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Comparative Study of Interleukin 28B Gene in Genotype 3 Chronic Hepatitis C Patients Before and After Treated With Interferon/Pegylated in Sindh

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Abstract: Chronic hepatitis C (HCV) caused by hepatitis C (HCV) virus, approximately estimated 170-200 million individuals in the world including Pakistan having 10 million HCV infected . The effective available treatment option is a combination of Pegylated interferon (PegIFN) and Ribavirin (RBV) . Recently Genome wide association studies (GAWAS)supporting two single nucleotide polymorphism SNPs adjacent interleukin 28B gene associated with spontaneous and treatment induced clearance of HCV patients. The aim of the present study was to analysis the impact of response associated interleukin 28B(IL28B) single nucleotide polymorphism (SNPs) rs12979860, rs8099917 in different treatment interval with one month (RVR) and after end of treatment (SVR) with Pegylated Interferon(PEG IFN) and Ribavirin in Hepatitis C genotype 3 patients. The present study enrolled 200 HCV RNA positive genotype 3 patients having both SNPs (rs12979860, rs8099917) were analyzed by Real Time Polymerase Chain Reaction (RT-PCR). The frequency distribution of IL28B genotypes were investigated by SNIPs (rs12979860 and rs8099917) in HCV RNA positive genotype 3 patients in Pakistani population.

1. INTRODUCTION

Hepatitis C (HCV) virus was cloned in 1989 (Choo *et al.,* 1989) approximately diameter 50-80 nm and belongs to Flavivaridae super family (Bostan and Mahmood, 2010; Catanese *et al.,* 2013; Knipe and Howley, 2001). HCV single stranded RNA virus having 9600 nucleotide genome (Moradpour *et al.,* 2007). Globally prevalence of cirrhosis about 27% and hepatocellular carcinoma round about 25% occur in hepatitis C infected patients (Perz *et al.,* 2006). Approximately 200 million peoples worldwide infected in HCV infections (Control and Prevention, 2012). The combination of Pegylated interferon (PEG IFN) and Ribavirin therapy are recommended for HCV treatment for many Asian countries (Al-Qahtani *et al.,* 2015; Gad *et al.,* 2009; This, 2002), the studies of Pakistani population in genotype 3 patients for 24 weeks and genotype 1 for 48 weeks (Ahmed *et al.,* 2011; Akram *et al.,* 2011; Aziz *et al.,* 2016; Aziz *et al.,* 2012; Aziz *et al.,*2011; Butt *et al.,*2009; Mahmood and Muhammad, 2011). The recent study demonstrated that Interleukin 28B (IL28B) gene encodes interferon lambda (IFN λ) having a strong association of spontaneous viral clearance and sustained virological response (SVR) in HCV treatment with PEG IFN and Ribavirin. (Allam *et al.,*2013; Aparicio *et al.,*2010; Huang *et al.,* 2012; Lange *et al.,* 2011). The present study aimed to find out the correlation of IL28B genotype polymorphism between responders and non responders having treatment of IFN/RBV therapy HCV genotype 3 patients and to rule out independent predictor for good response (favorable) in both the SNPs in HCV therapy patients.

2. MATERIAL AND METHODS

After the approval of ethical committee of Institute of Biochemistry, University of Sindh Jamshoro. This prospective cohort study was carried out in collaboration of Asian Institute of Medical Sciences (AIMS). Total 200 HCV RNA positive Genotype 3 patients with previous treatment history were recruited. The informed consent form was obtained. Peripheral blood samples were collected in EDTA tube for Genomic DNA and HCV viral RNA in Gel tube (03 ml and 02) for extraction.

2.1. RNA Extraction

Viral RNA was obtained from HCV serum specimen by Qiagene QIAamp viral RNA mini kit (QIAamp, Qiagen, Hilden, Germany) as per manufacture instruction.

2.2. Genomic DNA

Extraction of human genomic DNA obtained using

QIAamp DNA blood mini kit as per company instruction.

2.3. Amplification

Detection and Amplification had been done of HCV viral RNA by Real Time Rotor Gene Q (Qiagen) PCR.

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2.4. IL28B Genotype

AmpliSens PCR kit was used for IL28B single-nucleotide polymorphism (SNP) rs8099917 and rs12979860 with internal control (IC) was other target dye.

2.5. Liver function tests(LFT)

All of the biochemical tests were done by automation chemistry analyzer a Cobas C 311 (Roche) including serum albumin levels.

2.6. Statistical Analysis

SPSS v21.0 was used for data analysis, quantitative variables calculated in form of mean ± standard deviation, and chi-squared test and calculating OR with 95% CI and multi-regression also applied for the analysis .

2.7.Follow-up

All of the enrolled cases of this study were proper follow up having one month, three and six month(end of treatment).

3. RESULTS

In this study, two hundred enrolled HCV RNA positive genotype 3 patients for determine the correlation of IL28B genotype at SNP rs12979860 and rs8099917 with treatment response to IFN and Ribavirin therapy. In table 1 showed the statistical demographic data, biochemical parameters analysis , HCV viral loads and HCV genotypes .

Table 1: Statically Demographic data of the study .

BIOCHEMICAL TESTS	MEAN±STD-DEV
AMINOTRANSFERASE(ALT)	68.14±42.74
BILIRUBIN TOTAL	0.87±0.35
GAMMA GT	58.49±31.11
ALKALINE PHOSPHATASE	98.45±36.62
SERUM ALBUMIN	3.83±0.47

Abbreviations: γ-glutamyl transferase (GGT) , Alanine aminotransferase (ALT), Total Bilirubin(TBIL) , Albumin (ALB) , Alkaline phosphatase (ALP). The present study males were (54%) while females were (46%). The mean age for females were 35 ± 11 and for males were 39 ± 10 years respectively (**Fig.1)**.

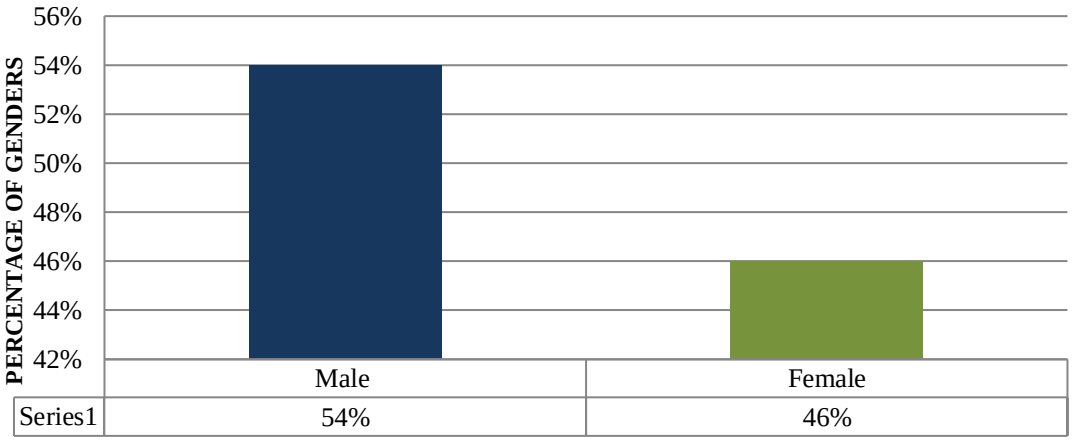

Fig. 1: Distribution of Genders.

ALT levels and HCV viral load were significantly elevated in the pretreated patient. Age (>40years and<40years) , viral load (>500000IU/ml and <500000IU/ml) and ALT(>40U/ml and <40U/ml) shows **(Table 2)**.

Table 2: HCV patients genotype3 treated PEG-IFN and Ribavirin associated variable characteristics with SVR.

VARIABLE	TOTAL NO OF TREATED PATIENTS	ACHIEVED SVR	SVR RATE %	*P*-Value
AGE,YEARS				
> 40 YEARS	97	60	61.8	**0.007**
< 40 YEARS	103	81	78.6	
GENDER				
MALE	108	66	61.2	**0.001**
FEMALE	92	75	81.5	
Pretreatment HCV viral load				
>500000 IU/ml	101	69	68.3	**0.299**
<500000 IU/ml	99	72	72.7	

Response rate Week 4 (RVR)				
RVR	110	106	96.5	**0.001**
NON-RVR	90	48	53.4	
rs12979860				
CC	98	72	73.5	**0.330**
NON CC/CT	102	69	67.6	
rs8099917				
TT	115	92	80.0	**0.000**
NON TG/GG	85	49	57.6	
Pretreatment ALT				
>40 U/ml	114	85	74.6	**0.098**
<40 U/ml	86	56	65.1	

IL28B polymorphisms SNPs rs8099917 and rs12979860 genotype frequencies distribution in 200 HCV RNA positive genotype 3 patients in Pakistani population. Homozygous TT in 115(57.5%), heterozygous GT 67(33.5%), and GG in 18(9.0%). The CC genotype of rs12979860 frequency were 98(49.0%), CT 81(40.5%), and TT 21(10.5%).

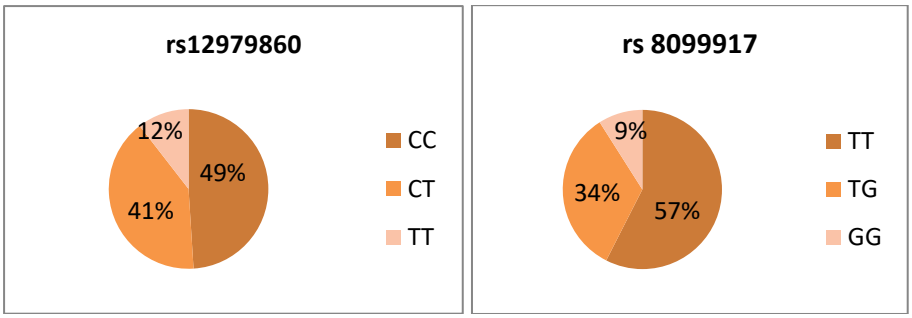

Fig 2 : Percentage of Patients According to IL28B Genotypes .

The frequencies according to Hardy Weinberg Equilibrium in respondent patients at locus rs12979860 was 50% of CC frequency, 41% of CT genotype while TT was 8.5%. Accordance to the Hardy–Weinberg equilibrium distribution of allelic frequencies were ($x2 = 0.02$, $P<0.05$). Similar analysis was performed for SNP rs8099917 TT allele was 64% ,TG 32% and GG 4% respectively. The allele frequencies at rs8099917 in responders were also found to be favorable in Hardy –Weinberg equilibrium with TT allele frequency at 0.8 and G allele frequency at 0.2 ($x2 = 0.58$, $P<0.05$).

Table 3: Frequency Distribution and Relation Between Respondent and Non-respondent According Hardy Weinberg Equilibrium.

rs8099917	**Respondent (%) n= 141**	**X**	**Non-respondent (%) n=59**	**X**	**Allelic distribution n=200**
TT	64%	0.58	36%	0.79	55%
TG	32%		48%		39%
GG	4%		16%		6%
rs12979860					
CC	50.4%	0.02	64%	0.58	48%
CT	41.1%		32%		43%
TT	8.5%		4%		9%

A total 200 HCV genotypes 3 patients were evaluated on SVR to IFN/RBV combination treatment 141 out of 200 patients were obtained SVR(70.5%) while RVR was achieved 110 (78.5%, *P*=0.0001) among SVR and RVR were significant higher in patients having major genotype TT as compare to genotype TG and GG showed in Table 4, SNP rs8099917 of the TT 65.2%, TG 29.7% and GG 4.7% genotype in responders while non responders TT 38.9%, TG 42.4% and GG 18.6 (*P*=0.0001). Similarly rs12979860 were found CC 51%, CT 40.4% and TT 8.5% in

responders while in non-responders CC 44.0%, CT40.6% and TT 15.3% . In SVR observed difference was non-significant *(P*= 0.330) showed in table -3. The multivariate regression analysis was performed to discriminate the significant effect of the following SNPs rs1297960 and rs8099917, (OR 17.74), Cl (95%) 2.19-143.58, *P*-value 0.007 showed in **(Table-4).**

Table 4*:* Comparison Of The Different Genotypes Of Interleukin28B (IL28B)Response IFN/RBV Treatment Between Responders And Non-Responders.

INTERLEUKIN28 B (IL28B) GENOTYPES		RESPONDERS		NON-RESPONDERS		TOTAL		*P*-=VALUE
		n	%	n	%	n	%	
rs8099917	TT	92	65.2%	23	38.98%	115	57.5%	**0.0001**
	TG	42	29.7%	25	42.39%	67	33.5%	
	GG	7	4.9%	11	18.64%	18	9.0%	
	TT	92	65.2%	23	38.98%	115	57.5%	**0.0009**
	TG+GG	49	34.8%	36	61.0%	85	42.5%	
rs12979860	CC	72	51.0%	26	44.0%	98	49.0%	**0.496**
	CT	57	40.4%	24	40.7%	81	40.5%	
	TT	12	8.5%	9	15.3%	21	10.5%	
	CC	72	51%	26	44.0%	98	49%	**0.438**
	CT+TT	69	48.9%	33	35.9%	102	51.0%	

Table5: Interferon (IFN)+Ribavirin (RBV) Therapy Response In 4 Week Associated IL28B SNPs rs12979860 And rs8099917.

RESPONDENT	NONRESPONDENT	*P*-value
rs12979860		
CC 61(55.4%)	CC 37 (41.1%)	0.077
CT 41(37.0%)	CT 40 (44.5%)	
TT 08 (7.2%)	TT +13 (14.5%)	
TOTAL 110	90	
rs8099917		
TT 82 (74.5%)	TT 32 (35.5%)	0.0001
TG 25 (22.7%)	TG 43 (81.1%)	
GG 03 (2.7%)	GG 15 (16.6%)	
TOTAL 110	90	

Further statistically investigated by SPSS, age and gender between pretreatment patients and post- treatment patients were obtained SVR significantly (*P*=0.007;*P*=0.001) with response of IFN/RBV therapy showed in Table 1. In addition ,we were found that the raised HCV viral load, serum Albumin and ALT level in patients pretreatment and their relationship between IL28B viral clearance response of IFN/RBV therapy with SVR. We did not observed the significant difference in normal and raised ALT and Albumin and high viral load , they all were non-significant showed **(Table 6).**

Table 6 Logistic Regression Multivariate Analysis For The Most Predictable Factors For Response To Treatment .

HCV THERAPY	OR	*P*-value	Cl (95%)
rs12979860	0.615	0.603	0.0978-3.908
rs8099917	17.74	0.007	2.19-143.58

Table 7 Previous Studies Which Reported SNP Allelic Association Of IL28B Polymorphisms And Favorable Allele In Different Population.

Origin of the study	Geno type	Polymorphisms SNPs	Alleles	Respondent (%)	Non-respondent (%)	Patients (*n*)	Favorable NPs	Ref / year
Pakistan	3	rs12980275	AA AG GG	62(62%) 25 (25%) 13 (13%)	45(37.5%) 56 (46.7%) 19 (15.8%)	220	AA	(Shaikh *et al.*, 2015) / 2015
		rs12979860	CC CT TT	19 (19%) 74 (74%) 7 (7%)	32 (26.7%) 70 (58.3%) 18 (15%)		CT	
		rs8099917	TT TG GG	66 (66%) 27 (27%) 7 (7%)	61 (50.8%) 57 (47.5%) 2 (1.7%)		TT	
Pakistan	3	rs8099917	TT TG GG	63 (60%) 38 (36.2%) 4 (3.8%)	18 (28.6%) 13 (34.2%) 2 (50.0%)	150	TT	(Aziz *et al.*, 2015) / 2014
		rs12979860	CC CT TT	57(54.3%) 39(37.1%) 9 (8.6%)	9 (15.8%) 17 (43.6%) 7 (77.8%)		CC	
Pakistan	3	rs12979860	CC CT TT	47(40.0%) 53(46.0%) 15 (13.0%)	4 (16.0%) 15 (60.0%) 6 (24.0%)		CT	(Imran *et al.*, 2015) / 2014
		rs8099917	TT TG GG	41 (35.6%) 55(47.8%) 19 (16.5%)	6 (24.0%) 13 (52.0%) 6 (24.0%)		TG	
India	3	rs12979860	CC CT TT	283(70.75%) 96(24.0%) 21 (5.25%)		400	CC	(Firdaus *et al.*, 2014) / 2014
		rs8099917	TT TG GG	310 (77.50%) 60(15%) 30 (7.50%)			TT	
India	3	rs8099917	TT TG GG	40 (78.43%) 10(19.60%) 1 (1.96%)	9 (42.85%) 11 52.38%) 1 (4.76%)	72	TT	(Chinnaswamy *et al.*, 2015) / 2014
India	3	rs12979860	CC CT TT	(61.1%) (30.5%) (8.5%)	(32.4%) (56.8%) (10.8%)	356	CC	(Gupta *et al.*, 2014) / 2014
Egypt	4	rs8099917	TT TG GG	42 (67.7%) 9(14.5%) 11 (17.7%)	12 (19.4%) 39 (62.9%) 11 (17.7%)	124	TT	(El Agrody *et al.*, 2015) / 2014
Egypt	4	rs8099917	TT TG GG	72 (80%) 12 (13.67%) 6 (6.33%)	30(33.33%) 54 (60.0%) 6 (6.33%)	180	TT	(Khattab *et al.*, 2016) / 2016
		Rs12980275	AA AG GG	75(83.33%) 3 (3.34%) 12(13.33%)	69(76.67%) 6 (6.67%) 15(16.66%)		AA	
Iran	1	rs12979860	CC CT TT	13(28.9%) 17(37.8%) 15 (33.3%)	2 (6.7%) 13(43.3%) 15(50.0%)	75	CT	(Zare *et al.*, 2016) / 2016
Iran	Mix genotype 1 & 3	rs12979860	CC CT TT	51(35.7%) 72(50.3%) 20 (14.0%)	11 (20.4%) 43 (79.6%) CT+TT	143	CT	(Behnava *et al.*, 2016) / 2016
		rs8099917	TT TG GG	82 (60.7%) 44(32.6%) 9 (6.7%)	25 (50.0%) 25 (50.0%) TG+GG		TT	
Pakistan	3	rs12979860	CC CT TT	72(51.0%) 57(40.4%) 12 (8.5%)	26 (44.0%) 24 (40.6%) 9 (15.3%)	200	---	
		rs8099917	TT TG GG	92 (65.2%) 42(29.7%) 7 (4.7%)	23 (38.9%) 25 (42.4%) 11 (18.6%)		TT	

Previous studies reported for the allele association of IL28B polymorphisms in different population found TT was the most predominant allele for IFN/RBV therapy as compared TG and GG and rs8099917 SNP was most respondent for viral clearance with IFN-RBV therapy as others SNPs in population showed in **(Table 7)**.

4. DISCUSSION

The gold standard treatment for chronic hepatitis C (CHC) is PEG IFN in combination with Ribavirin for HCV genotype 3 patients for 24 weeks (Dalgard *et al.*, 2004; This, 2002). The present study reported allelic frequencies of IL28B SNPs rs8099917 and rs12979860 in Pakistani HCV RNA positive genotype 3 patients assessed the impact of IL28B genotype response in therapy after end of treatment with PEGIFN /RBV. The distribution of frequency of IL28B SNPs were rs12979860 homozygous CC 98 (49.5%) , heterozygous CT 81(40.5%) and homozygous TT 21 (10.5%) while rs8099917 homozygous TT 115 (57.5) heterozygous TG 67 (33.5%) and homozygous GG 18 (9.0%). The most common frequencies in 200 HCV genotype 3 patient s population were CC and TT as followed CT and TG were similar as other Asian countries Taiwan, Korea and Japan 89.6%, 86.2% and 70.4% respectively. It is however the confirmation of allelic distribution of IL28B SNP rs8099917 TT was common in the present as well as in the former studies in different region s population .

The current study was defined to analyze , the effect of rs8099917 and rs12979860 observed SNP IL28B genotype rs8099917 TT (65.5%) was most predominant allele for predict of HCV therapy response accordance with Hardy Weinberg equilibrium contains high frequency and more strong positive response as compared TG and GG genotypes. It is in agreement of previous studies revealed high proportion of TT genotype with SVR in HCV genotype reported Shaikh et al 66% (Shaikh *et al.*, 2015), Aziz et al 60% (Aziz *et al.*, 2015), Firdus et al 77% (Firdaus *et al.*, 2014), Channaswamy et al 78% (Chinnaswamy *et al.*, 2015). The present study showed IL28B polymorphisms predicted SVR rates was achieved in 141 (70.5%) and RVR 110 (78.5%) higher response as compared SVR. We examined the association of IL28B gene response during RVR both SNPs rs120979860 and rs8099917 in HCV patients were achieved high response but also significant that were showing high relation between therapy and IL28B. It was as similar as Firdus et al observed 77% CC genotype at rs12979860 whilst the unfavorable CT 23% and similar SNP rs8099917 carried response rates were TT 73.2%, TG 19.2% and GG 7.6% in Indian population . Furthermore, the multivariate logistic regression analysis on rs8099917 was significant (odds ratio 17.74, Cl (95%) 2.19-143.58 *P*= 0.007). The response of the therapy were revealed by different studies demonstrated Olfat et al, Antaki et al (homozygous TT 80.4%, TT 60%) and significance analysis of TT genotype were achieved by Tanaka, et al, 2009 28, Thomas, et al, 2009 29 and Rauch et al, 2010. All of previous findings are confirmed the present study results that was significance on rs8099917 SNP TT having high frequency and favorable response in HCV treatment . In addition of co expression, the present study observed the higher rate of response in TT genotype 8099917 and the CC genotype genotype was not supported as previous studies Honda et al (Honda *et al.*, 2010) and Scherzer et al (Scherzer *et al.*, 2011) because of CC genotype varies among different population . Age and gender were associated with higher SVR rates. The current findings of the age was significant ($p = 0.007$) and gender *P*-value 0.001) were associated with SVR resemblance with previous studies by Idrees et al (Idrees and Riazuddin, 2009).

5. CONCLUSION

The present study have been identified a polymorphism of IL28B rs8099917 that is significantly associated during RVR and SVR with the response to IFN + Ribavirin for patients with chronic hepatitis C genotype 3 . No significance was found in the genotype and allele proportion of SNP rs12979860 , possible due to the small size of the sample.

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REFRENCES:

Ahmed W. U., A. Arif H. Qureshi S.E. Alam R. Ather S. Fariha, J. Waquar,. (2011) Factors influencing the response of interferon therapy in chronic hepatitis C patients. J Coll Physicians Surg Pak 21:69-73. DOI: 040579197 [pii]02.2011/JCPSP.6973 [doi].

Akram, M., M. Idrees, S. Zafar, A. Hussain, S. Butt, S.Afzal, I.U.Rehman, A. Liaqat S. Saleem M. Ali (2011) Effects of host and virus related factors on interferon-α+ ribavirin and pegylated-interferon+ ribavirin treatment outcomes in chronic Hepatitis C patients. Virology journal 8:234.

Al-Qahtani A., M. Al-Anazi A.A. Abdo, F.M. Sanai, W.Al-Hamoudi (2015) Correlation between genetic variations and serum level of interleukin 28B with virus genotypes and disease progression in chronic hepatitis C virus infection. Journal of immunology research.

Allam S.R., B. Krüger, A. Mehrotra T. Schiano B. Schröppel B. Murphy (2013) The association of IL28B polymorphism and graft survival in patients with hepatitis C undergoing liver transplantation. PLoS One 8:e54854.

Aparicio E., M. Parera, S. Franco N. Perez-Alvarez, C.Tural B. Clotet, M.A. Martínez (2010) IL28B SNP rs8099917 is strongly associated with pegylated interferon-α and ribavirin therapy treatment failure in HCV/HIV-1 coinfected patients. PLoS One 5:e13771.

Aziz H., A. Raza, J. Irfan (2016) Optimum predictors of therapeutic outcome in HCV patients in Pakistan. Journal of medical virology 88:100-108.

Aziz H., A.Raza, Y. Waheed, U. Gill, M.A. Gill. (2012) Analysis of variables and interactions among variables associated with a sustained virological response to pegylated interferon alfa-2a plus ribavirin in hepatitis C virus genotype 3-infected patients. Int J Infect Dis 16:e597-602. DOI: S1201-9712(12)00127-0 [pii]

Aziz H., A. Raza K. Ali, J.Z. Khattak, J. Irfan, M.I. Gill. (2015) Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy. Int J Infect Dis 30:91-7. DOI: S1201-9712(14)01703-2 [pii]
10.1016/j.ijid.2014.09.021 [doi].

Aziz H., L.Gil M.L., Waheed Y. Adeeb U. Raza A.Bilal I.,M.A. Athar (2011) Evaluation of prognostic factors for Peg Interferon alfa-2b plus ribavirin treatment on HCV infected patients in Pakistan. Infect Genet Evol 11:640-5. DOI: S1567-1348(11)00025-6 [pii]
10.1016/j.meegid.2011.01.022 [doi].

Behnava, B., H. Sharafi, M. Keshvari, A. Pouryasin, L. Mehrnoush, s. Salimi (2016) The role of polymorphisms near the IL28B gene on response to peg-interferon and ribavirin in thalassemic patients with hepatitis C. Hepatitis monthly 16.

Bostan N. and T. Mahmood. (2010) An overview about hepatitis C: a devastating virus. Critical reviews in microbiology 36:91-133.

Butt A.S., K. Mumtaz I. Aqeel, H.A. Shah, S. Hamid, W. Jafri. (2009) Sustained virological response to pegylated interferon and ribavirin in patients with genotype 3 HCV cirrhosis. Trop Gastroenterol 30: 207-12.

Catanese M.T., K. Uryu,M. Kopp,T.J. Edwards,L. Andrus (2013) Ultrastructural analysis of hepatitis C virus particles. Proceedings of the National Academy of Sciences 110:9505-9510.

Chinnaswamy S., K. Das, B. Bairagya, C.Bhattacharyya, A. Duseja, D. Amarapurkar, S. Chowdhury,A. Konar, (2015) Association of IL28B single nucleotide polymorphism rs8099917 with response to treatment in genotype 3 HCV-infected patients from India. Tropical Gastroenterology 35: 96-102.

Choo Q.-L., G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, M. Houghton (1989) Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepititis Genome. Science 244:359.

Control C.F.and D., Prevention. (2012) Updated CDC recommendations for the management of hepatitis B virus-infected health-care providers and students. MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports/Centers for Disease Control 61:1.

Dalgard O., K. Bjøro, K.B. Hellum, B. Myrvang, S. Ritland, K. Skaug N., Raknerud H.,Bell. (2004) Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 40:1260-1265.

El Agrody A.I., G.A.Khalil, M.M.Yousif, (2015) Interleukin 28b Gene Polymorphisms As A Predictor Of Treatment Response In Egyptians With Chronic Hepatitis C. Zagazig University Medical Journal 20.

Firdaus R., A. Biswas, K. Saha, A. Mukherjee, S. Chaudhuri (2014) Impact of host IL28B rs12979860, rs8099917 in interferon responsiveness and advanced liver disease in chronic genotype 3 hepatitis C patients. PLoS One 9:e99126.

Gad, H. H., C. Dellgren, Q. J. Hamming, S. Vends, (2009) Interferon-λ is functionally an interferon but structurally related to the interleukin-10 family. Journal of Biological Chemistry 284:20869-20875.

Gupta, A.C., N.Trehanpati, S. Sukriti, S. Hissar, V. Midha,(2014) Interleukin-28b CC genotype predicts early treatment response and CT/TT genotypes predicts non-response in patients infected with HCV genotype 3. Journal of medical virology 86:707-712.

Honda M., A. Sakai, T.Yamashita, Y. Nakamoto, E. Mizukoshi, Y. Sakai, M. Nakamura T. Shirasaki K. Horimoto (2010) Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C.

Gastroenterology 139:499-509. DOI: S0016-5085 (10)00657-8 [pii]
10.1053/j.gastro.2010.04.049 [doi].

Huang C.F., M.I.Yeh ,J.F. Huang J.F.Yang L.Y., His E. Juo (2012) Host interleukin-28B genetic variants versus viral kinetics in determining responses to standard-of-care for Asians with hepatitis C genotype 1. Antiviral research 93:239-244.

Idrees M., and S. Riazuddin (2009) A study of best positive predictors for sustained virologic response to interferon alpha plus ribavirin therapy in naive chronic hepatitis C patients. BMC Gastroenterol 9:5. DOI: 1471-230X-9-5 [pii]10.1186/1471-230X-9-5 [doi].

Imran M., S. Manzoor S. Azam S. Resham (2015) Genetic variant of IL28B rs12979860, as predictive marker of interferon-based therapy in Pakistani population. APMIS 123:342-349.

Khattab M.A., H.M. Abdelghany M.M. Ramzy R.M. Khairy, (2016) Impact of IL28B gene polymorphisms rs8099917 and rs12980275 on response to pegylated interferon-α/ribavirin therapy in chronic hepatitis C genotype 4 patients. Journal of biomedical research 30:40.

Knipe D., and P. Howley (2001) Flaviviridae: the virus and their replication. Fields virology, 4th ed. Lippincott-Raven, Philadelphia, PA:991-1029.

Lange C.M., D. Moradpour, A. Doehring M, Pascual H. Farnik (2011) Impact of donor and recipient IL28B rs12979860 genotypes on hepatitis C virus liver graft reinfection. Journal of hepatology 55:322-327.

Mahmood K and N. Muhammad. (2011) Side effects of combination of interferon plus ribavirin therapy in patients with chronic hepatitis C; an experience with 400 patients. Journal of Postgraduate Medical Institute (Peshawar-Pakistan) 21.

Moradpour D., F. Penin, C.M. Rice. (2007) Replication of hepatitis C virus. Nature Reviews. Microbiology 5:453.

Perz J.F., G. L. Armstrong L.A. Farrington, Y.J. Hutin (2006) The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. Journal of hepatology 45:529-538.

Scherzer T.M., H. Hofer A.F. Staettermayer K. Rutter. (2011) Early virologic response and IL28B polymorphisms in patients with chronic hepatitis C genotype 3 treated with peginterferon alfa-2a and ribavirin. J Hepatol 54:866-71. DOI: S0168-8278 (10)00931-1 [pii]
10.1016/j.jhep.2010.08.024 [doi].

Shaikh N., A.M. Waryah, B.R. Devrajani, M.I.Rajput. (2015) IL28B rs12980275 polymorphism shows association with response to treatment in Pakistani patients with chronic hepatitis C. Journal of medical virology 87:814-820.

This N. (2002) National Institutes of Health consensus development conference statement: management of hepatitis C: 2002—June 10-12,

Zare F., H. Hadinedoushan, M.A. Meybodi M. Dehghanmanshadi S.M. Bafghi M. Vakili (2016) The Effect of IL28B Gene Polymorphism on Treatment Response in Iranian Patients with Hepatitis C Virus Infection. Jundishapur Journal of Microbiology 9

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