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Interferon lambda-3 rs12979860 variants and response to pegylated interferon in chronic hepatitis-c genotype-3

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Interferon Lambda-3 rs12979860 Variants and Response to Pegylated Interferon in Chronic Hepatitis-C Genotype-3

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ABSTRACT

Objective: To assess the role of single nucleotide polymorphisms (SNPs) near the interferon lambda-3 (IFNλ3) (formal IL-28B) gene rs12979860 in predicting sustained virologic response (SVR) in hepatitis-C virus genotype-3 (HCV-3).
Study Design: Descriptive, analytical study.
Place and Duration of Study: Department of Medicine, The Aga Khan University Hospital, Karachi, from July 2012 to June 2014.
Methodology: Patients with HCV-3 were classified as sustained virologic response (SVR), relapsers and non-responders. SNP rs12979860 was determined by PCR-RFLP protocol. Differences between categorical variables were assessed by chi-square or Fisher's exact test, while those between continuous variables were evaluated using the Mann-Whitney U-test. Binary logistic regression analysis by forward conditional method was performed by using significant variables with p-values less than 0.05 as the criteria for model inclusion.
Results: Out of 115 patients, rs12979860 genotype-CC, CT, TT was found in 37 (32.2%), 70 (60.9%), and 8 (7%) patients. 72 patients were male with median age of 45 years. Cirrhosis was present in 32 patients. Patients with response failures (no response and relapse, n=36 and 29, respectively) had higher baseline gamma glutamyl transferase (GGT) level (p < 0.001), higher alanine aminotransferase (p=0.027) and cirrhosis (p=0.001) than patients with SVR. Genotype-CC was present in 16/65 in response failures compared to 21/50 who achieved SVR (p=0.048). Rapid virologic response (RVR) (p < 0.001), low GGT (p=0.001) and absence of cirrhosis (p=0.039) were the independent predictive factors for SVR. In patients who could not achieve RVR and in patients with cirrhosis, SVR was seen more in with genotype-CC (p=0.007 and 0.038).
Conclusion: In patients infected with HCV-3, IFNλ3 rs12979860, SNP has less impact on SVR.

Key Words: Hepatitis-C.   Interferon lambda-3.   Interleukin-28B.   Interferon.

INTRODUCTION

Studies have shown that genetic polymorphism (rs12979860) near the interferon-lambda-3 (IFNλ3) gene, on chromosome-19, formally known as interleukin 28B, is associated with variable responses to the pegylated interferon (Peg-IFN). The CC-genotype of IFNλ3 is associated with two to three fold increase in sustained virologic response (SVR) as compared with either CT or TT genotype in patients infected with hepatitis-C virus (HCV) genotype-1.[1,2]

A landmark study published in Nature-1, analyzed the data of more than 1,600 patients infected with HCV genotype-1 enrolled in another clinical trial.[3] SVR rates in patients with the CC-genotype of rs12979860 IFNλ3 were 69% in Caucasians, 48% in African-Americans, and 56% in Hispanics. For each group, these response rates were substantially higher (two folds) than for two other genotypes, TC and TT. Overall, the adjusted odds ratio for SVR associated with CC versus the other genotypes was 5.2 (95%; CI 4.1 to 6.7), the largest odds ratio of any pre-treatment predictor. The rs12979860-C variant is most frequently present in individuals from East Asia (allele frequency > 0.9) and least common in individuals of African origin (allele frequency 0.2 - 0.5).[4] In a US based study, the favourable CC genotype was observed in 37% of Caucasians, 29% of Hispanics, and 14% of African Americans tested.[2]

Though HCV genotype-3 (HCV-3) was considered as an 'easy-to-treat' virus with pegylated interferon (Peg-IFN) and ribavirin, the assumption was based on the initial studies that analyzed the combined data of genotype-2 and 3.[5] However, SVR rates appear to be lower in certain subgroups of people infected with HCV-3 compared with those with HCV-2. There are few available data regarding the association between IFNλ3 rs12979860 polymorphism and SVR to Peg-IFN plus ribavirin therapy in HCV-3 patients in Pakistani population.

The aim of this study was to find out frequency of IFNλ3 rs12979860 CC, TC and TT genotypes in patients infected with HCV-3, with SVR, non-response or relapse to the Peg-IFN and ribavirin therapy.

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METHODOLOGY

The study was conducted at the Department of Medicine, The Aga Khan University Hospital, Karachi, from July 2012 to June 2014.

Study population included HCV-3 patients who were treated with peg-IFN and ribavirin with compensated liver disease for whom longitudinal observations were available from the medical records. Patients were considered to have chronic hepatitis-C when HCV-RNA was detectable in patients reactive for anti-HCV antibodies for more than 6 months. Patients were categorized as responders, non-responders, and relapsers to the therapy. Exclusion criteria were previous treatment with standard or Peg-IFN, duration of treatment less than laid down criteria for response guided therapy, co-infection with hepatitis-B or HIV, decompensated cirrhosis, alcohol abuse, autoimmune disorders or hepatocellular carcinoma. Patients were also excluded when end-of-treatment and follow-up HCV RNA status was not documented.

The duration of treatment was 6 months for patients who had a rapid virologic response (RVR), i.e. clearance of the virus from the blood at 4 weeks of therapy, and 48 weeks who could not achieve RVR but had an early virologic response (EVR), i.e. negative serum HCV RNA at 12 weeks of therapy. Responders were the patients who had negative HCV RNA at the end of treatment and 6 months post-treatment (SVR). Patients who could not clear the virus at 12 weeks of treatment were considered as non-responders. Relapsers had end of treatment virologic response (ETR) but no SVR.

The study was approved by the Ethics Committee of the hospital. Informed consent was obtained from each patient. Baseline characteristics at the time of commencement of therapy were noted from the records, including age, gender, body mass index (BMI), HBsAg status, HCV RNA, qualitative or quantitative, and genotype and duration of therapy received. Blood samples were taken for HCV RNA and SNP. SNP of IFNλ3 rs12979860 was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocol as mentioned below. HCV RNA clearance was compared in lambda-3 rs12979860 "CC" with "non CC".

Seven ml blood was drawn into ethylene diamine tetra acetic acid (EDTA) tube. Genotyping for the IL-28B rs12979860 C/T polymorphism was performed by polymerase chain reaction based restriction fragment length polymorphism assay. Genomic DNA was extracted from whole blood samples by means of the QIA amp DNA blood mini-kit (Qiagen, Milan, Italy) according to manufacturer's instruction. A 242 base pair (bp) product was obtained with the forward primer 5'-GCTTATCGCATACGGCTAGG-3' and the reverse primer 5'-AGGCTCAGGGTCAATCACAG-3', as previously described.[6] PCR amplification was carried out in a total volume of 10 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, Tween-20 0.01%, 0.2 mM deoxyribonucleotides, 2-4 pmol of each primer, 2.0 mM MgCl$_2$, and 0.5 units hot-start Taq DNA polymerase (Promega, Madison, WI, USA). Samples containing 10 ng of genomic DNA were subjected to 40 cycles of denaturation (at 95°C for 30 seconds), annealing (at 62°C for 30 seconds), and elongation (at 72°C for 30 seconds) using a Perkin Elmer 9700 thermal cycler. In a total volume of 20 µl, 10 µl of the amplified products were digested with 1 unit of the Bst U-I restriction endonuclease (New England Bioloabs, Hitchin, UK) at 60°C overnight. The fragments digested were, respectively, 135 + 82 + 25 bp for the C allele and 160 + 82 bp for the T-allele variant. The fragments were resolved by electrophoresis in 3.5% agarose gel after staining with ethidium bromide. In 5 patients, the genomic region encompassing the IL-28B rs12979860 C/T polymorphism was sequenced with results confirming those obtained by the RFLP assay.

Serum HCV-RNA levels were quantified with the Real Time PCR COBAS AmpliPrep/COBAS TaqMan HCV Test 2.0 (Roche Molecular Systems, NJ, USA). The reverse transcription reaction (RT) was performed using first strand M-MLV reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, 5 µl out of the extracted RNA was added to 15 µl of RT mixture containing RT buffer, dNTP mix (10 mM each of four deoxynucleoside triphosphate stocks), 50 µM random hexamer primer and 75 U of M-MLV reverse transcriptase. The RT reaction mixture was incubated at 37°C for 120 minutes, and then at 85°C for 5 minutes to inactivate the enzyme. The resulting 20 µl of cDNA was kept at -20°C until use.

Statistical analyses were performed using SPSS, release 20 (IBM Corporation, Chicago, IL, USA). Categorical variables were expressed as frequencies (%) while continuous variables were presented as median with 25th-75th percentiles inter-quartile range (IQR). Differences between categorical variables were assessed by chi-square or Fisher's exact test, while those between continuous variables were evaluated using the Mann-Whitney U-test. Binary logistic regression analysis by forward conditional method was performed by using significant variables with p-values less than 0.05 as the criteria for model inclusion. For this model, continuous variables were converted into dichotomous variables using median values as cut off.

RESULTS

Out of 115 patients, 72 (62.3%) were male. Mean age was 43.4 ±10.4 years, median 45 years ranging from 18 - 67 years and interquartile range (IQR) of 36.0-50.0. Median body mass index (BMI) was 26.2 (IQR: 23.5-29.6), alanine aminotransferase (ALT) 67 (IQR: 44-101),

gamma glutamyl transferase (GGT) 51 (IQR: 32-83). Baseline quantitative HCV RNA levels were available only in 43 patients; median 7.84 x 10^5 (IQR: 2.11 x 10^5 - 2.91 x 10^6). Twenty-seven (23.5%) patients were diabetic. Cirrhosis of the liver as evident from histology, ultrasound or clinical examination was present in 32 (27.8%) patients. Patients with SVR were 50 (43.5%), non-responders 36 (31.3%), and relapses 29 (25.2%). IFNλ3 rs12979860 genotype CC, CT, TT was found in 37 (32.2%), 70 (60.9%), and 8 (7%) of patients. In carriers of rs12979860 genotype CC, SVR, relapse and non-response was seen in 21 (56.8%), 7 (18.9%), and 9 (24.3%) patients. These figures were 24 (34.3%), 21 (30.0%), and 25 (35.7%) for genotype CT; and 5 (62.5%), 1 (12.4%), 2 (25%) for genotype TT, respectively. Rapid virologic response (RVR) was not associated with IFNλ3 rs12979860 genotype as it was seen in 21/37 of CC genotype and 40/78 of non-CC genotype (p=0.583). RVR was linked with absence of clinical cirrhosis; achieved in 51/83 (61%) patients without cirrhosis versus 10/32 (31%) with cirrhosis (p=0.004). However, in patients who could not achieve RVR, SVR was seen more in patients with genotype CC; 5/16 (31%) patients with genotype CC compared to 1/37 (3%) patients with non-CC (p=0.007). There was no signigicant association of the presence of clinical cirrhosis with rs12979860 genotype (p=0. 564). Nevertheless, in patients with cirrhosis (n=32), SVR was achieved in 4/6 patients with genotype CC, compared with 5/26 with non-CC genotype (p=0.038).

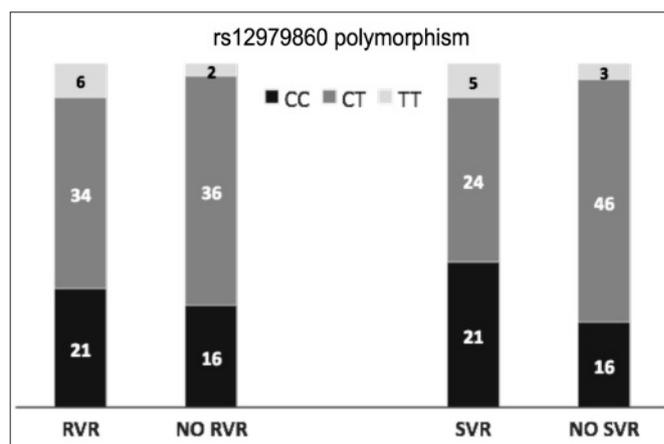There was no difference in the age, BMI, and HCV RNA level among patients with SVR and response failures

Figure 1: IFNλ3 rs12979860, CC or non-CC genotypes, and response to pegylated interferon in patients with hepatitis D
ETR = End of treatment virologic response; SVR = Sustained virologic response.

(non-response, or relapse) to the treatment (Table I). The median GGT of response failures was higher than responders (p < 0.001). They had higher ALT levels (p=0.027) and cirrhosis (p=0.001). Genotype CC was present in 16/65 (25%) response failure patients compared to 21/50 (42%) who achieved SVR (p=0.048). Logistic regression analysis showed that RVR (p < 0.001), low GGT (p=0.001) and absence of clinical cirrhosis (p=0.039) were the independent factors to have a SVR.

DISCUSSION

Host genetics play an indispensable part in the ability not only to clear acute hepatitis-C infection, but likewise

Table I: Characteristics of patients who received pegylated interferon plus ribavirin therapy.

	Sustained responders n=50	Non-responders and relapsers n=65	p-value	Odds ratio	95% Conf. Interval
Gender					
Male	33 (66%)	39 (60%)	0.510	1.29	0.56-3.00
Female	17 (34%)	26 (40%)			
Age (years)					
Median (IQR)	42 (32-50)	45 (37-50)	0.199		
Age < 45	28 (56%)	26 (40%)	0.088	1.91	0.85-4.32
Body mass index (kg/m^2)					
Median (IQR)	26.4 (23.5-29.0)	26.2 (23.8-29.2)	0.772		
BMI up to 26 kg/m^2	22 (44%)	28 (43%)	0.921	1.04	0.46-2.34
Absence of diabetes	41 (82%)	47 (72%)	0.224	1.75	0.65-4.75
Absence of cirrhosis	44 (88%)	39 (60%)	0.001*	4.89	1.68-14.89
ALT (IU/L)					
Median (IQR)	60 (39-91)	71 (47.5-114.5)	0.027		
ALT ≤ 67	29 (58%)	29 (45%)	0.155	1.71	0.76-3.87
GGT (IU/L)					
Median (IQR)	34 (27-54.3)	70 (44-121)	<.001*		
GGT ≤ 51	37 (74%)	21 (32%)	<.001*	5.96	2.45-14.78
Interferon λ3 type					
CC	21 (42%)	16 (25%)	0.048*	2.22	0.93-5.32
Non-CC	29 (58%)	49 (75%)			
Rapid virologic response	44 (88%)	17 (26%)	<.001*	20.71	6.85-65.99

*P-values by Fisher exact test / chi-square teat or Man-Whitney U test. *statistically significant p-values S.D.: standard deviation; IQR: interquartile range; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase.*

to achieve sustained virologic response (SVR) to interferon. In a study, carriers of the IFNλ3 rs12979860 C-allele who responded to interferon therapy, exhibited increased IFN-lambda levels.[7] Moreover, high IFN-lambda levels pre-disposed to spontaneous resolution of HCV infection. Thus, IFN-lambdas seem to play an important role in the control of hepatitis-C.

The role of IFNλ3 polymorphism in predicting response to interferon alpha based treatment in case of genotype 3 is debatable.[8-12] Nevertheless, a recent adequately powered study demonstrated that IFNλ3 genotypes are strong baseline predictors of SVR.[10] The writers concluded that confounders including cohort size explained to a great degree the controversy from previous stories. When the sample size is decent, the association between IL-28B and RVR or SVR can be appreciated in HCV-3.[11] Thus, it may be rational to evaluate IFNλ3 genotyping in patients with HCV-3 infection. Apparently the predictive value of rs12979860 CC in case of HCV-3 is less strong requiring a bigger sample size to become an independent predictor. This study of 115 patients indicates that rs12979860 SNP associates with SVR in HCV-3 infected patients. However, this variable got dropped during the regression analysis.

Distribution of rs12979860 genotypes in our general population is not known. In a study from Thailand, the distribution of IFNλ3, rs12979860 CC, CT, and TT in hepatitis-C patients of all viral genotypes was 84%, 12.4% and 3.6%, respectively.[8] In this study of HCV-3 patients, rs12979860 CC/CT/TT was found in 32.2%, 60.9% and 7% patients. Significantly lower frequencies of the favourable genotypes CC in these HCV-3 patients may be due to lower frequency of this genotype in the studied population or significant number of individuals with this genotype spontaneously clear the virus.

IFNλ3 genotype CC-genotype patients respond more promptly to treatment compared with the CT and TT genotypes in case of infection with HCV genotype-1. Moreover, CC genotype also predicted SVR in Caucasian patients who did not have RVR (66% vs. 31% and 24% respectively).[2] In this study done on genotype-3 patients, there was no difference in the RVR between genotype CC and non-CC. RVR was associated with absence of clinical cirrhosis (p-0.004). However, in patients who could not achieve RVR, SVR was seen in 5/16 patients with genotype-CC compared to 1/37 patients with non-CC (p=0.007). Other workers have also observed stronger impact of this polymorphism in this sub-set of patients.[13]

Few data are available about the value of IFNλ3 polymorphism in predicting SVR in patients with cirrhosis. Bruno et al. could not find its association with the clinical outcome in patients with HCV-3 induced compensated cirrhosis.[14] In this study, clinical cirrhosis

was present in 32 patients. SVR was less in patients with cirrhosis (p=0.001). SVR was achieved in 4/6 patients with genotype-CC, compared with 5/26 with non-CC genotype (p=0.038). But the number is too small and a larger study needed to accept or refute the role of this SNP in HCV-3 cirrhotic patients. So SVR appears to be lower in certain sub-groups of people infected with HCV-3 where the predictive value of IFNλ3 genotypes increases.

Suppiah et al. and Tanaka et al. identified a second polymorphism (rs8099917) in a similar region near the IL-28 gene, which was strongly linked with response to combination treatment with interferon and ribavirin in Australian and Japanese patients, all infected with viral genotype-1.[15,16] Higher rates of SVR are associated with rs8099917 TT-genotype.[17] Rauch et al. conducted a genome-wide association study (GWAS) including all viral genotypes 1-4, and found that the rs8099917 minor allele was linked with both progression in chronic hepatitis-C and failure to respond to treatment, with the most potent effects in patients infected with genotypes-1 or 4.[18] The gene polymorphsion rs8099917 was tested in only 25 patients. Its TT, TG and GG genotypes were in concordance with rs12979860 CC, CT and TT in all, except in 2 cases. Due to its strong correlation with rs12979860 in IL-28B, we thought there would not be any added benefit of testing this polymorphism in our patients. Similar correlation was found in another study done in patients of French ancestry in Canada.[19] These patients were not tested for IFN lambda-4. This polymorphism has been investigated as predictor of SVR in genotype-3 infected patients with conflicting results,[20,21] and testing for the SNP in this region as treatment predictor in Caucasian patients also does not offer any special advantage.[20]

Despite the availability of direct acting anti-viral drugs interferon based regimens will remain in the armamentarium for patients with HCV-3, due to non-availability and exuberant cost of DAAs or in combination with DAAs to achieve a better response rate. So the IFNλ3 SNP status in these patients may facilitate in decision-making for these patients. Though a meta-analysis failed to measure the impact of the IFNλ3 SNP in HCV-3 treatment outcome,[22] the bearing of the favourable genotypes-CC may be one of the pre-treatment predictors of the effectiveness of interferon based therapy in difficult-to-treat-patients infected with HCV-3.

CONCLUSION

Genetic analysis for IFNλ3 rs12979860 is only one of many factors that can influence response rates to pegylated interferon and ribavirin therapy in HCV-3 infection and should be interpreted in the context of other clinical factors predicting SVR including advanced

fibrosis and cirrhosis. IFNλ3 genotyping can aid in clinical decision-making for patients with HCV-3 who have cirrhosis or could not achieve RVR infection.

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