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Short communication

Circulating interferon-λ3 and post-vaccination antibodies against the surface antigen of hepatitis B virus in hemodialysis patients exposed to hepatitis E virus

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ABSTRACT

Responsiveness to the hepatitis B virus (HBV) vaccination in hemodialysis (HD) patients who had been exposed to the hepatitis E virus (HEV) and persistently generate antibodies against HEV remains unknown. Interferon (IFN)-λ3 positively correlates with the surface HBV antibodies (anti-HBs) in both healthy and HD subjects. We aimed to show whether HD patients differ in circulating IFN-λ3 and vaccine-induced anti-HBs titers concerning natural HEV immunization. HBV/HCV negative HD patients (31 HEV IgG positive, 45 HEV negative), HBV vaccinated and receiving booster doses as needed, had been tested for anti-HBs titers (CMIA) and IFN-λ3 concentrations (ELISA) in the blood collected before a dialysis session. There were no differences in circulating IFN-λ3 and anti-HBs titers between both groups. In responders to the HBV vaccine, there was a positive correlation between plasma IFN-λ3 levels and anti-HBs titers ($r = 0.505$, adjusted $P = 0.01$ in HEV exposed subjects; $r = 0.523$, adjusted $P = 0.001$ in controls). HEV past infection does not attenuate post-vaccination anti-HBs generation and does not influence a correlation between circulating IFN-λ3 levels and anti-HBs titers.

1. Introduction

In hemodialysis (HD) patients, the prevalence of hepatitis B virus (HBV) infection is still higher than in the general population. Among causative factors, inadequate response to HBV vaccination is one of the leaders. Responsiveness to HBV vaccination, defined as the generation of neutralizing antibodies against HBV surface antigen (anti-HBs) in amounts considered as protective (≥10 IU/l), is achieved in about 75% of HD subjects [1]. Recently, a strong positive correlation between the generation of anti-HBs and plasma interferon (IFN)-λ3 levels was demonstrated in HD patients and healthy subjects [2,3]. IFN-λ3 is involved in positive regulation of immune response and T cell activation [4]. IFN-λ3 and anti-HBs correlated also in HD subjects non-infected with HBV or hepatitis C virus (HCV), as well as, in those exposed to these viruses [2,3]. However, there is no data showing whether the association of IFN-λ3 and anti-HBs occurs in HD patients exposed to hepatitis E virus (HEV). Such information might be of importance, especially in HEV endemic areas, where the prevalence of HEV seromarkers reaches 50% [5]. Therefore, we aimed to show whether HD patients differ in circulating IFN-λ3 and vaccine-induced anti-HBs titers concerning past HEV infection.

2. Patients and methods

The study was carried out on 76 HD subjects living in the HEV endemic area (the Wielkopolska Voivodship, Poland). They were enrolled in the study as previously described for 90 patients [6]. Inclusion criteria for the current study were as follows: (1) age over 18 years, (2) stable clinical condition through at least two months before enrollment, (3) the established status as responder (anti-HBs titer ≥10 IU/l) to HBV vaccination or non-responder (anti-HBs titer < 10 IU/l) to HBV vaccination with the use of four primary vaccine doses containing 40 μg of

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HBV surface antigen (HBsAg) each, and at least three additional vaccine doses of 40 μg each. Exclusion criteria included: (1) corticosteroid/immunosuppressive therapy through at least two months before enrollment, (2) cachectic conditions causing decreases in serum proteins, (3) infection with HBV, HCV, or HIV before or during renal replacement therapy (RRT), (4) antiviral treatment against cytomegalovirus conducted before or at enrolment.

The 69 patients from 90 previously examined individuals [6] were included also in this study and the additional 7 subjects were enrolled. Twenty-one individuals investigated in the earlier study [6] were excluded due to HBV or HCV infection.

In the entire HD group, 60 individuals (78.9% of all) underwent HBV vaccination at the start of RRT. The remaining patients were vaccinated before RRT commencement. During dialysis treatment, anti-HBs titers were determined every 6 months. Vaccine booster doses (40 μg) were administered to responders on the mandatory basis to maintain anti-HBs > 10 IU/l if their anti-HBs titers decreased below 10 IU/l. However, there were patients who also received additional doses (20 μg) if their anti-HBs titers approaching 10 IU/l. Non-responders were not routinely receiving additional vaccine doses.

Blood samples were collected from 14th January 2014 to 18th September 2018. All patients enrolled in the study had taken a blood sample for laboratory determination. Plasma samples were stored at the end of the collection. In subjects diagnosed as non-responders before the beginning of the study (n = 9) and responders who appeared as those not requiring booster doses of HBV vaccine during the collection period after a primary vaccination finished before enrolment (n = 32), blood samples obtained at enrolment were taken for analysis. In the patients who started a primary vaccination during the study period at the RRT initiation and were diagnosed as non-responders as well as all receiving booster doses, blood probes obtained 1–2 months after the last dose of a primary vaccination or after the last booster dose were used in analyses.

HEV ORF2 antigen, anti-HEV IgM, and anti-HEV IgG were determined by an enzyme-linked immunosorbent assays (ELISA), using commercial kits (Wantai Biological Pharmacy Enterprise Co., Beijing, China). IFN-λ3 concentration was determined with ELISA kit (Human Interleukin 28B ELISA Kit, Sunred Biological Technology Co., Ltd., Shanghai, China). The quantification of anti-HBs was performed using Chemiluminescent Microparticle Immunoassay (CMIA) method (ABBOTT, Ireland).

Continuous variables were compared using the Mann-Whitney *U* test or Student's *t*-test, as appropriate. Dichotomous variables were tested for significance of differences by Pearson‘s chi-squared test or Fisher‘s exact test, as appropriate. Kruskal-Wallis test was used for comparing more than two independent samples. If this test showed significance, post-hoc Dunn’s tests (Benjamini-Hochberg corrected) were applied.

The study design was approved by the Institutional Review Board of Poznan University of Medical Sciences, Poland. Written informed consent was obtained from all study participants.

3. Results

All selected HD patients were anti-HEV IgM and HEV open reading frame 2 (ORF2) antigen negative. In 31 patients, past HEV exposure was diagnosed by anti-HEV IgG positivity and both anti-HEV IgM and HEV ORF2 antigen negativity. Anti-HEV IgG positive patients (n = 31) comprised the examined group, whereas anti-HEV IgG negative individuals (n = 45) served as controls. HEV exposed subjects showed longer renal RRT duration (Table 1). Therefore the total number of HBV vaccine doses and total HBV vaccine dose were both normalized using RRT duration. All non-responders to HBV vaccination (n = 15) were first time vaccinated at the start of RRT.

Non-responders to HBV vaccination showed lower plasma IFN-λ3 levels than responders. There were no differences between respective values of circulating IFN-λ3 and anti-HBs titers between responders or non-responders in HEV exposed and HEV not exposed groups. In patients able to develop protective titers of anti-HBs, there was a significant positive correlation between plasma IFN-λ3 levels and anti-HBs titers (Table 2).

4. Discussion

A correlation between circulating IFN-λ3 levels and anti-HBs titers was previously demonstrated, but the examined individuals were not tested for HEV exposure [2,3]. The current research shows that HEV past infection does not attenuate anti-HBs generation in individuals who can produce post-vaccination anti-HBs, and a correlation between IFN-λ3 levels and anti-HBs titers also occurs.

A relationship between IFN-λ3 levels and adaptive immunity is not firmly documented and provides non-consistent results [4]. The human immunodeficiency virus vaccine study revealed that IFN-λ3 is capable of enhancing adaptive immunity by the increase of antigen-specific IgG2a and the percentage of splenic $CD8^+$ T cells [7]. Also, the IFN-λ3 adjuvant reduced the number of $CD4^+/CD25^{hi}/FoxP3^+$ cells (Tregs) found in the spleens of vaccinated animals. What is clinically essential, IFN-λ3 induced 100% protection from mortality after a lethal influenza challenge in mice [7]. On the other hand, recombinant IFN-λ3 decreased influenza A H1N1 subtype-induced IgG production in peripheral blood mononuclear cells from solid organ transplant recipients, as well as, decreased H1N1-induced T helper 2 response, B cell activation, and IgG production in healthy volunteers [8].

As our study is cross-sectional, we can only notice the correlation between IFN-λ3 and anti-HBs titers. Whether IFN-λ3 is causally involved in the anti-HBs generation, acts in synergy with other factors necessary for anti-HBs production, like T-lymphocyte cytokines including IL-12 [9], or is a reaction on immunosuppressive factors simultaneously generated after antigen stimulation, like indoleamine 2,3-dioxygenase [10], remains to be elucidated in the further investigations.

Anti-HBs titers were similar in HEV-positive and HEV-negative patients. This similarity suggests that HEV past infection does not attenuate responsiveness to HBV booster doses which were comparable in both groups. However, response to a primary HBV vaccination increases with rising doses of vaccine, and higher vaccine doses may enhance revaccination response rate [11]. Normalization of HBV vaccine doses per duration of RRT did not reveal differences between HEV-positive and HEV-negative patients. Therefore, similar anti-HBs titers were obtained using similar vaccination schedule concerning the frequency of vaccine administration and injected vaccine antigen during the entire course of RRT. However, our data on combined HBV vaccine dosing have limited value due to the unknown time of HEV exposure. Knowing this time, normalization of HBV vaccine doses and total dose would be performed starting since the exposure time.

It is possible that response to primary HBV vaccination is also not influenced by past HEV infection. The percentage of non-responders was similar in both groups. By Mitsui et al. [12], 89.7% of HD patients is HEV-infected before the HD initiation. All non-responders to HBV vaccination were vaccinated first time at the start of RRT. Near 90% of them could already express past HEV exposition. If so, they were vaccinated as HEV positive subjects. With this assumption, a similar percentage of non-responders was revealed in HEV exposed and not exposed to HEV.

5. Conclusions

HD patients, exposed to HEV in the past (but not to HBV or HCV) and not showing persistent HEV infection, have similar anti-HBs titers and IFN-λ3 concentrations like HD patients without seromarkers indicating exposition to HEV, HBV, and HCV. The significant correlation between anti-HBs and plasma IFN-λ3 is observed also in HEV-exposed

Table 1
Characteristics of anti-HEV IgG positive patients and those negative for tested HEV seromarkers among HD patients negative for HBV or HCV serologic markers.

Data	Anti-HEV IgG positive patients n = 31	HEV negative patients n = 45	*P*-value
Clinical data			
Male gender, n, % of all	17 (54.8)	23 (51.1)	0.75
Age, years	66.5 (33.4–86.2)	68.7 (29.9–90.5)	0.40
Living in a rural area, n, % of all	16 (51.6)	17 (37.8)	0.23
RRT duration, years	7.3 ± 3.5	5.7 ± 3.1	0.04
History of parathyroidectomy, n, % of all	1 (3.2)	2 (4.4)	1.00
History of renal transplantation, n, % of all	5 (16.1)	9 (20)	0.67
Body mass index, kg/m^2	27.7 ± 3.5	26.4 ± 4.5	0.23
Causes of ESRD			
Diabetic nephropathy, n, % of all	9 (29)	11 (24.4)	0.66
Hypertensive nephropathy, n, % of all	6 (19.4)	9 (20)	0.94
Chronic glomerulonephritis, n, % of all	8 (25.8)	8 (17.8)	0.40
Chronic tubulointerstitial nephritis, n, % of all	4 (12.9)	5 (11.1)	1.00
Type of RRT			
HF-HD/HDF, n, % of all	19 (61.3)	20 (44.4)	0.15
PD as the first modality of RRT, n, % of all	1 (3.2)	2 (4.4)	1.00
Laboratory data			
Post vaccination anti-HBs titer, IU/L	220.5 (3.9–1000)	268 (0–1000)	0.69
Anti-HBs titer < 10 IU/l, n, % of all	6 (19.4)	9 (20.0)	0.94
Last HBV booster dose, μg	40 (20–40)	40 (20–40)	0.18
Total number of HBV vaccine doses (20 μg each) per 1 patient	5 (3–11)	5 (3–9)	0.92
Total number of HBV vaccine doses (20 μg each) per 1 patient-RRT year	0.61 (0.24–9.61)	0.81 (0.29–7.28)	0.15
Total HBV vaccine dose per 1 patient, μg	200 (60–380)	180 (60–360)	0.84
Total HBV vaccine dose per 1 patient-RRT year, μg	22.9 (7.8–192.1)	25.5 (9.9–291.2)	0.34
IFN-λ3, pg/ml	124 (15.9–232.7)	109 (33–208)	0.28
ALT, IU/l	13 (1–39)	17 (3–39)	0.18
AST, IU/l	15 (6–28)	15 (6–36)	0.83
GGT, IU/l	24 (9–513)	31 (5–139)	0.23
ALP, U/l	104 (54–443)	98.5 (41–1109.5)	0.88
Urea, mg/dl	114.7 (48.6–213)	105 (62.9–190.5)	0.91
C-reactive protein, mg/l	6.1 (0.8–142)	6.0 (0.8–104)	0.85
Albumin, g/dl	3.8 (3.2–4.7)	3.7 (2.0–4.8)	0.18

Abbreviations: ALP – alkaline phosphatase, ALT – alanine aminotransferase, Anti-HBs – antibodies to surface antigen of hepatitis B virus, Anti-HEV IgG – immunoglobulin G antibodies to hepatitis E virus, AST – aspartate aminotransferase, ESRD – end-stage renal disease, GGT – gamma-glutamyl transferase, HBV – hepatitis B virus, HCV – hepatitis C virus, HD – hemodialysis, HDF – hemodiafiltration, IFN – interferon, n – number of patients, PD – peritoneal dialysis, RRT – renal replacement therapy.
Conversion factors to SI units are as follows: for alanine aminotransferase – 1 IU/l = 0.0167 μkat/l, for albumin – 1 g/dl = 10 g/l, for alkaline phosphatase – 1 IU/l = 0.0167 μkat/l, for aspartate aminotransferase – 1 IU/l = 0.0167 μkat/l, for C-reactive protein – 1 mg/l = 9.524 nmol/l, for gamma-glutamyltransferase – 1 IU/l = 0.0167 μkat/l, for urea – 1 mg/dl = 0.1665 mmol/l.

Table 2
Circulating IFN-λ3 concentrations in non-infected and HEV-infected HD patients (n = 76) in relation to response to HBV vaccination.

No	Patient group	N A	IFN-λ3, pg/ml B	Anti-HBs titer, IU/l C	Spearman's rank correlation coefficient, *P*-value	Adjusted P-value[a]
1.	HD patients non-infected with HBV/HCV/HEV	45	109 (33–208)	268 (0–1000)	0.685, 2.1 E−7	2.3 E−6
2.	Responders to HBV vaccination	36 (80%)	122 (33–208)	324.5 (17.4–1000)	0.523, 0.001	0.001
3	Non-responders to HBV vaccination	9 (20%)	38 (35–77.4)	2 (0–3)	0.041, 0.9	0.7
4	HD patients exposed to HEV (anti-HEV IgG positive)	31	124 (15.9–232.7)	220.5 (3.9–1000)	0.687, 2.0 E−5	0.0001
5	Responders to HBV vaccination	25 (80.6%)	189.1 (37–232.7)	551 (14–1000)	0.505, 0.01	0.01
6	Non-responders to HBV vaccination	6 (19.4%)	54 (15.9–65)	6.5 (3.9–9.8)	−0.371, 0.468	1.0

P-values: A3 vs. A6 0.9; B1 vs. B4 0.3; C1 vs. C4 0.7; B2 vs. B3 0.0007; B2 vs. B5 0.2; B3 vs. B6 0.5; B5 vs. B6 0.0006; C2 vs. C3 < 0.00001; C2 vs. C5 0.5; C3 vs. C6 0.3; C5 vs. C6 0.0004.
[a] Adjustment for renal replacement duration and HD modality.

patients. The current data, like our previous studies [2,3], provide arguments that IFN-λ3 is a promising cytokine for further research on the role of IFN-λ3 in adaptive immunity, and worth further experiments concerning its application as a vaccine adjuvant.

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Declaration of Competing Interest

Authors have nothing to disclose.

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