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Research Article

IL28B rs12979860 gene polymorphism and sofosbuvir-based therapy response in HCV-infected Pakistani patients

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Abstract

Objectives: Interleukin-28B (IL-28B) gene polymorphisms play an important role in response prediction of direct-acting antivirals (DAAs) treatment, including Sofosbuvir and Daclatasvir with or without Ribavirin. The purpose of this study was to assess the IL-28B polymorphism SNP (rs12979860) and other clinical factors as response predictors for the sustained virological response (SVR) in chronic HCV-infected patients taking DAA therapy.

Methods: A cross-sectional and observational study was carried out among 104 HCV-infected patients who completed a course of Sofosbuvir and Daclatasvir along with Ribavirin. Patients were classified according to their response to therapy. Genotyping of IL-28B was determined through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, and HCV genotyping was identified by PCR method. We analyzed the response prediction of IL-28 gene polymorphism among patients receiving DAA therapy.

Results: Overall, IL-28B CC, CT, and TT genotypes were found in 56 (53.8%), 22 (21.2%), and 26 (25.0%) patients, respectively. Higher early virological response (EVR) and SVR were observed in patients with the rs12979860 CC alleles (82.1% and 75%) as compared to CT/TT alleles (54.2% and 20.8%). IL-28B CC genotype (OR=0.14; 95% CI=0.04-0.44; p=0.001) and EVR (OR=0.20; 95% CI=0.05-0.71; p=0.013) remained significantly associated with SVR in the multivariate regression analysis. However, the FIB-4 score (OR=4.24; 95% CI=1.46-11.75; p=0.008) is a strong predictor of non-SVR.

Conclusion: The antiviral efficacy of triple therapy (sofosbuvir, daclatasvir, and ribavirin) is influenced by the variability of the IL-28B gene, as well as the EVR and FIB-4 score. These variables also play a significant role in predicting the treatment response of patients with chronic HCV infection in Pakistan.

Keywords: Early virological response, HCV genotype, hepatitis C virus, IL-28B gene, sustained virological response

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With an estimated 71 million cases of chronic hepatitis C virus (HCV) infection worldwide, 80% of the burden is centered in lower-middle income nations, making this disease a serious global health concern [1]. Furthermore, chronic HCV is thought to be the primary risk factor for cirrhosis (15–35%), a condition that results in decompensated liver damage and eventual mortality [2]. In Pakistan, where a countrywide survey carried out in 2007–2008 estimated the prevalence of HCV at 4.8%, the virus is extremely prevalent. As part of its efforts to eradicate HCV infection, the World Health Organization (WHO) has set 2030 targets for a 65% decrease in HCV-associated death and an 80% decrease in international incidence [3].

For the past 20 years, combination therapy consisting of ribavirin and pegylated interferon alfa has been considered the gold standard treatment for HCV infection. However, this treat-

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ment plan was hampered by the frequent incidence of adverse effects that made it difficult to adhere to the prescribed course of action, which led to substantially elevated rates of treatment cessation and failure [4]. Direct-acting antiviral drugs (DAAs), like NS5A complex, NS5B polymerase, and NS3/4A protease inhibitors, have been available for a decade now and have shown promise in lowering viral levels in patients with different genotypes of HCV while remaining safe [5, 6].

The management of chronic hepatitis C is influenced by various parameters, including the level of HCV-RNA, the stage of hepatic fibrosis, and the genetic status of the host, despite the progress made in viral pathogenesis and treatment [7]. The IL-28 gene is also known as interferon-gamma 3 because it codes for the protein known as interferon-gamma 3, which is encoded by the cytokine gene Interleukin-28B (IL-28B), one of the host genetic factors [8, 9].

According to recent research, genetic variations in IL-28B may impact the way that various infectious diseases decrease HCV replication and produce the cytokines that are linked to it, which modify the host immune response [3, 10]. A SNP (rs12979860), which is situated just three kilobases upstream from the IL28B gene, has been identified by genome-wide association studies as a critical factor affecting the HCV clearance from infected patients [11, 12].

The disparities in treatment response rates observed between Caucasians, African-Americans, and Asians are mostly caused by one significant factor, which is the differences in allele frequencies among ethnic groups [13]. In HCV genotype-1, the IL-28B gene polymorphisms were found to be a significant predictor of both a sustained and rapid virological response to treatment. Similarly, compared to patients with non-CC genotypes, those with HCV genotype-3 who followed the treatment plan and had the CC genotype of rs12979860 were more than twice as likely to respond favorably to therapy [14]. It has been reported that the IL-28 CC gene polymorphism is more common in Pakistan than the CT/TT genotype, yet there are minor variances in the incidence of other genotypes in different parts of the country [15]. However, little research has been done on how this genetic difference affects how well DAA medication works in patients with HCV.

In this study, we aimed to evaluate the association between viral influential factors and IL-28B rs12979860 genetic variation in response to the DAAs therapy outcome in chronic HCV-infected patients.

Materials and Methods

This was a cross-sectional and observational study enrolling one hundred and four HCV-diagnosed patients who had received the specific combination therapy of sofosbuvir and daclatasvir from three different tertiary care hospitals in Islamabad, Pakistan, between April 2018 and August 2020. Before beginning this study, we obtained approval, by the Declaration of Helsinki and Nuremberg Code [16], from the Ethical Committee of Army Medical College, Rawalpindi (letter number 02/CREAM-A/Maleha). The 104 patients were divided according to their response to DAA therapy into a responder group (n=52) and a non-responder group (n=52). Informed consent was taken from each patient, and they were informed about the outcome of the study. Inclusion criteria were: 1) Positive with HCV-RNA with a lower limit of detection of 600 IU/mL, 2) Age not less than 18 years, and 3) Patients who have completed 12 weeks of sofosbuvir and daclatasvir combination therapy. Patients positive for HIV or hepatitis (A, B, D, or E) infection and evidence of metabolic, genetic, or autoimmune liver disease were excluded from this study.

All patients underwent a comprehensive medical assessment, which included gathering a detailed medical history, conducting clinical examinations, and performing anthropometric measurements such as body weight and height. Peripheral blood samples were collected and routine investigation was performed, including serum hemoglobin, albumin, platelets count, and liver function tests (serum bilirubin, ALT, AST & ALP) using kit Innoline by Martin Dow Marker Specialties (Pvt.) Ltd, and results were recorded carefully in the liver clinic of Holy Family Hospital, Pakistan.

FIB-4 index was calculated for each participant using: FIB-4=[age (yr) × AST (IU/L)/PLT (10^9/L) × √ALT(IU/L)]. Significant fibrosis was associated with a positive predictive value when the FIB-4 index was greater than 3.25, whereas severe fibrosis was associated with a negative predictive value when the index was less than 1.45 [17, 18].

All RNA preparation and HCV RNA determination steps were carried out under RNase-free conditions. HCV RNA quantification was determined by Smart Cycler II system (Applied Biosystem, Foster City, Calif; detection limit 20 IU/ml). Positive RT-PCR cases underwent HCV genotype detection using a specific HCV genotyping assay as described earlier [19]. An internal control was employed to amplify each sample, and both positive and negative controls were incorporated in each tested batch.

The major endpoint was the assessment of SVR, as responder patients who attained sustained virological response (defined as the absence of HCV RNA from serum by a sensitive PCR assay 12 weeks following discontinuation of therapy). Non-responder patients were with detectable (>2 log10) HCV RNA after 12 weeks of end of therapy. Relapse defined as patients with recurrence of HCV RNA levels during the follow-up evaluation after therapy is discontinued. Another term, EVR (early virological response), is defined as a ≥2 log reduction or complete absence of serum HCV RNA at week 12 of therapy compared with the baseline level [20].

PCR amplification and sequencing of rs12979860 single-nucleotide polymorphism was used for genotyping of IL28B. Blood samples from all patients were collected and subjected

to RNA extraction by RNA Mini prep Super Kit (Bio Basic Inc, Canada). First strand cDNA was synthesized by Revert-Aid Premium First Strand cDNA Synthesis Kit (Thermo Scientific Inc,#K1652, USA), using RNA as a template. The polymerase chain reaction-based restriction fragment length polymorphism assay was used for genotyping IL-28B rs12979860 SNP [21]. The TaqMan custom-designed primers and probes used for the genotyping procedure were; IL-28B forward and reverse primer: 5'-GCTTATCGCATACGGCTAGG-3' and 5'-AGGCTCAGGGTCAATCACAG-3'. The PCR mixture had a total volume of 20 µl, comprising 1 µl of DNA template (ranging from 120 to 480 ng), each primer (1 µl), and commercial Super Mix (10 µl), which included Taq polymerase, dNTPs, magnesium chloride, 10 x PCR buffer, and Syber green fluorescent dye. Before conducting the PCR reactions, genomic DNA extraction from whole blood samples was done by the non-enzymatic salting-out method. The mixture was loaded into 96-well MicroAmp Optical Reaction Plates (Applied Biosystems). All Real-time PCR reactions were carried out on the 7500 Fast Real-Time PCR System. The procedure involved initial denaturation for 5 minutes at 95°C, then 35 cycles at 95°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds with a final extension at 72°C for 10 min. On 2% agarose gel electrophoresis, bands of 160 and 82 bp indicated the TT genotype, 135, 82, and 25 bp bands indicated the CC genotype; 160, 135, 82, and 25 bp bands indicated the CT genotype and were visualized by a gel doc unit (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Statistical analyses were performed using SPSS v. 26.0. Qualitative variables were measured by number and percentage of patients, whereas mean and standard deviations (SDs) were used to describe quantitative data. Student's t-test was applied to compare independent samples from two groups, and the χ^2 test was performed to compare categorical data. The association between IL-28B polymorphisms and HCV genotyping with SVR, EVR, and relapse was evaluated using a one-way analysis of variance (ANOVA) test. Multivariate logistic regression analysis was performed to determine the role of genetic and bio-clinical variables as predictors of treatment outcomes. A 95% confidence interval (CI) was included in the calculation and reporting of odds ratios (ORs). A p-value with two tails less than 0.05 was deemed statistically significant.

Results

A total of 104 HCV-infected patients participated in the study with a male predominance of 68.3%. The average age was 42.15 years with a standard deviation of 3.78 years. Fifty-two (50%) patients failed to achieve SVR among the 104 total enrolled and have been labeled as non-responders. Among responders, 35 participants (67.7%) were male and 16 (32.7%) were females, however, among the non-responder group, 36 participants (69.2%) were males and 16 (30.8%) were females. The most prevalent IL-28B genotype and HCV genotype were genotype CC (53.8%), and genotype 3 (76%) in our test population.

When the study groups' laboratory parameters were taken into account, significant variations in serum ALT levels (p=0.046) and FIB-4 score (p<0.001) were found between the responder and non-responder groups. Other pretreatment factors that were found to be associated with achieving a positive virological response were low baseline HCV-RNA level <0.4 log6 IU/ml (p=0.020), HCV genotype (p=0.01), and EVR (p<0.001). There were no significant differences in gender, age, hemoglobin concentration, serum albumin, serum bilirubin, serum AST, BMI, and platelet count between these groups (p>0.05). More detailed information about these 104 patients can be found in Table 1.

Regarding IL-28B genotype frequency, a comparison was made between IL28B CC [56 out of 104 patients (53.8%)] and IL28B CT/TT [48 out of 104 patients (46.2%)]. The percentage of early response rate among patients with IL28B CC genotype was 82.1% as compared to 54.2% of the CT/TT genotypes (p=0.002). Important laboratory parameters including ALT and FIB-4 score had a highly statistically significant relationship between the IL-28B genotypes (p=0.001 and p=0.026 respectively). A statistically significant relation was determined between IL-28B (rs12979860) SNP and HCV genotype with 91.1% of IL-28B CC having HCV genotype 3 (p=0.001). However, the relationship between IL28B (rs12979860) and relapse in patients was insignificant with a 10.4% relapse rate among IL-28CT/TT patients and a 1.8% in IL28B CC (p=0.07). Furthermore, as depicted in Table 2, there was a statistically insignificant relation between the two groups when other biochemical parameters were compared such as BMI (p=0.808), hemoglobin concentration (p=0.057), serum bilirubin (p=0.399), serum albumin (p=0.941), platelet count (p=0.268) and serum AST (p=0.177).

Multivariate logistic regression analysis was conducted, which included IL-28B genotypes, HCV genotype, EVR, and various biochemical variables, such as serum ALT value, FIB-4 score, and HCV-RNA levels (which showed significant associations with SVR in the univariate analyses). FIB-4 score (OR=4.26; 95%CI=1.46–11.75) remained significantly associated with failure to achieve SVR to therapy, whereas EVR (OR=0.20; 95%CI=0.05–0.71) and IL-28B CC genotype (OR=0.14; 95%CI=0.04–0.44) remained significantly associated with response to DAA therapy as shown in Table 3.

Figure 1 depicts the frequency of IL28B genotype distribution according to response rate. It also gives a picture of the ongoing treatment virological response rate and relapse rate by HCV genotype. In SVR, the frequency of the IL-28B rs12979860 CC genotype was 42 out of 52 (80.8%), while in EVR, it was 46 out of 72 (63.9%). The frequency of HCV genotype 3 was considerably greater in the SVR (n=48/52, 92.3%) and EVR (n=61/72, 84.7%) groups.

Discussion

HCV is a significant global public health concern, but it is especially so in Pakistan. Pakistan has the highest death rate among the most severely affected countries. Direct-acting

Table 1. Univariate analysis of non-genetic & genetic characteristics of Pakistani patients with chronic hepatitis C infection

Characteristics	Responders n=52		Non-responders n=52		p
	n	%	n	%	
Gender					0.500
Male	35	67.3	36	69.2	
Female	17	32.7	16	30.8	
Age (years) (mean±SD)	40.04±5.43		42.52±7.745		0.062
min–max	32–62		30–62		
Hemoglobin (g/dl) (mean±SD)	13.9±1.69		13.89±1.88		0.880
min–max	11.0–17.2		10.9–17.9		
Albumin (g/L) (mean±SD)	4.37±0.43		4.29±0.53		0.479
min–max	3.40–4.92		3.20–5.00		
Bilirubin (mg/dl) (mean±SD)	0.71±0.28		0.80±0.29		0.136
min–max	0.36–1.34		0.36–1.56		
ALT (IU/L) (mean±SD)	33.71±11.53		40.40±20.21		0.046
min–max	14–82		16–134		
AST (IU/L) (mean±SD)	37.15±14.41		44.90±16.24		0.822
min–max	12–80		16–104		
BMI (kg/m^2) (mean±SD)	29.11±3.59		30.62±4.07		0.320
min–max	22.6–34.2		23.3–42.4		
Platelet (×10^9/L) (mean±SD)	209.15±71.40		208.25±69.44		0.094
min–max	102–356		128–387		
Pre-treatment viral load (log_{10} IU/ml)	1.35±0.64		1.79±1.1		0.020*
(mean±SD) min–max	0.65–5.24		0.21–8.65		
FIB-4 score (mean±SD)	0.92±0.48		1.59±0.46		<0.001**
min–max	0.33–2.08		0.33–1.97		
EVR					
Yes	46	88.5	26	50.0	<0.001**
No	06	11.5	26	50.8	
IL-28B gene polymorphism					
CC	42	80.8	14	26.9	<0.001
CT/ TT	10	19.2	38	73.1	
HCV genotype					
3	45	57.0	34	43.0	0.010
Non-3	07	28.0	18	72.0	

Data expressed as mean±SD or n (%) as appropriate. *: p<0.05: Statistically significant; **: p≤0.001: Highly significant statistically. SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; FIB-4: Fibrosis-4; EVR: Early virological response; IL28-B: Interleukin 28-B; HCV: Hepatitis C virus.

antivirals (DAAs), which have recently been developed, have dramatically changed HCV therapy by increasing virus eradication rates to around 90% in a variety of patients globally [22]. The global influence of DAAs on the total burden of disease remains limited, notwithstanding these advancements. A significant portion of people with long-term HCV infection remain at risk for serious liver problems such as hepatocellular carcinoma and liver cirrhosis [23].

The current study thoroughly examined the relationship between host genetic variations and clinical factors and the sustained virological response to DAAs. Differential clinical outcomes in response to DAAs against HCV infection are connected with factors related to viruses (such viral load) and hosts (SNPs). In this study, CC (53.8%) was the most common genotype for IL-28B rs12979860 among patients with chronic hepatitis C, followed by TT (25%) and CT (21.2%). These results corroborate those of Calisti et al. [24], who found that among chronic HCV patients, regardless of treatment-naive or experienced, the CC genotype was most common. More research is necessary to fully understand the connection because the precise mechanism is still unknown.

Table 2. Correlation of IL-28B genotypes with baseline demographics and clinical data of HCV-infected patients

Characteristics	IL-28BCC n=56		IL-28B CT/TT n=48		p
	n	%	n	%	
Gender					
Male	40	71.4	31	64.6	0.296
Female	16	28.6	17	35.4	
Age (years) (mean±SD)	41.27±6.53		41.29±7.11		0.986
min–max	32–62		30–62		
Hemoglobin (g/dl) (mean±SD)	13.6±1.64		14.28±1.89		0.057
min–max	10.9–17.9		10.9–17.9		
Albumin (g/L) (mean±SD)	4.34± 0.500		4.33±0.47		
min–max	3.30–5.00		3.20–5.00		0.941
Bilirubin (mg/dl) (mean±SD)	0.83±0.27		0.88±0.35		
min–max	0.36–1.34		0.36–1.56		0.399
ALT (IU/L) (mean±SD)	32.14±12.98		42.79±19.54		0.001
min–max	14–82		16–134		
AST (IU/L) (mean±SD)	40.54±14.66		44.81±17.40		0.177
min–max	12–80		16–104		
BMI (kg/m^2) (mean±SD)	30.04±3.23		29.85±4.41		0.808
min–max	22.6–36.4		22.8–42.4		
Platelet (×10^9/L) (mean±SD)	215.79±74.95		200.44±63.77		0.268
min–max	102–356		102–356		
Pre-treatment viral load (log$_{10}$ IU/ml)	1.414±1.07		1.75±0.79		0.078
(mean±SD) min–max	0.21–5.65		1.0–8.24		
HCV genotype					
3, no (%)	51	91.1	31	64.6	0.001
Non-3, no (%)	05	8.9	17	35.4	
FIB-4 score (mean±SD)	1.00±2.50		1.22±3.49		0.026
min-max	0.33–1.48		0.33 – 2.41		
EVR					
At week 4	46	82.1	26	54.2	0.002
No	10	17.9	22	45.8	
Response to treatment					
Yes	42	75	10	20.8	<0.001
No	14	25	38	79.2	
Relapse					
Yes	01	1.8	05	10.4	0.071
No	55	98.2	43	89.6	

Data expressed as mean SD or n (%) as appropriate. IL28-B: Interleukin 28-B; SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; HCV: hepatitis C virus; FIB-4: Fibrosis-4; EVR: Early virological response.

According to IL-28B polymorphism-based analyses, the IL-28B (rs12979860) CC variation was found to be significantly correlated with the rate of response to therapy (42/52, 80.8%) and EVR (46/72; 63.9%) when compared to patients infected with IL-28 CT and TT genotypes (p<0.001 and p=0.001, respectively). According to Abd Alla et al. [25], compared to non-CC genotypes, the pre-treatment detection of the IL-28B CC allele could indicate an increased probability of full clearance of the HCV. The TT genotype was significantly more expressed in relapse-prone patients (60%; p=0.116), indicating that the genotype is thought to be a risk factor for relapse following a first response to DAA treatment. According to Zaki SM, the current findings were consistent [1]. They demonstrated that the likelihood of non-response was considerably elevated by the TT and CT genotypes of IL-28B, respectively, by 10.364 and 8.768 folds. On the other hand, a previous finding demonstrated a different expression, with IL-28B CT being the most common genotype in the SVR group and demonstrating a statistically significant cor-

Table 3. Predictors of sustained virological response among HCV-infected patients after sofosbuvir and daclatasvir treatment

Predictors	ß	p	Odd ratio	95% CI for odds	
				Lower	Upper
IL-28B CC genotype	–1.94	0.001	0.14	0.04	0.44
ALT (IU/L)	0.003	0.83	0.99	0.96	1.02
HCV Genotype	–1.29	0.08	0.27	0.06	1.20
EVR	–1.57	0.013	0.20	0.05	0.71
FIB-4 Score	1.45	0.008	4.26	1.46	11.75
Pre-treatment viral load (log_{10} IU/ml)	0.10	0.75	1.11	0.64	1.93
Constant	1.58	0.23	4.88		

The reference category is non-responder. HCV: Hepatitis C virus; CI: Confidence interval; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; EVR: Early virological response; FIB-4: Fibrosis-4.

relation with triple therapy consisting of peg-interferon, sofosbuvir, and ribavirin [5, 26]. The discrepancies in treatment plans, HCV genotypes, and the features of the population under study are probably to blame for the inconsistent outcomes [27].

The FIB-4 is a noninvasive diagnostic tool to evaluate the presence of fibrotic liver. Our data confirmed that the patient's FIB4-scores are recognized as the strongest pre-treatment predictor of sustained viral clearance (p=0.008; OR=4.26). Presence of advanced fibrosis and cirrhosis seems to remain the strongest pretreatment predictor of response. In the study by Watanabe et al. [28], patients with high FIB-4 index developed hepatocellular carcinoma after DAAs therapy. Statistical analysis revealed that early virological response could be a predictive marker for the response rates of ongoing antiviral therapy (p=0.013; OR=0.20). Patients having low baseline viral load showed higher response rates as compared to patients who have higher baseline viral load (p=0.02). This finding points to a strong, independent correlation between SVR and the baseline viral load along with EVR which was also proved by similar other studies [29, 30]. Patients with HCV genotype 3 had a significantly higher frequency of rs12979860 genotype CC compared to those with HCV genotype non-3 (91.1% vs. 8.9%). Recent data, several previous studies reported that the patients with HCV genotype 3 had a higher incidence of IL-28B CC genotype than those with HCV genotype non-3 [31]. The underlying mechanism of this association may be either due to rs12979860 C allele carriers having higher rates of HCV genotype 3 infection, or that carriers of the CC genotype are more susceptible to HCV genotype 3a/b infection [14, 32].

In Pakistan, genotype-3 is the most common, followed by genotype-1. In the current study, HCV genotype-3 was found to be more prevalent among HCV-infected patients, a finding consistent with the study done by Tayyab et al. [33]. The frequency of HCV genotype 3 in patients attaining early virological response (61/72, 84.7%) and sustained virological response (48/52, 92.3%) is higher than other non-3 genotypes (p=0.001 and p=0.028,

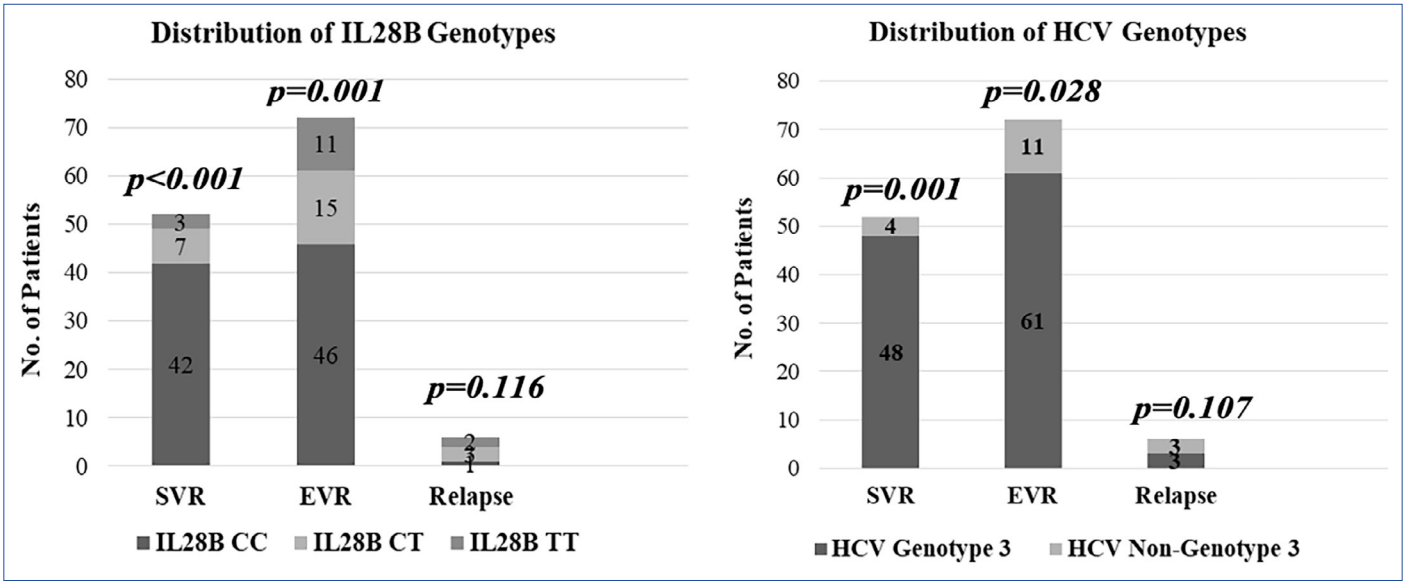

Figure 1. Distribution of IL-28B polymorphism and HCV genotype in patients with SVR, EVR, and Relapse.

IL28-B: Interleukin 28-B; HCV: Hepatitis C virus; SVR: sustained virological response; EVR: Early virological response.

respectively). These findings align with the outcomes reported by Mahmoud et al. [8], Junaid et al. [12], and Attallah et al. [34]. IFNλ3 triggers Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling after interacting to its receptor, triggers the expression of interferon-stimulated genes (ISGs) and ultimately establishes a potent antiviral condition [35].

This study had some limitations. We believed that HCV viremia reflects chronic infection rather than recent infection based on baseline data. A single-center sample collection method and a limited participant count were the study's shortcomings. Another drawback is that we did not assess the impact of IL28B gene polymorphisms on the emergence of various adverse effects of DAAs, particularly hepatocellular carcinoma and liver decompensation, or if IL-12 influenced the frequency of DAA-related adverse effects. The various extrahepatic effects of DAAs and the relationship between IL28B and IL-12 were not assessed. In addition, the effects of IL10 (rs1800896) polymorphisms could have been assessed for recent data in the Pakistani population as IL10 (rs1800896), along with IL28B (rs12979860), is also a good predictor of treatment response in chronic hepatitis C patients [3]. An association between the IL-28 B genotype and poor response to DAAs is required to be studied on a large scale, to formulate national health policies. It is essential to discover cost-effective and specific molecular factors responsible for virological response to antiviral therapy.

Conclusion

Our findings indicate that IL28-B variability influences the antiviral efficacy of DAAs and serves as a significant genetic predictive factor of treatment response in Pakistani HCV patients. The favorable CC genotype of IL-28B rs12979860 was higher and significantly associated with EVR and SVR in HCV-infected patients. Moreover, we found a strong association between high FIB-4 score and non-response to DAA therapy. These findings contribute to anticipating the response to therapy and have implications for reducing the cost of treatment in HCV patients. Altogether, IL-28B genotyping plays an essential role in the therapy choice algorithm.

Ethics Committee Approval: The study was approved by The Army Medical College, Rawalpindi Ethics Committee (No: 02/CREAM-A/Maleha, Date: 17/04/2014).

Authorship Contributions: Concept – M.A., A.J.; Design – M.A., A.S., B.A.; Supervision – A.S., S.A., S.S.; Materials – B.A., S.A., S.S.; Data collection &/or processing – M.A., A.J.; Analysis and/or interpretation – M.A., A.J.; Literature search – A.S., B.A., S.A.; Writing – M.A., A.J., A.S.; Critical review – B.A., S.A., S.S.

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