Interleukin-28B (rs12979860) gene variation and treatment outcome after peginterferon plus ribavirin therapy in patients with genotype 1 of hepatitis C virus

Hamid Kalantari, Tahmine Tavakoli, Bahram Bagherpour[1], Shahram Honarmand[2]

Department of Gastroenterology, Isfahan Liver Disease Research Center, Isfahan University of Medical Sciences, [1]Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, [2]Department of Pharmacology and Toxicology, Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: The success of treatment of chronic hepatitis C (CHC) with pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) is affected by several host, viral, and treatment factors. This study was designed to describe the association of interleukin (IL) 28B genotypes for rs12979860 with sustained virologic response (SVR) in patients with genotype 1 CHC infection treated with PEG-IFN α-2 and RBV. **Materials and Methods:** Interleukin-28B genotype in 100 studied patients was detected by tagman real-time polymerase chain reaction. Before treatment blood samples were obtained, then patients were treated for 48-week with a combination therapy using of the PEG-IFN α-2 and RBV. SVR evaluated 6 months after stopping therapy, and was defined as undetectable plasma hepatitis C virus-RNA. **Results:** Among studied patients, 65% were IL-28B CT, 27% CC, and 8% TT. In all studied patients, SVR was 58.3%, relapse 15.6%, and null virological response 26.1%. SVR rates were 76.9% in IL-28B-CC, 56.4% in IL-28B-CT, and 12.5% in IL-28B-TT patients. Relapse rates were 7.7% in IL-28B-CC, 12.9% in IL-28B-CT, and 62.5% in IL-28B-TT patients. There was a significant difference between response to treatment in patients IL-28B-CC, CT, and TT (P = 0.003). IL-28B genotype CC, (odds ratio = 0.053, 95% confidence interval; 0.005-0.54, P = 0.03), was the independent predicting factor. **Conclusion:** Interleukin-28B was an important predictor of CHC treatment outcome with Peg-IFN-α and RBV. IL-28B-CC seems to be more important than IL-28B-CT/TT in predicting positive treatment response.

Key words: Chronic hepatitis C, interleukin-28B, pegylated interferon-α, ribavirin

INTRODUCTION

Most infections with hepatitis C virus (HCV) do not resolve spontaneously. Chronic hepatitis C (CHC) virus is the most common cause of chronic liver disease.[1,2] It affects a significant proportion of the world, accounting for 3% of the total population.[3] Furthermore, it is estimated that affects approximately 130-170 million people worldwide.[4,5] WHO reported that every year >350,000 people die from liver disease caused by CHC and 3-4 million new infections occur every year.[6,7]

Pegylated interferon-α (PEG-IFN α-2) with ribavirin (RBV) is the standard treatment for patients with CHC. HCV genotype is a factor that can influence treatment decision and clinical outcomes. Among HCV genotypes, genotype 1, 2, and 3 are the most common HCV genotype in the world. However, in approximately half of the treated patients with HCV genotype 1 or 4,

this therapy does not eradicate HCV-infected and is associated with high failure rates due to its limited efficacy and significant adverse effects.[8,9] Sustained virologic response (SVR) rates of 47-54% are reported for this treatment for previously untreated patients.[10] In Iran, the rate of SVR was reported from 48% to 54%,[11-13] and the United States and Europe patients with CHC genotype 1, achieved an SVR 42-52%.[14]

It is clarified that the success of treatment is affected by several host, viral, and treatment factors. A reduced risk of long-term complications, such as cirrhosis and hepatocellular carcinoma, obesity, ethnicity, gender, and age can influence SVR.[15-17]

A host-specific genetic-based HCV response to treatment was described during 2009-2010.[18-20] In a study of 1600 CHC patients identified a single nucleotide polymorphism (SNP) located on chromosome 19, who

were homozygous for the major C allele at the rs12979860 SNP in proximity to interleukin (IL) 28B rs12979860 which was strongly associated with SVR. It was also shown that the CC genotype of IL-28B to be associated with a >2 fold increase in SVR as compared with the CT and TT genotypes.[18-20]

The study of genetic association in different worldwide population required to replicate. In the general population of Iran, the prevalence of HCV infection is <0.5%.[21] The most prevalent HCV genotype in Iran is genotype 1, and the number of nonresponders to PEG-IFN and RBV increases daily.[22] In a study, on 921 Iranian patients with CHC, the frequency of IL-28B rs12979860 CC, CT, and TT genotypes was reported 38%, 48.8%, and 13.2%, respectively.[22] Studies on relation between homozygous genotypes and SVR rates in Iranian CHC patients are limited, hence, the present study was designed to describe the frequency of the IL-28B genotypes for rs12979860 in a cohort of Iranian patients with genotype 1 CHC infections treated with PEG-IFN and RBV, and also evaluated the association of IL-28B genotypes for rs12979860 with SVR in this population and investigated whether the determination of IL-28B genotypes for rs12979860 would contribute to the prediction of SVR in these patients.

MATERIALS AND METHODS

This cross-sectional study is in compliance with the Helsinki Declaration, and the study protocol was approved by the institutional review boards of Isfahan University of medical science, Isfahan, Iran. After a full explanation of the study, written informed consent was obtained from all the participants of this study.

Between 2012 and 2013, one hundred patients with HCV genotype 1 infection that referred to hepatitis outpatient clinic of the Seyed Al-Shohadh Hospital of Isfahan University of Medical Sciences were enrolled in this cohort study. Patients of both sex, over or at age of 18 years old who had been detected of anti-HCV and HCV RNA within the serum were eligible if they had hemoglobin >10 g/dL, neutrophils >1500 cells/mm^3, platelets >7500 cells/mm^3, and no renal dysfunction (serum creatinine >1.5 mg/dL). Other inclusion criteria include no history of liver-function disorder, heart failure, uncontrollable diabetes, obstructive pulmonary disease, thyroid dysfunction, and no history of autoimmune hepatitis and pregnancy. Furthermore, patients with psychiatric disorders that may affect treatment adherence, those with end-stage kidney disease, suspected hepatocellular carcinoma, a serious high blood-pressure, a serious coronary artery disease, a serious psychological disease and who were co-infected with either hepatitis B virus or HIV or who had undergone liver transplantation

did not enter to the study. Patients who did not receive the full course of the planned treatment, or those for whom treatment response information was not available were excluded from the analysis.

Interleukin-28B genotype of SNPs rs12979860 was detected by tagman real-time polymerase chain reaction, and allele discrimination was achieved by detecting fluorescence using System SDS software (Applied Biosystems, Foster City, CA, USA).[19,23] The rs12979860 polymorphism detection results were designated in three genotype; CC, CT and TT.

Selected patients treated with a combination therapy using of the standard doses PEG-IFN α-2 (PEG-INTRON, Behestan Darou, Iran) and a weight-based dose of RBV (Rebetol), 1000 mg/day for <75 kg and 1200 mg/day for ≥75 kg, with the duration of 48-week.[24] Demographic, clinical, and laboratory data were collected in all patients. Before treatment, blood was collected in ethylenediaminetetraacetic acid tubes using standard procedures. Genomic DNA was extracted from 200 μL of the cell suspension with pure gene blood core c kit (Quagen, American) according to the manufacturer's instructions.

The scheduled visits were at baseline (variables included age, sex, body mass index (BMI), alanine aminotransferase (ALT), and HCV-RNA) and end of treatment. To evaluate SVR, a visit 6 months after stopping therapy was also conducted. Null virological response (NVR) was defined as detectable plasma HCV RNA at the end of treatment, and SVR was defined as undetectable plasma HCV RNA 6 months after the completion of therapy, and undetectable HCV RNA 6 months after the end of therapy without achieving SVR was considered as relapse.[25]

The collected data were analyzed statistically with SPSS software version 20 (SPSS Inc.; Chicago, IL, USA). Normality of data was assessed by Shapiro-Wilk normality test. All data were normally distributed ($P > 0.05$). Continuous variables were summarized as mean ± standard deviation and categorical variables as frequency and percentage. Individual characteristics among groups were compared using the one-way ANOVA (using Bonferroni as *Post-hoc* test) and independent sample *t*-test for continuous variables, and the Chi-squared test for categorical data. Multivariate logistic regression analysis was used to identify factors significantly associated response to treatment and SVR 6 months after the end of treatment. $P < 0.05$ was considered significant.
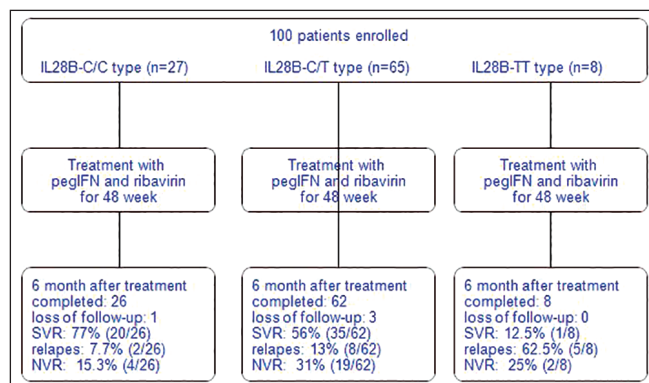
RESULTS

Figure 1 shows the study flow chart, of 100 enrolled patients the most prevalent IL-28B genotype was CT

(65%), followed by CC (27%) and TT (8%). During studied period, one patient with IL-28B-CC and three patients with IL-28B-CT were dropped out due to side effects (Fever, myalgia, fatigue, and general weakness). 6 months followed-up after completion of treatment, SVR rates were 77% in IL-28B-CC, 56% in IL-28B-CT, and 12.5% in IL-28B-TT.

The mean age of studied patients was 35.5 ± 12.5 years old. Eighty-one of patients (84%) were male, and 15 patients (16%) were female. Demographic and biochemical features of patients by IL-28B genotype are reported in Table 1. There were no significant differences between patients IL-28B genotype CC, CT, and TT for age, gender combination, level of ALT and HCV-RNA ($P > 0.05$). The mean of BMI was significantly lower in patients with IL-28B-TT than others ($P = 0.001$).

Table 2 shows the association between IL-28B genotypes with response to PEG-IFN-α/RBV treatment 6 months after completion of treatment. Relapse was observed in 15 (15.6%) of 96 patients, and the rates of SVR and NVR were 58.3% (56/96) and 20.1% (25/96) in studied patients. Response to treatment was significantly different among IL-28B-CC, CT, and TT genotypes ($P = 0.003$). Most of the patients with SVR had IL-28B-CC, most of the patients with NVR had IL-28B-CT and relapse was observed in most of patients with IL-28B-TT.

Characteristics and biochemical features of patients in regard to response to treatment 6 months after completion of treatment were reported in Table 3. There were no significant differences between treatment responses for mean of age, gender combination, BMI, and ALT ($P > 0.05$). Most of the patients (65%) with relapse or NVR had HCV-RNA lower than 10^6, whereas most of the patients with SVR (79%) had HCV-RNA than 10^6 ($P = 0.001$).

Results on factors associated with SVR 6 months after treatment by multivariate logistic regression are shown in Table 4. On multivariate analysis, at the end of treatment,

Table 1: Demographic and biochemical features of patients by IL-28B genotype

Variable	IL28B genotype			P
	CC	CT	TT	
Age (years)	35.2±13.1	33.4±12.7	40.5±7.2	0.31*
Gender				
Male	22 (84.6)	53 (85.5)	6 (75)	0.82†
Female	4 (15.4)	9 (14.5)	2 (25)	
BMI (kg/cm²)	26.6±1.2	27.5±1.7	25.8±1.5	0.001*
ALT	60.6±37.6	60.6±36.6	52.8±21.9	0.86*
HCV-RNA				
<10⁶	18 (69.2)	35 (56.5)	5 (62.5)	0.57†
≥10⁶	8 (38.8)	27 (43.5)	3 (37.5)	

Data are mean ± SD or number (%); BMI = Body mass index; SD = Standard deviation; ALT = Alanine aminotransferase; HCV = Hepatitis C virus; *P* values calculated by *one-way ANOVA and †Chi-square test

Table 2: The frequency of IL-28B genotype with response to PEG-IFN-α/RBV treatment

Response treatment	IL28B-CC (26)	IL28B-CT (62)	IL28B-TT (8)	P
SVR (*n*=56)	20 (76.9)	35 (56.4)	1 (12.5)	0.003
Relapse (*n*=15)	2 (7.7)	8 (12.9)	5 (62.5)	
NVR (*n*=25)	4 (15.4)	19 (30.7)	2 (25)	

Data are number (%); PEG-IFN-α = Pegylated interferon-α; SVR = Sustained virological response; NVR = Null virological response; RBV = Ribavirin; *P* values calculated by Chi-square test

Table 3: Demographic and biochemical features of patients by SVR and relapse

Variable	SVR (*n* = 56)	Relapse/NVR (*n* = 40)	P
Age (years)	36.5±13.3	33.1±11.7	0.18*
Gender			
Male	48 (86)	33 (82)	0.67†
Female	8 (14)	7 (18)	
BMI (kg/cm²)	26.9±1.4	27.2±1.1	0.26*
ALT	60.7±30.1	32.4±4.3	0.32*
HCV-RNA			
<10⁶	12 (21)	26 (65)	0.001†
≥10⁶	44 (79)	14 (35)	

Data are mean ± SD or number (%); SD = Standard deviation; ALT = Alanine aminotransferase; SVR = Sustained virological response; BMI = Body mass index; HCV = Hepatitis C virus; *P* values calculated by *Independent sample *t*-test and †Chi-square test

Table 4: Factors associated with SVR by multivariate logistic regression

Variable	SVR versus relapse/NVR	
	OR (95% CI)	P
Age	1.029 (0.993-1.066)	0.12
Gender (female)	0.774 (0.234-2.564)	0.67
ALT	1.008 (0.996-1.021)	0.21
IL28B genotype (CC)	0.053 (0.005-0.54)	0.013
IL28B genotype (CT)	0.14 (0.15-1.27)	0.081

SVR = Sustained virological response; NVR = Null virological response; ALT = Alanine aminotransferase; CI = Confidence interval; OR = Odds ratio

Figure 1: Study flowchart - NVR = Null virological response; SVR = Sustained virologic response

only IL-28B CC (odds ratio [OR] = 0.053, 95% confidence interval [CI]; 0.005-0.54, *P* = 0.013), was independent predicting factor for response to treatment. And other factors such as age, gender, ALT, and IL-28B CT were not significant predicting factors (*P* > 0.05).

DISCUSSION

Interleukin-28B variations were shown to be the strongest pretreatment predictor of virological response in HCV genotype 1 patients.[26] In the present study, we validated the clinical significance of the IL-28B polymorphisms on treatment outcome in patients with CHC genotypes 1, and our results showed that the prevalence of IL-28B genotypes was 65%, 27%, and 8% for CT, CC, and TT, respectively. 6 months after the end of the treatment, relapse and NVR rate was 41.7%, and the rate of SVR was 58.3%. SVR rate in patients with IL-28B-CC was 76.9%, in patients with IL-28B-CT was 56.4%, and in patients with IL-28B-TT was 12.5%. Among IL-28B genotypes, IL-28B CC with OR = 3.180, 95% CI; 1.109-9.117, was the only significant independent predicting factor.

Previous studies reported varies rate of SVR. In a retrospective study reported, 81.2% in all genotypes and 75.5% in genotype 1.[27] Kang *et al*., reported 69.5% in genotype 1,[28] and Lee *et al*., reported the rate of 68.4% in genotype 1.[29] Furthermore, SVR rates have been reported as 42-51% in genotype 1 in Western countries.[14,30,31] In the present study, 6 months after the end of 48-week treatment, the rate of SVR was 58.3%, which was lower to some previous reports[27-29] and was higher other reports in Western countries, African-Americans, and Asians patients.[30-33] Some reasons might be explained the discrepancy among studies, such as the high compliance percentage of patients available for follow-up observation after treatment type in studies with high SVR rates. A difference in race in studied population is another probable factor to explain the different in SVR rates. The IL-28B gene allele frequency was reported to vary according to ethnicity. It is indicated that a large proportion of the Asian population in some studies from different parts of Asia is of IL-28B genotype CC,[20,33] which explains the high SVR rates in patients in this area, whereas IL-28B TT genotype is reported more common in African-American patients with CHC who typically do not respond to PEG-IFN α-2 and RBV treatment.

In the present study, the IL-28B CT genotype, followed by the CC genotype, was the most prevalent. SVR rate was 76.9%, 56.4%, and 12.5%, in patients with IL-28B-CC, IL-28B-CT, and IL-28B-TT, respectively. In a multicenter German study, HCV genotype-1 patients were enrolled and treated with Peg-IFN and RBV, SVR was achieved in 85%, 58%, and 46% of patients with CC, CT, and TT IL-28B genotypes, respectively.[34] As shown, SVR rate in patients

with IL-28B-CC and IL-28B-CT is similar in these two studies but in German TT IL-28B genotypes SVR is reported higher than our results. Our findings agreed with another study that reported 79% of SVR in patients with IL-28B-CC and 24% of those with IL-28B-TT,[35] and agreed well with the observations by Ge *et al*., who reported 81% SVR in IL-28B-CC and 33% in IL-28B-TT genotypes.[19] Another study by Thompson *et al*., showed that in patients with the IL-28B-CC genotype, the SVR was 69%, compared to 33% in those with the IL-28B-CT genotype and 27% in those with the IL-28B-TT genotype.[36] In the same study by Stättermayer *et al*., the SVR rate was 79.1% in the CC genotype vs. 43.2% in patients with TT genotype.[37] Similar results were obtained in the group of patients in the study by Sporea *et al*., which reported 73.1% SVR rate in patients with IL-28B-CC genotype, 40.9% in patients with IL-28B-CT genotype, and 57.1% in patients with IL-28B-TT genotype.[38] These results investigated that in CHC infected patients, the IL-28B genotype is a potent predictor of response therapy. Thus, for genotype1-infected patients, tests for IL-28B rs12979860 polymorphisms may help guide the physician in treatment regimen and design and/or the patient's decision to undergo therapy.

The present study has a limitation by selection bias because this was conducted on patients in a single center. Furthermore, only selected patients who agreed to human gene sequencing of IL-28B and only one IL-28B genotyping of rs12979860 was assessed in the present study. Another limitation is the lack of data on biopsies findings because most of the studied patients refused biopsy, therefore, supplementary studies are suggested to assess the relation between genotyping, treatment response, and different alleles based on biopsies findings.

In summary, our results have shown that IL-28B (rs12979860) was an important predictor of CHC treatment outcome with PEG-IFN α-2 and RBV in patients with HCV genotype I infection in Isfahan, Iran. Patients with the IL-28B-CC genotype had higher rates of SVR than those with the IL-28B CT or TT genotype, and IL-28B-CC seems to be more important than IL-28B-CT/TT in predicting positive treatment response. However, to clarify the mechanism of IL-28B genotyping and its impact on treatment response, further studies are needed to be done.

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AUTHOR'S CONTRIBUTION

HK carried out the design and coordinated the study, participated in most of the experiments and prepared the

manuscript. TT provided assistance in the design of the study, coordinated and carried out all the experiments and participated in manuscript preparation. BB and ShH participated in most of the experiments and prepared the manuscript. All authors have read and approved the content of the manuscript.

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