFN-λ3 concentrations were similar to those previously shown in HD patients with favorable outcomes in terms of infections with hepatotropic viruses.[13] Whether individuals with chronic HEV infection show lower IFN-λ3 levels than those with resolved infections, remains to be elucidated in future research.

*IFNL4* rs8099917 and *IFNL4* rs12979860 are known to be associated with resolution of viral infections.[16] *IFNL4* rs12979860 shows

an association with HCV clearance due to its strong LD with *IFNL4* rs368234815, which regulates the generation of IFN-λ4.[14] Overexpression of IFN-λ4 suppresses IFN-λ3 induction and promoter activation.[47] Low intracellular expression of IFN-λ4 induces IFN-λ expression, leading to the preactivation of the JAK-STAT signaling and ISG expression.[48] Associations of *IFNL4* variants and their haplotypes with the resolution of HEV infection cannot be excluded in our study. Considering issues on LD interpretation in the human genome,[24,25,49] our LD analyzes, as measured by D', demonstrated the lack of recombination events between analyzed polymorphisms. The D' value equal to 1.0 was caused by the presence of only 3 of the 4 possible haplotypes for a pair of tested *IFNL4* variants, which forces D' to its maximum possible value. The $r^2$ value equal to 0.514 indicates that analyzed variants are in moderate LD and reflects the pairwise differences in frequencies of the linked alleles. In addition, the $r^2$ value between rs8099917 and rs12979860 suggests that these SNVs are not good proxies for each other and cannot be genotyped interchangeably.

The borderline significance of our results might be due to a small number of studied individuals. Additionally, the HEV-negative group could include patients who were infected with HEV many years ago and became anti-HEV IgG negative. The average negative seroconversion rate of anti-HEV IgG produced in response to asymptomatic HEV infection was estimated at 1.4% per year.[50] Therefore, past or future immune response to HEV infection was unknown in currently HEV-negative subjects. Genotyping patients with chronic HEV infection in comparison with those with spontaneous resolution might be more informative about the *IFNL4* association with HEV infection. However, a logistic regression analysis shows that harboring 2 major alleles of tested variants determines anti-HEV IgG positivity better than clinical data of HD patients. Therefore, homozygosity of major alleles in both tested *IFNL4* variants may promote spontaneous HEV resolution as it is shown for these polymorphisms concerning HCV infection in the general population[16] and in HD patients specifically.[18]

Activities of liver enzymes were higher in anti-HEV IgG–positive individuals compared with anti-HEV IgG–negative ones in some studies,[51,52] including HD patients.[53] Such a correlation was not shown in our HD patients. The lack of significant associations between liver enzyme activities and plasma C-reactive protein concentrations in terms of anti-HEV IgG positivity indirectly confirms that anti-HEV IgG is not associated with HEV-related chronic hepatitis.

In summary, from the clinical point of view, the most relevant finding is that anti-HEV IgG, which is a marker of the past HEV infection, is not associated either with deteriorated liver function or increased mortality of HD patients. The occurrence of anti-HBs, which are positive predictors of survival in HD patients,[54] is similar between patients who spontaneously eliminated HEV and those who are free from HEV seromarkers. Additionally, our study revealed that the favorable *IFNL4* polymorphisms and treatment with HF dialysis membranes are determinants of anti-HEV IgG positivity (spontaneous HEV resolution) among HD patients.

**SUPPLEMENTARY MATERIAL ONLINE** Supplementary material is available with the article at www.pamw.pl.

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**CONTRIBUTION STATEMENT** AEG and MB conceived the concept of the study. AEG designed the research. AEG, LN, and MŚ were involved in data collection. MŁ-Ż was engaged in HEV determination. AM and PPJ were responsible for genotyping. MŚ was responsible for statistical analysis. AEG, MŚ, and MB interpreted the data. AEG wrote the manuscript. All authors edited and approved the final version of the manuscript.



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