Polymorphisms in the *IL28B* gene (rs12979860, rs8099917) and the virological response to pegylated interferon therapy in hepatitis D virus patients

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Abstract

Aim : **Few data are available regarding the effects of interleukin 28B (*IL28B*) polymorphisms in chronic hepatitis D (CHD) patients. This study investigated the relationship between *IL28B* polymorphisms and the response of patients with CHD infections to pegylated interferon (PEG-IFN) therapy.**

Materials and methods : **A total of 101 CHD patients were selected, 80 of whom (46 males ; median age 41 years) satisfied the inclusion criteria and were enrolled in the study. Thirty-seven patients were treated with peg-IFNα for at least 12 months and were followed for a median of 18 months (range, 12-30 months). The primary treatment endpoint was the suppression of HDV replication, as documented by the loss of detectable *HDV* RNA in serum. Genotyping was used to analyse the *IL28B* polymorphisms rs12979860 and rs8099917 according to the virological response.**

Results : **After treatment, a sustained viral response (SVR) was achieved in 19 (51%) of the patients treated with PEG-INF. The *IL28B* genotypes in the 80 patients were as follows : CC in 36 (45%), CT in 33 (41%) and TT in 11 (14%) for rs12979860, and GG in 4 (5%), GT in 27 (34%) and TT in 49 (61%) for rs8099917. SVR was achieved in 5 (26%), 10 (53%) and 4 (21%) patients with CC, CT and TT at rs12979860, respectively, and one (5%), nine (47%) and nine (47%) patients with GG, GT and TT at rs8099917, respectively. There were differences in the SVR among genotypes (rs12979860 and rs8099917 ; chi-squared test, p = 0.047).**

Conclusion : IL28B **predicts the PEG-IFN response in patients with CHD infection.** (Acta gastroenterol. belg., **2016**, 79, **206-210**).

Key words : chronic hepatitis D, PEG-IFN therapy, IL28B.

Introduction

The incidence of hepatitis D virus (HDV) is decreasing in countries with long-standing vaccination programs for the prophylaxis of hepatitis B virus (HBV) infections. However, HDV remains a serious public health concern in developing countries (1). Although current studies estimate that 5% of HDV carriers are co-infected with Hepatitis B surface antigen (HBsAg) (2), a study conducted in southeast Turkey reported that the anti-HDV sero-positivities among patients with chronic hepatitis B (CHB) and hepatitis B-induced cirrhosis were 27% and 46%, respectively (3).

HDV is a defective virus that can infect only humans with acute or chronic HBV infections (4). This unique feature of HDV prevents anti-viral therapies from being as effective as they are for other hepatitis infections. Interferon-alpha (IFNα) is the only licensed drug whose short- and long-term effects against CHD has been evaluated (5-8). However, the success of treatment using IFNα is not yet satisfactory, and it can cause serious side effects. Therefore, the factors associated with the spontaneous or treatment-induced clearance of infections should be identified.

The studies conducted to date have revealed an association between single nucleotide polymorphisms (SNPs) near interleukin 28B (*IL28B*) and the response to therapy (9-12). It was demonstrated that good-response *IL28B* SNPs could increase the sustained viral response (SVR) rates by two-fold in patients with HCV genotypes 1 and 4, but were not associated with the response in HCV genotype 2 and 3 patients. This association was for not only treatment-induced clearance but also for the spontaneous clearance of HCV (9). *IL28B* SNPs were also associated with the treatment response of HBeAg-positive CHB patients to PEG-IFN (10). A recent study assessed the relationship between *IL28B* variations and the response of CHD patients to IFNα therapy (13). The investigators found that polymorphisms did not affect the antiviral response of patients with chronic HDV infection to IFNα. However, the limitation of an insufficient number of patients in this study created a need for further studies. Therefore, we conducted this study to investigate the possible association between favourable *IL28B* SNPs and the response of CHD patients to PEG-IFNα therapy.

Materials and Methods

Patient group

A total of 101 patients who visited Dicle University, Faculty of Medicine, Gastroenterology Clinic were anti-HDV and *HDV* RNA-positive and had received PEG-IFN therapy for a minimum of 12 months were reviewed retrospectively in this study. Twenty-one patients with either missing values or who did not sign a consent form were excluded. Thirty-seven of the remaining 80 patients

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were positive for both anti-HDV and HDV *RNA*, and 43 patients were positive only for anti-HDV. All patients (n = 80) were 16-79 years of age, had HDV and were HBsAg-positive. Patients with seropositive human immunodeficiency virus (HIV), HCV, other serious conditions (lung, heart or kidney disease), a prior history of interferon therapy, decompensated cirrhosis, malignant disease or a history of another hepatic disease were excluded from the study. The clinical data and basal laboratory values of the patients, as well as the values obtained at the end of the therapy and 6 months post-therapy, were recorded. *HDV* RNA values were reviewed to evaluate the end-of-treatment response (undetectable *HDV* RNA at the end of treatment) and SVR (undetectable *HDV* RNA 6 months after treatment discontinuation).

Twenty-one (57%) patients had liver biopsies, and thus histopathological data were available. Histopathological evaluations were performed to determine the Ishak scores (Modified Knodell score) (14). Eight of the remaining 16 patients did not undergo liver biopsy due to compensated cirrhosis, and eight patients started PEG-IFN therapy without biopsy.

The ethics committee of Abant Izzet Baysal University, Faculty of Medicine approved the study protocol, which was designed according to the Declaration of Helsinki. An informed consent form was obtained from each patient enrolled in the study.

Virological assessments

Serological markers (HBsAg, HBe antibodies, HBeAg and hepatitis B surface antigen [anti-HB] antibodies) were determined using qualitative micro-particle enzyme immunoassays (Organon Teknika BV, Boxtel, The Netherlands). Anti-HDV (Abbott Laboratories, Chicago, IL, USA), anti-HIV and anti-HCV antibodies were obtained from Organon Teknika. Serum HBV DNA was quantitated using real-time polymerase chain reaction (PCR) (AmpliPrep Cobas TaqMan HBV Test, Roche, Branchburg, NJ, USA) with a lower detection limit of 20 IU/mL. *HDV* RNA was detected to diagnose patients using qualitative reverse transcriptase nested PCR (Lightcycler 2.0 Instrument, Roche, Branchburg, NJ, USA) ; the lower detection limit for the assay was 1000 copies/mL (15). Haematological and biochemical parameters were analysed using an Abbott Aeroset auto-analyser.

Genetic analysis

Two centilitres of blood from each patient were isolated into haemogram tubes. Genomic DNA was then isolated from each blood sample using a DNA isolation kit (High Pure PCR Template Preparation Kit ; Roche). DNA isolation and PCR were performed at Abant Izzet Baysal University, Faculty of Medicine, Medical Genetics Department, Molecular Genetics Laboratory, Bolu, Turkey. The resulting genomic DNAs were analysed for polymorphisms at two different gene regions of *IL28B* (rs12979860 and rs8099917) using LightSNIP IL28B kits and a real-time PCR instrument (LıghtCycler480 Instrument II, Roche Diagnostic, Mannheim, Germany). DNA from each patient was amplified using PCR and then hybridised to probes specific to the gene regions to identify *IL28B* polymorphisms using real time PCR (RT-PCR).

RT-PCR melting curve analysis was used to identify homozygous wild-type, homozygous mutant and heterozygous genotypes. The heterozygous genotypes showed two peak values at different melting points, whereas both peak values in the homozygous wild-type and homozygous mutant genotypes were at the same melting point, because each gene region has a different number of peaks and peak values* as follows :

* *IL28B* rs12979860. There are two peaks and peak values for this gene region : 54.01, TT genotype ; 61.74, CC genotype ; 54.01-61.74, heterozygous genotype
* *IL28B* rs8099917. There are two peaks and peak values for this gene region : 51.00, TT genotype ; 58.76, GG genotype ; 51.00-58.76, heterozygous genotype

Statistical analysis

Statistical analyses were performed using SPSS (version 17 ; SPSS, Inc., Chicago, IL, USA). Continuous variables are presented as medians (ranges) and categorical variables as frequencies (percentage). Comparisons of continuous variables were performed using Mann-Whitney U tests, and differences between categorical variables were evaluated using chi-squared or the Fisher exact test, as appropriate. Logistic regression analysis was performed to identify the predictors associated with end of response and SVR. A p value less than 0.05 was considered statistically significant.

Results

The median age of the 37 patients who were eligible for this study was 41 years. Sixteen (43%) patients were female and 21 (57%) male. None of the patients had a history of interferon therapy. Thirteen (35%) patients had clinically compensated cirrhosis. The median duration of PEG-IFN (2a or 2b) therapy was 18 months (range, 12-30 months). The basal laboratory values of the patients are given in Table 1. Almost all patients (n = 35) were HBeAg negative. Therefore, impact of HBeAg status on therapy response could not be analyzed.

Histopathological evaluations of 21 patients revealed that five patients had cirrhosis. The median hepatic histology activity index (HAI) score of the remaining 16 patients was 3-13, and the median fibrosis score was 0-4.

The proportions of the 80 patients with the CC, CT and TT genotypes at rs12979860 were 45%, 41% and 14% respectively, whereas those of patients with the TT, GT and GG genotypes at rs8099917 were 61%, 34% and 5%, respectively (Table 2). The end-of-treatment response was achieved in 29%/25%/46% of patients with

Table 1. — **Demographic features and laboratory values of the patients (n = 37)**

Age (years) median (range)	41 (16-79)
Sex (F), n (%)	16 (43%)
Duration of Therapy (months), median (range)	18 (12-30)
Cirrhosis, n (%)	13 (35%)
Alcohol consumption, n (%)	0
Smoking, n (%)	14 (38%)
Diabetes mellitus, n (%)	3 (8%)
Glucose (mg/dl)	95 (70-220)
Creatinine (mg/dl)	0.73 (0.5-1.1)
Alanine aminotransferase (U/L)	56 (18-219)
Aspartate aminotransferase (U/L)	46 (19-169)
Albumin (g/dl)	3.9 (2.6-5)
INR	1.11 (0.8-1.88)
WBC ($\times 10^3/mm^3$)	6100 (3200-10500)
Haemoglobin (g/dl)	14.25 (11.1-16.5)
Platelet ($\times 10^3/mm^3$)	184 (121-207)
HBV DNA, IU/ml	0 (0-844000)

CC/TT/CT genotypes at rs12979860 ($p = 0.014$) and 8%/46%/46% of patients with GG/TT/GT genotypes at rs8099917 ($p = 0.020$) respectively. An SVR to PEG-IFN therapy was seen in 19 (51%) patients. Seventeen (46%), six (16%) and 14 (38%) patients had an *IL28B* rs12979860 genotype of CC, TT and CT, respectively. Conversely, two (5%), 23 (62%) and 12 (32%) patients had an *IL28B* rs8099917 genotype of GG, TT and GT. There were no statistically significant differences between the treated and untreated (anti-HDV[+], HDV RNA[–]) groups regarding *IL28* polymorphisms.

Twelve (67%) of the patients with no response to therapy had the CC genotype at rs12979860, whereas 10 (53%) of the 19 patients who showed an SVR to therapy had the CT genotype. There were statistically significant differences in the response to therapy among genotypes (chi-squared, $p = 0.047$). In contrast, 14 (78%) of 18 patients with no response to therapy had the TT polymorphism at rs8099917, and most of the patients with an SVR (9/19, 47%) also had the TT genotype (chi-squared, $p > 0.05$; Table 2). The proportion of rs12979860 CC genotypes was 67% among patients with no SVR to PEG-IFN therapy, whereas 74% of the patients with an SVR had a non-CC genotype ($p = 0.014$).

When other factors affecting the therapeutic response were evaluated, high alanine transaminase (ALT) and aspartate transaminase (AST) values prior to therapy were related to a poor SVR (Mann Whitney U-test ; $p = 0.022$ and $p = 0.031$, respectively). Age, gender, total bilirubin levels and international normalized ratio (INR) values were similar between patients with and without an SVR.

Univariate analysis did not show a correlation between SVR and ALT or AST levels. In contrast, logistic regression analysis revealed that a non-CC genotype at rs12979860 could predict a response to PEG-IFN therapy ($p = 0.017$; odds ratio 5.6 ; 95% confidence interval 1.360-23.059).

Discussion

HDV co-infects or super-infects with HBV, because it requires HBsAgs to form a novel HDV virion. Chronic infection with HDV and HBV results in the most dangerous form of viral hepatitis. IFNα therapy is the only FDA-approved therapy for HDV to date (16). PEG-IFN is administered weekly for 48 weeks followed by 6 months of observation post-therapy ; patients who remain negative for *HDV* RNA are considered to have eliminated the HDV. However, *HDV* RNA clearance was not as successful, and even if HDV clearance was seen, the result was unreliable due to non-standardised nucleic acid hybridization assays.

Two published studies analysed the effects of PEG-IFN for the treatment of CHD and reported achievement of an SVR in 21% and 43% of patients, respectively (17,18). Some studies have attempted to evaluate the appropriate treatment duration for patients receiving PEG-IFN (19,20). Nevertheless, the optimal treatment for HDV remains unclear.

IL28B polymorphisms affect both the response of patients to IFN therapy and the spontaneous clearance of HCV (9). Moreover, *IL28B* SNPs are associated with the response of HBeAg-positive CHB patients to PEG-INF (10). However, another study failed to show a relationship between *IFNL3* rs12979860 polymorphisms and the clearance of HBV infections (21).

Ubaldo *et al*. (22) failed to show a significant relationship between *IL28B* polymorphisms and spontaneous or IFN-induced *HDV* RNA clearance. However, the distribution of rs12979860 and rs8099917 genotypes in their study population deviated from Hardy-Weinberg equilibrium. Furthermore, although Ubaldo *et al*. found no significant difference in the rate of the rs12979860 CC genotype between IFN therapy responders and non-responders, Keshvari *et al*. (23) commented on this study

Table 2. — **Relationship between IL-28B genotype and virologic response to therapy**

	n		rs12979860		P		rs8099917		P
		CC	TT	CT		GG	TT	GT	
HDVRNA (-) anti-HDV (+)	43	19 (44%)	5 (12%)	19 (44%)		2 (5%)	26 (61%)	15 (35%)	
EoT (+)	24	7 (29%)	6 (25%)	11 (46%)	0.014* 0.005** 0.072 [π]	2 (8%)	11 (46%)	11 (46%)	0.020* 0.011 [π]
EoT (-)	13	10 (77%)	0	3 (23%)		0	12 (92%)	1 (8%)	
SVR	19	5 (26%)	4 (21%)	10 (53%)	0.047	1 (5%)	9 (47%)	9 (47%)	> 0.05
No SVR	18	12 (67%)	2 (11%)	4 (22%)		1 (6%)	14 (78%)	3 (17%)	

EoT, End of Treatment ; SVR, sustained virological response.
**Chi-squared*, ***Chi-squared* (cc versus noncc patients), [π]*fisher's exact* (tt versus nontt patients).

and noted a borderline association between the CC genotype and an IFN-induced response, even though it was not significant (24). In addition, treatment non-responders had a higher frequency of cirrhosis compared with treatment responders in this study population.

Another study investigating the clinical outcomes and IFN response rates among chronic HDV patients with different *IL28B* rs12979860 polymorphisms showed that although the IFN response could not be predicted by the *IL28B* genotype, there was a strong association between the *IL28B* TT genotype and unfavourable clinical outcomes such as HCC and liver decompensation (25). The reason for the different findings between this and the current study might be that a high range of treatment times was used for standard IFN therapy, whereas PEG-IFN was used for a minimum of 12 months in the current study.

A recent study that evaluated PEG-IFN therapy in CHD patients for a minimum of 12 months also failed to show a role of *IL28B* polymorphisms in IFN-induced or the spontaneous clearance of HDV infections (13). Consistent with the current study, the small number of patients might be the reason for the lack of statistical significance, as it affected the power of the study.

We conducted this study to investigate a possible association between *IL28B* SNPs and the response of CHD patients to PEG-IFNα therapy. Thirty-seven patients were enrolled in the study and analysed via DNA samples. All patients received PEG-INFα therapy for a minimum of 12 months. The data revealed that a non-CC genotype at rs12979860 predicted a response to PEG-IFN therapy, whereas there was no significant difference among the patients with different *IL28B* genotypes at rs8099917 regarding end-of-therapy response and SVR.

Previous studies are difficult to compare due to different therapeutic designs, a limited number of patients examined and non-standardised nucleic acid hybridisation assays. Another major problem is that even if *HDV* RNA clearance had been predicted by negative hybridisation assay results, such does not confirm eradication of the virus, since HDV can be transmitted to HBsAg carriers with natural infectivity thresholds that are much lower than current assays can detect (26,27). The contradictory results regarding the relationship between *IL28B* polymorphisms and the response to IFN treatment in CHD patients suggest a need for standardized prospective studies evaluating larger patient populations.

Conflict of interest

None.

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