

# Effect of IL-28 B Polymorphisms on Early Virological Response (EVR) in Chronic Hepatitis C Patients Treated with Interferon and Ribavirin

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## Abstract

**Background:** To determine the frequency of EVR in chronic hepatitis C (CHC) patients treated with Interferon and Ribavirin and to compare the effect of IL-28B SNP rs12979860 (CC and non CC genotypes) on frequency of EVR.

**Methods:** In this cross-sectional study 100 patients with Chronic Hepatitis C (CHC) with genotype 3 who received Interferon and Ribavirin in the standard doses were categorized in two groups depending upon the IL-28B SNP rs12979860 CC and non CC genotypes. Results of Qualitative PCR for HCV RNA after 12 weeks of treatment and EVR were entered. Frequency of EVR in the two groups (CC and non CC) was compared.

**Results:** Among the 100 patients with Chronic Hepatitis C treated with Interferon and Ribavirin, 72 patients achieved EVR (72%). Out of the 100 patients, 52 had CC genotype and 48 had non-CC genotype (40 with CT and 8 with TT genotype). In the CC group 47 out of 52 patients achieved EVR (90%) while in the non-CC group 25 out of 48 patients achieved EVR (52%). The p value in our study was 0.00

**Conclusion:** The frequency of EVR is 72% in Chronic Hepatitis C patients infected with genotype 3 treated with Interferon and Ribavirin which is comparable with Pegylated Interferon and Ribavirin. Patients with IL-28B SNP rs12979860 CC genotype have a better chance to achieve EVR (90%) as compared to the non-CC genotype (52%).

**Key Words:** Chronic hepatitis C (CHC), EVR, Interferon, Ribavirin, IL28B polymorphism

## Introduction

Hepatitis C is a global health problem affecting 3% of the total world population.<sup>1</sup> About 170 million people

are infected worldwide.<sup>2</sup> The prevalence of Hepatitis C in Pakistan is 4.7% which indicates a great burden of the disease.<sup>3</sup> Hepatitis C is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma.<sup>2,4-6</sup> Because of the high cost of treatment with PEG-IFN, Interferon is widely used in developing countries for treating Hepatitis C virus infection with good results.<sup>5,6</sup>

The treatment response varies among people with different ethnicity and better response rates have been studied in Asian patients.<sup>1,4</sup> Host genetic factors were considered to be important determinants for the treatment of CHC.<sup>1,4</sup> Recent genetic studies have resolved this issue and confirmed that there is a significant association between the single nucleotide polymorphisms (SNPs) of Interleukin-28B (IL28B) and Sustained virological response (SVR) when patients are treated with PegIFN/RBV.<sup>1,4,7,8</sup> In Hepatitis C patients receiving treatment better outcomes are seen with CC genotype then with CT and TT.<sup>8,10</sup>

Current treatment strategies for CHC treatment recommend to decide the duration of treatment on the basis of host and viral genotypes and viral responses during treatment.<sup>4</sup> Early virological response (EVR) is an important criteria for the decision to continue or terminate the treatment because very few patients who cannot achieve EVR can achieve sustained virological response (SVR).<sup>4,9</sup> The positive predictive value (PPV) of EVR associated with good treatment compliance is very high, achieving SVR in 75% of cases.<sup>9</sup>

Asians have C allele of rs12979860 of IL28B in highest frequency as compared to people with other ethnicity.<sup>4</sup> The CC genotype of rs12979860 has strong association with SVR in each population.<sup>4</sup> This genotype of IL28B is also associated with the spontaneous clearance of hepatitis C virus and with on treatment viral responses.<sup>4,7</sup> Gupta et al showed that

among the patients who achieved SVR, 61.1% had CC genotype and 38.9% had non-CC genotype.<sup>10</sup> A genetic testing for the SNP of IL28B rs12979860 has been suggested before deciding on treatment strategies because it significantly affects the treatment outcome.<sup>4</sup>

In a study conducted by Masood et al., EVR was achieved in 95.9% and ETR was achieved in 96.6% of chronic hepatitis C patients treated with Interferon and Ribavirin.<sup>5</sup> It has seen that peg interferon has better response than standard interferon for treatment of Hepatitis C positive patients.<sup>5</sup>

A large number of people infected with HCV infection are unaware of their infection. Those who have been diagnosed, treatment is not available because of the high cost of the treatment.<sup>11</sup> When given the treatment has high success rates even in the low income countries.<sup>12</sup> 33% of the patients with Chronic Hepatitis C develop liver cirrhosis or hepatocellular carcinoma.<sup>13</sup>

The limitations of treatment include high cost, the need for sophisticated laboratory tests and trained clinicians, as well as the limited efficacy and high toxicity of some of the medicines.<sup>14</sup>

### Patients and Methods

This cross sectional study was conducted at Centre for Liver and Digestive Diseases, Holy Family Hospital, Rawalpindi between September to February 2014. One hundred patients with Chronic Hepatitis C (CHC) with genotype 3 who received Interferon and Ribavirin in the standard doses (Interferon 3 million international units subcutaneously thrice weekly and Ribavirin 800 mg daily in divided doses) for 3 months were categorized in two groups depending upon the IL-28B SNP rs12979860 CC and non CC genotypes. Patients with Hb > 10g/dl, platelets > 100,000/mm<sup>3</sup> and TLC > 3000/mm<sup>3</sup> were included. Patients co-infected with Hepatitis B virus and who had ultrasonographic evidence of cirrhosis and portal hypertension were not included. Patients who developed complications during the treatment e.g. thrombocytopenia (Platelet count < 80,000/mm<sup>3</sup>), anemia (Hemoglobin < 8 g/dl) and neutropenia (ANC < 1000/mm<sup>3</sup>) were not included because treatment had to be withheld for variable period in those patients. Results of Qualitative PCR for HCV RNA after 12 weeks of treatment and early virological response (EVR) were entered. Frequency of EVR in the two groups (CC and non CC) was compared.

Analysis of IL 28B SNP rs12979860 was performed by PCR-RFLP protocol in whole blood samples taken from Hepatitis C patients included in the study. To compare frequency of EVR in CC and non-CC

genotype, Chi Square Test was applied (p-value <0.05 was taken statistically significant). Post stratification Chi Square test was applied keeping p-value <0.05 as statistically significant.

### Results

Amongst 100 patients, 46% were males while 54% were females. Mean age was 34.03 years (±8.91 years) and range was from 18 years to 55 year (Table 1). Fifty two had CC genotype and 48 had non-CC genotype (40 with CT and 8 with TT genotype). Females were slightly more than males in each group. The percentages of males in CC group was 47.9% compared to 52.1% females and in non CC genotype group, males were 44.2% comparable to 55.8% females. Twenty one (43.8%) were up to 30 years of age in Non CC group comparable to 27 (56.3%) patients with ages more than 30 years. Amongst 52 patients with CC genotype, 19 (36.5%) were up to 30 years of age compared to 33 (63.5%) patients of ages more than 30 years (Table 2). The attainment of Early Virological Response (EVR) was observed in 72% of the 100 study participants. Among females 38 (70.4%) attained EVR and among males 34 (73.9%) achieved EVR (Table 2) Majority of the patients 47 (90.4%) with CC genotype attained EVR, In the non CC genotype group of patients, 25 (52.1%) patients attained EVR. This association was observed to be highly statistically significant based on Pearson's chi square test (d.f. 1,  $\chi$  statistic 18.16) with a p-value of 0.00 (Table 2). Even though no statistically significant association was observed between gender and attainment of EVR (d.f. 1,  $\chi$  statistic 1.60, p-value 0.20) as well as age group and attainment of EVR (d.f. 1,  $\chi$  statistic 0.15, p-value 0.69).

**Table 1: Age groups of the study participants according to the gender**

Age (years)	Gender		Total
	Male (No (%))	Female No (%)	
Upto 30	18(45.0%)	22(55.0%)	40(100%)
Above 30	28(46.7%)	32(53.3%)	60(100%)
Total	46%	54%	100%

**Table 2: Post stratification Chi-Square test**

Categories based on gender or age	Genotype	EVR not attained	EVR attained	Pearsons Chi Square
Males	Non CC	10(43.4%)	13(56.52%)	p-value 0.007
	CC	2(8.69%)	21(91.30%)	
Females	Non CC	13(52%)	12(48%)	p-value 0.001
	CC	3(10.34%)	26(89.65%)	
Age up to 30 years	Non CC	13(61.90%)	8(38.1%)	p-value 0.000
	CC	1(5.26%)	18(94.73%)	
Age above 30 years	Non CC	10(37%)	17(63%)	p-value 0.02
	CC	4(12.12%)	29(87.87%)	

## Discussion

Present study results are consistent with the international values which describes SVR of 70-80% in genotype 3 infected patients treated with PEG IFN and Ribavirin. The results are different from a Pakistani study conducted which showed an EVR rate of 95.9% in chronic hepatitis C patients.<sup>5</sup> Another Pakistani study showed an SVR rate of 75-81% in genotype 2 and 3 infected patients.<sup>6</sup> This study also shows that Interferon and Ribavirin combination therapy is still an acceptable choice to treat chronic Hepatitis C patients with genotype 2 and 3 in our region. Another study by Garcia et al who analyzed 1014 patients showed an EVR rate of 87% in total patients and 96% in genotype 2 and 3 infected patients but that study was conducted with PEG IFN and RBV.<sup>9</sup>

Our study observed a significant role of IL 28B polymorphism on EVR in chronic hepatitis C patients infected with Genotype 3 treated. Thomas et al. reported the rs12979860 C allele frequency in worldwide population, in that C allele frequency was 23% to 55% in African populations, 52% to 80% in European population, 75% to 98% in Southwest Asians, and 90% to 100% in East Asian populations.<sup>15</sup> Our study showed that 52% of the Pakistani population have favourable genotype of IL 28B (CC genotype) and thus can achieve good response after Interferon and Ribavirin therapy that is a response rate of 90%. An Indian study by Gupta et al showed CC genotype in 61% of Indian population with RVR rate of 72% in the total population studied.<sup>10</sup> All of these patients were infected with genotype 3.

Interferon is still used in low-income countries like Pakistan to treat chronic hepatitis C with fairly good results. Masood et al. showed that EVR was achieved in 95.9% and ETR was achieved in 96.6% of chronic hepatitis C patients treated with Interferon and Ribavirin.<sup>5</sup> It is important as these patients who will respond will have reduced risk of developing liver cirrhosis and hepatocellular carcinoma which are among the leading cause of death in our set up. Our study has shown a response rate of 72% in all the patients and a very high response rate of 90% in patients with the CC Genotype. Since the predictive ability of IL28B genotype is strong enough. Therefore, it is reasonable to recommend a pre-treatment genotyping for SVR prediction.

## Conclusion

1. The frequency of EVR is 72% in Chronic Hepatitis C patients infected with genotype 3 treated with Interferon and Ribavirin which is the same as

described in literature with Pegylated Interferon and Ribavirin.

2. Host genetic factors like IL 28B polymorphism guide individualized treatment strategies and aid in determining the best treatment plan for each patient

3. Patients with IL-28B SNP rs12979860 CC genotype have a much better chance to achieve EVR (90%) as compared to the non-CC genotype (52%) .

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