Original Article

The Role of Biochemical Variations and Genotype Testing in Determining the Virological Response of Patients Infected with Hepatitis C Virus

Abid Shoukat, Mosin S Khan[1], Syed Mudassar[1], Zaffar Kawoosa, Altaf H Shah, Showkat Ali Zargar

Departments of Gastroenterology and [1]Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India

First two authors have contributed equally

Abstract

Background: In hepatitis C virus (HCV), infection viral and *IL28B* genotype along with many clinical and biochemical factors can influence response rates to pegylated interferon plus ribavirin (Peg-IFN-a/R) therapy and progression to chronic hepatitis C (CHC). **Aims:** The present study was conducted to determine the effect of biochemical and risk factors on treatment outcome in CHC patients in relation to their viral and host genotype. **Settings and Design:** The present study was a prospective Pe- IFN efficacy study consisting of Peg-IFN-a/R therapy for 24–48 weeks including 250 HCV infected patients. **Materials and Methods:** Biochemical parameters were determined by Beckman Coulter AU680 automated analyzer. HCV and Interleukin 28B (*IL28B*) genotyping were carried out by polymerase chain reaction-restriction fragment length polymorphism and viral load was determined by quantitative real-time PCR. **Results:** Wild outnumbered the variant genotypes in *rs*12979860, *rs*12980275, and *rs*8099917 SNP of *IL28B* gene. Sustained virological response (SVR) SVR and viral genotype were significantly associated with age, hepatic steatosis, low-grade varices, and serum aspartate transaminase levels (at the end of treatment) ($P < 0.05$). In addition, SVR was significantly influenced by body mass index (BMI), insulin resistance, serum low-density lipoprotein , and ferritin levels ($P < 0.05$). Viral genotype 1 infected patients had higher serum cholesterol and triglyceride levels ($P < 0.05$). **Conclusions:** Although the *IL28B* sequence variation is the major factor that can influence response rates to antiviral therapy, viral and biochemical factors also have a definite role to play in the diagnosis, etiology, and treatment outcome in HCV-infected patients.

Keywords: Biochemical, chronic hepatitis C, IL28B, pegylated interferon, viral genotype

Introduction

Hepatitis C virus (HCV) is a positive-stranded RNA virus that chronically infects 130–150 million people comprising of nearly 3% of the world population. Approximately 7,00,000 people die each year from hepatitis C-related liver diseases. Infection with HCV induces a wide range of innate and adaptive immune responses that achieve permanent control of HCV in 20% to 50% of infected individuals.[1] Most HCV infections persist (70%–80%), and about 30% of individuals with a persistent infection develop chronic liver diseases, including cirrhosis, and hepatocellular carcinoma (HCC).[2] A variety of host, viral, and environmental factors are associated with the rate of progression of fibrosis and cirrhosis. Obesity is a metabolic risk factor for the development of HCC in the setting of chronic hepatitis C (CHC).[3] Males, diabetic patients, smokers, and patients with increased hepatic iron are more likely to have advanced fibrosis.[4] Lipid metabolism is profoundly disturbed in HCV infection. Hepatic steatosis as well as superimposed steatohepatitis is important risk factors for fibrosis progression in CHC. In individuals with CHC, viral load and elevated serum alanine transaminase (ALT) levels may have clinical relevance.[3]

The goal of treating CHC patients with peginterferon plus ribavirin and direct acting antivirals (DAA's) is to eradicate the virus so as to attain SVR (SVR12 and SVR24); defined as viremia after

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Address for correspondence: Prof. Showkat Ali Zargar, Department of Gastroenterology, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar - 190 011, Jammu and Kashmir, India.
E-mail: syed.mudassar@skims.ac.in

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completion of antiviral therapy for chronic HCV infection.[5,6] Although there are DDA's such as telaprevir and boceprevir (first generation); faldaprevir, simeprevir, and sofosbuvir (second generation) used nowadays for treatment of HCV infections, this treatment modality is expensive and not available at all medical centers. Still, long-acting pegylated interferon-α plus oral treatment with ribavirin (Peg-IFN-a/R) is considerably less potent than newer DAAs, and the end-of-treatment response is generally substantially higher than SVR24.[6] Virus-specific characteristics but also clinical and biochemical parameters are among the baseline predictors of response to HCV treatment. Metabolic factors, such as high body mass index (BMI), presence and severity of liver steatosis, increasing homeostasis model assessment of insulin resistance score and older age, advanced stage of fibrosis and high viral load at baseline have been reported as negative predictors of response.[5,7] Of known pretreatment variables, a powerful predictor of response to treatment is the viral genotype. Genotypes 2 and 3 have got a better outcome than genotypes 1 and 4 with an SVR of 75%–85% versus 45%–55%, respectively.[8] Pretreatment ALT levels were found to be correlated positively with SVR.[9]

Among the baseline predictors of response, the pretreatment host genetic polymorphisms have been the subject of recent, major studies. Several independent studies have consistently shown that single nucleotide polymorphisms (SNPs) near *IL28B*, which encodes the type III IFN-λ3 are strongly associated with the response to treatment of CHC and is assumed to explain the heterogeneity in HCV clearance across individuals.[10] In particular, the homozygous wild genotypes TT at marker rs8099917, CC at marker rs12979860 and AA at marker rs12980275 are all associated with favorable treatment outcomes. These data have been confirmed in populations of different ancestry and HCV genotypes, and in various clinical scenarios.[11]

Although, *IL28B* genotype is only one of many factors that can influence response rates to Peg-IFN-a/R therapy in HCV infection and progression to CHC it should be interpreted in the context of other clinical and biochemical factors. With this background in mind, the present study was conceived to study the correlation of *IL28B* polymorphism with clinical, biochemical and viral characteristics in patients with CHC in relation to treatment outcome (SVR) in the only tertiary care hospital in the valley of Kashmir.

Materials and Methods

The present work was a prospective Peg IFN efficacy study conducted in the Department of Gastroenterology and Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Jammu and Kashmir, India between July 2012 and July 2015. The study was approved by the Ethical Committee of SKIMS.

Inclusion and exclusion criteria

The patients of any age group or sex with HCV-RNA level higher than 50 U/ml and not on treatment for HCV infection were included in the study. Patients with hemoglobin <7 g/dl, an absolute neutrophil count <500/mm^3, platelet count <50,000/mm^3, decompensated liver disease, active malignancies, severe psychiatric illness, the presence of HCC, the presence of autoimmune disorder, and on immunosuppressive drugs were excluded from the study.

Sample size

The study included 250 HCV-infected patients. Blood samples were collected in clot activator vials for HCV serology and other serum biochemical parameters. Blood collected in ethylenediaminetetraacetic acid vials was used for HCV RNA levels, viral and host genotyping. A complete history was taken from all the patients before testing. All the procedures were performed after taking the proper consent from the patients and their caretakers.

Hepatitis C virus serology and genotyping

Serum samples were screened for Hepatitis C and anti-HCV antibodies using commercially available ELISA kits (HCV: Anti-HCV (ELISA): Murex, Biotech, Kyalami, South Africa). HCV genotyping was done using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) employing the method of Chinchai *et al*.[12]

Hepatitis C virus RNA levels and treatment responses

The HCV RNA was determined using a COBAS Amplicor HCV Monitor, v2.0 (Roche Diagnostics, Branchburg, NJ, USA) with a precision value of 50 IU/ml before, during and after the treatment.

Biochemical parameters

Estimation of biochemical parameters like serum fasting blood glucose, albumin, cholesterol, triglyceride, low-density lipoprotein (LDL), bilirubin, ALT, aspartate transaminase (AST), ALP, ferritin, hemogram, vitamin D, vitamin B_{12} and TSH before the start of treatment. Investigations were performed on fully automated biochemistry analyzer (Beckman Coulter AU 680, USA) and Immunoassay Analyzer (Beckman Coulter Access II, USA). Some of the parameters such as ALT, AST, total leukocytic count (TLC), Hb, and platelet count were estimated at the end of the therapy to estimate the efficacy of therapy and complications associated with the drugs.

Other Investigations

All patients were subjected to esophagogastroduodenoscopy (EGD) and ultrasonography. EGD was performed with an Olympus (Olympus, Japan) endoscope to look for varices. Abdominal ultrasonography was performed with the Aloka SSD 260 Ultrasonographer (Aloka Science and Humanity, Tokyo, Japan, with a 5 or 7.5 MHz transducer) on all patients.

IL28B gene polymorphism

PCR-RFLP was established for polymorphic analysis of *rs*12979860, *rs*12980275 and *rs*8099917 SNPs in the promoter region of *IL28B* gene.[13] Restriction digestion products for each were separated on 3% agarose gels stained with ethidium bromide for visualization on a ultraviolet trans-illuminator.

Statistical analysis

All qualitative variables were expressed as mean ± standard deviation Patient's baseline characteristics, and outcome measures were compared with the use of Student's *t*-test for

parametric data, the Mann–Whitney U-test for nonparametric data for qualitative variables and Pearson's Chi-square test or Fisher's exact test for proportions. Multivariate analysis was performed by binary logistic regression for the parameters significant on univariate analysis. Statistical analysis was performed using the statistical software package SPSS (SPSS version 20 Inc., Chicago, IL, USA).

Results

During the study period of 3 years, 250 HCV-infected patients were enrolled in the study. Table 1 summarizes the various methods for detection of HCV. Of the 250 patients, males were 170 (68.0%) and 80 (32.0%) were females. Majority of the patients were <50 years of age (74%; 185 of 250) and <70 kg of weight (69.8%; 117 of 250). Most of the patients had normal BMI (18.5–24.99 kg/m^2; 63.6%) followed by preobese class (25–29.99 kg/m^2; 25.2%), obese Class I (4.8%) underweight class (4.8%), obese Class II (1.6%) and underweight (4.8%). Patients were enrolled from almost every district of the state with a higher frequency of rural patients (55.6%; 139 of 250) [Figure 1]. Genotype 3 was the predominant genotype seen in 187 (74.8%) patients. Table 2 depicts the frequency distribution of selected demographic and risk factors in 250 HCV-infected patients. Table 3 depicts the levels of various physical parameters, biochemical factors altered due to complications associated with the drugs (Hb, TLC, Plt, PT, INR, etc.,) and the factors which influence outcome to treatment (HCV viral load, serum ferritin, vitamin D, vitamin B_{12} levels, triglyceride (TG), LDL, cholesterol, ALT, AST, etc).

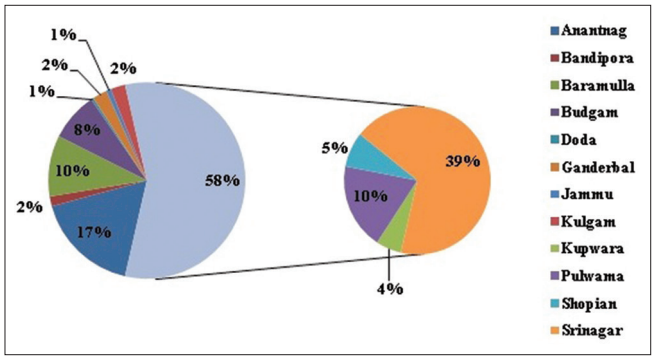

Figure 1: District wise distribution of hepatitis C virus infected patients

Table 1: Modes of detection of hepatitis C virus infection

Mode of detection	Frequency (%)
Before blood donation	75 (30.0)
Evaluation for FL	27 (10.8)
Family screening	4 (1.6)
Liver disease	3 (1.2)
Routine screening	47 (18.8)
Screening before surgery	46 (18.4)
Transaminitis	46 (18.4)
Others	2 (0.8)

FL: Fatty liver

Table 2: Frequency distribution analysis of selected demographic and risk factors in hepatitis C patients (*n*=250)

Characteristics	Cases, *n* (%)
Age group	
<50	185 (74.0)
≥50	65 (26.0)
Sex	
Female	80 (32.0)
Male	170 (68.0)
Weight (kg)	
≥70	73 (29.2)
<70	117 (69.8)
Genotype	
1	63 (25.2)
3	187 (74.8)
SVR	
No	8 (3.2)
Yes	242 (96.8)
USG	
Group I and II FL	127 (50.8)
Normal	123 (49.2)
EGD	
LGV	13 (5.2)
Normal	237 (94.8)
DM	
No	213 (85.2)
Yes	37 (14.8)
Bilirubin	
Elevated	12 (5.0)
Normal	238 (95.0)
ALT	
Elevated	211 (84.4)
Normal	39 (15.6)
AST	
Elevated	199 (79.6)
Normal	51 (20.4)
ALP	
Elevated	8 (3.2)
Normal	242 (96.8)
Cholesterol	
Elevated	78 (31.2)
Normal	172 (68.8)
TG	
Elevated	58 (23.2)
Normal	192 (76.8)
LDL	
Elevated	37 (14.8)
Normal	213 (85.2)
TSH	
Elevated	8 (3.2)
Normal	242 (96.8)
Hb	
Low	32 (12.8)
Normal	218 (87.2)

Contd...

Table 2: Contd...

Characteristics	Cases, *n* (%)
TLC	
Low	11 (4.4)
Normal	239 (95.6)
Platelets	
Low	161 (64.4)
Normal	89 (35.6)
Ferritin	
Elevated	155 (62.0)
Normal	95 (38.0)
Vitamin D	
Low	58 (23.2)
Normal	192 (76.8)
Vitamin B_{12}	
Low	2 (0.8)
Normal	248 (99.2)
AST last	
Elevated	114 (45.6)
Normal	136 (54.4)
ALT last	
Elevated	139 (55.6)
Normal	111 (44.4)
Hb last	
Low	216 (86.4)
Normal	34 (13.6)
TLC last	
Low	135 (54.0)
Normal	115 (56.0)
Platelets last	
Low	232 (92.8)
Normal	18 (7.2)

SVR: Sustained virological response, USG: Ultra sonography, EGD: Esophagogastroduodenoscopy, DM: Diabetes mellitus, ALP: Alkaline phosphatase, Hb: Hemoglobin, TLC: Total leukocytic count, PT: Prothrombin time, INR: International normalized ratio , ALT: Alanine transaminase; AST: Aspartate transaminase, Last: At the end of treatment, LDL: Low density lipoprotein, TG: Triglyceride, LGV: Low grade varices

In case of *IL28B rs*12979860 polymorphism, 136 patients (54.4%) had wild genotype (CC) while as variant genotype (CT + TT) was seen in 114 patients (45.6%) [Table 4]. In *rs*12980275 SNP 132 patients (52.8%) had AA genotype, whereas 118 patients (47.2%) had variant (AG + GG) genotype. Among the patients having nonalcoholic fatty liver disease (NAFLD) only 44% were having AA genotype compared to 61.8% of patients without NAFLD ($P < 0.05$) [Table 4]. In case of *rs*8099917 SNP wild genotype (TT) was present in 59.6% compared to 40.4% having variant genotype (TG + GG). Wild-type genotype (TT) was present in 87.5% of patients with initially elevated levels of ALP with a significant association ($P < 0.05$). Association between *IL28B* genotypes, demographic, and biochemical characteristics of HCV patients is shown in Table 4.

Most of the patients having <50 years of age and <70 kg of weight achieved SVR ($P < 0.05$). Patients having normal baseline LDL levels responded very well to antiviral therapy

Table 3: Levels of various patient characteristics and biochemical parameters

Parameter	Mean±SD	Range
Height (m)	1.65±0.15	1.5-1.87
Weight (kg)	64.5±17.1	35-96
BMI (kg/m^2)	23.54±3.6	16-35
Age (years)	41.2±12.5	14-70
Hb (g/dL)	13.35±1.51	9.2-17.9
TLC (1000/mm^3)	6.09±1.34	3.5-10.6
Platelets (1000/mm^3)	133.4±47.2	68-293
Bilirubin (mg/dL)	1.1±0.4	0.4-3.6
ALT (IU/L)	106±30.2	32-360
AST (IU/L)	80.1±21.2	25-308
TG (mg/dL)	157.4±52.3	54-329
LDL (IU/L)	103.8±25.4	34-194
Cholesterol (mg/dl)	186.1±35.8	108-296
PT	14.3±0.87	12-18
INR	1.07±0.09	1-1.4
Globulin (mg/dL)	2.9±0.34	2-3.9
Albumin (mg/dL)	3.8±0.3	2.9-4.7
Vitamin B12 (ng/L)	424.1±151.9	200-882
Vitamin D (nmol/dL)	67.1±18.9	12-121
Ferritin (ng/mL)	349.1±138.9	67-793
TLC last (1000/mm^3)	3.9±0.4	1.7-9.2
Platelets last (1000/mm^3)	92.2±25.9	40-207
Hb last (g/dL)	9.8±0.6	6.9-14.7
ALT last (IU/L)	54.5±26.3	21-225
AST last (IU/L)	49.2±24.3	20-231
HCV RNA baseline (lac IU/mL)	13.8±0.7	0.04-198.6
HCV RNA at 4 weeks (lac IU/mL)	0.09±0.001	0-7.8
HCV RNA at 12 weeks (lac IU/mL)	0.34±0.01	0-69.7

BMI: Basal metabolic index, TLC: Total leukocytic count, PT: Prothrombin time, INR: International normalized ratio, ALT: Alanine transaminase; AST: Aspartate transaminase, HCV: Hepatitis C virus, RNA: Ribonucleic acid, Hb: Hemoglobin, LDL: Low density lipoprotein, TG: Triglyceride, Last: At the end of treatment

compared to patients having elevated levels ($P < 0.05$). All the patients with normal ferritin levels achieved SVR ($P < 0.05$). Nearly 99% of patients who had normal AST levels after 24 weeks of treatment achieved SVR ($P < 0.05$). The association between SVR and majority of biochemical and risk factors of HCV patients is shown in Table 5.

Among the patients having <50 years of age 80.5% (149 of 185) were infected with genotype 3, while as only 19.5% of patients with virus having genotype 1 ($P < 0.05$). Among the patients infected with genotype 3 majority were having normal cholesterol and TG levels compared to elevated cholesterol and TG levels in patients infected with genotype 1 virus ($P < 0.05$). One hundred and ten of 187 (58.8%) patients with genotype 3 infection have normal AST levels at the end of treatment compared to patients with genotype 1 virus (26 of 63; 41.2%) ($P < 0.05$). The various parameters and laboratory investigations in relation to viral genotypes are shown in Table 5.

Table 6 depicts the multivariate analysis of various parameters with respect to viral and host genotype. Viral genotype was

Table 4: Association between rs12979860 (C to T) rs12980275 (A to G) and rs8099917 (T to G) genotypes, demographic and biochemical characteristics of hepatitis C virus patients

Characteristics	Cases (*n*=250), *n* (%)	rs12979860 (C to T)			rs12980275 (A to G)			rs8099917 (T to G)		
		CC, *n* (%) 136 (54.4)	CT + TT, *n* (%) 114 (45.6)	*P*	AA, *n* (%) 132 (52.8)	AG + GG, *n* (%) 118 (47.2)	*P*	TT, *n* (%) 149 (59.6)	TG + GG, *n* (%) 101 (40.4)	*P*
Age group										
<50	185 (74.0)	105 (56.8)	80 (43.2)	0.13	97 (52.4)	88 (47.6)	0.5	108 (58.4)	77 (41.6)	0.3
≥50	65 (26.0)	31 (47.7)	34 (52.3)		35 (53.8)	30 (46.2)		41 (63.1)	24 (36.9)	
Sex										
Female	80 (32.0)	49 (61.3)	31 (38.7)	0.08	42 (52.5)	38 (47.5)	0.5	49 (61.3)	31 (38.8)	0.4
Male	170 (68.0)	87 (51.2)	83 (48.8)		90 (52.9)	80 (47.1)		100 (58.8)	70 (41.2)	
Weight (kg)										
≥70	73 (29.2)	34 (46.6)	39 (53.4)	0.07	31 (42.5)	42 (57.5)	0.02	44 (60.3)	29 (39.7)	0.5
<70	117 (69.8)	102 (57.6)	75 (42.4)		101 (57.1)	76 (42.9)		105 (59.3)	72 (40.7)	
SVR										
No	8 (3.2)	1 (12.5)	7 (87.5)	0.02	0	8 (100)	0.002	0	8 (100)	0.001
Yes	242 (96.8)	135 (55.8)	107 (44.2)		132 (54.5)	110 (45.5)		149 (61.6)	93 (38.4)	
Genotype										
1	63 (25.2)	27 (42.9)	36 (57.1)	0.012	33 (52.4)	30 (47.6)	0.1	34 (54.0)	29 (46.0)	0.06
3	187 (74.8)	109 (58.3)	78 (41.7)		99 (52.9)	88 (47.1)		115 (61.5)	72 (38.5)	
USG										
FL	127 (50.8)	70 (55.1)	57 (44.9)	0.5	56 (44.1)	71 (55.8)	0.004	75 (59.1)	52 (40.9)	0.4
N	123 (49.2)	66 (53.7)	57 (46.3)		76 (61.8)	47 (38.2)		74 (60.2)	49 (39.8)	
EGD										
LGV	13 (5.2)	6 (46.2)	7 (53.8)	0.3	7 (53.8)	6 (46.2)	0.6	7 (53.8)	6 (46.2)	0.4
N	237 (94.8)	130 (54.9)	107 (45.1)		125 (52.7)	112 (47.3)		142 (59.9)	95 (40.1)	
DM										
No	213 (85.2)	115 (54.0)	98 (46.0)	0.5	112 (52.6)	101 (47.4)	0.5	127 (59.6)	86 (40.4)	0.5
Yes	37 (14.8)	21 (56.8)	16 (43.2)		20 (54.1)	17 (45.9)		22 (59.5)	15 (40.5)	
Bilirubin										
E	12 (5.0)	5 (41.7)	7 (58.3)	0.2	7 (58.3)	5 (41.7)	0.4	7 (58.3)	5 (41.7)	0.5
N	238 (95.0)	131 (55.0)	107 (45.0)		125 (52.5)	113 (47.5)		142 (59.7)	96 (40.3)	
ALT										
E	211 (84.4)	112 (53.1)	90 (46.9)	0.2	110 (52.1)	101 (47.9)	0.3	128 (60.7)	83 (39.3)	0.2
N	39 (15.6)	24 (61.5)	15 (38.5)		22 (56.4)	17 (43.6)		21 (53.8)	18 (46.2)	
AST										
E	199 (79.6)	104 (52.3)	95 (47.7)	0.1	103 (51.8)	96 (48.2)	0.3	122 (61.3)	77 (38.7)	0.2
N	51 (20.4)	32 (62.7)	19 (37.3)		29 (56.9)	22 (43.1)		27 (52.9)	24 (47.1)	
ALP										
E	8 (3.2)	8 (100)	0	0.07	4 (50.0)	4 (50.0)	0.6	7 (87.5)	1 (12.5)	0.09
N	242 (96.8)	128 (52.9)	114 (47.1)		128 (52.9)	114 (47.1)		142 (58.7)	100 (41.3)	
Cholesterol										
E	78 (31.2)	40 (51.3)	38 (48.7)	0.3	38 (48.7)	40 (51.3)	0.2	41 (52.6)	37 (47.4)	0.08
N	172 (68.8)	96 (55.8)	76 (44.2)		94 (54.7)	78 (45.3)		108 (62.8)	104 (37.2)	
TG										
E	58 (23.2)	32 (52.2)	26 (44.8)	0.5	29 (50.0)	29 (50.0)	0.3	29 (50.0)	29 (50.0)	0.06
N	192 (76.8)	104 (54.2)	88 (45.8)		103 (53.6)	89 (46.4)		120 (62.5)	72 (37.5)	
LDL										
E	37 (14.8)	21 (56.8)	16 (43.2)	0.5	19 (51.4)	18 (48.6)	0.5	21 (56.8)	16 (43.2)	0.4
N	213 (85.2)	115 (54.0)	98 (46.0)		113 (53.1)	100 (46.9)		128 (60.1)	85 (39.9)	
TSH										
E	8 (3.2)	6 (75.0)	2 (25)	0.2	6 (75.0)	2 (25.0)	0.1	5 (62.5)	3 (37.5)	0.5
N	242 (96.8)	130 (53.7)	112 (46.3)		126 (52.1)	116 (47.9)		144 (59.5)	98 (40.5)	
Hb										
L	32 (12.8)	20 (62.5)	12 (37.5)	0.4	19 (59.3)	13 (40.7)	0.4	18 (56.2)	14 (43.8)	0.6
N	218 (87.2)	116 (53.2)	102 (46.8)		113 (51.8)	105 (48.2)		131 (60.0)	87 (40.0)	

Contd...

Table 4: Contd...

Characteristics	Cases (*n*=250), *n* (%)	rs12979860 (C to T)			rs12980275 (A to G)			rs8099917 (T to G)		
		CC, *n* (%) 136 (54.4)	CT + TT, *n* (%) 114 (45.6)	*P*	AA, *n* (%) 132 (52.8)	AG + GG, *n* (%) 118 (47.2)	*P*	TT, *n* (%) 149 (59.6)	TG + GG, *n* (%) 101 (40.4)	*P*
TLC										
L	11 (4.4)	6 (54.5)	5 (55.5)	0.8	6 (54.5)	5 (55.5)	0.9	8 (72.7)	3 (27.3)	0.2
N	239 (95.6)	130 (54.3)	119 (55.7)		126 (52.7)	113 (47.3)		141 (59.0)	98 (41.0)	
Platelets										
L	161 (64.4)	88 (54.6)	73 (55.4)	0.9	82 (51.0)	79 (49.0)	0.4	108 (67.0)	53 (33.0)	0.001
N	89 (35.6)	48 (54.0)	41 (56.0)		50 (56.1)	39 (43.9)		41 (46.0)	48 (54.0)	
Ferritin										
E	155 (62.0)	83 (53.5)	72 (56.5)	0.7	89 (57.4)	66 (42.6)	0.06	88 (56.7)	67 (43.3)	0.2
N	95 (38.0)	53 (55.7)	42 (54.3)		43 (45.2)	52 (54.8)		61 (64.2)	34 (35.8)	
Vitamin D										
L	58 (23.2)	35 (60.3)	23 (39.7)	0.2	30 (51.7)	28 (48.3)	0.8	32 (55.1)	26 (44.9)	0.4
N	192 (76.8)	101 (52.6)	91 (47.4)		102 (53.1)	90 (46.9)		117 (61.0)	75 (39.0)	
Vitamin B_{12}										
L	2 (0.8)	0	2 (100)	0.3	0	2 (100)	0.2	1 (50.0)	1 (50.0)	0.6
N	248 (99.2)	136 (54.8)	112 (55.2)		132 (53.2)	116 (46.7)		148 (59.6)	100 (40.4)	
AST Last										
E	114 (45.6)	56 (49.1)	58 (50.9)	0.08	71 (51.1)	68 (48.9)	0.3	85 (61.2)	54 (38.8)	0.3
N	136 (54.4)	80 (58.8)	56 (41.2)		61 (55.0)	50 (45.0)		64 (57.7)	47 (42.3)	
ALT last										
E	139 (55.6)	69 (49.6)	70 (50.4)	0.6	61 (53.5)	53 (46.5)	0.5	71 (62.3)	43 (37.7)	0.3
N	111 (44.4)	67 (60.4)	44 (39.6)		71 (52.2)	65 (47.8)		78 (57.4)	58 (42.6)	
Hb last										
L	216 (86.4)	116 (53.7)	100 (56.3)	0.5	116 (53.7)	100 (46.3)	0.4	127 (58.7)	89 (41.3)	0.3
N	34 (13.6)	20 (58.8)	14 (41.2)		16 (47.0)	18 (53.0)		22 (64.7)	12 (35.3)	
TLC last										
L	135 (54.0)	76 (56.2)	59 (43.8)	0.5	66 (48.8)	69 (51.2)	0.17	82 (60.7)	53 (39.3)	0.6
N	115 (56.0)	60 (52.1)	55 (57.9)		66 (57.3)	49 (42.7)		67 (58.2)	48 (41.2)	
Platelets last										
L	232 (92.8)	124 (53.4)	108 (56.6)	0.2	120 (51.7)	112 (49.3)	0.2	138 (59.4)	94 (41.6)	0.8
N	18 (7.2)	12 (66.7)	6 (33.3)		12 (66.6)	6 (33.3)		11 (61.1)	7 (38.9)	

SVR: Sustained virological response, USG: Ultra sonography, EGD: Esophagogastroduodenoscopy, FL: Fatty liver, LGV: Low grade varices, E: Elevated, N: Normal, DM: Diabetes mellitus, ALP: Alkaline phosphatase, Hb: Hemoglobin, TLC: Total leukocytic count, PT: Prothrombin time, INR: International normalized ratio , ALT: Alanine transaminase: AST: Aspartate transaminase, Last: At the end of treatment, TG: Triglyceride

Table 5: Association of various biochemical and risk factors of hepatitis C virus patients with sustained virological response and viral genotype

Overall genotype	Cases (*n*=250), *n* (%)	SVR		OR (95%CI)	*P*	Genotype		OR (95%CI)	*P*
		No 8 (3.2)	Yes 242 (96.8)			1 63 (25.2)	3 187 (74.8)		
Age group									
<50	185 (74.0)	2 (1.1)	183 (98.9)	0.1 (0.02-0.5)	0.005	36 (19.5)	149 (80.5)	0.3 (0.2-0.6)	0.0004
≥50	65 (26.0)	6 (9.2)	59 (90.8)			27 (41.5)	38 (58.5)		
Sex									
Female	80 (32.0)	1 (1.2)	79 (98.8)	0.3 (0.03-2.4)	0.2	20 (25.0)	60 (75.0)	0.9 (0.5-1.8)	0.5
Male	170 (68.0)	7 (4.1)	163 (95.9)			43 (25.3)	127 (74.7)		
Weight (kg)									
≥70	73 (29.2)	5 (6.8)	68 (93.2)	4.2 (1.0-18.0)	0.04	23 (31.5)	50 (68.5)	1.5 (0.8-2.8)	0.09
<70	117 (69.8)	3 (1.7)	174 (98.3)			40 (22.6)	137 (77.4)		
USG									
FL	127 (50.8)	8 (6.3)	119 (93.7)	9.3 (1.1-74.5)	0.01	40 (31.5)	87 (68.5)	1.9 (1.1-3.5)	0.01
N	123 (49.2)	0	123 (100.0)			23 (18.7)	100 (81.3)		

Contd...

Table 5: Contd...

Overall genotype	Cases (*n*=250), *n* (%)	SVR		OR (95%CI)	*P*	Genotype		OR (95%CI)	*P*
		No 8 (3.2)	Yes 242 (96.8)			1 63 (25.2)	3 187 (74.8)		
EGD									
LGV	13 (5.2)	3 (23.1)	10 (76.9)	14.0 (2.9-66.5)	0.005	9 (69.2)	04 (30.8)	7.6 (2.2-75.7)	0.001
N	237 (94.8)	5 (2.1)	232 (97.9)			54 (22.8)	183 (77.2)		
DM									
No	213 (85.2)	4 (1.9)	209 (98.1)	0.1 (0.03-0.6)	0.02	54 (25.4)	159 (74.6)	1.0 (0.4-2.3)	0.5
Yes	37 (14.8)	4 (10.8)	33 (89.2)			09 (24.3)	28 (75.7)		
Bilirubin									
E	12 (5.0)	0	12 (100.0)	1.9 (0.2-16.0)	0.4	5 (41.7)	7 (58.3)	2.2 (0.6-7.2)	0.1
N	238 (95.0)	8 (3.4)	230 (96.6)			58 (24.4)	180 (78.6)		
ALT									
E	211 (84.4)	8 (3.8)	203 (96.2)	1.7 (0.2-14.3)	0.4	56 (26.5)	155 (73.5)	1.6 (0.6-3.90	0.1
N	39 (15.6)	0	39 (100)			07 (17.9)	32 (82.1)		
AST									
E	199 (79.6)	7 (3.5)	192 (96.5)	1.8 (0.2-15.1)	0.5	54 (27.1)	145 (72.9)	1.7 (0.7-3.8)	0.1
N	51 (20.4)	1 (2.0)	50 (98.0)			9 (17.6)	42 (82.4)		
ALP									
E	08 (3.2)	0	8 (100.0)	2.9 (0.3-25.4)	0.3	1 (12.5)	7 (87.5)	0.4 (0.05-3.4)	0.3
N	242 (96.8)	8 (3.3)	234 (96.7)			62 (25.6)	180 (74.4)		
Cholesterol									
E	78 (31.2)	5 (6.4)	73 (93.6)	3.8 (0.9-16.5)	0.06	28 (35.9)	50 (64.1)	2.1 (1.2-3.9)	0.008
N	172 (68.8)	3 (1.7)	169 (98.3)			35 (20.3)	137 (79.7)		
TG									
E	58 (23.2)	4 (6.9)	54 (93.1)	3.4 (0.8-14.3)	0.08	24 (41.4)	34 (58.6)	2.7 (1.4-5.1)	0.001
N	192 (76.8)	4 (2.1)	188 (97.9)			39 (20.3)	153 (79.7)		
LDL									
E	37 (14.8)	4 (10.8)	33 (89.2)	6.3 (1.5-26.5)	0.01	9 (24.3)	28 (75.7)	0.9 (0.4-2.1)	0.5
N	213 (85.2)	4 (1.9)	209 (98.1)			54 (25.4)	159 (74.6)		
TSH									
E	08 (3.2)	0	8 (100.0)	2.9 (0.3-25.4)	0.3	2 (25.0)	6 (75.0)	1.0 (0.2-5.0)	0.6
N	242 (96.8)	8 (3.3)	234 (96.7)			61 (25.2)	181 (74.8)		
Hb									
L	32 (12.8)	2 (6.2)	30 (93.8)	0.4 (0.08-2.2)	0.2	11 (34.3)	21 (65.7)	0.5 (0.2-1.3)	0.1
N	218 (87.2)	6 (2.7)	212 (97.3)			52 (23.8)	166 (76.2)		
TLC									
L	11 (4.4)	0	11 (100)	0.4 (0.05-	0.4	3 (27.2)	8 (72.7)	0.9 (0.2-3.4)	0.5
N	239 (95.6)	8 (3.3)	231 (96.7)	3.9)		60 (25.1)	179 (74.9)		
Platelets									
L	161 (64.4)	7 (4.3)	154 (95.7)	0.25 (0.03-2.0)	0.1	43 (26.7)	118 (73.3)	0.7 (0.4-1.4)	0.4
N	89 (35.6)	1 (1.1)	88 (98.9)			20 (22.4)	69 (77.6)		
Ferritin									
E	155 (62.0)	8 (5.1)	147 (94.9)	0.17 (0.02-1.3)	0.05	44 (28.3)	111 (71.7)	0.6 (0.3-1.1)	0.1
N	95 (38.0)	0	95 (100)			19 (20.0)	76 (80.0)		
Vitamin D									
L	58 (23.2)	2 (3.4)	56 (96.7)	0.9 (0.1-4.6)	0.5	15 (25.8)	43 (74.2)	0.9 (0.4-1.8)	0.8
N	192 (76.8)	6 (3.1)	186 (96.9)			48 (25.0)	144 (75.0)		
Vitamin B12									
L	02 (0.8)	0	02 (100)	0.1 (0.01-1.1)	0.14	01 (50.0)	01 (50.0)	0.3 (0.02-5.4)	0.4
N	248 (99.2)	8 (3.2)	240 (96.8)			62 (25.0)	186 (75.0)		
AST last									
E	114 (45.6)	7 (6.1)	107 (93.9)	8.8 (1.0-72.8)	0.01	37 (32.5)	77 (67.5)	2.0 (1.1-3.6)	0.01
N	136 (54.4)	1 (0.7)	35 (99.3)			26 (19.1)	110 (80.9)		
ALT last									

Contd...

Table 5: Contd...

Overall genotype	Cases ($n=250$), n (%)	SVR		OR (95%CI)	*P*	Genotype		OR (95%CI)	*P*
		No 8 (3.2)	Yes 242 (96.8)			1 63 (25.2)	3 187 (74.8)		
E	139 (55.6)	7 (5.0)	132 (95.0)	5.8 (0.7-48.1)	0.06	34 (24.5)	105 (75.5)	0.9 (0.5-1.6)	0.4
N	111 (44.4)	1 (0.9)	10 (99.1)			29 (26.1)	82 (73.9)		
Hb last									
L	216 (86.4)	7 (3.2)	209 (96.8)	0.9 (0.1-7.5)	0.7	58 (26.8)	158 (73.2)	0.4 (0.17-1.2)	0.1
N	34 (13.6)	1 (2.9)	33 (97.1)			05 (14.7)	29 (85.3)		
TLC last									
L	135 (54.0)	5 (3.7)	130 (96.3)	0.6 (0.16-2.9)	0.4	36 (26.6)	99 (73.4)	0.8 (0.4-1.5)	0.3
N	115 (56.0)	3 2.6)	112 (97.4)			27 (23.4)	88 (76.6)		
Platelets last									
L	232 (92.8)	8 (3.4)	224 (96.6)	1.3 (0.15-10.9)	0.5	57 (24.5)	175 (75.5)	1.5 (0.5-4.2)	0.2
N	18 (7.2)	0	18 (100)			6 (33.3)	12 (66.7)		

SVR: Sustained virological response, USG: Ultra sonography, EGD: Esophagogastroduodenoscopy, FL: Fatty liver, LGV: Low grade varices, E: Elevated, N: Normal, DM: Diabetes mellitus, ALP: Alkaline phosphatase, Hb: Hemoglobin, TLC: Total leukocytic count, PT: Prothrombin time, INR: International normalized ratio, ALT: Alanine transaminase, AST: Aspartate transaminase, Last: At the end of treatment, OR: Odds ratio, CI: Confidence interval, TG: Triglyceride

significantly associated with EGD, DM, TG, ALT Last, and AST ($P < 0.05$). All the three SNPs were significantly associated with each other ($P < 0.05$).

Discussion

The basic aim of this study was to find out the association of viral and host genotypes with biochemical and other risk factors of HCV infected patients in relation to their treatment outcome. Outnumbering of favorable genotypes, good responders, genotype 3 virus, normal lipid profiles, nondiabetics along with normal vitamin status has been the major observations in this study. Similarly, apart from being significantly associated with each other a positive association of polymorphic SNPs with SVR, viral genotype, BMI, NAFLD, liver enzymes was observed along with a positive association of SVR and Viral genotype with age, NAFLD, diabetes mellitus, low-grade varices and lipid profile. Although many inferences have been made from this study, there is still a lot to be known from the investigations of this kind.

Since screening methods were the major source of detection of CHC in our study and probably males frequently participate in screening camps than females, it was no surprise that majority of our HCV infected patients were males (68%; 170 of 250) which is also consistent with many studies from India and abroad[14] [Table 1].

History of diabetes mellitus was present in 37 (14.8%) patients which is comparable to around 15% reported by Loguercio *et al*.[15] but higher than 8.2% reported by Tohra *et al*.[16] Recently, the presence and severity of hepatic steatosis have emerged as an important marker of progressive liver disease as well as virological response to anti-HCV therapy.[17] The LDL receptors could permit the entry of HCV in hepatocytes leading to decreased serum LDL levels.[18] Moreover, HCV replication could decrease intrahepatic cholesterol synthesis[18] supporting the presence of normal lipid profile in our set of patients [Table 2].

Majority of studies support the association of SNPs near the *IL28B* gene region encoding IFN lambda 3 with virological response and spontaneous elimination of the virus.[19] Little is known about the IFN-λ family, but the evidence is mounting to support a role for them in the immune response to viral infections. Therefore, associations made between IL28B variants and HCV clearance in large-scale genetic studies provide an exciting mechanistic link between innate immunity and viral clearance. In this study, we genotyped three *IL28B* polymorphisms in 250 HCV infected patients in which the regularity of wild (favorable) allele was greater than variant alleles in all the analyzed SNPs giving the preliminary thought about the encouraging antiviral therapy response of HCV infected patients of valley. In case of rs12979860 SNP frequency of wild genotype (CC) was 54.4% which is higher than reported by previous studies.[20] On elucidation of *rs*12980275 SNP, the frequency of wild genotype (AA) was 52.8% which is higher than the frequency of 39% reported by Fattovich *et al*.[21] 59.6% in our patients were wild (TT) for *rs*8099917 SNP which is comparable to 56% reported by Fattovich *et al*.[22] and many other studies[23] [Table 4].

The study showed significant association of *IL28B rs*12979860 (CC) and *rs*8099917 (TT) favorable alleles with HCV genotype 2/3 in agreement with several studies.[24] Favorable genotypes of *rs*12979860, *rs*12980275, and *rs*8099917 were found to be significantly associated with SVR ($P < 0.05$) which has previously been validated.[19] The presence of variant alleles of *IL28B* SNPs has been linked to severe hepatic steatosis[21] in treaty with our study where we observed the higher percentage of variant allele for *rs*12980275 SNP ($P < 0.05$) in patients with NAFLD. Surprisingly, wild allele of rs8099917 SNP was strongly associated with elevated baseline ALP levels and decreased platelet count ($P < 0.05$) which is in contrast to many studies[25] [Table 4].

Table 6: Multivariate analysis viral genotype and single nucleotide polymorphisms with various parameters by logistic regression

Variables	Wald	*P*	OR (95% CI)
Viral genotype			
Age in years			
<50	6.505	0.011	0.36 (0.17-79)
≥50			
EGD			
LGV	3.660	0.056	4.59 (0.96-1.90)
Normal			
DM			
No	5.225	0.022	0.28 (0.09-0.83)
Yes			
TG (mg/dL)			
Elevated	9.776	0.002	3.30 (1.56-6.98)
Normal			
ALT last (U/L)			
Elevated	10.462	0.001	0.14 (0.04-0.46)
Normal			
AST last (U/L)			
Elevated	10.063	0.002	6.44 (2.03-20.39)
Normal			
rs12979860 (C to T)			
AST last (U/L)			
Elevated	4.36	0.03	0.56 (0.33-0.96)
Normal			
rs12980275			
AA	5.77	0.01	0.51 (0.30-0.88)
AG + GG			
rs8099917			
TT	6.18	0.01	0.50 (0.29-0.86)
TG + GG			
rs12980275 (A to G)			
USG group			
Group I and II FL	6.36	0.01	0.50 (0.29-0.85)
Normal			
rs8099917			
TT	4.30	0.03	0.55 (0.32-0.96)
TG + GG			
rs12979860			
CC	5.18	0.02	0.53 (0.31-0.91)
CT + TT			
rs8099917 (T to G)			
AST (U/L)			
Elevated	3.88	0.04	1.97 (1.00-3.87)
Normal			
rs12979860			
CC	7.25	0.00	0.46 (0.26-0.81)
CT + TT			
rs12980275			
AA	3.92	0.04	0.57 (0.33-0.99)
AG + GG			

OR: Odds ratio, CI: Confidence interval,
EGD: Esophagogastroduodenoscopy, LGV: Low grade varices,
DM: Diabetes mellitus, ALT: Alanine transaminase, AST: Aspartate transaminase, TG: Triglyceride, FL: Fatty liver

Of the 250, 238 (96.8%) went on to attain SVR [Table 5]. Young patients (<50 years) were found to have an excellent outcome on treatment in compliance with many studies.[26] Overweight and increased BMI is a negative predictor of SVR in HCV-infected patients.[15] Since the mean weight and BMI was lower in our patients, the SVR was achieved in the majority of cases. Different studies have revealed that NAFLD, higher grades of fatty liver and hepatic steatosis are associated with poor SVR[27] in harmony with our observations. Almost all the patients with normal EGD attained SVR in our study supporting the presence of varices as an independent factor associated with poor SVR.[28] The presence of insulin resistance and other factors associated with it such as diabetes mellitus and high LDL levels have been shown to have a negative impact on achieving SVR[29] in coherence with our study. As obese patients are known to have poor lymphatic circulation, this effect can limit the serum levels of pegylated IFN-a, leading to a reduction in SVR rates.[30] Almost all the patients with SVR had normal AST levels at the end of treatment ($P < 0.05$) which does not support the majority of studies established previously.[31] Although many studies have indicated that low Vitamin D and Vitamin B_{12} levels are associated with poor treatment outcome,[32] we did not find any correlation between vitamin levels and SVR.

When stratified according to viral genotype the majority of patients were infected with genotype 3 alike many studies.[30] In contrast to the majority of studies,[33] there was a significantly higher percentage of patients with ≥50 years of age in genotype 3 than genotype 1 (58.5% vs. 41.5%; $P = 0.0004$). Nonalcoholic steatohepatitis (NASH) is a more advanced form of NAFLD in which there is inflammation in and around the fatty liver cells. NASH is now considered to be one of the main causes of cirrhosis.[34] Among the patients with NAFLD, 31.5% were infected with genotype 1 virus as against 18.7% of patients in non-NAFLD group infected with the same genotype ($P = 0.01$) which is in contradiction to previous studies showing a strong association between genotype 3 and steatosis.[34] High serum cholesterol and serum triglyceride were associated with genotype 1 ($P = 0.008$ and 0.001). Ching-Sheng *et al.* demonstrated the association of elevated lipid profile with viral genotype 2.[35] Genotype 3 HCV has been correlated with dyslipidemia.[36] However, the differential associations of lipid profiles between different viral genotypes remain largely unknown and deserve further studies. Of the various laboratory parameters which were significantly higher in genotype 1 compared to genotype 3 included AST levels (at the end of treatment; $P = 0.01$) in accordance with many studies[37] [Table 5].

Conclusions

Although the *IL28B* sequence variation is one of the major factors that can influence response rates to antiviral therapy, viral and biochemical factors also have a definite role to play in the diagnosis, etiology, and treatment outcome in HCV infected patients. A greater understanding of the mechanism behind the association of host and viral factors should provide insight into viral/host interactions leading to opportunities for improved anti-HCV therapeutics and more effective individualized therapy.

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Conflicts of interest

There are no conflicts of interest.

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