Original Paper

Kidney & Blood Pressure Research

Kidney Blood Press Res 2012;35:1–8
DOI: 10.1159/000329932

Received: December 23, 2010
Accepted: June 1, 2011
Published online: August 10, 2011

# *Interleukin-18* Promoter Polymorphism and Development of Antibodies to Surface Antigen of Hepatitis B Virus in Hemodialysis Patients

Alicja E. Grzegorzewska[a] Piotr Wobszal[a, b] Paweł P. Jagodziński[b]

[a]Department of Nephrology, Transplantology and Internal Diseases, and [b]Department of Biochemistry and Molecular Biology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland

**Key Words**
Antibodies to surface antigen of hepatitis B virus · Hemodialysis · *Interleukin-18* gene

**Abstract**
***Background:*** Interleukin (IL)-18 is involved in hepatitis B virus (HBV) clearance and augments antibodies against surface antigen of HBV (anti-HBsAg) production during DNA vaccination. The IL-18 –1297C>T (rs360719) polymorphism may modulate the IL-18 expression. ***Aim:*** To determine the potential association of IL-18 –1297C>T polymorphism with development of anti-HBsAg in hemodialysis (HD) patients. ***Methods:*** The frequency of IL-18 –1297C>T alleles and genotypes was identified by polymerase chain reaction restriction fragment length polymorphism in 435 HD patients. Group 1 (n = 219) developed an anti-HBsAg titer >10 IU/l as a result of vaccination or HBV transmission. Group 2 (n = 216) included patients who did not develop an anti-HBsAg titer >10 IU/l in response to at least one full series of vaccination or HBV transmission. The significance of genotype frequency was tested using the Fisher exact test. ***Results:*** In group 1, the frequencies of –1297CC, –1297CT and –1297TT genotypes were 7.3, 39.7 and 53.0%, respectively, and in group 2 they were 1.9, 42.1 and 56.0%, respectively. The odds ratio for CC versus CT + TT was 0.239 (95% CI 0.079–0.728, p = 0.010), and for CC versus TT it was 0.240 (95% CI 0.078–0.738, p = 0.009). ***Conclusion:*** In HD patients, the IL-18 –1297CC genotype may play a role in anti-HBsAg development in response to HBV surface antigen.


## Introduction

Patients treated with intermittent hemodialysis (HD) due to end-stage renal disease are at risk of infection with blood-transmitted viruses, like hepatitis B virus (HBV). Infected patients develop antibodies against core antigen of HBV (anti-HBcAg), which persist for the entire life as an established marker of current or previous infection. The prevalence of anti-HBcAg in the chronic dialysis population ranges from 0 to 54% [1–4]. Anti-HBcAg may be accompanied by antibodies against surface antigen of HBV (anti-HBsAg) or in 14–31% of cases may remain isolated [4, 5], even in patients who eliminated surface antigen of HBV (HBsAg) and are not considered as HBV carriers.

The use of protein-based vaccines made up of recombinant HBsAg significantly decreased prevalence and incidence of HBV carriers in dialysis facilities [6]. Vaccination with an anti-HBsAg titer conferring protection (over 10 IU/l [7]) was reported in 54–86% of HD patients using a recombinant vaccine [8–10]. Ineffective vaccination is

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com


1420–4096/12/0351–0001$38.00/0

Accessible online at:
www.karger.com/kbr

Prof. Alicja E. Grzegorzewska
Department of Nephrology, Transplantology and Internal Diseases
Karol Marcinkowski University of Medical Sciences, Al. Przybyszewskiego 49
PL–60-355 Poznań (Poland)
Tel. +48 61 869 1700, E-Mail alicja_grzegorzewska@yahoo.com

predictive of prevalence and incidence of both HBsAg positivity [11] and anti-HBcAg positivity [4, 12]. Aforementioned data indicate that certain proportions of HD patients do not have any appreciable immune response to HBsAg, either in cases of natural exposure or a planned immunization.

Proinflammatory, Th1-promoting and regulatory cytokines are critical signals required for the development of an effective adaptive immune response. These cytokines could mediate immunomodulatory effects by altering the magnitude and direction of the host immune responses and could improve the vaccine efficacy [13, 14]. Interleukin (IL)-18 has been shown to exert innate and acquired immune responses, especially to mediate both Th1- and Th2-driven immune responses [15–19]. The anti-HBV activity of Th1 lymphocytes was strongly induced by IL-12 + IL-18, and resulted in interferon (IFN)-γ, IL-4, IL-5 and IL-10 production by CD4 T lymphocytes isolated from peripheral blood of children with chronic hepatitis B [20]. In combination with IL-12, IL-18 induces IFN-γ production also in β-cells and natural killer cells, promoting Th1 type immune responses [21, 22]. IFN-γ appears to play an important role in T cell-mediated viral clearance. IL-18 also acts as an immune adjuvant to induce the specific humoral immune response [23].

The expression of IL-18 mRNA was recently related to the –1297C>T (rs360719) polymorphism of the *IL-18* gene by Sánchez et al. [24]. Their study indicates that inhibitory transcription factor OCT-1 binds to the allele T but not to the allele C at position –1297 (rs360719) [24]. The rs360719 T allele was identified as a possible major repressor site in the *IL-18* promoter. This suppression would result in reduced IL-18 production. The rs360719 C-allele cannot bind OCT-1, leading to an increase in *IL-18* transcription.

Our aim was to determine in HD patients the potential association between the *IL-18* –1297C>T promoter polymorphism and development of anti-HBsAg in response to HBsAg administered in anti-HBV vaccine (a planned immunization) or acquired due to HBV transmission (a natural immunization).

## Patients and Methods

*Patients*

Studies were carried out in the spring-summer time of 2009 in patients treated in dialysis centers in the Wielkopolska region of Poland.

Since 2004, all patients starting intermittent HD in Poland undergo obligatory vaccination against HBV as soon as possible after obtaining a negative result of blood testing on HBsAg and detection of an anti-HBsAg titer below 10 IU/l considered as negative. This obligatory testing is performed at the first HD session. However, some patients were vaccinated much earlier before the official introduction of vaccination to dialysis centers; those who started HD before 2004 and were not vaccinated but remained HBsAg and anti-HBsAg negative underwent vaccination after commencement of routine vaccinations. Since 2009, results of blood testing on total anti-HBcAg are also considered in the vaccination strategy. HBsAg-negative patients with positive testing for both anti-HBcAg and anti-HBsAg are not vaccinated. HBsAg-negative patients showing anti-HBcAg positivity but anti-HBsAg negativity (so called isolated anti-HBcAg positivity) are usually not vaccinated either, but in some centers they are given a full vaccination series or at least one vaccine dose. According to standard rules [25], an anti-HBsAg titer is checked after 4–8 weeks from the last vaccine dose. When an anti-HBsAg titer remains below 10 IU/l, vaccination against HBV is usually repeated, especially when the clinical status of the patient is better than that observed at the previous vaccination. Due to fluctuations of anti-HBsAg in HD patients, a blood testing for anti-HBsAg is obligatorily repeated every 6 months and decisions for booster doses of vaccine are made.

Patients enrolled in the study had to fulfill the following criteria:

(1) Treatment with intermittent HD due to end-stage renal disease
(2) No signs and symptoms of acute infection with blood-borne viruses
(3) A known anti-HBsAg titer (all available results of each patient were analyzed)
(4) In patients without serological signs of HBV transmission, at least one full vaccination series against HBV with a checked anti-HBsAg titer after 4–8 weeks from the last vaccine dose
(5) Agreement of participation in the study

A response to HBsAg was considered positive when an anti-HBsAg titer exceeded 10 IU/l. Such a titer is assumed to be protective in vaccinated patients [7].

When checking data of patients with collected blood samples, the number of participants with an anti-HBsAg titer over 10 IU/l was over 200; only patients with negative results of testing for anti-HBsAg fulfilling aforementioned criteria were enrolled.

For patients in a relationship, only one person could participate in the study.

Patients included in the study (n = 435) were divided into two groups. Group 1 (HBsAg responders, n = 219) consisted of patients who developed an anti-HBsAg titer >10 IU/l as a result of vaccination (patients with total anti-HBcAg negative, n = 125) or as a result of HBV transmission (patients with total anti-HBcAg positive, n = 94). In some HBsAg-negative anti-HBcAg-positive patients, anti-HBsAg production could be enhanced or induced by vaccination when a result of anti-HBcAg testing was not known before vaccination. Patients naturally infected in the past redeveloped protective anti-HBsAg titers more easily [26, 27]. In some patients with a long course of renal disease, a history of vaccination effectiveness revealed periods with or without anti-HBsAg >10 IU/l. If a patient had anti-HBsAg >10 IU/l in the past but lost it during follow-up, she/he was considered as constitutionally able to respond to HBsAg, and was included in group 1. In this study, 19 patients of group 1 were anti-HBsAg negative at

the moment of blood sampling for analysis. Group 2 (HBsAg nonresponders, n = 216) included patients who did not develop an anti-HBsAg titer >10 IU/l in response to at least one full series of vaccination (patients with total anti-HBcAg negative, n = 176) or HBV transmission (patients with total anti-HBcAg positive, n = 40). Their available documents did not reveal any anti-HBsAg >10 IU/l.

Registered blood donors (n = 240) from the Wielkopolska region of Poland, qualified for blood donation according to the criteria of the Polish Ministry of Health [28], served as controls for both groups of HD patients. Among controls, there were 143 women (age 36 ± 8 years) and 97 men (age 60 ± 4 years). The serum alanine aminotransferase activity of controls was not higher than twice the upper normal limit of the applied laboratory method. All controls showed negative blood testing for HBsAg and HBV DNA as well as for seromarkers of infection with hepatitis C virus. Unfortunately, the vaccination rate against HBV and the anti-HBsAg titer were not known in these healthy persons.

*Laboratory Methods*

HBsAg and anti-HBcAg were determined by the Microparticle Enzyme Immunoassay (MEIA) technology (AxSYM; Abbott Laboratories, Abbott Park, Ill., USA). For detection of anti-HBsAg and antibodies to hepatitis C virus, the MEIA technology was also used (Abbott, Wiesbaden, Germany). HBV DNA was determined using a qualitative test Cobas Amplicor HBV Monitor; RNA of HCV was tested using Cobas Amplicor Hepatitis C Virus Test, version 2.0 (both Roche Diagnostics Ltd., Rotkreutz, Switzerland). Serum activities of liver enzymes were determined by routine laboratory methods.

*IL-18 Genotyping*

DNA was isolated from peripheral leukocytes using a standard salting out procedure. The *IL-18* –1297C/T (rs360719) polymorphic variant was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was performed employing primer pair 5′-CAACAGTGATTACAAAGGAAGT-3′ and 5′-TAAATGGGTAGGAATAAGTGAGA-3′. The PCR-amplified fragments of *IL-18* 474 bp in length were digested with the endonuclease *Nla*III (CATG/; New England Biolabs, Ipswich, Mass., USA). The *IL-18* C allele remained uncut, whereas the *IL-18* T allele was cleaved into 295 and 179 bp. DNA digestion products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The PCR-RFLP analysis was repeated for all patients and controls samples. The conformity of the *IL-18* –1297C/T polymorphism was also conducted by commercial sequencing analysis. All PCR products were sequenced for the *IL-18* –1297C/C genotype, half for the C/T genotype, and 10% for the T/T genotype in both groups and controls. The rate of concordance between RFLP analysis and sequencing was 99.8%.

*Statistical Methods*

The prevalence of *IL-18* genotypes in groups 1 and 2 and in controls was examined for deviation from the Hardy-Weinberg equilibrium. The Fisher exact test was employed to show differences in genotypic and allelic distribution between the examined groups. The analysis of differences in genotype frequencies between both groups was performed for the hypothetical model of inheritance: recessive (CC vs. CT + TT), dominant (CC + CT vs. TT) and additive (CC vs. TT). A p value $<0.05$ was considered statistically significant. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Power analysis was conducted employing the Fisher exact test, which was available at an online internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/main/powersamplesize.

The normality of distribution of the variables shown in table 1 was checked by the Shapiro-Wilk test. Descriptive statistics are presented as percentage for categorical variables, as mean with one standard deviation for normally distributed continuous variables or as median with range for not normally distributed continuous variables. The prevalence of variables was assessed by the $\chi^2$ test or Yates $\chi^2$ test, as appropriate. Results were compared using Student's t test for nonpaired data if distribution of variables was normal or the Mann-Whitney U test for other than normal distributions.

The logistic regression analysis (the hierarchical model) was used to show variables associated with anti-HBsAg development. OR and CI at the levels ±95% were shown for significant predictors. A p value $<0.05$ was judged to be significant.

*Ethical Issues*

This study was approved by the Institutional Review Board of Karol Marcinkowski University of Medical Sciences in Poznań, Poland.

## Results

Table 1 shows the demographic, clinical and laboratory data of the entire group (n = 435) and of patients grouped by an anti-HBsAg titer >10 UI/l (group 1) and ≤10 UI/l (group 2). All patients were Caucasian. Differences in prevalence of HBV seromarkers between groups 1 and 2 depended on the applied method of group separation. Patients who developed anti-HBsAg showed more frequently chronic glomerulonephritis and less frequently diabetic nephropathy as causes of end-stage renal disease, were younger and were longer on renal replacement therapy (RRT) compared to nonresponders.

Distribution of the –1297C/T (rs360719) *IL-18* promoter polymorphisms in both groups of HD patients and in controls is shown in table 2. There was no statistical deviation from the Hardy-Weinberg equilibrium in the genotype frequencies of HD patients in group 1 (expected frequencies: –1297CC 7.3%, –1297CT 39.7%, –1297TT 53.0%), HD patients in group 2 (expected frequencies: –1297CC 5.3%, –1297CT 45.3%, –1297TT 59.4%) and controls (expected frequencies: –1297CC 8.5%, –1297CT 41.3%, –1297TT 50.2%).

Patients in group 1 (responders) did not differ significantly in the distribution of *IL-18* genotypes and alleles compared to controls, whereas in group 2 patients (nonresponders), the frequency of –1297CC (rs360719) was

**Table 1.** Demographic, clinical and laboratory data of the entire group and of patients grouped by a titer of anti-HBsAg >10 UI/l (group 1) and ≤10 UI/l (group 2)

| Parameter | All patients (n = 435) | Group 1 (n = 219) | Group 2 (n = 216) | p value (group 1 vs. 2) |
|---|---|---|---|---|
| Men/women | 243/192 (55.9/44.1) | 122/97 (55.7/44.3) | 121/95 (56.0/44.0) | 0.9480 |
| Age, years | 64.2 (15.1–93.1) | 62.1 (15.1–93.1) | 68.8 (19.3–92.5) | 0.0200 |
| Age >30/≤30 years | 418/17 (96.1/3.9) | 209/10 (95.4/4.6) | 209/7 (96.8/3.2) | 0.6413 |
| RRT duration, years | 2.00 (0.04–29.16) | 3.00 (0.05–29.16) | 1.30 (0.04–24.21) | 0.0149 |
| Diabetic nephropathy | 115 (26.4) | 48 (21.9) | 67 (31.0) | 0.0410 |
| Chronic glomerulonephritis | 81 (18.6) | 52 (23.7) | 29 (13.4) | 0.0083 |
| Hypertensive nephropathy | 60 (13.8) | 26 (11.9) | 34 (15.7) | 0.3026 |
| Chronic tubulointerstitial nephritis | 55 (12.6) | 32 (14.6) | 23 (10.6) | 0.2716 |
| History of acute hepatitis | 22 (5.1) | 13 (5.9) | 9 (4.2) | 0.5331 |
| Positive/negative HBsAg | 15/420 (3.4/96.6) | 0/219 (0/100) | 15/201 (6.9/93.1) | 0.0002 |
| Positive/negative HBV DNA | 11/85 (11.5/88.5) | 1/64 (1.5/98.5) | 10/21 (32.3/67.7) | <0.0001 |
| Positive/negative anti-HBcAg | 134/301 (30.8/69.2) | 94/125 (42.9/57.1) | 40/176 (18.5/81.5) | <0.0001 |
| Isolated positive anti-HBcAg | 40 (9.2) | 0 | 40 (100) | <0.0001 |
| Full vaccination series against HBV with developed anti-HBsAg titer >10 IU/l | 125 (28.7) | 125 (57.1) | 0 | <0.0001 |
| Positive/negative anti-HCV | 56/379 (12.9/87.1) | 28/191 (12.8/87.2) | 28/188 (13.0/87.0) | 0.9559 |
| Positive/negative HCV RNA | 34/391 (8.0/92.0) | 20/195 (9.3/90.7) | 14/196 (6.7/93.3) | 0.4108 |
| ALT, U/l | 13 (2–209) | 13 (2–209) | 13 (2–126) | 0.2798 |
| AST, U/l | 15 (3–106) | 15 (3–106) | 14 (4–97) | 0.5389 |
| GGT, U/l | 28 (4–1,315) | 27 (4–1,315) | 29 (5–308) | 0.3931 |

Data are expressed as medians. Figures in parentheses indicate ranges or percentages.

ALT = Alanine aminotransferase; anti-HCV = antibodies to hepatitis C virus; AST = aspartate aminotransferase; GGT = γ-glutamyltranspeptidase.

significantly lower than that in controls in both the CC versus CT + TT (sample power 77.8%) and CC versus TT models (sample power 94.0%).

The frequency of the *IL-18* –1297CC genotype was approximately 4 times higher in responders to HBsAg (group 1) compared to nonresponders (group 2). In patients bearing the CC genotype, there were 80.0% of persons with an anti-HBsAg titer >10 IU/l. This prevalence was higher than that shown in persons with the CT genotype (48.9%, p = 0.009) or the TT genotype (48.7%, p = 0.009). Patients bearing the –1297CC genotype could on average exhibit a 4.18-fold increased chance of developing anti-HBsAg in response to vaccine HBV surface antigen or HBV natural exposure. The power analysis amounted to 78% for the CC versus CT + TT genotypes.

The logistic regression analysis performed for the entire group of HD patients included gender, age, 4 most frequently observed causes of end-stage renal disease (table 1), RRT vintage, liver enzyme activities and the –1297C>T (rs360719) polymorphism of the *IL-18* gene. The CC genotype, chronic glomerulonephritis as a cause of chronic renal failure and RRT vintage were positively associated with anti-HBsAg development, whereas metrical age and diabetic nephropathy as a cause of chronic renal failure showed negative association with anti-HBsAg formation (table 3).

Independent variables which in this study were not associated with anti-HBsAg titer >10 IU/l were gender, liver enzymes activities and some other causes of end-stage renal disease (hypertensive nephropathy, chronic tubulointerstitial nephritis).

## Discussion

Immune and nonimmune low responsiveness to viral antigens shown in HD patients as compared to persons without impaired renal function, resulting in the former group in a lower frequency of anti-HBsAg production in response to HBsAg, is of great clinical importance. HD patients who do not develop anti-HBsAg after HBV exposure may remain HBV carriers and can infect other

**Table 2.** Distribution of the –1297C/T (rs360719) *IL-18* promoter polymorphisms in hemodialysis patients with a titer of anti-HBsAg >10 UI/l (group 1) and ≤10 UI/l (group 2) as well as in controls

| Group | Genotype distribution absolute number | | | Allele absolute number | | OR (95% CI) | p value |
|---|---|---|---|---|---|---|---|
| | CC | CT | TT | C | T | | |
| Group 1 (n = 219) | 16 (7.3%) | 87 (39.7%) | 116 (50.3%) | 119 (27.1%) | 319 (72.9%) | **Group 2 vs. group 1** | |
| | | | | | | CC vs. CT + TT: 0.239 (0.078–0.728) | 0.010 |
| Group 2 (n = 216) | | | | | | CC + CT vs. TT: 0.884 (0.606–1.290) | 0.587 |
| | 4 (1.9%) | 91 (42.1%) | 121 (56.1%) | 99 (22.9%) | 333 (77.1%) | CC vs. TT: 0.240 (0.078–0.738) | 0.009 |
| | | | | | | C vs. T: 0.9058 (0.797–1.084) | 0.171 |
| | | | | | | **Group 1 vs. controls** | 0.692 |
| | | | | | | CC vs. CT + TT: 0.822 (0.417–1.619) | 0.651 |
| | | | | | | CC + CT vs. TT: 0.903 (0.626–1.303) | 0.639 |
| | | | | | | CC vs. TT: 0.795 (0.395–1.598) | 0.550 |
| | | | | | | C vs. T: 0.9060 (0.679–1.209) | |
| Controls (n = 240) | 21 (8.8%) | 98 (40.8%) | 121 (50.4%) | 140 (29.2%) | 340 (70.8%) | **Group 2 vs. controls** | |
| | | | | | | CC vs. CT + TT: 0.197 (0.066–0.582) | 0.002 |
| | | | | | | CC + CT vs. TT: 0.798 (0.552–1.155) | 0.270 |
| | | | | | | CC vs. TT: 0.191 (0.063–0.572) | 0.001 |
| | | | | | | C vs. T: 0.722 (0.536–0.973) | 0.038 |

**Table 3.** Results of the logistic regression analysis in the entire group of hemodialysis patients

| Characteristics of the model | | Variables significantly associated with an anti-HBsAg titer >10 IU/l | OR (95% CI) | p value |
|---|---|---|---|---|
| $\chi^2$ | p value | | | |
| 15.229 | 0.000 | Chronic glomerulonephritis | 0.504 (0.304–0.835) | 0.008 |
| | | CC genotype | 0.244 (0.080–0.751) | 0.014 |
| 12.230 | 0.002 | Diabetic nephropathy | 1.583 (1.024–2.447) | 0.038 |
| | | CC genotype | 0.244 (0.080–0.748) | 0.013 |
| 13.087 | 0.001 | Metrical age | 1.014 (1.002–1.027) | 0.024 |
| | | CC genotype | 0.242 (0.079–0.743) | 0.013 |
| 13.739 | 0.001 | RRT vintage | 0.946 (0.902–0.991) | 0.020 |
| | | CC genotype | 0.244 (0.080–0.746) | 0.013 |

Dummy variables were used for an anti-HBsAg titer (titer ≤10 IU/l = 0, titer >10 IU/l = 1), causes of end-stage renal disease (positive diagnosis = 1, negative diagnosis = 0) and CC genotype (present = 1, not present = 0).

people. HD patients who do not respond to vaccination against HBV are susceptible to HBV infection.

Reasons for low responsiveness to viral antigens are multifactorial, and may also be genetically related. An HLA-linked immune suppression gene for HBsAg controlling the nonresponsiveness to HBsAg through HBsAg-specific suppressor T cells was described [29]. In the Japanese population, the extended HLA haplotype was shown to control nonimmune responsiveness to HBsAg [30]. The HLA-DR1 gene was found as an immune response gene for HBsAg in both Caucasian and Japanese populations [30, 31].

Positive response for vaccines was shown to be associated with increased IFN-γ production [32, 33]. As IL-18 is involved in IFN-γ production [21–23], it was used to modulate DNA vaccines against HBV [33–35]. Channarong et al. [34] have constructed a recombinant plasmid carrying the gene encoding HBsAg linked to DNA segment encoding full-length murine IL-18. All vaccinated mice revealed significant serum anti-HBsAg IgG re-

sponse after two intramuscular injections of the vaccines as compared to the level of mice vaccinated without DNA segment encoding IL-18. We suggest that vaccines encoding IL-18 could be especially indicated for nonresponders bearing other *IL-18* genotypes than –1297 CC (rs360719).

Natural expression of IL-18 is dependent on the genetic pattern. Sánchez et al. [24] have found a significant increase in the relative expression of IL-18 mRNA in individuals carrying the rs360719 C allele. Persons with the *IL-18 –1297* CC or the *IL-18–1297* CT genotypes compared with those carrying the *IL-18 –1297* TT genotype showed an increased susceptibility to systemic lupus erythematosus [24] and to giant-cell arteritis [36]. Both of these studies indicate an increased risk of inflammatory/autoimmune diseases related to the CC or CT genotypes. In our study, we have shown a positive association of the *IL-18 –1297* CC genotype with anti-HBsAg production in HD patients. The hemodialyzed responders for HBsAg showed a significantly higher –1297CC (rs360719) genotype frequency as compared to the hemodialyzed nonresponders, which may suggest an association of *IL-18* polymorphism with anti-HBsAg production. A lower –1297CC (rs360719) genotype frequency in nonresponders could result in a decreased expression of IL-18 levels, which negatively influences Th1 type immune responses [21, 22] involved in production of antibodies. On the other hand, recombinant HBsAg induces inhibition of IL-18 expression [37], which may be of importance in the situation of genetically decreased expression of IL-18 levels. According to the results of our study, patients bearing the –1297CC genotype may exhibit an approximately 4-fold increased chance of developing anti-HBsAg in response to vaccine HBsAg or HBV infection. Moreover, the CC genotype was independently associated with anti-HBsAg development.

Nonresponsiveness to HBsAg in HD patients is obviously not only related to genetic aspects. The *IL-18* –1297 CC (rs360719) polymorphism was present in only 7.3% of dialyzed responders, which indicates an influence of other strong factors which promote anti-HBsAg development. Our study indicates that bearing the *IL-18* –1297 CC genotype is not obligatory for anti-HBsAg development, but facilitates anti-HBsAg formation. Patients with the better clinical status, surviving longer, could have a greater ability for immunological response to vaccination. In fact, responders to HBsAg spent more time on RRT, usually underwent two full vaccination series against HBV and were given booster doses of vaccine, which finally could result in an anti-HBs titer >10 IU/l. Additionally, patients with a long time on RRT underwent natural selection with elimination of weaker patients, usually suffering from multiple comorbidities. Surviving patients could have a greater ability for immunological response to vaccination. On the other hand, diabetes mellitus usually affects patients' clinical status more negatively than primary renal diseases, like chronic glomerulonephritis, and impairs the immunological response to HBV vaccine [38]. The seroconversion rate to anti-HBsAg positivity after vaccination was 84% in HD patients below 40 years and only 33% in those ≥60 years [39]. A positive association between anti-HBsAg development and RRT vintage and chronic glomerulonephritis as a cause of end-stage renal disease, as well as a negative association of anti-HBsAg generation with age and diabetic nephropathy were also revealed in our study.

There are some limitations to our study. Acquired low immune responsiveness could cause difficulties in proper categorization of patients into the desired groups. In our group of nonresponders, some patients may have been immunized well before HD commencement, but they were not vaccinated at that time. Predialysis patients showing a better preserved renal function develop protective anti-HBsAg titer more easily than dialyzed patients [40]. These patients may carry the –1297CC (rs360719) *IL-18* genotype, which is suggested by our results to be related to responsiveness to HBsAg.

Our study did not include a control group of healthy persons with a known anti-HBsAg status after anti-HBV vaccination or natural HBV transmission with a determined *IL-18* genotype. To our knowledge, such a group has not been described in the literature either. To have at least an approximate reference of *IL-18* genotypes obtained in HD patients to those shown in healthy subjects, we have compared our results of HD patients with *IL-18* genotypes with those of blood donors from the Wielkopolska region of Poland and with available data of other authors [24, 41]. In both the CC versus CT + TT model and the CC versus TT model, the frequency of –1297CC (rs360719) in HD responders was not significantly different than that in the control Caucasian population described by others [24, 41] and in our study. It may be suggested that this group of HD patients had a similar gene regulation of IL-18 expression as control persons, probably showing responsiveness to various HBV vaccines of about 85–90% [30, 42]. In hemodialyzed nonresponders, the frequency of –1297CC (rs360719) in both the recessive model (CC vs. CT + TT) and the additive model (CC vs. TT) was lower than that in our own control population, in healthy subjects living in the South Moravia re-

gion of the Czech Republic [41] and in Spain, Italy and Argentina [24].

In conclusion, in HD patients, the *IL-18* –1297CC genotype may play a role in anti-HBsAg development in response to HBV surface antigen. This study indicates a new possible explanation for unresponsiveness to HBsAg in HD patients.


## Acknowledgement

We especially thank the many participating physicians for their efforts in collecting data and making this study possible.


## References


1 Oesterreicher C, Hammer J, Koch U, Pfeffel F, Sunder-Plassmann G, Petermann D, Müller C: HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis. Kidney Int 1995;48:1967–1971.

2 Minuk GY, Sun DF, Greenberg R, Zhang M, Hawkins K, Uhanova J, Gutkin A, Bernstein K, Giulivi A, Osiowy C: Occult hepatitis B virus infection in a North American adult hemodialysis patient population. Hepatology 2004;40:1072–1077.

3 Fabrizi F, Messa PG, Lunghi G, Aucella F, Bisegna S, Mangano S, Villa M, Barbisoni F, Rusconi E, Martin P: Occult hepatitis B virus infection in dialysis patients: a multicentre survey. Aliment Pharmacol Ther 2005;21:1341–1347.

4 Grzegorzewska AE, Kurzawska-Firlej D, Ratajewski W, Frankiewicz D, Niepolski L, Kaczmarek A: Antibodies to core antigen of hepatitis B virus in patients on renal replacement therapy: association with demographic, clinical and laboratory data. Nephron Clin Pract 2010;114:c194–c203.

5 Lai KN, Tam JS, Lai FM, Lin HJ: Isolated presence of antibody to hepatitis B core antigen in dialysis patients: occurrence of subclinical hepatitis? Am J Kidney Dis 1989;13:370–376.

6 Miller ER, Alter MJ, Tokars JI: Protective effect of hepatitis B vaccine in chronic hemodialysis patients. Am J Kidney Dis 1999;33:356–360.

7 European Best Practice Guidelines. Prevention and management of HBV, HCV and HIV in HD patients. Nephrol Dial Transplant 2002;17:78–87.

8 Bruguera M, Cremades M, Mayor A, Sánchez Tapias JM, Rodés J: Immunogenicity of a recombinant hepatitis B vaccine in haemodialysis patients. Postgrad Med J 1987;63 (suppl 2):155–158.

9 Mitwalli A: Responsiveness to hepatitis B vaccine in immunocompromised patients by doubling the dose scheduling. Nephron 1996;73:417–420.

10 Sorkhi H, Roushan MR, Al Hashemi GH, Dooki MR, Bai S: Response to hepatitis B virus vaccination in haemodialysis patients with and without hepatitis C infection. East Mediterr Health J 2008;14:798–803.

11 Cendoroglo Neto M, Draibe SA, Silva AE, Ferraz ML, Granato C, Pereira CA, Sesso RC, Gaspar AM, Ajzen H: Incidence of and risk factors for hepatitis B virus and hepatitis C virus infection among haemodialysis and CAPD patients: evidence for environmental transmission. Nephrol Dial Transplant 1995;10:240–246.

12 Grzegorzewska AE, Kaczmarek-Leki V, Młot-Michalska M, Niepolski L: Seroconversion rate to positivity for antibodies against core antigen of hepatitis B virus and duration of renal replacement therapy. Nephrol Dial Transplant 2011;26:970–976.

13 Scheerlinck JY: Genetic adjuvants for DNA vaccines. Vaccine 2001;19:2647–2656.

14 Yen H, Scheerlinck JY: Co-delivery of plasmid-encoded cytokines modulates the immune response to a DNA vaccine delivered by in vivo electroporation. Vaccine 2007;25:2575–2582.

15 Gracie JA, Robertson SE, McInnes IB: Interleukin-18. J Leukoc Biol 2003;73:213–224.

16 McInnes IB, Gracie JA, Leung BP, Wei XQ, Liew FY: Interleukin 18: a pleiotropic participant in chronic inflammation. Immunol Today 2000;21:312–315.

17 Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K: Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol 1998;70:281–312.

18 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001;19:423–474.

19 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 2001;12:53–72.

20 Szkaradkiewicz A, Jopek A, Wysocki J: Effects of IL-12 and IL-18 on HBcAg-specific cytokine production by CD4 T lymphocytes of children with chronic hepatitis B infection. Antiviral Res 2005;66:23–27.

21 Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S, Nakanishi K: IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. J Immunol 1998;161:3400–3407.

22 Tominaga K, Yoshimoto T, Torigoe K, Kurimoto M, Matsui K, Hada T, Okamura H, Nakanishi K: IL-12 synergizes with IL-18 or IL-1beta for IFN-gamma production from human T cells. Int Immunol 2000;12:151–160.

23 Kim JJ, Nottingham LK, Tsai A, Lee DJ, Maguire HC, Oh J, Dentchev T, Manson KH, Wyand MS, Agadjanyan MG, Ugen KE, Weiner DB: Antigen-specific humoral and cellular immune responses can be modulated in rhesus macaques through the use of IFN-gamma, IL-12, or IL-18 gene adjuvants. J Med Primatol 1999;28:214–223.

24 Sánchez E, Palomino-Morales RJ, Ortego-Centeno N, Jiménez-Alonso J, González-Gay MA, López-Nevot MA, Sánchez-Román J, de Ramón E, González-Escribano MF, Pons-Estel BA, D'Alfonso S, Sebastiani GD, Italian collaborative group, Alarcón-Riquelme ME, Martín J: Identification of a new putative functional *IL18* gene variant through an association study in systemic lupus erythematosus. Hum Mol Genet 2009;18:3739–3748.

25 Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR Recomm Rep 2001;50 (RR-5):1–43.

26 Tsouchnikas I, Dounousi E, Xanthopoulou K, Papakonstantinou S, Thomoglou V, Tsakiris D: Loss of hepatitis B immunity in hemodialysis patients acquired either naturally or after vaccination. Clin Nephrol 2007;68:228–234.

27 Barałkiewicz G, Juszczyk J, Grzegorzewska A, Czarnecki R: The results of the vaccination against hepatitis B ('Engerix B') in dialysed uraemic patients (in Polish). Hepatologia Pol 1996;3:11–14.

28 Załączniki do rozporządzenia Ministra Zdrowia z dnia 18 kwietnia 2005 r. Warsaw, Polish Ministry of Health, 2005.

29 Watanabe H, Matsushita S, Kamikawaji N, Hirayama K, Okumura M, Sasazuki T: Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to hepatitis B surface antigen via CD8+ suppressor T cells. Hum Immunol 1988;22:9–17.

30 Hatae K, Kimura A, Okubo R, Watanabe H, Erlich HA, Ueda K, Nishimura Y, Sasazuki T: Genetic control of nonresponsiveness to hepatitis B virus vaccine by an extended HLA haplotype. Eur J Immunol 1992;22: 1899–1905.
31 Walker M, Szumuness W, Stevens C, Rubinstein P: Genetics of anti-HBs responsiveness. I. HLA-DR7 and non-responsiveness to hepatitis vaccination. Transfusion 1981;21:601.
32 Petrescu L, Stancu S, Tardei G, Santimbrean C, Penescu M, Mircescu G: Tuberculin skin test, interferon-gamma assay, and T cells subpopulations in hemodialysis patients. J Ren Nutr 2010;20(suppl 5):S109–S117.
33 Tang LL, Liu KZ: Recent advances in DNA vaccine of hepatitis virus. Hepatobiliary Pancreat Dis Int 2002;1:228–231.
34 Channarong S, Mitrevej A, Sinchaipanid N, Usuwantim K, Kulkeaw K, Chaicumpa W: Cloning, protein expression and immunogenicity of HBs-murine IL-18 fusion DNA vaccine. Asian Pac J Allergy Immunol 2007;25: 233–242.
35 Chen JZ, Zhu HH, Liu KZ, Chen Z: Enhancing cellular immune response to HBV M DNA vaccine in mice by codelivery of interleukin-18 recombinant. J Zhejiang Univ Sci 2004;5:467–471.
36 Palomino-Morales RJ, Vazquez-Rodriguez TR, Torres O, Morado IC, Castañeda S, Miranda-Filloy JA, Callejas-Rubio JL, Fernandez-Gutierrez B, Gonzalez-Gay MA, Martin J: Association between IL-18 gene polymorphisms and biopsy-proven giant cell arteritis. Arthritis Res Ther 2010;12:R51.
37 Cheng J, Imanishi H, Morisaki H, Liu W, Nakamura H, Morisaki T, Hada T: Recombinant HBsAg inhibits LPS-induced COX-2 expression and IL-18 production by interfering with the NFkB pathway in a human monocytic cell line, THP-1. J Hepatol 2005; 43:465–471.
38 Fabrizi F, Dixit V, Martin P, Messa P: Meta-analysis: the impact of diabetes mellitus on the immunological response to hepatitis B virus vaccine in dialysis patients. Aliment Pharmacol Ther 2011;33:815–821.
39 Shatat HZ, Kotkat AM, Farghaly AG: Immune response to hepatitis B vaccine in haemodialysis patients. J Egypt Public Health Assoc 2000;75:257–275.
40 DaRoza G, Loewen A, Djurdjev O, Love J, Kempston C, Burnett S, Kiaii M, Taylor PA, Levin A: Stage of chronic kidney disease predicts seroconversion after hepatitis B immunization: earlier is better. Am J Kidney Dis 2003;42:1184–1192.
41 Izakovicova Holla L, Hrdlicková B, Schüller M, Buckova D, Kindlova D, Izakovic V, Vasku A: Haplotype analysis of the interleukin-18 gene in Czech patients with allergic disorders. Hum Immunol 2010;71:592–597.
42 American Academy of Pediatrics: Hepatitis B, in Peter G (ed): Report of the Committee on Infectious Diseases. Red Book, ed 24. Elk Grove Village, American Academy of Pediatrics, 1997.