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Original Article

Impact of *IL28B* genetic variant's and viral genotype on treatment outcome of hepatitis C infected patients

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Abstract

Introduction: Viral genotype and variation in host genes involved in the immune response may predict the treatment response in patients infected with HCV. The present study was designed to determine the distribution pattern of HCV and host genotypes in Chronic Hepatitis C (CHC) patients and their association with virological response and other risk factors.

Methodology: Two hundred and fifty (n = 250) HCV positive patients were included in the study. HCV and Interleukin 28B (*IL28B*) genotyping was carried out by PCR-RFLP.

Results: Viral genotype 3 was the predominant genotype seen in 187 (74.8%) patients. Wild genotype predominated in *rs*12979860, *rs*12980275 and *rs*8099917 SNP of *IL28B* gene. A significant difference was found in end stage virological response (EVR) between HCV genotype 1 infected patients with wild and variant genotype for *rs*12980275 and *rs*8099917 SNPs respectively ($P < 0.05$). On multivariate analysis all the SNPs were found to be associated with each other ($P < 0.05$) with *rs*12980275 SNP associated with history of Jaundice ($P < 0.05$). Viral genotype 3 was significantly associated with age (< 50 years) and rapid virological response (RVR) while as viral genotype 1 was significantly associated with history of surgery on multivariate analysis ($P < 0.05$).

Conclusions: The viral genotype and *IL28B* polymorphisms are important factors to personalize antiviral therapy of patients with CHC.

Key words: Hepatitis C; chronic hepatitis C; IL28B; viral genotype; PCR-RFLP; hepatocellular carcinoma.

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Introduction

It is now well established that HCV infection affects all countries and chronic infection with HCV is a global health problem that affects more than 180 million people worldwide, approximately 3% of the worldwide population is infected with the HCV [1,2]. In Asia, the prevalence of HCV ranges between 2% and 5% [1,3]. It is estimated that in India approximately 1.8-2.5% of the population is presently infected by HCV and about 20 million people are already having HCV infection [3]. HCV infection leads to chronic liver disease in 60–70% of patients, 5–20% will develop cirrhosis, and 1–5% will die of complications including hepatocellular carcinoma (HCC) [4,5]. Direct acting antivirals like protease inhibitors are expensive and not available at all medical centers, therefore the most effective current standard therapy for chronic HCV infection consists of long-acting pegylated interferon-α plus oral treatment with ribavirin (Peg-IFN-a/R) [6,7].

Virus-specific characteristics (viral load, genotype) and clinical parameters (age, gender, BMI, Stage of liver fibrosis and liver enzymes) are among the baseline predictors of response to HCV treatment [8]. Moreover, host genetic variation may influence the response to HCV treatment and is assumed to explain the heterogeneity in HCV clearance across individuals. Several independent studies have consistently shown that single nucleotide polymorphisms (SNPs) near *IL28B,* which encodes the type III interferon IFN-λ3 are strongly associated with the response to treatment of chronic hepatitis C [9,10] and affects the spontaneous and induced clearance of HCV and progression to CHC [11]. The mechanism by which SNPs influence the outcome of HCV infection and its treatment is completely explained. The first and largest genome wide association study (GWAS) identified a top discovery SNP, rs12979860, which is located on chromosome 19q13, 3kb upstream of the *IL28B* gene, which codes for IFN-λ-3 [9,12]. Following this

discovery, other SNP, rs8099917 has been identified and is in linkage disequilibrium with the previous SNP. In particular, the homozygous genotypes CC at marker *rs*12979860, AA at marker *rs*12980275 and TT at marker *rs*8099917 are all associated with favorable treatment outcomes [13]. These data have been confirmed in multiple ethnic groups, populations of different ancestry and HCV genotypes, and in various clinical scenarios [14]. Multiple clinical cohort studies have validated the findings of the GWA studies and regression modelling has shown that the *IL28B* polymorphism is the strongest baseline predictor of SVR in patients treated with Peg-IFN-a/R [15,16]. *IL28B* genotype testing has become part of the standard of care as it can predict which patients are more likely to respond to treatment [2].

A sizeable chunk of patients with hepatitis C are being treated in our clinical setting at out tertiary care hospital where we observe that clinical response is quite good in majority of patients. With this background in mind, the present study was conceived to study the rate of response and predictors of response based on clinical, viral and genetic characteristics in patients with chronic HCV infection who were treated with a combination of Peg-IFN-a/R in a superspeciality hospital in the valley of Kashmir.

Methodology

Patient Samples

The present study was a prospective non-randomized open labeled clinical trial conducted between July 2013 and July 2016 in the Department of Gastroenterology and Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Kashmir, India. All consecutive patients (n = 250) above age of 14 years with hepatitis C infection were enrolled. The diagnosis of HCV was established by the presence of HCV-RNA level greater than 50 IU/mL. The study was approved by the Ethical Committee of SKIMS, Soura-190011, Srinagar, India. Written informed consent was obtained from all patients before taking their blood samples.

HCV 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). Identification of HCV genotype was carried out by PCR-RFLP using the method of Chinchai *et al.* [17]. In HCV RNA positive samples, subtypes were determined by performing PCR using type specific primers for the core region of the HCV genome [18].

HCV 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.

Intervention

The patients underwent interventional treatment in accordance with the standard protocol consisting of 180µg of peg interferon α-2a or 1.5µg/kilogram of body weight of pegylated interferon α-2b administered subcutaneously once per week. In genotype 1 and 4 patients, 1000 mg and 1200 mg of ribavirin was administered orally in daily doses in patients < 75 kg and > 75 kg of weight respectively. In genotype 2 and 3 patients, ribavirin was administered orally in 800 mg daily doses.

IL28B gene polymorphism

High-molecular-weight DNA was isolated from blood samples using DNA extraction kit (Zymo Research, USA). The amplification reaction was carried out in 25 µL reaction volume in a 0.2 mL PCR tubes to amplify *IL28B* gene encompassing the polymorphisms under study. Polymerase chain reaction was set up using Genomic DNA: 250 ng/µL; 10X PCR buffer: 100 mM Tris-HCl, pH 8.3; 500 Mm KCl; 15 mM $MgCl_2$; 0.1% gelatin and 1% Triton X100; Deoxyribonucleotide triphosphate: 10 mM each dATP, dCTP, dGTP and dTTP; Primers: 10 µM in sterile deionized water and Taq DNA polymerase: 5U/µL. Table 1 shows three different sets of primers for amplification of respective exons of *IL28B* gene, annealing temperature, restriction enzymes and

Table 1. Primers and restriction enzymes used for screening the various SNPs in the upstream of IL28B gene.

SNP	Primer sequence	A_T (°C)	Product (bp)	RE*	DP**(bp)
rs12979860 (C to T)	F-5'-AGGGCCCCTAACCTCTGCACAGTCT-3' R-5'-GCTGAGGGACCGCTACGTAAGTCACC-3'	58	403	*BstUI*	184,105,89 and 25 (CC) 184,130 and 89 (TT)
rs12980275 (A to G)	F-5'-GAGAGCAAGAGGAGGGAAGGAA-3' R-5'-GTGTGCCATTAGCCAGTCAGAT-3'	58	441	*sl*I	121 and 320 (AA) 121, 30 and 290 (GG)
rs8099917 (T to G)	F-5'-TTCACCATCCTCCTCTCATCCCTCAT-3' R-5'-TCCTAAATTGACGGGCCATCTGTTTC-3'	58	401	*Mae*III	105,110 and 186 (TT) 105,110,39 and 147 (GG)

* RE: Restriction enzyme **DP: Digestion product.

Table 3. Levels of various demographic and baseline parameters.

S. No.	Parameter	Mean ± SD	Range
1	Height (meters)	1.65±0.15	1.5-1.87
2	Weight (Kgs)	64.5±17.1	35-96
3	BMI (Kg/m^2)	23.54±3.6	16-35
4	Age (years)	41.2±12.5	14-70
5	HCV RNA baseline (lac IU/ml)	13.8±0.7	0.04-198.6
6	HCV RNA at 4 weeks (lac IU/ml)	0.09±0.001	0-7.8
7	HCV RNA at 12 weeks (lac IU/ml)	0.34±0.01	0-69.7

Table 4. Association between rs12979860 (C to T) rs12980275 (A to G) and rs8099917 (T to G) Genotypes and Clinico-pathological Characteristics of Hepatitis C (HCV) infected patients.

Characteristics		Cases n (%)	rs12979860 (C to T)			rs12980275 (A to G)			rs8099917 (T to G)		
			CC n (%)	CT+TT n (%)	P value	AA n (%)	AG+GG n (%)	P value	TT n (%)	TG+GG n (%)	P value
		n = 250	**136(54.4)**	**114 (45.6)**		**132 (52.8)**	**118 (47.2)**		**149 (59.6)**	**101 (40.4)**	
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)	
Gender	F	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
	M	170 (68.0)	87 (51.2)	83 (48.8)		90 (52.9)	80 (47.1)		100 (58.8)	70 (41.2)	
Weight in Kgs	> 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)	
Dwelling	R	139 (55.6)	71 (51.1)	68 (48.9)	0.1	79 (56.8)	60 (43.2)	0.09	82 (59.0)	57 (41.0)	0.5
	U	111 (44.4)	65 (58.6)	46 (41.1)		53 (47.7)	58 (52.3)		67 (60.4)	44 (39.4)	
Smoking	No	157 (62.8)	85 (54.1)	72 (45.9)	0.5	86 (54.8)	71 (45.2)	0.2	93 (59.2)	64 (40.8)	0.5
	Yes	93 (37.2)	51 (54.8)	42 (45.2)		46 (49.5)	47 (50.5)		56 (60.2)	37 (39.8)	
Drug abuse	No	240 (96.0)	129 (53.8)	111 (46.2)	0.3	126 (52.5)	114 (47.5)	0.4	141 (58.8)	99 (41.2)	0.1
	Yes	10 (4.0)	07 (70.0)	03 (30.0)		06 (60.0)	04 (40.0)		08 (80.0)	02 (20.0)	
Alcohol use	No	240 (96.0)	132 (55.0)	108 (45.0)	0.27	126 (52.5)	114 (47.5)	0.4	142 (59.2)	98 (40.8)	0.4
	Yes	10 (4.0)	04 (40.0)	06 (60.0)		06 (60.0)	04 (40.0)		07 (70.0)	03 (30.0)	
Blood transfusion	No	169 (67.6)	93 (55.0)	76 (45.0)	0.4	91 (53.8)	78 (46.2)	0.3	102 (60.4)	67 (39.6)	0.4
	Yes	81 (32.4)	43 (53.1)	38 (46.9)		41 (50.6)	40 (49.4)		47 (58.0)	34 (42.0)	
Family history	No	220 (88.0)	119 (54.1)	101 (45.9)	0.4	114 (51.8)	106 (48.2)	0.2	126 (57.3)	94 (42.7)	**0.03**
	Yes	30 (12.0)	17 (56.7)	13 (43.3)		18 (60.0)	12 (40.0)		23 (76.7)	07 (23.3)	
Tooth extraction	No	195 (78.0)	27 (49.1)	28 (50.9)	0.2	28 (60.0)	27 (40.0)	0.4	30 (54.5)	25 (45.5)	0.2
	Yes	55 (22.0)	109 (55.9)	86 (44.1)		104 (53.3)	98 (46.7)		119 (61.0)	76 (39.0)	
Needle prick	No	234 (93.6)	126 (53.8)	108 (46.2)	0.34	125 (53.4)	109 (46.6)	0.3	140 (59.8)	94 (40.2)	0.5
	Yes	16 (6.4)	10 (62.5)	06 (37.5)		07 (43.8)	09 (56.2)		09 (56.2)	07 (43.8)	
History of jaundice	No	191 (76.4)	100 (52.4)	91 (47.6)	0.15	108 (56.5)	83 (43.5)	**0.02**	115 (60.2)	76 (39.8)	0.4
	Yes	59 (23.6)	36 (61.0)	23 (39)		24 (40.7)	35 (59.3)		34 (57.6)	25 (42.4)	
Sharing razor	No	246 (98.4)	135 (54.9)	111 (45.1)	0.3	131 (53.3)	115 (46.7)	0.3	147 (59.8)	99 (40.2)	0.5
	Yes	04 (1.6)	01 (25.0)	03 (75.0)		01 (25)	03 (75.0)		02 (50.0)	02 (50.0)	
Use of same needle	No	241 (96.4)	130 (53.7)	111 (46.1)	0.3	127 (52.7)	114 (47.3)	0.6	142 (58.9)	99 (41.1)	0.2
	Yes	09 (3.6)	06 (66.7)	03 (33.3)		05 (55.6)	04 (44.4)		07 (77.8)	02 (22.2)	
Surgery in past	No	128 (51.2)	71 (55.5)	57 (44.5)	0.4	67 (52.3)	61 (47.7)	0.5	73 (57.0)	55 (43.0)	0.2
	Yes	122 (48.8)	65 (53.3)	57 (46.7)		65 (53.3)	57 (46.7)		76 (62.3)	46 (37.7)	
Interferon	α2a	82 (32.8)	45 (54.9)	37 (45.1)	0.5	43 (52.4)	39 (47.6)	0.5	47 (57.3)	35 (42.7)	0.3
	α 2b	168 (67.2)	91 (54.2)	77 (45.8)		89 (53.0)	79 (47.0)		102 (60.7)	66 (39.3)	
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)	
Sexual activity	MP	06 (2.4)	03 (50.0)	03 (50.0)	0.5	04 (66.7)	02 (33.3)	0.4	06 (100)	00 (00)	**0.04**
	SP	244 (97.6)	133 (54.5)	111 (45.5)		128 (52.5)	116 (47.5)		143 (58.6)	101 (41.4)	
RVR	No	65 (26.0)	30 (46.2)	35 (53.8)	0.08	29 (44.6)	36 (55.4)	0.08	28 (43.1)	37 (56.9)	**0.001**
	Yes	185 (74.0)	106 (57.3)	79 (42.7)		103 (55.7)	82 (44.3)		121 (65.4)	64 (34.6)	
EVR	No	10 (4.0)	03 (30.0)	07 (70.0)	0.1	01 (10.0)	09 (90.0)	**0.006**	01 (10.0)	09 (90.0)	**0.002**
	Yes	240 (96.0)	133 (55.4)	107 (44.6)		131 (54.6)	109 (45.4)		148 (61.7)	92 (38.3)	
ETR	No	02 (1.0)	00 (00)	02 (100)	0.2	00 (0.0)	02 (100)	0.2	00 (00)	02 (100)	0.1
	Yes	248 (99.0)	136 (54.8)	112 (45.2)		132 (53.2)	116 (46.8)		149 (60.1)	99 (39.9)	
SVR	No	08 (3.2)	01 (12.5)	07 (87.5)	**0.02**	00 (0.0)	08 (100)	**0.002**	00 (00)	08 (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)	

RVR: Rapid virological response, EVR: End stage virological response, SVR: Sustained virological response, ETR: End of treatment response, F: Female, M: Male, R: Rural, U: Urban, MP: Multiple partners, SP: Single partner.

Table 5. Association between rs8099917 (T to G), rs12980275 (A to G) and rs8099917 (T to G) genotypes and sustained virological response (SVR), rapid virological response (RVR), end stage virological response (EVR) with respect to viral genotype 1 and 3.

Viral genotype	Virological Response		rs12979860 (C to T)			rs12980275 (A to G)			rs8099917 (T to G)		
			CC (%)	CT+TT (%)	*P* value	AA (%)	AG+GG (%)	*P* value	TT (%)	TG+GG (%)	*P* value
1	SVR	No	00 (0.0)	04 (100.0)	0.09	00 (0.0)	04 (100.0)	**0.04**	00 (0.0)	04 (100.0)	**0.04**
		Yes	27 (45.8)	32 (54.2)		33 (55.9)	26 (44.1)		34 (57.6)	25 (42.4)	
3	SVR	No	01 (25)	03 (75)	0.2	00 (0.0)	04 (100.0)	**0.04**	00 (0.0)	04 (100.0)	**0.02**
		Yes	108 (59.0)	75 (41.0)		99 (54.1)	84 (45.9)		115 (62.8)	68 (37.2)	
1	RVR	No	10 (37.0)	17 (63.0)	0.2	11 (40.7)	16 (59.3)	0.08	10 (37.0)	17 (63.0)	**0.01**
		Yes	17 (47.2)	19 (52.8)		22 (61.1)	14 (38.9)		24 (66.7)	12 (33.3)	
3	RVR	No	20 (52.6)	18 (47.4)	0.2	18 (47.4)	20 (52.6)	0.2	18 (47.4)	20 (52.6)	**0.03**
		Yes	89 (59.7)	60 (40.3)		81 (54.4)	68 (45.6)		97 (65.1)	52 (34.9)	
1	EVR	No	02 (28.6)	05 (71.4)	0.3	00 (0.0)	07 (100.0)	**0.004**	00 (0.0)	07 (100.0)	**0.003**
		Yes	25 (44.6)	31 (55.4)		33 (58.9)	23 (41.1)		34 (60.7)	22 (39.3)	
3	EVR	No	01 (33.3)	02 (66.7)	0.3	01 (33.3)	02 (66.7)	0.4	01 (33.3)	02 (66.7)	0.3
		Yes	108 (58.7)	76 (41.3)		98 (53.3)	86 (46.7)		114 (62.0)	70 (38.0)	

SVR: Sustained virological response, RVR: Rapid virological response, EVR: End stage virological response, ETR: End of treatment response.

Table 6. Association of various parameters of Hepatitis C (HCV) Patients with Sustained Virological Response (SVR) and Viral Genotype.

		Cases n (%)	SVR		OR (95%CI)	P value	Genotype		OR (95%CI)	P Value
			No	Yes			1	3		
Overall genotype		**n = 250**	**08 (3.2)**	**242 (96.8)**			**63 (25.2)**	**187 (74.8)**		
Age group	< 50	185 (74.0)	02 (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)	06 (9.2)	59 (90.8)			27 (41.5)	38 (58.5)		
Gender	F	80 (32.0)	01 (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
	M	170 (68.0)	07 (4.1)	163 (95.9)			43 (25.3)	127 (74.7)		
Weight in Kgs	> 70	73 (29.2)	05 (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)	03 (1.7)	174 (98.3)			40 (22.6)	137 (77.4)		
Dwelling	R	139 (55.6)	03 (2.2)	136 (97.8)	0.4 (0.1-2.0)	0.2	37 (26.6)	102 (73.4)	1.1 (0.6-2.1)	0.3
	U	111 (44.4)	05 (4.5)	106 (95.5)			26 (23.4)	85 (76.6)		
Smoking	No	157 (62.8)	03 (1.9)	154 (98.1)	0.3 (0.08-1.4)	0.1	40 (25.5)	117 (74.5)	1.0 (0.5-1.8)	0.5
	Yes	93 (37.2)	05 (5.4)	88 (94.6)			23 (24.7)	70 (75.3)		
Drug abuse	No	240 (96.0)	08 (3.3)	232 (96.7)	0.4 (0.04-3.6)	0.7	62 (25.8)	178 (74.2)	3.1 (0.3-25.2)	0.2
	Yes	10 (4.0)	00 (0.0)	10 (100.0)			01 (10.0)	09 (90.0)		
Alcohol use	No	240 (96.0)	06 (2.5)	234 (97.5)	0.1 (0.01-0.6)	**0.03**	61 (25.4)	179 (74.6)	1.3 (0.2-6.5)	0.5
	Yes	10 (4.0)	02 (20.0)	08 (80.0)			02 (20.0)	08 (80.0)		
Blood transfusion	No	169 (67.6)	06 (3.6)	163 (96.54)	1.4 (0.3-7.3)	0.5	38 (22.5)	131 (77.5)	0.6 (0.3-1.1)	0.1
	Yes	81 (32.4)	02 (2.5)	79 (97.5)			25 (30.9)	56 (69.1)		
Family history	No	220 (88.0)	08 (3.6)	212 (96.4)	1.3 (0.16-10.6)	0.6	54 (24.5)	166 (75.5)	0.7 (0.3-1.7)	0.3
	Yes	30 (12.0)	00 (0.0)	30 (100.0)			09 (30.0)	21 (70.0)		
Dental extraction	No	195 (78.0)	04 (7.3)	51 (92.7)	3.7 (0.9-15.4)	0.07	11 (20.0)	44 (80.0)	0.7 (0.3-1.4)	0.2
	Yes	55 (22.0)	04 (2.1)	191 (97.9)			52 (26.7)	143 (73.3)		
Needle prick	No	234 (93.6)	06 (2.6)	228 (97.4)	0.2 (0.03-0.9)	0.08	59 (25.2)	175 (74.8)	1.0 (0.3-3.2)	0.6
	Yes	16 (6.4)	02 (12.5)	14 (87.5)			04 (25.0)	12 (75.0)		
History of jaundice	No	191 (76.4)	07 (3.7)	184 (96.3)	2.2 (0.2-18.3)	0.4	48 (25.1)	143 (74.9)	1.0 (0.5-1.9)	0.5
	Yes	59 (23.6)	01 (1.7)	58 (98.3)			15 (25.4)	44 (74.6)		
Sharing razor	No	246 (98.4)	08 (3.3)	238 (96.7)	0.2 (0.01-1.7)	0.3	63 (25.6)	183 (74.4)	1.7 (0.2-15.1)	0.5
	Yes	04 (1.6)	00 (0.0)	04 (100.0)			00 (0.0)	04 (100.0)		
Use of same needle	No	241 (96.4)	08 (3.3)	233 (96.7)	0.3 (0.04-3.3)	0.3	63 (26.1)	178 (73.9)	3.5 (0.4-28.4)	0.17
	Yes	09 (3.6)	00 (0.0)	09 (100.0)			00 (0.0)	09 (100.)		
Surgery in past	No	128 (51.2)	03 (2.3)	125 (97.7)	0.5 (0.1-2.4)	0.3	23 (18.0)	105 (82.0)	0.5 (0.2-0.8)	**0.005**
	Yes	122 (48.8)	05 (4.1)	117 (95.9)			40 (32.8)	82 (67.2)		
Interferon	α2a	82 (32.8)	04 (4.9)	78 (95.1)	2.1 (0.5-8.6)	0.2	14 (17.1)	68 (82.9)	0.5 (0.2-0.9)	**0.02**
	α2b	168 (67.2)	04 (2.4)	164 (97.6)			49 (29.2)	119 (70.8)		
Genotype	1	63 (25.2)	04 (6.3)	59 (93.7)	3.1 (0.7-12.7)	0.1	-	-	-	-
	3	187 (74.8)	04 (2.1)	183 (97.9)						
Sexual activity	MP	06 (2.4)	00 (0.0)	06 (96.7)	3.7 (0.4-33.0)	0.8	00 (0.0)	06 (100.0)	0.4 (0.05-3.3)	0.3
	SP	244 (97.6)	08 (3.3)	236 (100.0)			63 (25.8)	181 (74.2)		
RVR	No	65 (26.0)	06 (9.2)	59 (90.8)	9.3 (1.8-47.3)	**0.005**	27 (41.5)	38 (58.5)	2.9 (1.5-5.4)	**0.001**
	Yes	185 (74.0)	02 (1.1)	183 (98.9)			36 (19.5)	149 (80.5)		
EVR	No	10 (4.0)	06 (60.0)	04 (40.0)	178 (27.2-920)	**0.001**	07 (70.0)	03 (30.0)	7.6 (1.9-30.6)	**0.003**
	Yes	240 (96.0)	02 (0.8)	238 (99.2)			56 (23.3)	184 (76.7)		
ETR	No	02 (1.0)	02 (100)	00 (0.0)	104 (9.5-205)	**0.001**	01 (50.0)	01 (50.0)	3.0 (1.0-48.6)	0.4
	Yes	248 (99.0)	06 (2.4)	242 (97.6)			62 (25.0)	188 (75.0)		
SVR	No	08 (3.2)	-	-	-	-	04 (50.0)	04 (50.0)	3.1 (0.7-12.7)	0.1
	Yes	242 (96.8)					59 (24.4)	183 (75.6)		

RVR: Rapid virological response, EVR: End stage virological response, SVR: Sustained virological response, ETR: End of treatment response, R: Rural, U: Urban, F: Female, M: Male, MP: Multiple partners, SP: Single partner.

Variant genotypes of *rs*12980275 (AG+GG) and *rs*8099917 (TG+GG) were significantly associated with SVR in patients infected with genotype 1 as well as genotype 3 (P < 0.05). In case of *rs*8099917 SNP the variant genotypes were also significantly associated with SVR in patients infected with both viral genotypes (P < 0.05). All the non-EVR patients infected with only genotype 1 virus had variant genotype for *rs*12980275 and *rs*8099917 SNP (P < 0.05) (Table 5).

Most of the patients with < 50 years of age and < 70 kilograms of weight achieved SVR (P < 0.05). The patients having history of alcohol consumption were relatively poor responders (P < 0.05). Almost 99.0% of patients achieving RVR and EVR went on to achieve SVR (P < 0.05). 149 of 185 (80.5%) patients with < 50 years of age were infected with genotype 3 as against 19.5% patients infected with genotype 1 virus (P < 0.05). About 32% (40 of 122) patients having history of surgery in past were infected with genotype 1 and 68% were infected with genotype 3 (P < 0.05). Most of the patients infected with genotype 3 achieved RVR and EVR as compared to the patients infected with viral genotype 1 (P < 0.05). The various parameters in relation to SVR and viral genotypes are shown in Table 6.

Table 7 depicts the multivariate analysis of various parameters with respect to viral and host genotype by logistic regression. Viral genotype was significantly associated with age, Rapid virological response and history of surgery in past (P < 0.05). All the three SNPs were significantly associated with each other (P < 0.05). *rs*12980275 SNP was associated with history of Jaundice (P < 0.05).

Discussion

Hepatitis C infection is a leading cause of chronic hepatitis, cirrhosis and liver cancer and a primary indication for liver transplantation [4,5]. Currently, standard of care (SOC) for all HCV genotypes continues to be (Peg-IFN-a/R) for 24 weeks for genotypes 2 and 3 or 48 weeks for genotypes 1 and 4 due to the scarcity and expensiveness of oral antivirals in India. [6].

Of the 250 patients infected with HCV, alcohol intake and drug abuse was extremely uncommon in our patients (10 of 250; 04%) because of social and religious prohibitions. This is in sharp contrast to all other major studies from other parts of world including India where alcohol consumption varied from 50% to 74% [19,20] (Table 2).

Recent studies have demonstrated that single nucleotide polymorphisms (SNPs) near the *IL28B* gene are strongly associated with HCV patient's response to Peg-IFN-a/R treatment and spontaneous elimination of the virus in different populations [21,22]. A reasonable hypothesis might involve differential levels of

Table 7. Multivariate analysis of viral genotype and SNPs with various parameters by logistic regression.

Variables		Wald	P value	Odds ratio (95% C.I)
Viral genotype				
Age in years	< 50 ≥ 50	6.505	0.011	0.36 (0.17-79)
RVR	No Yes	7.258	0.007	0.38 (0.19 - 0.77)
Surgery in past	No Yes	5.376	0.020	2.26 (1.13 - 4.50)
***rs*12979860 (C to T)**				
rs12980275	AA AG+GG	5.77	0.01	0.51 (0.30 - 0.88)
rs8099917	TT TG+GG	6.18	0.01	0.50 (0.29 - 0.86)
***rs*12980275 (A to G)**				
rs8099917	TT TG+GG	4.30	0.03	0.55 (0.32 - 0.96)
History of jaundice	No Yes	4.67	0.03	0.50 (0.26 - 0.93)
rs12979860	CC CT+TT	5.18	0.02	0.53 (0.31 - 0.91)
***rs*8099917 (T to G)**				
rs12979860	CC CT+TT	7.25	0.001	0.46 (0.26 - 0.81)
rs12980275	AA AG+GG	3.92	0.04	0.57 (0.33 - 0.99)

RVR: Rapid virological response.

intrahepatic interferon-stimulated gene (ISG) expression according to *IL28B* genotype, where the good response variant was associated with lower levels of intrahepatic ISG expression, and therefore slightly higher levels of circulating serum HCV RNA. The mechanistic basis for this association is unknown, and it remains unclear whether it is driven by the host, the virus, or both [23].

In our study, we genotyped all three *IL28B* polymorphisms. Determination of *rs*12979860 *IL28B* genotypes has shown that CC genotype was found in 54.4% (136 of 250) of patients and variant genotype (CT+TT) was present in 45.6% of patients. In two independent studies distribution of *rs*12979860 CC genotype was 26.8% and 21%, respectively while as Fattoviich *et al.* reported 39% CC genotype [24-26]. In case of *rs*12980275 the frequency of wild and variant genotype (AA) was 52.8% which is higher than the frequency of 39% reported by Fattoviich *et al.* [26]. The frequency of *rs*8099917 TT genotype was 59.6% presented in our patients, comparable to 56% reported by Fattoviich *et al.* [26] (Table 3). So in this study, the incidence of favourable/wild genotypes was more frequent than other studies [16,27].

In this study, the effect of *IL28B* polymorphisms was evaluated comparing the favourable (wild) genotypes with the unfavourable (variant) ones. A positive correlation was found between wild genotype (TT) of *rs*8099917 SNP and RVR ($P < 0.05$) while as a positive correlation was also found between wild genotypes of *rs*12980275 and *rs*8099917 and EVR ($P < 0.05$). A strong association was found between favourable genotype of all the three SNPs of *IL28B* gene and SVR ($P < 0.05$) (Table 3). A number of studies have validated the effect of the favourable genotypes on RVR and SVR [24-27].

On analysing the effects of *IL28B* polymorphisms on SVR with respect to genotype 1 and 3 separately, the effect of the genotype *rs*12980275 and *rs*8099917 on SVR was found to be statistically significant in both viral genotypes ($P < 0.05$). Thus the *IL28B* gene polymorphism closely associates with the natural course and treatment response of CHC in different populations, irrespective of HCV genotype. However, when stratified according to EVR the effect of the genotype *rs*12980275 and *rs*8099917 was found to be statistically significant in case of patients infected with genotype 1 as compared to genotype 3 virus ($P < 0.05$) (Table 4).

Of the 250 patients, 238 (96.8%) went on to have undetectable HCV RNA after 6 months of treatment i.e. attained SVR (Table 5). Almost all the patients with < 50 years of age were found to achieve SVR ($P = 0.005$) in accordance with many studies [28-29]. In consistence with many studies it was observed that overweight individuals (≥ 70 kilograms) with CHC are less likely to achieve SVR ($P = 0.04$) [28,30]. 20% of patients with history of alcohol intake didn't achieve SVR compared to only 2.5% of non-alcoholics falling in non-SVR group. We found a significant impact of alcohol intake on SVR ($P = 0.03$) in accordance with previous reported studies [19,31]. Since it has been well validated from previous studies across the globe that alcoholism is associated with a poor treatment outcome, the virtual absence of alcohol intake in our patients may be an important factor for the high SVR in our population. Secondly, almost all the non-SVR group had a variant version of alleles in all the 3 SNPs; hampering the clearance of HCV as compared to SVR group where majority of alleles were wild for all the three SNPs of IL28 gene. Lastly, the Kashmir has an ethnic population with only inter-regional marriages which gives it a conserved gene pool; different from the rest of the country which might play an important role in the HCV clearance and perhaps the genetic basis of which needs to be elucidated in future. Although 98% of patients with genotype 3 achieved SVR, genotype 1 patients also showed very high SVR of 94% ($P>0.05$). So interestingly, in our study treatment response was independent of viral genotype which is in contradiction with majority of studies which associate genotype 1 and 4 with lower SVR [32-34].

RVR was achieved by 74.0% of patients which is considerably higher than most studies [32,33]. In all, 183 of 185 patients (98.9%) who achieved RVR went on to attain SVR ($P = 0.05$) which is in consistence with most of the studies [35,36]. In patients infected with HCV, the achievement of EVR is of major importance for the overall therapy success. All guidelines recommend to perform a quantitative assessment of HCV RNA at week 12 of treatment. EVR is highly predictive of SVR and provides a valuable tool to decide on continuation and duration of treatment, as well as providing patients with an additional motivation to adhere to treatment [37-39]. In this study, majority of the patients had attained EVR (240 out of 250; 96.0%). Such an extremely high rates of EVR has not been reported in any of the previous studies [32,33].

Viral genotype 1 was seen in 63 patients (25.2%) and genotype 3 was seen in 187 patients (74.8%) while as genotype 2, 4, 5 and 6 were absent in our cases (Table 5). Similar frequency of viral genotypes has been reported by many studies from India [40,41]. There was a significantly lower percentage of patients with ≥ 50

years of age in genotype 1 than genotype 3 (41.5% vs. 58.5%) which is in contrast to majority of studies where patients with genotype 1 or 2 were significantly older [42,43]. Majority of patients with genotype 1 were having past history of surgery compared to genotype 3 patients (40 of 63 vs. 82 of 187; P = 0.005). This is in agreement with other studies from different countries where majority of the cases with genotype 1 had history of hospitalization for major/minor surgery [44,45]. Of the 185 patients achieving RVR (74.0%) only 19.5% (36) belonged to genotype 1 while 80.5% (149) had genotype 3 (P = 0.001) and all the patients with genotype 3 went on to achieve complete EVR (P = 0.003). In other sense 36 out of 63 patients (57.1%) with genotype 1 while 149 out of 187 (79.6%) with genotype 3 achieved RVR which is considerably higher than most studies [32-35,46,47]. Although a few studies have recorded higher RVR but their overall SVR rates were lower than this study [48,49].

Conclusion

Viral Genotype 3 and *IL28B* wild genotype predominates in the HCV infected patients of this closed population. Although SVR was achieved by majority of patients and SVR was independent of viral genotype, *IL28B* polymorphisms might serve as important factor to personalize antiviral therapy of patients with CHC. However, reproduction of genetic results in various clinical settings is a prerequisite for establishing such therapy regimens.

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Authors' contributions

AS and MSK contributed equally in the designing the sampling protocol and performing the molecular and biochemical testing; AS, ZK and AHS recruited the patients; MSK and RAD performed the analysis and the interpretation of all the data; MSK and SM were involved in drafting the manuscript and revising it critically. SAZ participated in its design and coordination and provided all the infrastructure to carry out the experiments and patient's examination. All authors contributed to and approved the submitted manuscript.

References

1. World Health Organization WHO) (2016) Hepatitis C Fact Sheet. Available: http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-c. Accessed 17 May 2017.
2. Bellanti F, Vendemiale G, Altomare E, Serviddio G (2012) The impact of interferon lambda 3 gene polymorphism on natural course and treatment of hepatitis C. Clin Dev Immunol 1: 1-9.
3. Ponamgi SP, Chandra M. Naresh Kumar Y, Rahamathullah S, Narasu L, Habibullah CM, Khaja MN (2009) Genotype analysis and assessment of antigenic sensitivity for recombinant HCV proteins by indigenous SIBA for detection of Hepatitis C Virus infection: A comparison with 3rd EIA and RT-PCR. Indian J Biotechnol 8: 33-39.
4. Koziel M, Peters M (2007) Viral hepatitis in HIV infection. N Engl J Med 356: 1445-1454.
5. Wise M, Balek S, Fineli L, Bell BP, Sorvillo F (2008) Changing trends in hepatitis C-related mortality in the United States, 1995 to 2004. Hepatol 47: 1128-1135.
6. Ghany MG, Strader DB, Strader DB, Thomas DL, Seeff LB (2009) Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 49: 1335-13374.
7. Muir AJ, Bornstein JD, Killenberg PG (2004) Peg interferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. New Engl J Med 350: 2265-2271.
8. Kau A, Vermehren J, Sarrazin C (2008) Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol 49: 634-651.
9. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461: 399-401.
10. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J (2009) IL28B is associated with response to chronic hepatitis C interferon-A and ribavirin therapy. Nat Genet 41: 1100-1104.
11. Koziel M, Peters M (2007) Viral hepatitis in HIV infection. N Engl J Med 356: 1445–1454.
12. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41: 1105-1109.
13. Derbala M, Rizk NM, Al-Kaabi S, John A, Sharma M, El-dweik N, Yakoob R, Pasic F, Almohanadi M, Alejji K, Abdelmola A, Butt M (2013) The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients. Virology 444: 292-300.
14. De Nicola S, Aghemo A, Rumi MG, Galmozzi E, Valenti L, Soffredini R, De Francesco R, Prati GM, D'Ambrosio R, Cheroni C, Donato MF, Colombo M (2012) Interleukin 28B polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. Hepatology 55: 336-342.
15. Jiménez-Sousa MA, Fernández-Rodríguez A, Guzmán-Fulgencio M, García-Álvarez M, Resino S (2013) Meta-

analysis: implications of interleukin-28B polymorphisms in spontaneous and treatment-related clearance for patients with hepatitis C BMC Med 8: 6-15.
16. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG (2010) Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in hepatitis C virus-1patients. Gastroenterology 139: 120-129.
17. Chinchai T, Labout J, Noppornpanth S, Theamboonlers A, Haagmans BL, Osterhaus AD, Poovorawan Y (2003) Comparative study of different methods to genotype hepatitis C virus type 6 variants. J. Virol. Methods 109: 195-201.
18. Ohno T, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY (1997) New hepatitis C virus genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 4, 5a and 6a. J. Clin. Microbiol. 35: 201-207.
19. Tohra SK, Taneja S, Ghosh S, Sharma BK, Duseja A, Dhiman RK, Das A, Chawla YK (2011) Prediction of sustained virological response to combination therapy with pegylated interferon alfa and ribavirin in patients with genotype 3 chronic hepatitis C. Dig Dis Sci 56: 2449–2455.
20. Sood A, Midha V, Mehta V, Sharma S, Mittal R, Thara A, Sood N, Kaur A (2010) How sustained is sustained viral response in patients with hepatitis C virus infection? Indian J Gastroenterol 29: 112-115.
21. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY (2010) Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138: 1338–1345.
22. Thomas D, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M (2009) Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461: 798–801.
23. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, Hong L, McKenzie A, Patel K, Shianna KV, McHutchison JG, Goldstein DB, Afdhal N (2010) IL28B Genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology 52: 1888-1896.
24. Mach T, Ciesla A, Sanak M, Glowacki MK, Warunek W, Owczarek D (2012) The importance of IL28B polymorphism in response to pegylated Interferon α and ribavirin in chronic hepatitis caused by HCV Genotype 1b. Prz. Gastroenterol. 7:38-42.
25. Kozlowski P, Pogorzelska J, Łapiński TW, Kowalczuk O, Nikliński J, Flisiak R (2012) The occurrence of HCV genotypes and single polynucleotide polymorphisms of rs12979860 among HCV infected patients in northeastern Poland J Przegl Epidemiol 66: 335-339.
26. Fattovich G, Covolo L, Bibert S, Askarieh G, Lagging M, Clément S, Malerba G, Pasino M, Guido M, Puoti M, Gaeta GB, Santantonio T, Raimondo G, Bruno R, Bochud PY, Donato F, Negro F (2011) ITAHEC Study Group IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C. Aliment Pharmacol Ther 33: 1162-1172.
27. McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG (2010) Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology 138 :2307-2314.
28. Rosen HR (2011) Clinical practice-chronic hepatitis C infection. N Engl J Med 364: 2429-2438.
29. Asselah T, Estrabaud E, Bieche I, Lapalus M, De Muynck S, Vidaud M, Saadoun D, Soumelis V, Marcellin P (2010) Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int 30: 1259-1269.
30. Loguercio C, Federico A, Masarone M, Torella R, Blanco Cdel V, Persico M (2008) The impact of diet on liver fibrosis and on response to interferon therapy in patients with HCV-related chronic hepatitis. Am J Gastroenterol 103: 3159-3166.
31. Ohnishi K, Matsuo S, Matsutani K, Itahashi M, Kakihara K, Suzuki K, Ito S, Fujiwara K (1996) Interferon therapy for chronic hepatitis C in habitual drinkers: comparison with chronic hepatitis C in infrequent drinkers. Am J Gastroenterol 91: 1374-1379.
32. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK (2001) Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 358: 958-965.
33. Borroni G, Andreoletti M, Casiraghi MA, Ceriani R, Guerzoni P, Omazzi B, Terreni N, Salerno F (2008) Effectiveness of pegylated interferon/ribavirin combination in 'real world' patients with chronic hepatitis C virus infection. Aliment Pharmacol Ther 27: 790-797.
34. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM (2004) Peginterferon-alpha-2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 140: 346-355.
35. Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Solá R, Shafran SD, Barange K, Lin A, Soman A, Zeuzem S (2007) Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 357: 124-123.
36. Dalgard O, Bjøro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlund E, Reichard O, Myrvang B, Sundelöf B, Ritland S, Hellum K, Frydén A, Florholmen J, Verbaan H (2008) Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology 47: 35–42.
37. Lin CY, Sheen IS, Chen JY, Huang CW, Huang CH, Jeng WJ, Chen WT (2013) Various predictors of sustained virologic response in different age groups of patients with genotype-1 chronic hepatitis C. J Clin Gastroenterol 47: 794-799.
38. García-Samaniego J, Sheen IS, Chen JY, Huang CW, Huang CH, Jeng WJ, Chen WT (2013) Factors associated with early virological response to peginterferon-α-2a/ribavirin in chronic hepatitis C. World J Gastroenterol 19: 1943-1952.

39. Nachnani JS, Gidwani R, Sadeddin E, Clarkston WK, Fiorella R, Alba LM (2007) Clinicopathological predictors to predict sustained viral response rates in patients with chronic hepatitis C infection. Indian J Gastroenterol 26: 279-282.
40. Singh S, Malhotra V, Sarin SK (2004) Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in India. Indian J Med Res 119: 145-148.
41. Chakravarti A, Dogra G, Verma V, Srivastava AP (2011) Distribution pattern of HCV genotypes & its association with viral load. Indian J Med Res 133: 326-331.
42. Iqbal S, Khalil-Ur-Rahman, Sheikh MA, Arshad M (2014) Response of different HCV genotypes to interferon therapy in different age groups of chronic Hepatitis-C patients. J Ayub Med Coll Abbottabad 26: 310-315.
43. Haushofer AC, Kopty C, Hauer R, Brunner H, Halbmayer WM (2001) HCV genotypes and age distribution in patients of Vienna and surrounding areas. J Clin Virol 20: 41-47.
44. Skamperle M, Seme K, Lunar MM, Maver PJ, Tomažič J, Vovko TD, Pečavar B, Matičič M, Poljak M (2014) Prevalence, genotype distribution, and risk factors for hepatitis C infection among HIV-infected individuals in Slovenia: a 1986–2013 update. Acta Dermatovenerol Alp Pannonica Adriat 23: 25–26.
45. Daw MA, El-Bouzedi A, Dau AA (2015) Geographic distribution of HCV genotypes in Libya and analysis of risk factors involved in their transmission. BMC Res. Notes 8: 367-376.
46. Lagging M, Langeland N, Pedersen C, Färkkilä M, Buhl MR, Mørch K, Dhillon AP, Alsiö A, Hellstrand K, Westin J, Norkrans G (2008) Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. Hepatology 47: 1837–1845.
47. Mecenate F, Pellicelli AM, Barbaro G, Romano M, Barlattani A, Mazzoni E, Bonaventura ME, Nosotti L, Arcuri P, Picardi A, Barbarini G, D'Ambrosio C, Paffetti A, Andreoli A, Soccorsi F (2010) Short versus standard treatment with pegylated interferon alfa-2A plus ribavirin in patients with hepatitis C virus genotype 2 or 3: the cleo trial. BMC Gastroenterol 19: 10–21.
48. Yu ML, Dai CY, Huang JF, Hou NJ, Lee LP, Hsieh MY, Chiu CF, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL (2007) A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. Gut 56: 553–559.
49. Von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Häussinger D, Herrmann E, Zeuzem S (2005) Peginterferon- alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 129: 522–527.

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