Effects of interleukin-12 gene polymorphism on response to hepatitis B vaccination among hemodialysis Egyptian patients

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Background
Hepatitis B virus (HBV) infection is a major health problem among hemodialysis (HD) patients. Interleukin (IL)-12 gene polymorphisms may be associated with immune response variability to recombinant HBV vaccines. The aim was to determine the correlation between IL-12 gene polymorphism and hepatitis B surface-antibody (HBs-Ab) titer in response to HBV vaccine among HD Egyptian patients.
Patients and methods
Seventy patients receiving long-term HD and 20 age-matched and sex-matched healthy controls were enrolled. All participants were non-HBV vaccinated and seronegative for HBV and HIV. Recombinant HBV vaccine was given (three-dose scheduled). Thereafter, HBs-Ab titer and IL-12 gene polymorphism were evaluated 8 weeks after the last vaccination dose.
Results
There was no response (HBs-Ab$<$10 μIU/ml) in 20% of HD patients and 10% of the controls. HBs-Ab titers showed no significant correlation with duration of HD, BMI, serum albumin, hemoglobin, leucocytic count, parathyroid hormone level, or IL-12 gene polymorphism. Responders to vaccination had significantly lower transferrin saturation and significantly higher levels of urea reduction ratio, K_t/V and lymphopenia. IL-12B genotype frequency was as follows: AA (58.3 vs. 55.6%), AC (37.5%) and CC (4.2 vs. 0%) in responders of either HD or control participants, respectively ($P$$>$0.05 for all).
Conclusion
There was no significant association between IL-12B gene polymorphism and HBs-Ab response in Egyptian HD patients. In HD patients, lymphocytopnea, diabetes mellitus (DM), high transferrin saturation and inefficient HD were associated with HBV vaccine hyporesponsiveness.

Keywords:
hepatitis B virus vaccines, hemodialysis, interleukin-12 gene polymorphism

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Introduction

Viral hepatitis is considered a major hazard for both patients and medical staff in hemodialysis (HD) units, with an increased risk of parenterally transmitted infections due to repeated blood transfusions, invasive procedures and immunosuppression. Virions were found on environmental surfaces in the HD facilities in the absence of visible blood [1]. Hepatitis B virus (HBV) infection is a major threat for HD patients, and its presentation and clinical course among them is different from the general population. Patients on chronic HD had occult B infection, with a prevalence of 4.1% in Minia, Assuit and Upper Egypt. While in Lower Egypt, the prevalence of occult B infection among HD patients with and without hepatitis C virus (HCV) coinfection was 6.3 and 3.8%, respectively [2].

Control of HBV infection has been a continuous challenge in HD units. The use of disposable dialyzers, electronic machines, the routine viral screening of blood donors, the use of fistulae instead of arteriovenous shunts, grafts, and cuffed venous catheters, and the use of recombinant erythropoietin (EPO) that reduced rates of blood transfusions are some of the precautionary measures to be taken. These procedures together with hand-washing, wearing gloves, gowns and face shields, active vaccination and segregation of HBV carriers are now considered

a standard practice. Routine vaccination against HBV infection is mandatory [3].

Seroconversion rates were inversely related to the chronic kidney disease stage. More than 40% of patients undergoing HD who respond to primary vaccination will have undetectable hepatitis B surface-antibody (HBs-Ab) levels 3 years after vaccination. Chaves *et al.* [4] studied the effect of revaccination of nonresponder HD patients and the duration of protection. They found that among weak responders (HBs-Ab level 10.0–99.9 mIU/ml), protective HBs-Ab levels persisted for 12 months after vaccination in 44%, whereas, among strong responders (HBs-Ab level≥100 mIU/ml), protective HBs-Ab levels persisted for 12 months in 92%, and for 24 months after vaccination in 68%.

Many factors affect vaccine efficacy in patients on long-term HD, such as age, immune compromise because of uremia, diabetes, efficacy of HD, B7-2 (CD86)-defective expression on their monocytes, and HIV seropositivity [5].

Uremia is associated with an immunosuppression state. Uremic patients have a state of hypercytokinemia, which may be related to proinflammatory cytokine accumulation as a result of its decreased excretion by the diseased kidney and/or increased formation induced by oxidative stress, uremic toxins, volume overload and other comorbidities. This alteration of adaptive immunity is mainly attributed to impaired functions of the T-lymphocytes and the antigen-presenting cells (APCs). There is increased Th1/Th2 ratio despite the sustained maturation of Th lymphocytes due to the elevated Th1 levels in HD patients. This can be explained by the increased production of interleukin (IL)-12, which acts on T lymphocytes, increasing interferon-γ production and decreasing the production of IL-4, promoting their differentiation into Th1 type. Th1 cells produce many cytokines, notably tumor necrosis factor-α, IL-12, and IFN-γ, while Th2 cells produce mainly IL-4 and IL-5, which have a varied impact on the immune response [6].

As T-cell activation by APCs is dependent on toll-like receptors (TLRs), end-stage renal disease (ESRD), especially in HD patients, is associated with B-cell lymphopenia. This may be explained by an increased susceptibility to B-cell death by apoptosis. Immunoglobulin (Ig) levels and serum IgG, IgM and IgA production were documented to be disturbed in HD patients. Hence, a large part of immune alterations in patients with ESRD may be related to protein-energy wasting. There is decrease in antigen-presenting abilities of dendritic cells and macrophages in uremic patients through alterations in costimulatory molecules (CD80, CD86), which are regulated by TLRs; it seems apparent to be a disorder in the expression of TLR and/or the activity causing impairment of APC functions [7].

The impaired expression and upregulation of B7-2 by the monocytes of HD patients were considered important features of the cellular immune defect leading to decreased costimulation and effector activation of T cells and contribute to a molecular explanation for the impaired response to the HBV vaccination among them [8].

Cytokines, especially IL-12 and IL-18, play a major immunoregulatory role in the immune response to HBV antigens and its clearance during natural infection and after vaccination [9]. Gene polymorphisms in the immunoregulatory cytokines IL-2, IL-4, IL-10, IL-12B, and IL-18 are correlated with variable immune response to recombinant HBV vaccines [10]. The aim of this study was to determine the correlations between IL-12 gene polymorphism and HBV antibodies in response to HBV vaccination among HD Egyptian patients.

Patients and methods

Patients

Seventy adult consecutive Egyptian outpatients attending the Department of Internal Medicine at Mansoura General Hospital with a confirmed diagnosis of chronic renal failure and receiving long-term HD with no history of previous HBV vaccination were initially enrolled in this study. Another 20 age-matched and sex-matched healthy controls (HCs) were also enrolled. The age range was 18–70 years. Male to female ratio was 1.09; 47/43. The study was initiated in October 2014 and continued through 2016. The study was approved by the Ethical Commission and Institutional Review Board of Mansoura Faculty of Medicine in Egypt (MFM-IRB; R.19.06.535). A written informed conscious consent was obtained from all participants before their participation.

Inclusion criteria

Patients with ESRD and on long-term HD and who had no history of previous HBV vaccination were included in the study. Exclusion criteria were as

follows: extremes of age (<18 and > 70 years), history of cancer of any type within the last 5 years, history of solid organ or bone marrow transplantation, antiviral treatment, patients with other systems' failure, thyroid disorders, systemic infection (e.g. HIV, bacterial and fungal), chronic hepatitis B or D, primary biliary cirrhosis, metabolic liver disease and autoimmune hepatitis. All participants were assigned to the following groups:

(1) Group 1: HD patients with HCV infection and diabetes mellitus (DM) (12 patients).
(2) Group 2: HD patients with HCV infection and who were nondiabetic (24 patients).
(3) Group 3: HD patients without HCV infection and who were diabetic (10 patients).
(4) Group 4: HD patients without HCV infection and who were nondiabetic (24 patients).
(5) Group 5: age-matched and sex-matched HCs (20 individuals).

Methods

Initially, all participants completed a detailed questionnaire with regard to diet, habits, and drugs, and submitted to thorough history taking with detailed physical examinations and relevant medical history. At the day of study inclusion, three milliliters of venous blood were obtained from all participants, and the serum samples were centrifuged at 3500 rpm, then aliquoted and stored at −70°C until assayed. Laboratory parameters were analyzed, and patients also underwent ultrasound and computed tomography scan. Adequacy of HD was assessed by using the urea reduction ratio (URR) and the K_t/V formula [11].

All patients and controls were given the recombinant HBV vaccine, intramuscularly in the deltoid muscle (at a schedule of 0, 1, 6 months). Determination of serum HBs-Ab titer was tested 8 weeks from the last dose of the HBV vaccine using commercially available human enzyme-linked immunosorbent assay kit according to manufacturer guidelines (Ref. SAB.CE; Dia. Pro. Diagnostic Bioprobes Srl, Milano, Italy). HBs-Ab titer of at least 10 mIU/ml was considered to be protective. A nonresponse was defined as HBs-Ab titers less than 10 mIU/ml.

DNA extraction and detection of IL-12 gene polymorphism (IL-2B 3′UTR A>C; rs321227) were carried out by molecular biology technique using PCR amplification followed by restriction fragment length polymorphism assay [12]. Genomic DNA was prepared from 2 ml EDTA-collected peripheral blood leukocytes (stored at −80°C until use), either by salting out or using Qiagen protocol QIAamp DNA Blood Mini Kit (cat no. 51104; Qiagen GmbH, Hilden, Germany). Extracted DNA was amplified using the PCR with sequence-specific primers; forward: 5′ TTA AAG ACA CAA CGG AAT AGA C 3′, reverse: 5′ TGC TTT ATC AAC ACC ATC TCC 3′. The amplified products were then digested with TaqI restriction endonucleases overnight at 37°C, electrophoresed on 2% horizontal agarose gel, stained with 0.5 mg/ml of ethidium bromide and visualized under UVB-illumination using the E-Gel Precast Agarose Electrophoresis System (Invitrogen Life Technologies, Paisley, UK) [13].

Statistical analyses

Data were analyzed using SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA). Quantitative (continuous) data were expressed as mean±SD in parametric data or as median interquartile range (IQR) in nonparametric data, while qualitative data and categorical variables were expressed as number and percentage. Categorical variables were compared using the χ^2-test or Fisher's exact test. Subgroups were compared using the McNemar test. Kruskal–Wallis one-way analysis of variance followed by Mann–Whitney test was applied for multiple comparisons in nonparametric data. Hardy–Weinberg (H–W) equilibrium was used to assess the independent segregation of alleles [14]. This H–W equilibrium was then assessed using χ^2-test or Fisher's exact test when appropriate. Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Carriage rate was calculated as the number of individuals carrying at least one copy of test allele divided by the total number of individuals. Variables that achieved statistical significance with the univariate analysis were included in multiple regression analysis with a forward stepwise (likelihood ratio) to evaluate the independent factors affecting HBs-Ab titer. Moreover, the odds ratios with 95% confidence intervals (CIs) of the association between HBs-Ab titer and IL-12B genotypic frequencies were estimated using multiple logistic regression analysis after controlling for other covariates [15]. For all statistical studies, P value less than 0.05 was considered to be statistically significant.

Results

The demographic and clinical characteristics of all HD patients' groups are presented in Table 1. There were

Table 1 Demographic and clinical characteristics of hemodialysis patients

Parameters	HCs (20)	HD (70)	Group 1 (12)	Group 2 (24)	Group 3 (10)	Group 4 (24)	ANOVA
Age (years)	55.4±12.7	56.4±13.9	62.92±9.87	55.71±14.61	60.6±6.96	52.17±15.88	0.11
Sex (male/female)	9/11	34/36	4/8	16/8	4/6	10/4	0.17
BMI (kg/m^2)	22.8±4.3	23.7±5.42	24.17±5.78	23.25±5.61	24.00±5.7	23.91±5.19	0.87
Blood transfusion (yes/no)	0	49/21	9/3	18/6	7/3	15/9	0.58
EPO: yes	0	70	12	24	10	24	0.78
Duration of HD	–	4 (0.5–18)	1.25 (1–7)	5 (2–18)	1.25 (1–6)	3 (0.5–12)	0.065

The test used: one-way analysis of variance (ANOVA) for data expressed in mean±SD and χ^2-test for data expressed in frequency. EPO, erythropoietin; HD, hemodialysis.

Table 2 HBs-Ab titer among various groups

	N	Comorbidity	Median (range)	%Of titer≥10 mIU/ml (responders)
HCs (20)	20	–	359.4 (495–2.2)[b,d]	18/20 (90)
HD patients	70		296.5 (520–2.1)	56/70 (80)
P value			0.078	0.3
Subgroup 1	12	HCV, DM	218.5 (379–2.1)	14
Subgroup 2	24	HCV	220.2 (520–3.6)	16
Subgroup 3	10	DM	228 (361–2.1)	13
Subgroup 4	24	No HCV, no DM	368 (496–4.3)[a,c]	47[a,c]
P (ANOVA)			0.02	0.04

Kruskal–Wallis one-way analysis of variance (ANOVA) test was used followed by Mann–Whitney for pairwise comparisons. DM, diabetes mellitus; HBs-Ab, hepatitis B surface-antibody; HCs, healthy controls; HCV, hepatitis C virus; HD, hemodialysis. [a]Significance between groups 1 and 4. [b]Significance between subgroup 1 and control. [c]Significance between subgroups 3 and 4. [d]Significance between subgroup 3 and control.

no statistically significant differences as regards age, sex, blood transfusion, or EPO administration ($P>$ 0.05).

The HBs-Ab titer among various groups is shown in Table 2. There were no significant differences in the level of HBs-Ab titer among HD patients and control participants (P=0.078). Moreover, the percentage of responder participants with HBs-Ab titer more than 10 mIU/ml was statistically nonsignificantly high in the control group than in HD patients (90 vs. 80%, respectively; P=0.3). However, the median levels of HBs-Ab titer were statistically significantly higher among the HC group (mIU/ml) and subgroup of 4 HD patients (negative for both HCV and DM) than those of patients of subgroups 1 and 3 (359.4, 368 vs. 218.5, 228 mIU/ml, respectively; P=0.02).

Comparing the demographic, clinical, and laboratory data of responders and nonresponders among HD patients (Table 3), it was observed that there were no statistically significant differences with regard to the age, sex, BMI, liver functions, hemoglobin concentration, total leukocytic count, parathyroid hormone, blood transfusion or EPO administration and duration of HD ($P>$ 0.05). In contrast, there was a statistically significant difference between responder and nonresponder patients as regards lymphopenia, transferrin saturation (TSAT), K_t/V, and URR ($P<$ 0.05).

Primer sequences of IL-12B polymorphisms were demonstrated in Table 4. The Base change was 3′UTR A>C. The PCR amplified fragments of IL-12B were 557 bp in length and were isolated and digested with the endonuclease TaqI (T/CGA) (New England Biolabs, Ipswich, Massachusetts, USA). The IL-12B 3′UTR A allele remained uncut, whereas the IL-12B 3′UTR C allele was cleaved into 454 and 103 bp fragments. Moreover, the PCR product bands at 557 bp and IL-12B gene polymorphism bands at 557, 454 and 103 bp are demonstrated in Fig. 1. Lanes 1–6 show PCR products at base pair 557. Lane 7 shows DNA ladder (marker). Lanes 8, 9, 13, 16 and 18 show AC genotypes (bands at base pair 454 and 557). Lane 10 shows no bands. Lanes 11, 12, 15, 17, 19 and 20 show AA genotypes (bands at base pair 557).

IL-12B genotypic frequencies in HD patients and HCs are shown in Table 5. According to H–W equilibrium, there were no significant differences in genotypic or allelic frequencies of IL-12B in HD patients and HCs. Genotype frequencies were 58.57 versus 50% for genotype AA, 37.14 versus 50% for genotype AC, and 4.29 versus 0% for genotype CC in the HD and HC groups, respectively. In responders of HD and controls (antibody titer≥10 mIU/ml), 58.93 versus 55.6% had the genotype AA, 37.5 versus 44.4% had genotype AC and 3.57 versus 0.0% had genotype CC, respectively. Using the carriage of genotype AA as

Table 3 Other factors affecting the antibody response in the hemodialysis group (*n*=70)

Parameter (mean±SD)	Responders (56)	Nonresponders (14)	*P* value
HBs-Ab titer (mIU/ml)	≥10	< 10	
Age	54.37±14.94	61.17±12.67	0.15
Duration of HD	3.0 (0.50–18.00)	4.5 (1.00–13.00)	0.4
BMI (kg/m^2)	27.06±5.67	25.15±27.06	0.3
Blood transfusion (positive/negative)	41 (73.2)/15 (26.8)	8 (57.1)/6 (42.9)	0.3
EPO [*N* (%)]	56 (100)	14 (100)	
ALT	18.40±9.88	18.00±18.40	0.9
AST	19.33±7.61	23.67±19.33	0.11
Total protein	7.27±0.58	7.03±7.27	0.3
Serum albumin	3.99±0.36	3.89±3.99	0.4
Hemoglobin (g/dl)	9.51±1.57	9.52±9.51	0.98
WBCs (normal/leucopenia) [*N* (%)]	55 (98.2)/1 (1.8)	14 (100)/0	0.6
Lymphocytes (normal/lymphopenia) [*N* (%)]	46 (82.1)/10 (17.9)	3 (21.4)/11 (78.6)	<0.0001
Transferrin (TSAT) (%)	27.33±7.18	34.19±27.33	0.005
PTH [*N* (%)]			
< 150	17 (30.4)	7 (50)	0.17
150–300	18 (32.1)	6 (42.9)	
> 300	21 (37.5)	1 (7.1)	
URR (%)	63.64±9.11	52.73±63.64	0.002
K_t/V	1.17±0.19	0.94±1.17	0.002

Tests used: Student's *t*-test for parametric data, Mann–Whitney and χ^2-test for nonparametric data expressed in frequency. HBs-Ab, hepatitis B surface-antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EPO: erythropoietin; HB, hemoglobin; HD, hemodialysis; *K*, dialyzer clearance of urea; PTH, parathyroid hormone; *t*, dialysis time; TSAT, transferrin saturation; URR, urea reduction ratio; *V*, volume of distribution of urea; WBCs, white blood cells.

Table 4 Primer sequences of interleukin-12B polymorphisms

IL-12B (rs3212227): 557 bp	Forward primer	5′ TAAAGACACAACGGAATAGAC 3′
	Reverse primer	5′ TGCTTTATCAACACCATCTCC 3′
	Base fragments	AA: 557 bpAC: 557, 454, 103 bpCC: 454, 103 bp
	Base change	3′UTR A>C
	Endonuclease	TaqI (T/CGA)

IL, interleukin.

a reference, no statistically significant differences were observed when comparing both HD and HC groups [*P*=0.37, 95% CI: 0.63 (0.23–1.73)] and when comparing the responders in either groups [*P*=0.69, 95% CI: 1.24 (0.41–3.75)].

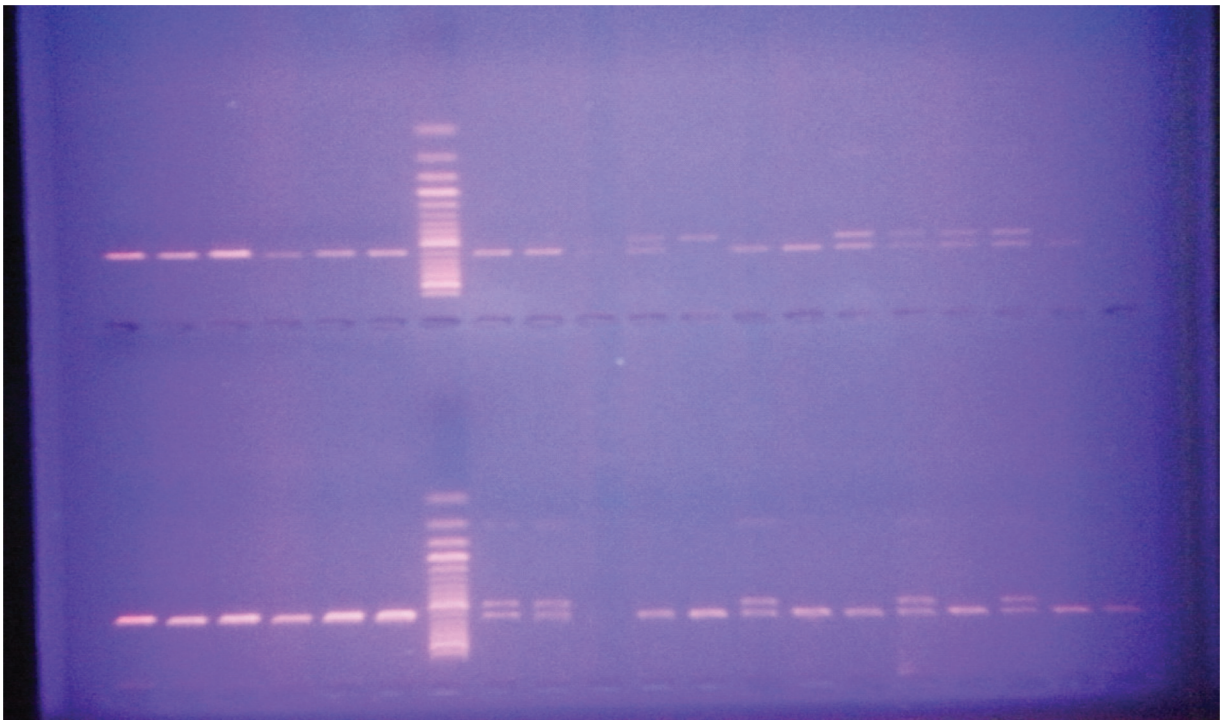
All nonresponding HC participants (100%) carried the genotype variant AC, while nonresponding HD patients carried the genotype variant AA in 57.14% and the genotype variant CC in 42.86%. In Table 6, when using the carriage of genotype AA as a reference, comparing the responders and nonresponders in HD patients revealed no statistically significant differences [*P*=0.87, 95% CI: 0.9 (0.2–3.3)].

Discussion

HBV infections can be prevented or controlled by the host humoral immune response (HBs-Ab), elicited either naturally or by vaccination. Cytokines, especially IL-10 and IL-18, were associated with HBs-Ab development in HD patients and promotion of IFN-γ production, which stimulates phagocytic cells to produce IL-12 and other proinflammatory cytokines. IL-12, produced mainly by activated APCs, is significantly elevated in HD patients; however, the constitutive IFN-γ release may be undetectable in HD patients, leading to Th1 lymphocyte immunodeficiency [16].

In this study, the duration of HD was statistically significantly longer in nondiabetic HD patients. This may be explained by the decreasing effect of diabetes on life expectancy. Similar to previous studies, hypertensive nephropathy was the most frequent known etiology for HD in Egypt [17]. Moreover, diabetic patients with HCV under HD had significantly lower serum albumin levels explained by microalbuminuria of diabetic nephropathy and compromised synthetic liver functions, protein malnutrition, fluid overload, and the chronic inflammatory state.

Figure 1



PCR product bands at 557 base pair (bp) and interleukin-12B gene polymorphism bands at 557, 454 and 103 bp.

Table 5 Interleukin-12B genotypic frequencies in hemodialysis patients (70) and healthy controls (20)

IL-12B gene polymorphisms			HD patients (70)	HCs (20)	*P* value	OR (95% CI)
All participants (90)	Genotype	AA	41 (58.57)	10 (50.0)	–	1 (ref.)
A=2 (AA)+AC C=2 (CC)+AC		AC	26 (37.14)	10 (50.0)	0.37	0.63 (0.23–1.73)
		CC	3 (4.29)	0	1.00	0
	Allele	A	108 (77.0)	30 (75.0)	–	1 (ref.)
		C	32 (23.0)	10 (25.0)	0.77	0.89 (0.39–2.01)
Responders (56/70 patients, 80%)	Genotype	AA	33 (58.93)	10 (55.6)	–	1 (ref.)
		AC	21 (37.50)	8 (44.4)	0.69	1.24 (0.41–3.75)
		CC	2 (3.57)	0	1.00	0
	Allele	A	87 (77.68)	28 (78.0)	–	1 (ref.)
		C	25 (22.32)	8 (22.0)	0.93	0.96 (0.38–2.41)
Nonresponders (14/20 patients, 20%)	Genotype	AA	8 (57.14)	0	–	1 (ref.)
	6 (42.86)	AC	6 (42.86)	2 (100.0)	0.244	0.15 (0.001–3.71)
	0	CC	0	0	1.0	0
	Allele	A	22 (78.57)	2 (50.0)	–	1 (ref.)
		C	6 (21.43)	2 (50.0)	0.233	0.26 (0.03–2.36)

CI, confidence interval; HCs, healthy controls; HD, hemodialysis; IL, interleukin; OR, odds ratio.

In HD patients, there were significant low TSAT and mean hemoglobin level. Anemia in HD patients is a multifactorial process due to relative EPO deficiency, uremic-induced inhibitors of erythropoiesis, shortened erythrocyte survival, inflammation and cytokines release, inadequate HD, aluminum intoxication, and disordered iron homeostasis. Causes of iron deficiency anemia in HD may include chronic blood loss, poor nutrition, malabsorption and functional iron deficiency anemia [18].

Protection with hepatitis-B vaccination is considered to be achieved when the HBs-Ab titer is at least 10 mIU/ml. Patients undergoing HD showed a lower response to HB vaccine than the general population, and the seroconversion rates were inversely related to the chronic kidney disease stage. ESRD patients have a reduced response to HB vaccine, and more than 40% of patients undergoing HD who respond to primary vaccination will have undetectable HBs-Ab levels 3 years after vaccination. Protective HBs-Ab levels

Table 6 Interleukin-12B genotypic frequencies in responders and nonresponders of hemodialysis patients

IL-12B gene polymorphisms	Responders (56 patients)	Nonresponders (14 patients)	*P* value	OR (95% CI)
Genotype				
AA	33 (58.93)	8 (57.14)	–	1 (ref.)
AC	21 (37.50)	6 (42.86)	0.87	0.9 (0.2–3.3)
CC	2 (3.57)	0	1.00	–
Allele				
A	87 (77.68)	22 (78.57)	–	1 (ref.)
C	25 (22.32)	6 (21.43)	0.83	1.13 (0.38–3.37)

CI, confidence interval; IL, interleukin; OR, odds ratio.

persisted for 12 months post-HBV vaccination in only 44% of weak responders (HBs-Ab level 10.0–99.9 mIU/ml) and in 92% among strong responders (HBs-Ab level≥100 mIU/ml) [19].

Similar to previous studies, we observed a higher antibody response in HCs than in HD patients (90 vs. 80% obtained HBs-Ab titer≥10 mIU/ml and 85 vs. 63.3% obtained HBs-Ab titer> 100 mIU/ml, respectively). Moreover, the higher response noticed in HD patients was statistically significant in nondiabetic and non-HCV HD patients. This low responsiveness in HD patients may be related to uremic toxin accumulation, the increase of systemic and vascular inflammatory biomarker concentration, advanced glycation end products, and the use of the three-dose schedule instead of the four-dose schedule recommended for HD patients.

Strategies to improve the response to HBV vaccine in HD patients could include early vaccination, efficient dialysis, intradermal injection of the vaccine, adjuvant vaccines (HB-AS04, HB-AS02), immunostimulants (levamisole, granulocyte-macrophage colony-stimulating factor, thymopentin, recombinant interferon-α2), and the use of third-generation vaccines (pre-S1 and pre-S2 antigens) [20].

The difference in responsiveness between HD subgroups may be attributed to numerous changes in cellular and humoral immune responses described in nonuremic patients with DM. The presence of HLA-DR3 and DR7 in diabetic individuals may be implicated in this impaired immunological response. A meta-analysis of 12 studies involving 1002 patients on long-term dialysis showed also a significant decrease in response rates among the diabetic versus the nondiabetic HD patients [5].

HCV does not interfere with the development of a protective antibody response after vaccination, although lower titers of HBs-Ab have been reported after vaccination of HCV-positive patients in comparison with HCV-negative patients [21]. In contrast, Navarro *et al.* [22] noted that HCV infection might reduce the effectiveness of the hepatitis B vaccine in HD patients. A meta-analysis of eight studies on 520 HD patients noted that there were no significant decreases in immune response among HCV-positive versus HCV-negative HD patients [23].

In this study, there was no significant association between IL-12B gene polymorphism and HBs-Ab development in either the HD patients or in the HC group ($P>$ 0.05). Moreover, responders of HD patients had the genotype variant AA (58.93%), AC (37.5%) and CC (3.57%), while the nonresponder HD patients had genotype variant AA (57.14%), AC (42.86%) and CC (0%) ($P>$ 0.05).

Grzegorzewska *et al.* [24] found no association between IL-12A or IL-12B gene polymorphism and the developed HBs-Ab titer in the HD patients. On the contrary, Wang *et al.* [25], found an association between IL-12B gene polymorphism and the nonresponse to full-dose HBV vaccination. IL-18 gene polymorphism could be associated with the development of HBs-Ab titer in HD patients, either alone or combined with IL-12A or IL-12B polymorphism. Peripheral blood mononuclear cells from high responders showed elevated production of IL-2, IL-12, and interferon-γ. These variable observations may be related to differences in sample size, race, ethnicity, or the use of different molecular biological techniques.

In this study, age, sex, duration of HD therapy, BMI, previous blood transfusion, hemoglobin, total protein, albumin level, total leukocytic count, HCV-Ab positivity and parathyroid hormone level had no effect on the response to HBV vaccine. In contrast, it had been reported that age negatively influenced the response to the HBV vaccine in HD patients in a meta-analysis of 31 clinical trials and was attributed to the age-associated changes in immune status [26].In this study, lymphopenia was noticed in 30% of our patients,

and it was significantly associated with impaired responsiveness to HBV vaccine ($P<0.0001$). ESRD, especially in HD patients, was associated with B-cell lymphopenia [27].

All the patients included in our study were on recombinant human erythropoietin (rHuEpo) therapy. In this study, significant higher TAST values were observed in HD patients not responding to HB vaccine ($P=0.005$). Impaired responsiveness to HB vaccine was noted with the administration of intravenous iron during the period of vaccination in HD patients on rHuEpo. In concordance to a meta-analysis of 11 studies on 862 patients, we failed to detect a significant relationship between the use of rHuEpo and the response to HBV vaccine. It was thought that rHuEpo therapy has humoral and cellular immunomodulating effects, improving the response to HBV vaccine [28].

This may be caused by the deleterious effects of iron on CD4+ and CD16+ human lymphocyte cell population that may be mediated by intracellular reactive oxygen species' generation.

There were significant lower mean URR% and K_t/V values in HD patients (61.46±11.13 and 1.12±0.23, respectively). The responders to HBV vaccination had significantly higher URR% and K_t/V in comparison with nonresponders (63.64±9.11 and 1.17±0.19 vs. 52.73±63.64 and 0.94±1.17, respectively, $P<0.05$). The positive effect of efficient HD on the response to HB vaccination was also reported by Dede *et al.* [29]. This may be explained by restoration of the impaired B7-2 (CD 86) expression on monocytes of dialysis patients by efficient dialysis.

Conclusion

There was no significant association between IL-12B gene polymorphism and HBs-Ab response in Egyptian HD patients. In HD patients, lymphocytopnea, DM, high TSAT and inefficient HD were associated with HBV vaccine hyporesponsiveness.

Recommendation

Ongoing research should include larger sample size with four-dose HBV vaccine schedule and examine the combined effect of gene polymorphisms of other cytokines on the response to HBV vaccine in HD patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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