

ORIGINAL ARTICLE

PREDICTIVE POTENTIAL OF IL28B GENE IN HCV PATIENTS, RESISTANT TO DACLATASVIR AND SOFOSBUVIR IN KPK POPULATION

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Background: Recently various combinations of direct acting antivirals (DAAs) have been tried successfully. The Sofosbuvir + Daclatasvir combination has been used with promising results. Recently, resistance has been noticed against DAAs. Therefore, polymorphism at particular sites in the interleukin 28B gene are under study to find possible association with resistance. This study was aimed at finding out any association of SNPs rs8099917 and rs12979860 (IL28B gene) with response and resistance to treatment in HCV genotype 3 patients in Khyber Pakhtunkhwa. **Methods:** This cross sectional, Analytical study was conducted at Gastroenterology/hepatology OPD of Prime Teaching Hospital, Peshawar Medical College. Collected Samples were stored at -20° C in PCR Lab of the College. DNA extraction and genotyping was carried out at BJ Molecular Biology Lab in Rawalpindi. Data was analyzed by using SPSS version 21. Chi-Square Test was used to see the statistically significant differences between rs8099917 T/G and rs12979860 T/C model. **Results:** In the IL28-B gene, single nucleotide polymorphism at rs12979860 T/C model, we observed that there are 37.5% CC homozygous, 12.5% TT homozygous and 50% CT heterozygous genotypes in resistant patients and 42.85% CC homozygous, 28.57% TT and 28.57% CT genotype in responder group. In rs12979860 T/C model, genotype of IL28-B in the responder and resistant group significantly varies at p -value =0.00572. **Conclusion:** We conclude that in SNP at rs12979860, CC genotype is associated with clearance of HCV, while CT genotype was more prevalent in the resistant group and associated with chronicity.

Keywords: IL28B; Hepatitis C; Genotype 3a

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INTRODUCTION

Hepatitis C has remained a global concern for decades now and remains a great problem, especially in the underdeveloped and developing countries. Around 58 million people are suffering from chronic hepatitis C virus infection globally with an addition of 1.5 million cases every year. A majority of these chronically infected patients further develop cirrhosis or liver cancer.¹

HCV resistance to direct antivirals is a new phenomenon and an emerging field of research. Resistance to DAAs is developing against currently available DAA combinations. Most common resistance-associated substitutions (RASs) are in NS3 and NS5A region for genotypes 1a and 3. The 9.5 kilo base RNA genome of HCV produces over a billion copies on a daily basis under the action of RNA polymerases. On average, this process results in 1-3 errors per replication cycle. Some of these errors result in non-replicable (Dead) progeny viruses. Sometimes, these errors of transcription occur in important coding regions such as NS3 and

NS5A which may lead to decreased susceptibility to one or more antivirals.²

Over the past decade, direct-acting antivirals (DAAs) have been used in various combinations and several studies have reported 90-95% sustained virological response (SVR) rates.³⁻⁶

A major reason for treatment failure with DAAs in the majority of cases is virological resistance. This in turn is dependent on HCV genotype and the combination of DAAs used. If the facility is available, baseline testing for resistance may help optimize therapy in genotype 3 or other rare sub-genotypes.⁷ An Egyptian study concluded that combination therapy with Sofosbuvir + Daclatasvir was effective and well accepted in HCV patients. They did not recommend routine testing for resistance associated variants (RAVs) because they can occur naturally.⁸

A recent study in Italy observed a higher prevalence of resistance associated substitutions (RASs) in cases of treatment failure with various classes and combinations of DAAs and suggested

that genotypic studies may be useful in the selection of second line regimen.⁹

Over past 5–10 years, studies were carried out on single nucleotide polymorphism (SNPs) in IL28B gene, and it was observed that it is associated with viral clearance. In this regard, single nucleotide polymorphisms in IL28B gene has been implicated as showing decreased responsiveness to Sofosbuvir and other DAAs. In recent developments, pan-genotypic regimens have been proven effective against HCV genotype 3b but the resistant cases of 3a for daclatasvir and sofosbuvir are emerging and are of great concern.¹⁰ Goossens¹¹ pointed out that there is an emerging resistance of Sofosbuvir and other DAAs against genotype 3 of HCV, which is alarming.

IL28B gene is located on chromosome 19q13, it is produced in response to viral infections by many immune cells which include alveolar epithelial cells, neuronal cells and hepatocytes. IL28B (IFN- λ) is strongly related to viral clearance in chronic HCV patients.¹² IL28B is a part of innate immunity, involved in the immune response against many viruses, which also include Hepatitis C. There are three subtypes, also known as genotypes, CC, CT, and TT. The immune response of CC genotype towards HCV infection is stronger than CT and TT genotypes. People carrying CC genotype are more likely to clear HCV infection within months of being infected which is way better than people having other IL28B genotypes (CT and TT).¹³

The upstream haplotype that is associated with spontaneous viral clearance of HCV at SNPs rs8099917 and rs12979860 is CT, while the haplotype that is persistent at these SNPs is TG.¹⁴ Although SVR to interferon in combination with DAAs is good, however, due to complications and difficulty of injectable therapy, the oral pan-genotypic DAAs are being used as an effective alternative. It is anticipated that IL28B genotyping has a good predictive potential.¹⁵

Interleukin production limits itself as soon as the need is over. They are produced by the body cells in response to pathogen invasion and to the antigens that elicit pro-inflammatory responses. They mediate immune responses and have both autocrine and paracrine functions. The messenger RNA that codes for most interleukins is unstable and their synthesis is short-lived. Once these molecules are formed, they are rapidly secreted. IL28 is produced by regulatory T-cells which later acts on melanocytes and keratinocytes. IL28 helps in presenting viral antigens to CD8+T lymphocytes.¹⁶ Many studies conducted on polymorphisms in the IL28B gene regions encoding interferon lambda 3 (IFN- λ 3), suggest interferon-

based HCV clearance. The TT and CC types show a high percentage of spontaneous viral clearance corresponding to approximately 89% positive predictive value. In the Pakistani population, the most predictive of HCV viral clearance was found to be CC genotype predominantly.^{17–21}

In our study, we searched for different genotypes of IL28B in chronic HCV patients who are developing resistance to sofosbuvir 400mg and daclatasvir 80mg combination therapy in our population. We focused on single nucleotide polymorphism (SNPs) at two locations; rs12979860 and rs8099917 simultaneously. The aims of this study were;

- a. To find out various IL28B genotypes in chronic hepatitis C, genotype 3a patients, who are resistant or responders to Sofosbuvir and daclatasvir combination therapy.
- b. To determine whether we can predict the outcome of treatment based on the frequency of these genotypes.

MATERIAL AND METHODS

This analytical, cross-sectional study was conducted in the gastroenterology and hepatology clinic of a prime teaching hospital, Peshawar medical college, Peshawar, Pakistan, from 1st September 2020 to 31st March 2021. The study was conducted after approval by the Institutional Review Board (IRB) of Peshawar Medical College, under the auspices of Riphah International University.

Patients with positive HCV RNA after the completion of 12 weeks of treatment were picked by purposive sampling over a period of 6 months. The ages ranged between 18 to 70 years. Patients having HCV, and co-infection with HBV and diabetes were not included in the study. 3ml venous blood was collected in EDTA-containing tubes observing aseptic techniques. The samples were stored at -20 degrees centigrade in the PCR lab of Peshawar Medical College. The blood samples of patients whose PCR became negative by 12th week of treatment with the sofosbuvir + daclatasvir combination were labelled as responders. We could get only seven samples in this time frame. However, we were interested more in patients showing resistance to this combination therapy, so that we could look for a specific pattern of single nucleotide polymorphism in such cases. We were able to collect eight cases, whose PCR was still positive for HCV RNA after 12 weeks of treatment. The blood samples of these patients were labelled as “resistant”.

DNA was extracted by the optimized protocol of BJ Molecular Biology Lab in Rawalpindi, Pakistan.

Primers were manufactured by Macrogen, South Korea and optimized in BJ Lab. In each Lyophilized primer recommended sterile double distilled water is added to make it re-suspend. The final concentration should be 100µM. to prepare a 10µM Working solution of primers we diluted the original stock primers with 1:10 ratio by using sterile ddH₂O. and the final concentration of Primers in a PCR reaction would be 1:25 per reaction. The set of primers as mentioned above are optimized for annealing before PCR Amplification. Gradient PCR is used for optimization and T_m 62 is confirmed as the annealing temperature for all primers.

Two SNPs (rs8099917 and rs12979860) were chosen for genotyping. DNA was extracted from peripheral blood using a standard CTAB (cetyltrimethylammonium bromide) method. Genotyping for the IL28B was performed by TETRA-ARM PCR-based assay. Forward and reverse primer pair was used for each SNP, which were designed in such a way that if SNP rs8099917 is present the ~500bp will show and if the desired SNP is not present there will be no primer annealing

due to mismatch at 5' end of the primer and no band will be observed on gel. If SNP rs12979860 is present, the allele-specific primers will bound to the template and 242bp band will appear on gel while if no SNP is present there will be no band. The outer set of primers was used as a positive control.

RESULTS

The following online tools are used to check the binding of primers with the desired product. <https://blast.ncbi.nlm.nih.gov/Blast.cgi> is used for checking primer specification and similarly, websites USCS.com and bioinformatics.org are used for Insilico PCR products.

Insilico PCR using our primers sets. The desired product length of 242bp, 500bp was obtained. Purified PCR products were run on 1.5% agarose gel. 30ml of 1.5% gel is prepared by adding 0.45g of agarose in 1x TBE buffer. The solution is then heated for 1 minute in the microwave and cooled down a bit before adding 3 µl Ethidium Bromide. 5µl PCR purified Samples are then loaded on the gel after adding 3µl loading dye in it.

select all 100 sequences selected		GenBank	Graphics	Distance tree of results	MSA View			
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Eukaryotic synthetic construct chromosome 19	eukaryotic synt...	44.1	44.1	100%	0.091	100.00%	64242768	CP034522.1
Eukaryotic synthetic construct chromosome 19	eukaryotic synt...	44.1	44.1	100%	0.091	100.00%	64242768	CP034497.1
Homo sapiens interferon lambda 4 (gene/pseudogene) (IFNL4). RefSeqGene on chromosome 19	Homo sapiens	44.1	44.1	100%	0.091	100.00%	9543	NG_055295.1
Homo sapiens isolate CHM13 chromosome 19	Homo sapiens	44.1	44.1	100%	0.091	100.00%	61707364	CP068259.2
Acanthopagrus latus isolate v.2019 genome assembly, chromosome: 20	Acanthopagrus...	44.1	44.1	100%	0.091	100.00%	24488360	LR884479.1
Homo sapiens DNA, chromosome 19, nearly complete genome	Homo sapiens	44.1	44.1	100%	0.091	100.00%	59105444	AP023479.1
Homo sapiens chromosome 19 clone CTC-246B18, complete sequence	Homo sapiens	44.1	44.1	100%	0.091	100.00%	138538	AC011445.6
Myripristis murdjan genome assembly, chromosome: 11	Myripristis mur...	42.1	42.1	95%	0.36	100.00%	31823041	LR597560.1
Solea senegalensis genome assembly, chromosome: C	Solea senegal...	42.1	108	95%	0.36	100.00%	33076413	QW185634.1
Immersiporthe knoxdavisiana isolate CMW 37318 chromosome 9	Immersiporthe ...	42.1	42.1	95%	0.36	100.00%	2481546	CP088214.1
Onychomys torridus genome assembly, chromosome: 17	Onychomys tor...	42.1	577	100%	0.36	100.00%	64256681	LR877204.1
Onychomys torridus genome assembly, chromosome: 16	Onychomys tor...	42.1	349	100%	0.36	100.00%	68548656	LR877203.1
Onychomys torridus genome assembly, chromosome: 14	Onychomys tor...	42.1	714	100%	0.36	100.00%	84754330	LR877201.1
Scleropages formosus genome assembly, chromosome: 3	Scleropages fo...	40.1	104	90%	1.4	100.00%	40850809	LR584068.1
Anabas testudineus genome assembly, chromosome: 8	Anabas testudi...	40.1	40.1	90%	1.4	100.00%	21832553	LR132046.1

Figure-1: Blast Results

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PCR Products results

>242 bp product from linear template Untitled, base 815 to base 1056 ( f - r ).
GCTTATCGCATAACGGCTAGGCCCCCTCGCCAGGGCCCCCTAACCTCTGCACAGTCTGGGAT
TCCTGGACGTGGATGGGTACTGGCAGCGCACGGTTCGTGCCTGTCGTGTACTGAACCAGGG
AGCTCCCCGAAGGCGGAACCAGGGTTGAATTGCACTCCGCGCTCCCCAGCAAAGCCCC
TCGCCCCGACCTGGAGCCGAGTCTCCCGGCAGGGCTCCCTTCTGTGATTGACCCTGAGC
CT
    
```

Figure-2: PCR product results

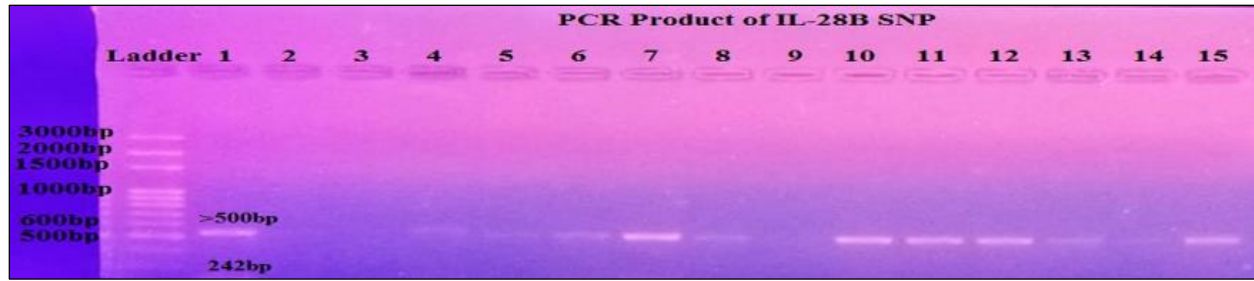


Figure-3: shows PCR product of IL28B SNP

Table-1: DNA samples number and tests performed

Sample No.	Primer Optimization	Insilico PCR	Blast	TETRA ARM PCR	Confirmatory Gel-electrophoresis
1-7	Yes	yes	Yes	yes	yes
8-15	Yes	yes	Yes	yes	yes

Table-2: Primer Details

Name	Forward	Reverse	T(a)	Product size
rs12979860	GCTTATCGCATACGGCTAGG	AGGCTCAGGGTCAATCACAG	62	242/268
rs8099917	TCCTCTCATCCCTCATCCCACT	ACATCCACACCCTCAACCCT	62	500/420

Table-3: Genotyping of SNP rs12979860 (CT) model, Responder & Resistant to Sofosbuvir & Daclatasvir

Responders					
S. No.	Patient Code	IL28B Genotypes	Frequency of Genotypes	χ^2	p-Value on the basis of χ^2 (responders and resistant)
1.	A	CC	CC allele = 3/7 (42.85%) TT allele = 2/7 (28.57%) CT allele = 2/7 (28.57%)	10.326	0.005722**
2.	B	TT			
3.	C	TT			
4.	D	CC			
5.	E	CC			
6.	F	CT			
7.	G	CT			
Resistant					
S. No.	Patient Code	IL28B Genotypes	Frequency of Genotypes	χ^2 (responders and resistant)	Relative Risk (responders & resistant)
8.	H	CC	CC allele = 3/8 (37.5%) TT allele = 1/8 (12.5%) CT allele = 4/8 (50%)	10.326	CC&TT/CT model Relative Risk (RR) = 1.11 95% confidence interval = [0.8397, 1.4628] CC/CT&TT model = Relative Risk (RR) = 0.6508 95% confidence interval = [0.4988, 0.8492]
9.	I	TT			
10.	J	CC			
11.	K	CC			
12.	L	CT			
13.	M	CT			
14.	N	CT			
15.	O	CT			

Table-4: SNP rs8099917 (T/G) model responders & resistant to sofosbuvir & daclatasvir

Responders					
Sr. No.	Patient Code	IL28B Genotypes	Genotype frequency	χ^2	p-Value based on χ^2 (responders and resistant)
1.	A	TT	GG allele = 2/7 (28.57%) TT allele = 2/7 (28.57%) TG allele = 3/7 (42.86%)	10.638	0.0049**
2.	B	GG			
3.	C	GG			
4.	D	TG			
5.	E	TG			
6.	F	TG			
7.	G	TT			
Resistant					
S. No.	Patient Code	IL28B Genotypes	Genotype frequency	χ^2 (responders and resistant)	Relative Risk (responders and resistant)
8.	H	TG	GG allele = 2/8 (25%) TT allele = 4/8 (50%) TG allele = 2/8 (25%)	10.638	GG&TG/TT model Relative Risk (RR) = 1.1043 95% confidence interval = [0.8193, 1.4885] GG/TG&TT model = Relative Risk (RR) = 1.59 95% confidence interval = [1.1539, 2.2143]
9.	I	GG			
10.	J	TT			
11.	K	TT			
12.	L	TT			
13.	M	TG			
14.	N	GG			
15.	O	TT			

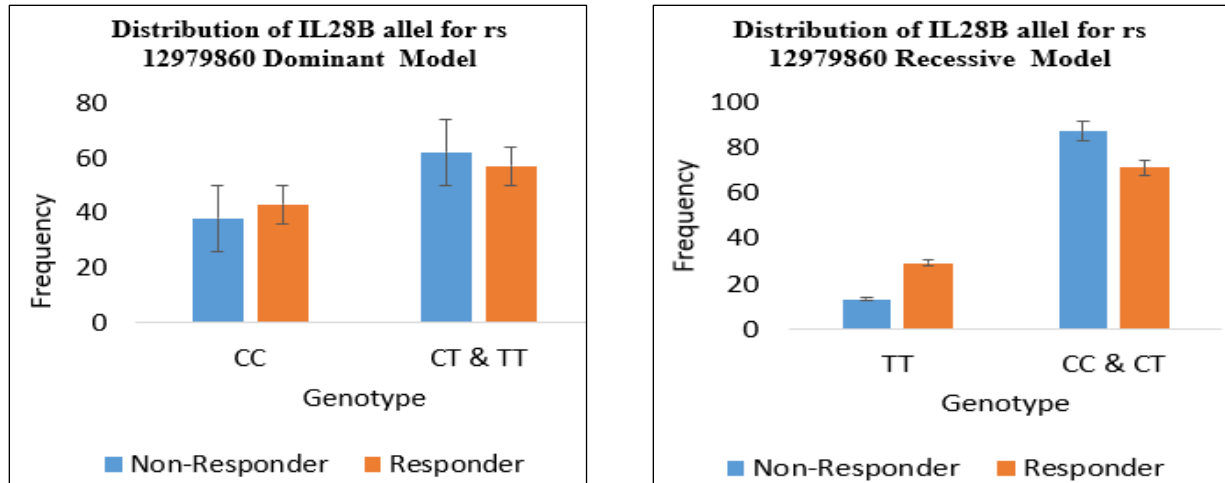


Figure-4 (A & B): Frequency distribution of IL28 for SNP RS 12979860 in responders and non-responder group

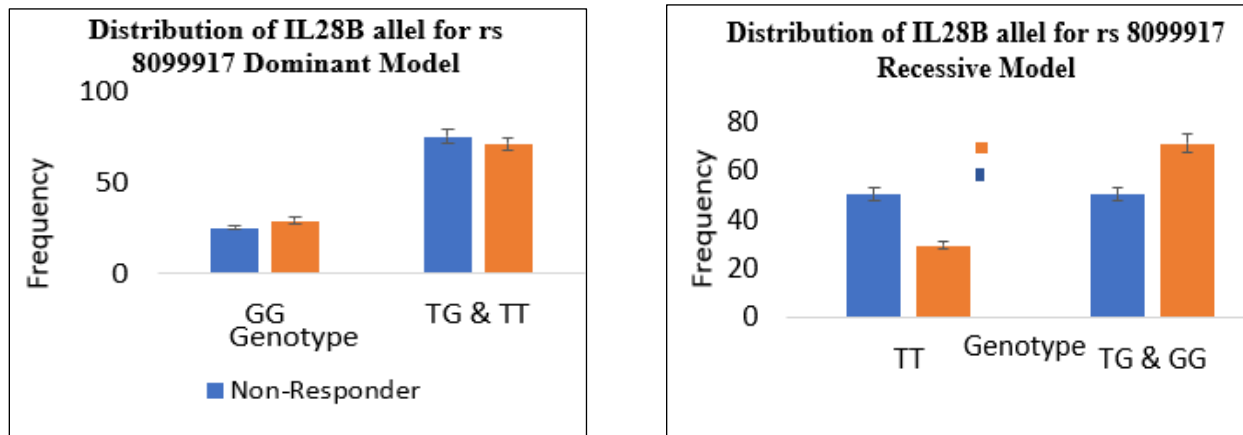


Figure-4 (C & D): Frequency distribution of IL28 for SNP Rs 8099917 in responders and non-responder group.

DISCUSSION

The best possible prediction of SVR to antiviral therapy in patients with chronic hepatitis C virus infection could be highly useful in terms of length and cost of treatment. Direct acting antivirals (DAAs) such as the first and second-generation protease inhibitors telaprevir, boceprevir, and simeprevir, as well as the polymerase inhibitor sofosbuvir, and NS5A inhibitor daclatasvir, in various combinations, are currently some of the options for highly effective combination therapies, with or without interferon. Even more DAAs are in the production pipeline, which may be approved shortly for combination regimens without including interferon. However, approval and affordability in developing countries may be delayed.²²

In our study, the frequency distribution of IL28-B single nucleotide polymorphism SNP rs12979860 T/C model (Table 3) in resistant and responders to therapeutic drugs Sofosbuvir & Daclatasvir varies significantly. We found that there are 37.5% CC homozygous, 12.5% TT homozygous and 50 % CT heterozygous genotypes in

resistant patients and 42.85% CC homozygous, 28.57% TT homozygous and 28.57 % CT heterozygous genotype in responder group. The relative risk is calculated in both the dominant model (CC&TC/TT) and recessive model (CC/TC&TT). Relative Risk (RR) in CC&TT/TC model among responder and resistant is 1.11 at 95% confidence interval [0.8397, 1.4628], whereas Relative Risk (RR) for CC/TC&TT model is 0.6508 at 95% confidence interval [0.4988, 0.8492]. Resultantly the genotype of IL28-B in the responder and resistant group significantly varies at p -value=0.00572.

In a study conducted in Uruguay, it was observed that in the HCV infected cohort, the frequencies for the rs12979860 genotypes were 29.5% CC, 47.4% CT and 23.1% TT compared to 45.7%, 42.4% and 11.9% respectively in the control group, evidenced to be statistically significant ($p < 0.05$).²³ Our results are comparable with the Uruguayan Study for rs12979860 T/C model. An Iranian study observed that in IL-28B at rs12979860 SNP CC genotype had higher frequency in spontaneously cleared patients in comparison with chronic HCV patients.²⁴ In Spontaneous clearance cases,

genotype CC frequency was 65.1% as compared to our study having 42.85%. The difference is because of the reason, that their figures are showing the frequency of mixed genotypes, while our study indicates HCV genotype 3a only. In chronic HCV cases, this frequency was 33.86% vs 37.5% in our study, which is comparable.²⁴ Similarly, in the Uruguayan study, frequencies for the rs8099917 TT, GT and GG genotypes were 57.7%, 28.2% and 14.1%, respectively in HCV patients as compared to 60.9%, 33.7% and 5.45 in their control group. Therefore, they concluded that within the Uruguayan population, rs12979860 might be a better predictor than rs8099917, at least in terms of occurrence of chronic HCV infection.²³

We also observed in our study that the frequency distribution of IL28-B single nucleotide polymorphism rs8099917 T/G model (Table-4) in resistant cases to therapeutic drugs Sofosbuvir & Daclatasvir is noticeable. We witnessed that there are 25% GG homozygous, 50% TT homozygous and 25% GT heterozygous genotypes in resistant patients and 28.57% GG homozygous, 28.57% TT homozygous and 42.85% GT heterozygous genotype in responder group. The proportion of subjects (2*3) who reported being responded to differs by (Chi sq value=10.638) response to Sofosbuvir & Daclatasvir. The relative risk is calculated in both the dominant model (GG&TG/TT) and recessive model (CC/TC&TT). Relative Risk (RR) in CC&TT/TC model among responder and resistant is 1.11 at 95% confidence interval [0.8397, 1.4628] whereas Relative Risk (RR) for CC/TC&TT model is 1.59 at 95% confidence interval [1.1539, 2.2143]. Resultantly the genotype of IL28-B in responder and resistant group significantly differs at p -value =0.0049.

A study conducted in Salt Lake City USA, found that CT, TG, and TT genotypes were observed in all five ethnic populations. rs12979860/rs8099917 were detected in whites, Asians, Middle Easterners, Hispanics, and African Americans, at the following frequencies: CC/TT was (39.2%, 78.9%, 40.0%, 33.9%, and 16.8%), CT/TT was (20.8%, 0%, 40%, 9.3%, and 37.0%), TT/TT was (2.4%, 0%, 0%, 3.4%, and 35.3%), CT/TG was (24.0%, 19.7%, 20%, 39.8%, and 3.4%), TT/TG was (8.0%, 1.4%, 0%, 3.4%, and 5.9%), and TT/GG was (5.6%, 0%, 0%, 10.2%, and 1.7%), respectively.²⁵ The highlighted figures are for Asians, which are in contrast to our study. In our population, we observed that in T/G model (rs8099917) TT allele was 28.57%, TG 42.85% and GG 28.57%. This study shows a lot of variations in different ethnic groups, and hence, prompted us to investigate the frequency of these genotypes in our population.

Another study reported, that in a German control population, distribution of IL28B rs12979860 C/C and IFN-L4 ss469415590 TT/TT was comparable

to our study with 46% and 47%, respectively, whereas IL28B rs8099917 T/T was slightly higher (67%). In the Egyptian control cohort, the frequencies of these SNPs were comparable with the German control population (47% for rs12979860 CC, 70% for rs8099917 TT, and 45% for ss469415590 TT/TT).²⁶ Study by Aziz H. *et al.*¹⁷ found 3 types of genotypes rs8099917:60% homozygous TT, 36.2% heterozygous GT and 3.8% GG. 54.3% CC genotype rs12979860, 37.1% CT and 8.6% TT. Overall, SVR was achieved in 68.6% of patients. Patients with the favourable genotype CC of rs12979860 had a higher SVR of 84.2%, compared to 56.4% and 22.2% for the minor genotypes CT and TT, respectively ($p=0.0001$). They discovered no significant link between SVR and antiviral treatment in patients with genotype TT (rs8099917) (71.9%, $p=0.36$). Patients with major genotype TT had a significantly higher rate of rapid virological response (88.9%, $p=0.04$). These findings indicate that the IL28B polymorphism is strongly linked to SVR to therapy in the Pakistani population infected with HCV genotype 3. Patients with HCV who are homozygous C/C have a higher chance of SVR. Furthermore, patients who carry T/T (rs8099917) have a higher chance of RVR.

CONCLUSION

1. In rs12979860 T/C model genotypes of IL28-B in responder and resistant group significantly vary at p -value=0.00572.
2. Homozygous genotype CC at rs12979860 was found to be more prevalent (42.8%) in responder group, compared to resistant group (37.5%) and can be predictive for spontaneous clearance of the virus as well as SVR after the use of DAAs.
3. Heterozygous CT allele at rs12979860, was more predominant in Non –Responder group (50%) as compared to responder group (28.57%). Therefore, it is concluded that this genotype may be predictive of chronicity.
4. Polymorphism at rs8099917 was not clearly demarcated in the two groups, hence outcome of therapy could not be predicted with confidence.

Recommendation: A large scale multi centered studies are required in future, enrolling large number of participants to enable these studies to be applied to the general population.

AUTHORS' CONTRIBUTION

SS: Principal investigator collection of samples and processing at BJ Labs. SM; Manuscript writing and revision. MH: Review of manuscript. Research supervisor. NUH: Concept and provision of cases. Co-supervisor. MK: Procurement of samples.

The authors declare that there is no conflict of interest.

REFERENCES

1. WHO fact sheet 2021. Hepatitis C. [Internet]. [cited 2023 Jan]. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>
2. American Association for the Study of Liver Diseases and American Society of Infectious diseases society of America. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C.2017.
3. Kowdley KV, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, *et al.* Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014;370(20):1879–88.
4. Feld JJ, Jacobson IM, Hézode C, Asselah T, Ruane PJ, Gruener N, *et al.* Sofosbuvir velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infections. *N Engl J Med* 2015;373(27):2607.
5. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, *et al.* Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014;370(3):211–21.
6. Wyles DL, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, *et al.* Daclatasvir plus sofosbuvir for HCV in patients coinfectd with HIV-1. *N Engl J Med* 2015;373(8):714–25.
7. Sarrazin C. Treatment failure with DAA therapy: Importance of resistance. *J Hepatol* 2021;74(6):1472–82.
8. Ramadan A, Karam H, Abdel-Monem SS, Ahmed AM, Omar RM, Ahmed HR, *et al.* Evaluation of the safety and resistance associated variants of sofosbuvir/daclatasvir among egyptian patients with hepatitis C virus: a prospective study. *Bull Pharm Sci* 2022;45(2):1143–53.
9. Di Stefano M, Faleo G, Farhan Mohamed AM, Morella S, Bruno SR, Tundo P, *et al.* Resistance Associated Mutations in HCV Patients Failing DAA Treatment. *New Microbiol* 2021;44(1):12–8.
10. Raza H, Ahmad T, Afzal MS. HCV, Interferon Therapy Response, Direct Acting Antiviral Therapy Revolution and Pakistan: Future Perspectives. *Asian Pac J Cancer Prev* 2015;16(13):5583–4.
11. Goossens N, Negro F. Is genotype 3 of the hepatitis C virus the new villain? *Hepatology* 2014;59(6):2403–12.
12. Imran M, Manzoor S, Ashraf J, Khalid M, Tariq M, Khaliq HM, *et al.* Role of viral and host factors in interferon based therapy of hepatitis C virus infection. *Virology* 2013; 10:299.
13. Calisti G, Tavares A, Macartney MJ, McCormick A, Labbett W, Jacobs M, *et al.* IL28B genotype predicts response to chronic hepatitis C triple therapy with telaprevir or boceprevir in treatment naïve and treatment-experienced patients other than prior partial- and null-responders. *Springerplus* 2015; 4:357.
14. Murray MF. Chapter 39-susceptibility and response to infection. Emery and rimoin's principles and practice of medical genetics. 2013. [Internet]. [cited 2023 Jan]. Available from: <https://www.sciencedirect.com/topics/medicine-and-dentistry/interleukin-28b>
15. Afzal MS, Predictive potential of IL-28B genetic testing for interferon based hepatitis C virus therapy in Pakistan: Current scenario and future perspective. *World J Hepatol* 2016;8(26):1116–8.
16. Justiz Vaillant AA, Qurie A. Interleukin. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2022 Jan-. [cited 2023 Jan]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK499840/>
17. Aziz H, Raza A, Ali K, Khattak JZ, Irfan J, Gill ML. 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* 2015; 30:91–7.
18. Hashmi AH, Ahmad N, Riaz S, Ali L, Siddiqi S, Khan KM, *et al.* Genotype CC rs12979860 is providing protection against infection rather than assisting in treatment response for HCV genotype 3a infection. *Genes Immun* 2014;15(6):430–2.
19. Khubaib B, Saleem S, Idrees M, Afzal S, Wasim M. The genotype CC of IL-28B SNP rs12979860 is significantly associated with a sustained virological response in chronic HCVinfected Pakistani patients. *J Dig Dis* 2015;16(5):293–8.
20. Shaikh N, Waryah AM, Devrajani BR, Rajput MI, Hayat AS, Shaikh S. IL28B rs12980275 polymorphism shows association with response to treatment in Pakistani patients with chronic hepatitis C. *J Med Virol* 2015;87(5):814–20.
21. Imran M, Manzoor S, Azam S, Resham S. Genetic variant of IL28B rs12979860, as predictive marker of interferon-based therapy in Pakistani population. *APMIS* 2015;123(4):342–49.
22. Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H. *Hepatology - Clinical textbook*. 10th Edition. [Internet]. Hamburg: Medizin Fokus Verlag, chapter 12, 2017; p.133–69. [cited 2023 Jan]. Available from: hepatologytextbook.com/download/hepatology2020.pdf
23. Echeverría N, Chiodi D, López P, Ciceron AS, Angulo J, López-Lastra M, *et al.* IL28B gene polymorphism rs12979860, but not rs8099917, contributes to the occurrence of chronic HCV infection in Uruguayan patients. *Virology* 2018; 15:40.
24. Moghimi M, Tavakoli F, Doosti M, Ahmadi-Vasmehjani A, Akhondi-Meybodi M. Correlation between interleukin-28 gene polymorphism with interleukin-28 cytokine levels and viral genotypes among HCV patients in Yazd, Iran. *BMC Res Notes* 2019; 12:626.
25. Melis R, Fauron C, McMillin G, Lyon E, Shirts B, Hubley LM, *et al.* Simultaneous Genotyping of rs12979860 and rs8099917 Variants Near the IL28B Locus Associated with HCV Clearance and Treatment Response. *J Mol Diagn* 2011;13(4):446–51.
26. Susser S, Herrmann E, Lange C, Hamdi N, Müller T, Berg T, *et al.* Predictive Value of Interferon-Lambda Gene Polymorphisms for Treatment Responsein Chronic Hepatitis C. *PLoS One* 2014;9(11): e112592.

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