ORIGINAL ARTICLE

IL28B POLYMORPHISM AS A PREDICTOR OF ANTIVIRAL RESPONSE IN CHRONIC HEPATITIS C GENOTYPE 3

Javed Iqbal Farooqi[1], Rukhsana Javed Farooqi[2], Nowshad Khan[3], Riaz Muhammad[1], Noorullah Khan[1], Abdur Rehman[1], Zafar Ali[1], Intekhab Alam[1], Mohammad Jamshed Khan[1], Muhammad Asghar[1], Farooq Ahmad[1]

[1]Department of Medicine, PGMI, Lady Reading Hospital, Peshawar, [2]Khyber Teaching Hospital Peshawar and [3]Department of Medicine, Gomal Medical College, D.I.Khan, Pakistan

ABSTRACT

Background: Genetic variation in the interleukin 28B (*IL28B*) gene has been associated with response to Interferon-α & Ribavirin (IFN+Riba) therapy in hepatitis C virus (HCV) genotype 1-infected patients; there is limited data for HCV patients with genotype 3. We evaluated the effects of *IL28B* polymorphisms on response to treatment with IFN+Riba in patients with HCV genotype 3.

Material & Methods: This cross-sectional study was conducted in Medical A Ward, PGMI, Lady Reading Hospital and Author's Private Hepatology Clinic, Peshawar, Pakistan from July 2013 to June 2014. *IL28B* host genotypes (CC, CT and TT) were analyzed in 184 patients of chronic hepatitis C genotype 3, for association with outcome of antiviral therapy with IFN+Riba, using SPSS version 16.0.

Results: The frequencies of the *IL28B* genotypes were as follows: CC, 24.5%; CT, 60.3%; and TT, 15.2%; Non-CC genotype was significantly associated with the Non-Responder status of the studied patients of HCV genotype 3 irrespective of their gender and genotype subtype status, with statistically sagnifiant association between virological and clinical profile (p=0.001).

Conclusion: An *IL28B* polymorphism (Non-CC genotype) was associated with Virological non-Response in patients infected with genotype 3 HCV who did not achieve a RVR. Analysis of *IL28B* genotype might be used to guide treatment for these patients.

KEY WORDS: IL28B polymorphism; Hepatitis C virus; Genotype 3; Sustained virological response.

This article may be cited as: Farooqi JI, Farooqi RJ, Khan N, Muhammad R, Khan N, Rehman A, et al. IL28B polymorphism as a predictor of antiviral response in chronic hepatitis C genotype 3. Gomal J Med Sci 2014; 12:133-7.

INTRODUCTION

Hepatitis C infection is a major global public health problem[1-6] as well as in Pakistan.[7-17] IFN+Riba has been the standard of care for hepatitis C over the last two decades or so, with an efficacy of 60-85% depending upon HCV genotype.[18,19] Outcome of IFN+Riba depends upon several viral and host-related factors. These include viral genotype, serum HCV RNA level, stage of hepatic fibrosis, body mass index (BMI) of the patient, presence or absence of diabetes mellitus (insulin resistance), and co-infection with hepatitis B and HIV.[20] We know that on-treatment viral kinetics, especially rapid virological response (RVR) can predict sustained virological response (SVR) stronger than these baseline predictors.[21]

Host genetic factors have recently been identified to influence treatment responsiveness in patients with chronic hepatitis C. In September 2009, Ge et al[22] in a genome-wide association study (GWAS) found that a single nucleotide polymorphism (SNP) at rs12979860 near *IL28* gene on chromosome 19, encoding interferon-lambda-3, is the strongest host genetic predictor of SVR in hepatitis C genotype 1. There are three genotypes of *IL28B*: CC, CT and TT. The CC genotype of *IL28B* is associated with two to three fold increases in sustained virologic response (SVR) as compared with either CT or TT genotype.[22,23]

Initially, most of the studies concentrated on the HCV genotype 1, which is the most frequent type in Western countries, and resulted in general agreement that *IL28B* non-CC genotypes are associated

Corresponding Author:
Dr. Javed Iqbal Farooqi
Associate Professor
Department of Medicine
PGMI, Lady Reading Hospital
Peshawar, Pakistan
E-mail: dr_farooqi@hotmail.com

with low SVR rates as compared to CC genotype. Later on HCV genotype 2, 3 and 4 were also studied for the impact of *IL28B* polymorphisms on the outcome of Anti-Viral Treatment (AVT). *IL28B* polymorphisms have the same impact on the outcome of AVT in HCV genotype 4 as that in HCV genotype1,[24,25] whereas conflicting results have been reported in HCV genotype 2 or 3. Some of the studies[26,27] has shown strong association of *IL28B* polymorphisms with the outcome of AVT in HCV genotype 2 and 3, whereas others[28,29] have failed to show any association.

HCV genotype 3 is the most common genotype in Pakistan, found in more than 85% of our HCV patients.[30,31] Association of *IL28B* polymorphisms with the outcome of AVT in HCV genotype 3 in Pakistan has not been studied so far. Therefore, we aimed to study *IL28B* genotypes (CC, CT and TT) in our patients of HCV genotype 3 who did not respond to 6 months combination therapy of IFN+Riba, and to establish their association with the lack of response in these patients.

MATERIAL AND METHODS

This cross-sectional study was conducted in Medical A Ward, PGMI, Lady Reading Hospital and Author's Private Hepatology Clinic, Peshawar, Pakistan from July 2013 to June 2014. All patients with chronic hepatitis C genotype 3 who did not respond to Standard-Interferon+Ribavirin, and then Pegylated-Interferon + Ribavirin Therapy for 6 months each, were included in the study by convenience sampling. Patients, with clinical evidence of liver cirrhosis, co-infection with HBV and diabetes mellitus were excluded from the study. Analysis of *IL28B* (rs12979860) polymorphism was performed by PCR-RFLP (Restriction Fragment Length Polymorphism) protocol by a Reference Lab.

According to HCV genotype, patients were divided in two groups: genotype 3a and genotype 3b. According to host genotype *IL28B* polymorphism, patients were divided into three groups: *IL28B* genotype CC, CT, and TT.

SPSS Version 16.0 was used to analyze the data. After entry, descriptive statistics was used to calculate Mean ± SD for numerical variables like age (in years) and ALT levels (U/L). Frequencies and percentages were calculated for gender, HCV and *IL28B* genotypic profile of patients. Chi-square (in Crosstabs analysis) was used to identify significant association between *IL28B* genotypes with the outcome of AVT in both gender and HCV genotype-3 subtypes.

RESULTS

A total of 184 patients were assessed during the study period. Among them, 71(38.6%) were males and 113 (61.4%) were females, with male to female ratio of 1:1.6. Mean age was 40.86 ± 9.943 years, ranged from 19 to 66 years. Majority of patients belonged to age-group of 31-40 years, followed by age-group of 41-50 years. (Table 1)

Table 1: Age-wise distribution of patients with chronic hepatitis C genotype 3.

Age group (years)	Male		Female		Total	
<20	1		0		1	0.5%
21-30	0		31		31	16.8%
31-40	34		35		69	37.5%
41-50	29		19		48	26.1%
51-60	6		28		34	18.5%
>60	1		0		1	0.5%
Total	71	38.6%	113	61.4%	184	100%

Table 2: ALT levels of patients with chronic hepatitis C genotype 3.

ALT group (U/L)	Male		Female		Total	
<40	4		12		16	8.7%
41-80	55		61		116	63%
81-120	7		25		32	17.4%
121-160	3		8		11	6.0%
161-200	1		2		3	1.6%
>200	1		5		6	3.3%
Total	71	38.6%	113	61.4%	184	100%

Table 3: Gender-wise distribution of IL28B profile of patients with chronic hepatitis C genotype 3.

IL28B genotypes	Male		Female		Total	
CC	27		18		45	24.5%
CT	35		76		111	60.3%
TT	9		19		28	15.2%
Total	71	38.6%	113	61.4%	184	100%

Table 4: HCV 3 subtype-wise distribution of IL28B profile of patients with chronic hepatitis C genotype 3.

IL28B genotypes	Genotype 3a		Genotype 3b		Total	
CC	36		9		45	24.5%
CT	79		32		111	60.3%
TT	17		11		28	15.2%
Total	71	38.6%	113	61.4%	184	100%

Table 5: Correlation of IL28B genotypes with gender and HCV genotype subtypes with chronic hepatitis C genotype 3.

IL28B genotypes	Gender		HCV-Genotype 3 subtypes	
	Male	Female	3a	3b
CC	27	18	36	9
Non-CC	44	95	96	139
p-value	*0.001	*0.001	*0.001	*0.001

The mean serum ALT was 73.52 ± 39.383 U/L, ranged from 17 to 255 U/L. Majority of patients belonged to ALT-Group of 41-80 U/L, followed by ALT-Group of 81-120 U/L. (Table 2)

Among the two subtypes of HCV genotype 3, subtype 3a was found in 132 (71.7%) patients (54 males and 78 females), whereas subtype 3b was found in 52 (28.35%) patients (17 males and 35 females). Among *IL28B* genotypes, CT was the most common, found in 111 (60.3%) patients, irrespective of gender (Table-3) and HCV genotype 3 subtypes (Table-4). Majority of the patients belonged to Non-CC genotype. (Table-5) Pearson Chi-Square test showed that *IL28B* Non-CC genotype is significantly associated with the "non-responder" status of the studied HCV genotype 3 patients irrespective of their gender status and genotype 3 subtype status (p=0.001), and similarly statistically sagnifiantassociation was seen between virological and clinical profile (p=0.001).

DISCUSSION

To our knowledge this is the first study of the role and relevance of *IL28B* genetic polymorphism to IFN+Riba treatment outcome in the setting of chronic hepatitis C genotype 3 patients in Pakistan. As mentioned before, in contrast to HCV genotype 1, only a few studies are available where association of *IL28B* polymorphisms has been analyzed with the outcome of AVT (IFN+Riba) on a limited number of HCV genotype 3 patients, with conflicting results. Mangia et al,[26] evaluated the effects of *IL-28B* polymorphisms on response to treatment with Peg-interferon and Ribavirin in 268 patients of Caucasian origin, out of these only 55 patients were HCV genotype 3. The frequencies of the *IL28B* genotypes were as follows: CC 37%; CT 48%; and TT 15%; 82% of patients with the CC genotype achieved a sustained virologic response (SVR), compared with 75% with the CT and 58% with the TT genotypes (p=0.004). Differences between *IL28B* genotypes were greatest among patients who failed to attain RVR (VD24 SVR rates: CC 87%; CT 67%; and TT 29%; p=0.0002). Among patients with RVRs (61%), the *IL28B* genotype was not associated with SVR (>70% for all *IL28B* genotypes). In a multivariable logistic regression model, *IL28B* genotype predicted SVR (Odds ratio 1.76; 95% confidence interval, 1.16-2.7). Sarrazin et al[27] analyzed 267 patients with chronic hepatitis C genotype 2/3 for association of three *IL28B* host genotypes (rs8099917, rs12980275 and rs12979860) with sustained virologic response (SVR) to antiviral therapy with Pegylated- interferon-alfa and Ribavirin and with respect to epidemiological, biochemical, and virological parameters. For comparison, hepatitis C genotype 1 patients (n = 378) and healthy controls (n = 200) were also included. The rs12979860 CC genotype,

lower age, and genotype 2 were significantly associated with SVR in HCV genotype 2/3-infected patients ($p = 0.01$, $p = 0.03$ and $p = 0.03$, respectively). No association was observed for rs8099917 and rs12980275. In addition, an SVR in patients with rapid virologic response (RVR) was associated with the rs12979860 CC genotype ($p = 0.05$), while for non-RVR no association was found. Furthermore, a significant association with a higher baseline viral load was observed for all three *IL28B* genotypes in genotype 1/2/3-infected patients. Finally, increasing frequencies of the rs12979860 CC genotypes were observed in genotype 1- (33.9%), genotype 3- (38.9%), and genotype 2-infected (51.9%) patients in comparison with healthy controls (49.0%) ($p < 0.01$).

We analyzed 184 patients with chronic hepatitis C genotype 3; the number of patients in our study was higher than Mangia et al[26] and Sarrazin et al.[27] All of these 184 patients were "non-responders" to standard Interferon+Ribavirin initially and later on repeat therapy of Pegylated-Interferon+Ribavirin. Each therapy was given for 6 months. RVR was not achieved with either therapy in any of these patients. Out of these, 132 (71.7%) patients were having HCV genotype *3a* and 52 (28.35%) patients were having HCV genotype *3b.* Among *IL28B* genotypes, CT was the most common, found in 111 (60.3%) patients. Our data indicate that the *IL28B* polymorphism is relevant to treatment outcome in HCV genotype 3. We found that *IL28B* Non-CC genotype is significantly associated with the Non-Responder status of the studied HCV genotype 3 patients, irrespective of their gender status and genotype subtype status (p=0.001), and similarly the strength of association between virological and clinical profile is very strong (p=0.001). We found CT genotype to be the most common *IL28B genotype*. Our study confirms the findings reported by Mangia et al[26] and Sarrazin et al,[27] none of our patients had achieved rapid virological response (RVR), suggesting that the major effect of this genetic variant was in patients who did not achieve a rapid response at week 4, where *IL28B* genotype had a strong influence on the rate of SVR. Grebely et al[32] discussed the potential role of *IL28B* genotypes in treatment decision making in hepatitis C. In view of our findings as well, it seems logical that in patients who fail to achieve RVR, early therapeutic intervention may be recommended for individuals with unfavorable *IL28B* genotypes.

CONCLUSION

Our findings strongly suggest *IL28B* polymorphism as a predictor of antiviral response in chronic hepatitis C genotype 3 like previously observed in HCV genotype 1, and particularly in patients who did not achieve RVR with IFN+Riba. In the era of oral anti-HCV drugs, further prospective randomized studies are needed to investigate whether patients without RVR carrying the *IL28B* non-CC genotypes benefit from the oral anti-HCV drugs.

REFRENCES

1. http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003
2. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007; 13:2436–41.
3. Cornberg M, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C, et al. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. Liver Int 2011; 31 Suppl 2:30–60.
4. Global Burden of Hepatitis C Working Group. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol 2004; 44:20–9.
5. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5:558–67.
6. Alavian SM. New globally faces of hepatitis B and C in the world. Gastro Hepat FBB 2011; 4:171-4.
7. Luby SP, Qamruddin K, Shah AA, Omair A, Pahsa O, Khan AJ, et al. The relationship between therapeutic injections and high prevalence of hepatitis C infection in Hafizabad, Pakistan. Epidemiol Infect 1997; 119:349–56.
8. Pasha O, Luby SP, Khan AJ, Shah SA, McCormick JB, Fisher-Hoch SP. Household members of hepatitis C virus-infected people in Hafizabad, Pakistan: infection by injections from health care providers. Epidemiol Infect 1999; 123:515–8.
9. Shazi L, Abbas Z. Comparison of risk factors for hepatitis B and C in patients visiting a gastroenterology clinic. J Coll Physicians Surg Pak 2006; 16:104–7.
10. Ambreen S, Mohammad A, Umar M, et al, Nine year audit liver disease burden and liver related mortality audit in tertiary care hospital of Rawalpindi Medical College. Pak J Gastroenterol 2008; 22:31-4.
11. Khokhar N, Niazi S. Chronic liver disease related mortality pattern in northern Pakistan. J Coll Physicians Surg Pak 2003; 13:495-7.
12. Umar M, Khaar HB, Khan AA, et al. Diagnosis, management, and prevention of hepatitis C in Pakistan. Pak J Gastroenterol 2009; 23:8-19.
13. Umar M, Khaar HT, Khurram M, Hasan Z. Anti-HCV antibody positivity of various sections of Pakistani patients. J Coll Physicians Surg Pak 2009; 19:737-41.
14. Umar M, Bushra H, et al. Hepatitis C in Pakistan: A review of available data. Hepat Mon 2010; 10:205-14.
15. Khan AA, Sarwar S. Response to combination therapy in Hepatitis virus C genotype 2 and 3. J Coll Physicians Surg Pak 2009; 19:473-7.

16. Batool U, Qureshi S. Declining sustained virological response in hepatitis C. J Coll Physicians Surg Pak 2006; 16:187-91.

17. Umar M, Bilal MI. Hepatitis C, A Mega Menace: A Pakistani Perspective. J Pak Med Stud 2012; 2: 68-72.

18. Alavian SM, Tabatabaei SV, Keshvari M, Behnava B, Miri SM, Elizee PK, et al. peginterferon alpha-2a and ribavirin treatment of patients with haemophilia and hepatitis C virus infection: a single centre study of 367 cases. Liver Int 2010; 30:1173-80.

19. Alavian SM. Are the real HCV infection features in Iranian patients the same as what is expected? Hepat Mon 2005; 5:3-5.

20. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of Hepatitis C Virus Infection. J Hepatol 2011; 55:245-64.

21. Poordad FF. The role of rapid virological response in determining Treatment duration for chronic hepatitis C [Review Article]. Aliment Pharmacol Ther 2010; 31:1251-7.

22. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009; 461:399-401.

23. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pre-treatment predictor of sustained virologic response in hepatitis C virus. Gastroenterology 2010; 139:120-9.

24. Asselah T, De Muynck S, Broet P, Masliah-planchon J, Blanluet M, Bieche I, et al. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. J Hepatol 2011; 56:527-32.

25. De Nicola S, Aghemo A, Rumi MG, Galmozzi E, Valenti L, Soffredini R, et al. An IL28B polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. Hepatology 2012;55:336-42.

26. Mangia A, Thompson AJ, Santoro R, piazzolla V, Tillmann HL, Patel K, et al. An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. Gastroenterology 2010; 139:821-7.

27. Sarrazin C, Susser S, Doehring A, Lange CM, Muller T, Schlecker C, et al. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. J Hepatol 2011; 54:415-21.

28. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010; 138:1338-45.

29. Moghaddam A, Melum E, Reinton N, Ring-Larsen H, Verbaan H, Bjoro K, et al. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. Hepatology 2011; 53:746-54.

30. Hussain A, Nasir MI, Siddiqui AA, Ahmed A. Prevalence of HCV genotypes in patients reporting in tertiary health care hospital of Karachi. Pak J Pharma 2011; 28:23-9.

31. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J 2011; 8:433.

32. Grebely J, Petoumenos K, Hellard M, Matthews GV, Suppiah V, Applegate T, et al; ATACH Study Group. Potential role for interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. Hepatology 2010; 52:1216-24.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.