

## Interleukin 28B genotype in chronic HCV patients in relation to response to treatment by pegylated interferon and ribavirin

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**Abstract: Background:** Genome wide association studies have recently revealed that several single-nucleotide polymorphisms (SNPs) in the interleukin (IL) 28B genes can predict the sustained virological response (SVR) to pegylated interferon  $\alpha$ 2a/b plus ribavirin in hepatitis C virus (HCV) genotype 1 patients. However, data for patients infected with HCV genotype 4 (HCV G4) are limited. **Objectives:** The objective of this study was to study the effect of interleukin-28B genotype as a predictor of response of HCV patients to treatment by pegylated interferon and ribavirin & Compare IL-28B polymorphism as a predictor of response to other possible predicting factors. **Patients and methods:** We performed our study in the form of retrospective studies on one hundred patients treated with Reiferon Retard (pegylated interferon  $\alpha$  2a given at a dose 160  $\mu$ g /week) and ribavirin on weight based dosing from National Liver Institute HCV outpatient clinics Menoufiya University. Patients were divided into 3 groups according to response to treatment (responders, non responders and relapsers). IL28B SNPs (rs12979860) was identified by polymerase chain reaction and DNA sequencing. SVR was statistically correlated with various clinical, histopathological and genetic parameters. **Results:** IL28B Genotype showing that 19 patients (19 %) were of genotype C/C, 57 of patients (57%) were of genotype C/T and 24 patients (24%) were of genotype T/T. Also we noted that (14/19) 73.5% of cases having CC genotype, (16/57) 28% of those having CT genotype and only (5/24) 20% of those having TT genotype achieved SVR. Dyslipidemia showed no statistically difference in achieving SVR between those who have Dyslipidemia and those who do not have. Advanced fibrosis, positive PCR of HCV RNA at twelve week, high BMI, diabetes mellitus, older age and male gender were associated with non response to treatment with pegylated interferon and ribavirin. **Conclusions:** IL28 B is the strongest predictor of treatment in Egyptian population but alone should not be considered to predict treatment outcomes as there are many cofactors affecting treatment response and Analysis of IL28B genotype might be used to guide treatment for these patients. [Ahmed Shaban Mohamed, Elsayed Mohamed Abdelaal, Hassan Ahmed Elzohry, Imam Abdel Latif Waked and Hala Hany El Said. **Interleukin 28B genotype in HCV patients in relation to response to treatment by pegylated interferon and ribavirin.** *J Am Sci* 2015;11(5):23-33]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 4

**Key words:** Hepatitis C Virus (HCV), PCR of HCV RNA, pegylated interferon  $\alpha$  2a, IL 28 B genotype (C/C, C/T&T/T) ribavirin, Body mass index (BMI)

### 1. Background

Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease, cirrhosis, and Hepatocellular carcinoma. The strong homogeneity of HCV subtypes found in Egypt (mostly genotype 4) suggests an epidemic spread of HCV (1).

The current standard of care for chronic infection with HCV is 24 or 48 weeks of therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV). Response to therapy is variable and viral and host characteristics can influence whether patients achieve a sustained virological response (SVR) (2).

Viral genotype is a predictor of response. Patient genetic ancestry is also a factor in treatment outcome. Other factors predictive of response to PEG-IFN and RBV include hepatitis C viral load as well as patient age, sex, weight, liver fibrosis stage, and adherence to

therapy. Because PEG-IFN and RBV can cause burdensome adverse effects and treatment is prolonged, clinicians often weigh the various viral and host characteristics for each patient before initiating treatment (2).

Variation in IL28B appears to influence the kinetics of viral response to therapy. In 2009, reports from three genome-wide association studies described several highly correlated common single nucleotide polymorphisms (SNPs) in the vicinity of three IFN-lambda genes as being highly predictive of response to PEG-IFN and RBV therapy in patients with genotype 1 HCV. The three genes encode IFN- lambda 1 (IL29), IFN- lambda 2 (IL28A), and IFN- lambda 3 (IL28B) (3-4).

The same set of SNPs was subsequently associated with natural clearance of HCV (5).

Polymorphism rs12979860, which is upstream of

the IL28B gene on chromosome 19, was strongly associated with SVR. The C allele at rs12979860 was positively associated with SVR. The association between the rs12979860 SNP and SVR appears to be clinically relevant (6).

The presence of only one C allele conferred little benefit in treatment response, as was true in the analyses performed by Ge *et al.* and in the studies of spontaneous clearance reported by Thomas *et al.* (3-5).

After initiation of PEG-IFN and RBV therapy, patients with the C/C genotype at rs12979860 have a greater HCV RNA decline from days 0-28 than patients with the C/T or T/T genotype (6).

## 2.Objectives

The objective of this study was evaluating the effect of interleukin-28B genotype as a predictor of response of HCV patients to treatment by pegylated interferon and ribavirin & Compare IL-28B polymorphism as a predictor of response to other possible predicting factors.

## 3. Patients and methods

### Selection of Patients:

This study enrolled 100 patients from National Liver Institute HCV outpatient clinic, Menoufiya University, Egypt in a retrospective manner on those who received treatment with Reiferon Retard which is pegylated interferon  $\alpha$  2a by MENAPHARM in a dose of 160  $\mu$ g once per week and ribavirin on weight based dosing. All patients with chronic HCV infection by positive serum HCV-RNA PCR and liver biopsy examination for which scoring by ISHAK score was done were included in our study while Patients with other causes of chronic liver disease were excluded (e.g. chronic HBV) also we excluded patients with autoimmune disorders, pregnancy, limiting psychiatric, ophthalmological or cardiological disorders, those had platelet count less than 75,000/mm<sup>3</sup>, neutrophil counts less than 1500 cells/mm<sup>3</sup>, hemoglobin levels less than 10 g/dl. All patients were subjected to:

A. Thorough history taking and complete clinical examination.

B. Collection of data: age, weight and height to calculate body mass index (BMI), liver function tests done before start of treatment, Complete blood picture, quantitative PCR of HCV before, during, at the end of treatment and six months after end of treatment to evaluate response to treatment, liver biopsy done before treatment including stage of fibrosis, grade of activity and steatosis if present.

C. Sampling was performed after informed consent was obtained from each patient included in the study to use the samples and clinical data for research purposes after being informed about the

nature of the study. The study protocol conforms to the most recent ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the National Liver Institute (NLI) human research committee.

### DNA Collection, Extraction and IL28B Genotyping:

Blood was collected into EDTA tubes. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quality was assessed by calculating the absorbance ratio OD260nm/280nm using NanoDrop model ND-1000 (Wilmington, USA). DNA samples were subjected to DNA quantization and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). All equipment was calibrated. DNA quantization the NanoDrop spectrophotometer was blanked; a spectrum was taken as a reference material (blank) and was stored in the memory as an array of light intensity by wavelength. Measurement of a sample was taken; the intensity of light that had transmitted through the sample was recorded. The sample intensities along with the blank intensities were used to calculate the sample absorbance. The calculation of the concentration was automated. IL28B variant rs12979860 was detected using an allelic discrimination (AD) assay; a multiplexed (more than one primer/probe pair per reaction) endpoint (data is collected at the end of the PCR process) assay that detects variants of a single nucleic acid sequence in TaqMan® Gene Expression Assays. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence. For each sample in an AD assay, a unique pair of fluorescent dye detectors is used, for example, two TaqMan® MGB probes that target a SNP site. One fluorescent dye detector is a perfect match for the wild type and the other fluorescent dye detector is a perfect match for the polymorphism (allele 2).

### Patients' Classification:

Patients were classified according to their response to pegylated interferon and ribavirin therapy into three groups. Group I: 36 nonresponders (NR), including those who had detectable HCV RNA at weeks 12, 24, 48. Group II: 29relapsers who had detectable HCV RNA 24 weeks after stoppage of treatment. Group III: 35 patients with sustained virological response (SVR) with undetectable HCV RNA six months after stopping treatment.

## 4.Statistical Analysis

Data was statistically analyzed using SPSS (Statistical Package for Social Science) program version 13 for windows and Epi info program version

for all the analysis  $p$  value  $< 0.05$  was considered statistically significant. Data are shown as mean, standard deviation, frequency and percent. Chi square test was done for qualitative variable analysis and  $p$ -value  $< 0.05$  was considered significant. Student t-test was done for normally distributed quantitative variables to measure mean and standard deviation and  $p$ -value  $< 0.05$  was considered significant. Mann-Whitney test was done for quantitative variables which are not normally distributed and  $p$ -value  $< 0.05$  was considered significant. Univariate logistic regression was performed on each variable and each statistically significant variable was documented. Multivariate logistic regression analysis model was performed on factors which were significantly different in a univariate analysis between patients of both groups in order to determine adjusted Odds ratio (OR) and 95% confidence interval (CI) to detect the factors that are independently associated with the presence of the factor under the study.

## 5.Results

The baseline characteristics of the 100 studied patients were showing that the mean age was  $41.5 \pm 8.4$ , body mass index (BMI) mean was  $26.1 \pm 3$ , total bilirubin before treatment was of mean  $0.8 \pm 0.2$ , mean of direct bilirubin  $0.3 \pm 0.1$ , mean of albumin was  $4.1 \pm 0.2$ , mean AST level before start of treatment was  $39.2 \pm 26.0$ , mean of ALT before treatment was  $38.3 \pm 22.6$ , mean of Prothrombin concentration before treatment was  $90.2 \pm 9.1$ , the mean of PCR before treatment was  $1.9E6 \pm 7.8E6$  while mean of PCR at week 12 was  $7.2E4 \pm 2.6E5$ , patients receiving treatment till 24 injections were 80 patients with mean and standard deviation  $2.9E4 \pm 1.5E5$ , patients receiving treatment till 48 injections were 67 patients

with mean  $1.5E4 \pm 6.9E4$ , mean of HCV RNA PCR relapsers was  $1.2E5 \pm 7.4E5$ , 71 patients (71%) were male and 29 (29%) were female. As regarding diabetes: non diabetic patients in studied group were 81 patients (81%) and diabetic patients were 19 patients (19%). As regarding liver biopsy: Steatosis was detected in 8 patients (8%) while absent in 92 patients (92%). Fibrosis stage by ISHAK score showing 8 patients (8%) were with stage F1 fibrosis, F2 represented in 29 patients (29%) liver biopsy, 37 patients (37%) with stage F3, 23 patients (23%) with stage F4 and 3 patients (3%) with stage F5. Grade of activity: 13 patients (13%) were with activity grade 3/18 (minimal activity), 55 patients (55%) were with mild activity, 32 patients (32%) were with moderate activity. As regarding Dyslipidemia, 52 patients (52%) were in Dyslipidemia state and 48 patients (48%) were not, IL28B Genotype showing that 19 patients (19%) were of genotype C/C, 57 of patients (57%) were of genotype C/T and 24 patients (24%) were of genotype T/T.

Table 1. Comparison of the numerical variables according to the type of PegINF/RBV response showing no significant difference between the 3 group (35 responders, 36 non responders and 29 patients relapsers) as regarding 1) age, 2) Bilirubin level before treatment shows no difference in response to treatment and 3) Prothrombin concentration.

But there is significant difference as regarding 1) Body mass index (BMI) is highly significant as low BMI is associated with higher response. 2) Elevated ALT level before treatment is associated higher with non response to treatment. 3) Rapid decline in PCR highly significantly correlates with response to treatment.

**Table 1. Comparison of the numerical variables according to the type of PegINF/RBV response according to achieving SVR:**

	Responder (35)		Nonresponder (36)		Relapser (29)		
	M	±SD	M	±SD	M	±SD	
Age (years) <sup>§</sup>	39.0	9.7	42.6	7.1	43.1	7.8	>0.05
BMI	23.7	2.3	27.9	2.6	26.8	2.2	<b>0.001</b>
Bilirubin T. (mg/dL)	0.8	0.3	0.8	0.2	0.8	0.2	>0.05
Bilirubin D. (mg/dL)	0.3	0.1	0.3	0.1	0.3	0.1	>0.05
Albumin (g/dL)	4.1	0.2	4.1	0.2	4.1	0.2	>0.05
AST (U/L) <sup>§</sup>	32.6	15.2	45.6	34.0	39.1	23.6	<b>0.02</b>
ALT (U/L) <sup>§</sup>	31.7	14.5	46.6	28.6	36.0	19.7	<b>0.04</b>
Prothrombin %	89.5	9.3	89.4	9.8	92.0	7.9	>0.05
HCV PCR 0 (IU)	2.200.000	8.300.000	800.000	1.600.000	3.000.000	11.000.000	>0.05
HCV PCR 12 (IU) <sup>§</sup>	2.100	6.400	200.000	400.000	1.600	4.300	<b>0.001</b>

ALT: Alanine Aminotransferase / AST: Aspartate Aminotransferase/ PCR0: Polymerase Chain Reaction for HCV RNA at beginning of treatment / PCR12: Polymerase Chain Reaction for HCV RNA at week 12 of treatment / N: Number / BMI: body mass index

**Table 2. Comparison of the nominal variables according to the type of PegINF/RBV response according to achieving SVR**

Variables		Type of PegINF/RBV response			P
		Responder (35)	Nonresponder (36)	Relapser (29)	
		N (%)	N (%)	N (%)	
Sex	Male	29	21	21	>0.05
	Female	6	15	8	
Diabetes Mellitus	Non-diabetics	34	22	25	0.001
	Diabetics	1	14	4	
IL28 B Mutation	C/C	14	2	3	0.002
	C/T	16	22	19	
	T/T	5	12	7	
Liver biopsy Findings	Steatosis	Absent	34	31	>0.05
		Detected	1	5	
	Fibrosis	F1	1	5	0.05
		F2	17	6	
		F3	13	13	
		F4	4	11	
		F5	0	1	
	Activity	minimal	5	5	>0.05
		Mild	21	19	
		moderate	9	12	
Dyslipidemia	Negative	17	17	14	>0.05
	Positive	18	19	15	

Table 2 shows Comparison of the nominal variables according to the type of PegINF/RBV responses showing that there is no significant difference between the 3 groups as regarding 1) sex, 2) liver steatosis, 3) grade of activity in liver biopsy nor 4) lipid profile abnormality.

But:

- 1) According to IL28B genotype patients with C/C genotype were associated with response to

treatment and on the other hand patients with T/T genotype were associated with non response.

- 2) Diabetes mellitus is highly significant as diabetic patients are highly associated with non response.
- 3) Also low grade fibrosis stage on ISHAK classification was associated with response to treatment.

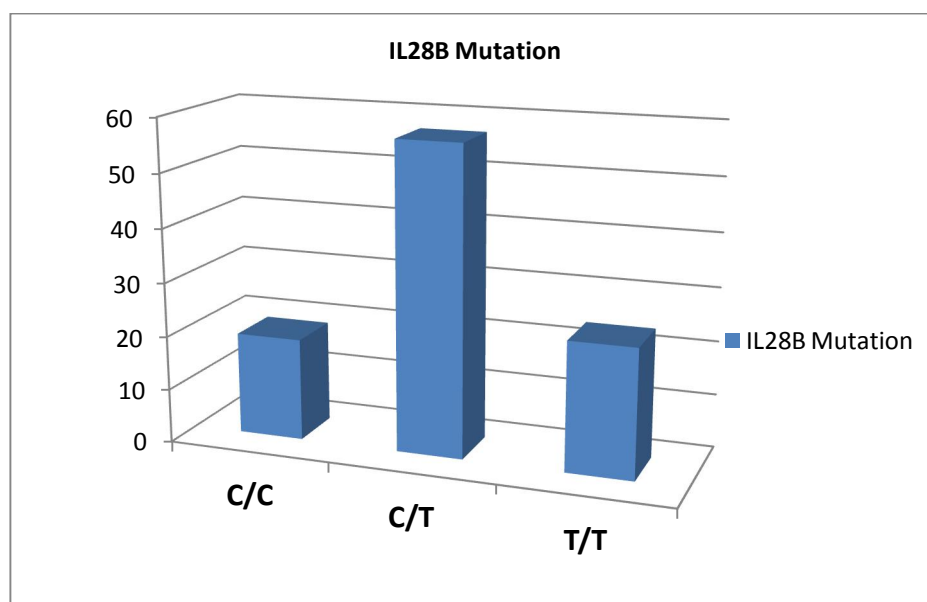


Figure (1) shows prevalence of IL28B genotype in studied group as described C/T genotype was the most prevalent genotype (57%), T/T genotype (24%) and C/C genotype was of the lowest prevalence (19%).

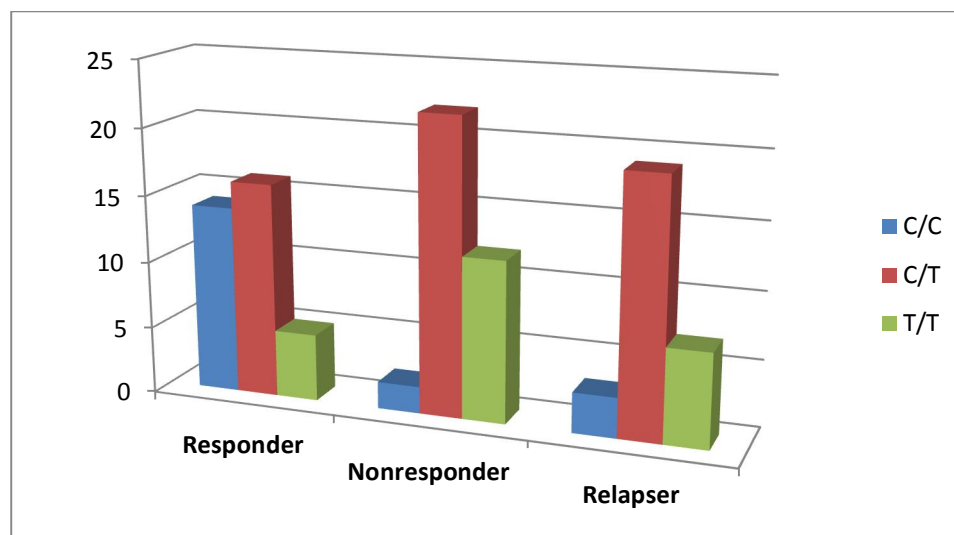


Figure (2) Correlation between IL28B genotype and response to treatment:

In C/C genotype responders were seen more frequently than non responders and relapsers.

In T/T genotype non responders were frequent more than relapsers than responders.

While in C/T genotype non responders were frequent more than relapsers than responders.

**Table 3. Comparison of the numerical variables according to the type of IL28B gene mutations:**

Variables	Type of IL28B gene mutations						P
	C/C (19)		C/T (57)		T/T (24)		
Age (years)	42.1	9.1	41.6	8.4	40.6	8.2	>0.05
BMI	24.5	2.6	26.6	2.8	26.2	3.4	<b>0.03</b>
Bilirubin T. (mg/dL)	0.8	0.3	0.8	0.3	0.8	0.2	>0.05
Bilirubin D. (mg/dL)	0.3	0.1	0.3	0.1	0.3	0.1	>0.05
Albumin (g/dL)	4.1	0.2	4.1	0.2	4.1	0.2	>0.05
AST (U/L) §	29.2	6.0	41.1	26.4	42.5	32.8	<b>0.02</b>
ALT (U/L) §	27.7	4.7	40.0	22.4	42.8	29.2	<b>0.04</b>
Prothrombin %	91.6	7.5	90.6	8.3	88.0	11.6	>0.05
HCV PCR 0 (IU)	3600000	14000000	1800000	6600000	830000	1200000	>0.05
HCV PCR 12 (IU)	2100	6000	80000	200000	110000	430000	>0.05

Table 3 shows Comparison of the numerical variables according to the type of IL28B gene mutations showing that C/C genotype of IL28B was associated statistically with 1) low BMI and 2) low ALT level while there was no difference statistically between the three genotypes as regarding 1) age, 2) bilirubin level, 3) Prothrombin concentration and 4) HCV load.

Table 4 shows Comparison of the nominal variables according to the type of IL28B gene mutation showing that there is highly association between IL28B genotype mutation and response to treatment.

But no significant correlation between genotypes of IL28B and other variants as 1) sex, 2) diabetes mellitus, 3) liver biopsy (steatosis, stage of fibrosis and grade of activity) or 4) Dyslipidemia.

Table 5 & Figure 3: shows Univariate logistic regression analysis for detection of predictors of nonresponse to PegINF/RBV therapy showing that

- The advance of age is more likely to be associated with nonresponse to PegINF/RBV therapy (odds: 1.05, 95% CI:1-1.11 ).
- The more the increase of the BMI, the more probability of nonresponse to PegINF/RBV therapy.
- The more the increase of the ALT levels, the more probability of nonresponse to PegINF/RBV therapy.
- Positive HCV PCR count at week 12 is more likely to be associated with nonresponse to PegINF/RBV therapy.

The presence of IL28B mutation C/C is less likely to be associated with nonresponse to PegINF/RBV therapy.

Table 4. Comparison of the nominal variables according to the type of IL28B gene mutation.

Variables		Type of IL28B gene mutations			P	
		C/C (19)	C/T (57)	T/T (24)		
		N	N	N		
Sex	Male	16	39	16	>0.05	
	Female	3	18	8		
Diabetes Mellitus	Non-diabetics	18	45	18	>0.05	
	Diabetics	1	12	6		
Liver biopsy Findings	Steatosis	Absent	18	54	20	>0.05
		Detected	1	3	4	
	Fibrosis	F1	0	5	3	>0.05
		F2	8	17	4	
		F3	8	20	9	
		F4	2	14	7	
		F5	1	1	1	
	Activity	Minimal	1	10	2	>0.05
		Mild	12	29	14	
		Moderate	6	18	8	
PegINF/RBV response.	Responder	14	16	5	<b>0.002</b>	
	Nonresponder	2	22	12		
	Relapser	3	19	7		
PegINF/RBV response (SVR).	Responder	14	16	5	<b>0.001</b>	
	Nonresponder	5	41	19		
Dyslipidemia	Negative	8	29	11	>0.05	
	Positive	11	28	13		

Table 5. Univariate logistic regression analysis for detection of predictors of nonresponse to PegINF/RBV therapy.

Variables in the Equation	B	Wald	Sig.	Exp(B) Odds ratio	95% C.I. for EXP(B)	
					Lower	Upper
Age	0.158	5.424	<b>0.02</b>	1.2	1.025	1.339
Sex(male)	-1.165-	0.530	0.47	0.3	0.014	7.181
Bil.T	0.798	0.233	0.63	2.222	0.087	56.627
Albumin	2.097	1.283	0.26	8.141	0.216	306.618
AST	0.138	3.932	<b>0.05</b>	1.1	1.002	1.315
ALT	-0.172	4.117	<b>0.04</b>	0.8	0.713	.994
PCR.0	0.001	0.001	0.98	1.000	1.000	1.000
PCR.12	0.001	5.285	<b>0.02</b>	1	1.000	1.000
IL28.Mutation		7.294	0.03			
IL28.Mutation(C/C)	-3.363-	5.753	<b>0.02</b>	0.04	0.002	.541
IL28.Mutation(C/T)	-.342-	0.097	0.76	0.710	0.082	6.145
LB.Steatosis(-ve)	1.081	0.200	0.65	2.947	0.026	336.485
LB.Fibrosis		7.627	0.11			
LB.Fibrosis(F1)	-19.300-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F2)	-26.272-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F3)	-23.432-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F4)	-22.522-	0.001	1.00	0.001	0.001	.
Dyslipidemia(-ve)	-.862-	0.488	0.48	0.422	0.038	4.743



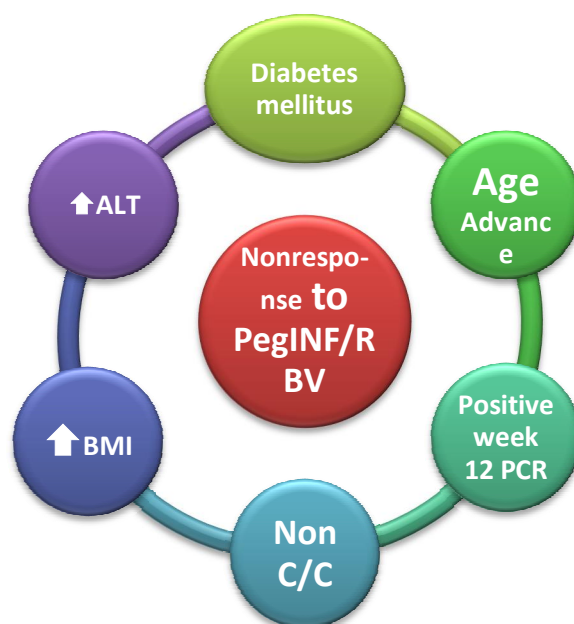


Figure 3. Univariate logistic regression analysis for detection of predictors of nonresponse to PegINF/RBV therapy

**Table 6. Multivariate logistic regression analysis for detection of predictors of nonresponse to PegINF/RBV therapy.**

Variables in the Equation	B	Wald	Sig.	Exp(B) Odds ratio	95% C.I. for EXP(B)	
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Albumin	2.097	1.283	0.26	8.141	0.216	306.618
AST	0.138	3.932	<b>0.05</b>	1.1	1.002	1.315
ALT	-0.172	4.117	<b>0.04</b>	0.8	0.713	.994
PCR.0	0.001	0.001	0.98	1.000	1.000	1.000
PCR.12	0.001	5.285	<b>0.02</b>	1	1.000	1.000
IL28.Mutation		7.294	0.03			
IL28.Mutation(C/C)	-3.363-	5.753	<b>0.02</b>	0.04	0.002	.541
IL28.Mutation(C/T)	-.342-	0.097	0.76	0.710	0.082	6.145
LB.Steatosis(-ve)	1.081	0.200	0.65	2.947	0.026	336.485
LB.Fibrosis		7.627	0.11			
LB.Fibrosis(F1)	-19.300-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F2)	-26.272-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F3)	-23.432-	0.001	1.00	0.001	0.001	.
LB.Fibrosis(F4)	-22.522-	0.001	1.00	0.001	0.001	.
Dyslipidemia(-ve)	-.862-	0.488	0.48	0.422	0.038	4.743
Constant	14.899	0.001	1.00	2954874.702		

Figure 4 shows the multivariate logistic regression analysis for detection of predictors of nonresponse to PegINF/RBV therapy.

Our results were able to identify the advance of age is more likely to be associated with nonresponse to PegINF/RBV therapy.

- The more the increase of the ALT levels, the more probability of nonresponse to PegINF/RBV therapy.
- Positive HCV PCR count at week 12 was more likely to be associated with nonresponse to PegINF/RBV therapy.
- The presence of IL28B mutation C/C was less likely to be associated with nonresponse to PegINF/RBV therapy.

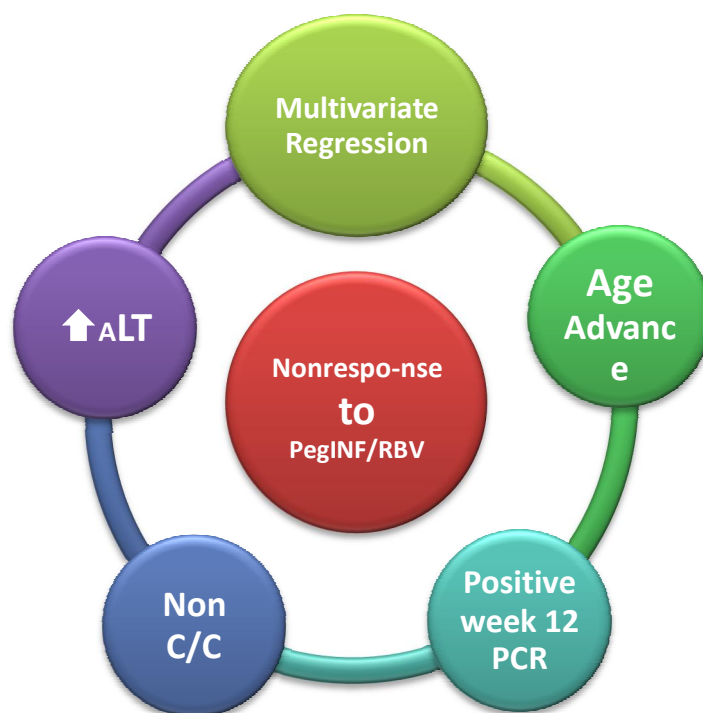


Figure 4: Multivariate logistic regression analysis for detection of predictors of nonresponse to PegIFN/RBV therapy.

## 6. Discussion

As regarding IL28B Genotype showed that 19 patients (19 %) were of genotype C/C, 57 of patients (57%) were of genotype C/T and 24 patients (24%) were of genotype T/T this was in accordance with Clark PJ et al who observed that different ethnic groups have marked differential distribution of IL28B polymorphisms. For instance, the favorable CC allele of rs12979860 is least frequent in African-Americans and most frequent in Asians. Allele frequencies differ between ethnic groups, largely explaining the observed differences in response rates between Caucasians, African Americans and Asians (7). and De Nicola S *et al.* study prevalence of IL28B in 103 HCV-4 patients at two liver centers at the Maggiore Hospital Milan (Italy) found that 23% were genotype CC, 63% CT, and 14% TT (8) also we found that there was difference statistically between the three genotypes of IL28B as 14 of total 19 CC genotype achieved SVR (73.5%), 16 patients of total 57 CT genotype achieved SVR (28%) while only 20% of TT genotype achieved SVR (5 cases of total 24) That was different from Asselah *et al.* who studied the effect of IL28B on 82 HCV-4 patients, showed that The response rates were 81.8%, 46.5%, and 29.4% for genotype CC, CT, and TT, respectively (9). In another study of Ayman A *et al.*, study IL28B effect in 129 treatment-naïve patients showed that patients carrying CT and CC genotypes

achieved SVR equally (47%) in comparison to TT genotype (5.6%) (10) But Stättermayer AF *et al.* showed that SVR rates were similar between those with C/C and who carry the T allele (80.5% vs 74.4%). However, IL28B genotype may be included in treatment algorithms to tailor therapy among patients who have failed to achieve RVR and a lack of RVR in patients with unfavorable IL28B genotypes should not be considered as an unethical stopping rule; however, premature therapy termination should be considered in cases with severe treatment side effects, poor motivation or severe comorbidities (11). Also De Nicola S *et al.* found that 88% of CC patients achieved an SVR versus 37% of CT/TT. CC patients more often had a rapid virologic response (RVR) than CT/TT patients (50% versus 29%) (8). Dyslipidemia in our study showed no statistically difference in achieving SVR but Dyslipidemia was seen more frequently in those who did not achieve SVR. This may be explained as dyslipidemia was frequently associated with insulin resistance. This is in accordance with Romero-Gomez M *et al.* who showed that sustained virological response in patients with genotype 1 and insulin resistance occurred in 23 of 70 (32.8%) patients, vs. 26 of 43 (60.5%) genotype 1 patients without insulin resistance (11).

As regarding age our study showed that the mean age of studied group was 41.5, mean of age in



responders was 39.0, 42.6 in non responders and 43.1 in relapsers. So there was statistically significant relation between young age and response to treatment in the Univariate logistic regression analysis and Multivariate logistic regression analysis. Responders group (achieved SVR) are younger than non responders (did not achieved SVR). This was in agreeing with Kau A *et al.* who showed that higher SVR rates were obtained in patients younger than 40–45 years old (12). Also with Alberti A *et al.* who showed that the probability of response to interferon in terms of maintaining normal ALT after withdrawal was significantly higher in patients aged below 45 years (13). In our study by multivariate logistic regression and univariate logistic regression analysis we found that no statistically correlation between gender and response outcomes. this was in accordance with Alberti A *et al.* showed that the probability of response to interferon in terms of maintaining normal ALT after withdrawal did not appear to be influenced by sex (13). Also Kau A *et al.* proved that Female patients had been shown to achieve higher SVR rates than males in two studies using the old combination of standard IFN and RBV. This may be explained by the low number of females in our study. but in both of the pegIFN/RBV registration trials, no statistically significant correlation was found between sex and SVR(12).

The mean of body mass index (BMI) in our study was 26.1, 23.7 in those who achieved SVR and 27.4 in those who did not achieve SVR so there is statistically correlation between those with low BMI and response to treatment. This is in agreeing with Bressler BL *et al.* who showed that obesity when defined as a BMI greater than 30 kg/m (2), is an independent (of genotype and cirrhosis) negative predictor of response to hepatitis C treatment (14). Also with Manns MP who showed that Caucasians who have a higher body weight or BMI results in a lower SVR rate (15). Berg *et al.* showed that a high BMI was inversely correlated with SVR in both IFN and PEG IFN treated individuals (16). Camps J *et al.* showed that Response was attained in 75% of non-obese patients (BMI <30), compared with only 50% of obese patients (17). A number of studies have found that patients with a higher body weight have reduced response rates following antiviral therapy (18, 15 & 19).

Low ALT levels in our study showed statistical correlation with those who achieved SVR. This is in agreeing with Serfaty L *et al.* that showed that pretreatment ALT and  $\gamma$ -glutamyltransferase levels tend to be lower in responders (20). García-Samaniego J *et al.* who showed that patients who failed to achieve EVR were older, had higher ALT (21). However, in another study in HCV genotypes 2

and 3 infected patients such a correlation was observed (22). In other studies elevated ALT levels (threefold higher than the upper limit of normal) was associated with a good response to treatment (11). This difference from our study can be explained by the observation that most non responders in our study with elevated ALT did not reach 3 fold level and most non responders group were diabetics which can explain elevated ALT.

Our study showed no statistically significant correlation between baseline viral load and treatment outcomes. This was in accordance with Ferenci P *et al.* who showed that obtaining a rapid virologic response (RVR, defined as HCV RNA being undetectable at week 4 of therapy) was the strongest predictor of SVR, no matter what adverse baseline predictors may have been present (23). And different from Jensen DM *et al.* who showed that Patients with a baseline HCV RNA of less than 200,000 IU/ml or 200,000-600,000 IU/ml were more likely to achieve a RVR than those with HCV RNA greater than 600,000 IU/ml (24). Also Jacobson IM *et al.* and Huang CF *et al.* showed that factors such as high viral load, older age, black race and advanced fibrosis or cirrhosis negatively influence SVR rates (26)(25). Our study showed that the rapid decline in PCR at week 12 was statistically significant and highly associated with a good response. This was in accordance Fried MW *et al.* and Davis GL *et al.* who showed that Absence of an early virologic response (EVR) has the best negative predictive value for treatment outcome (27&19). Another study showed that early viral kinetics are the strongest predictor of achieving SVR: obtaining a rapid virologic response (RVR, defined as HCV RNA being undetectable at week 4 of therapy) was the strongest predictor of SVR, no matter what adverse baseline predictors may have been present (23).

In our study Diabetes mellitus was statistically associated with non response as 19% of studied group were diabetics and 81% were non diabetics, only 5.2% of diabetic patients responded to treatment while 94.8 did not achieve SVR this can be explained as most diabetic patient have insulin resistance which was associated with poor response to treatment. This was in accordance Romero-Gomez M *et al.* who showed that Insulin resistance was independently associated with poor treatment response, especially in genotype 1-infected patients (11).

In our study we noticed that the stage of hepatic fibrosis was highly associated with response to treatment as low grade fibrosis patients were more likely associated with treatment response and advanced stage of fibrosis patients were more associated with non response As those who achieved SVR were noticed more in those who have F2 or F3

stage of fibrosis by ISHAK staging while those who did not achieved SVR had F4 and F5 stage of fibrosis according to ISHAK staging while in F1 stage of fibrosis non responders who did not achieved SVR were more than responders as most patients who start treatment with F1 stage of fibrosis according to ISHAK stage had elevated enzymes and elevated enzymes in our study was associated with non response, most of those patients were diabetics (5 diabetics and 3 non diabetics) so they were associated with non response. This was in accordance *Poynard et al* showed that presence of advanced liver fibrosis and cirrhosis has long been recognized to be associated with lower response rates to IFN-based treatment *also* the majority of fibrosis progression occurred in those aged fifty years or older (28).

### 7.Conclusion:

Our study showed that (14/19) 73.5% of cases having CC genotype, (16/57) 28% of those having CT genotype and only (5/24) 20% of those having TT genotype achieved SVR. Dyslipidemia showed no statistically difference in achieving SVR between those who have Dyslipidemia and those who do not have. Advanced fibrosis, positive PCR of HCV RNA at twelve week, high BMI, diabetes mellitus, older age and male gender were associated with non response to treatment with pegylated interferon and ribavirin. So IL28 B is the strongest predictor of treatment in Egyptian population but alone should not be considered to predict treatment outcomes as there are many cofactors affecting treatment response and Analysis of IL28B genotype might be used to guide treatment for these patients.

**Conflict of interest:** Non Declare

### Acknowledgement

The authors wish to thank all patients and their families, nurses, study contributors and investigators for their active participation in this study

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### References

1. Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009;29:74–81.
2. Muir AJ, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N Engl J Med*. 2004;350:2265-2271.
3. Ge D, Fellay J, Thompson AJ, *et al*. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–401.
4. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML. IL28B is associated with response to chronic hepatitis C interferon-alpha and RBV therapy. *Nat Genet* 2009;41:1100-1104.
5. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O’Huigin C, *et al*. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009;461:798-801.
6. Thompson AJ, Muir AJ, Sulkowski MS, *et al*. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology*. 2010;139:120-129.
7. Clark PJ, Thompson AJ, McHutchison JG. IL28B genomic-based treatment paradigms for patients with chronic hepatitis C infection: the future of personalized HCV therapies. *Am J Gastroenterol* 2011; 106(1):38–45.
8. De Nicola S, Aghemo A, Rumi MG *et al*. An *IL28B* polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. *Hepatology* 2012; 55: 336–342.
9. Asselah T, De Muynck S, Broet P, *et al*. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. (2012) *J Hepatol* 2012;56(3):527–532.
10. Ayman A. Abdo, Mohammed N. Al-Ahdal, Saira S. Khalid, *et al*. IL28B polymorphisms predict the virological response to standard therapy in patients with chronic hepatitis C virus genotype 4 infection.(2012) *HepatolInt* DOI 10.1007/s12072-013-9421-8.
11. Stättermayer AF, Stauber R, Hofer H *et al*. Impact of *IL28B* genotype on the early and sustained virologic response in treatment-naïve patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2011; 9: 344–350.
12. Romero-Gomez M, Del Mar Vilorio M, Andrade RJ, *et al*. Insulin resistance impairs sustained response rate to peg-interferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; 128: 636-641.
13. Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. *J*. 2008. *Hepatol*. 49(4), 634–651.
14. Alberti A, Chemello L, Bonetti P, *et al*. Treatment with interferon(s) of community-

- acquired chronic hepatitis and cirrhosis type C. *J Hepatol* 1993;17(suppl 3):S122–6.
15. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003;38:639–644.
  16. Manns MP, McHutchison JG, Gordon SC, *et al.* Peg- interferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958-965.
  17. Berg T, von Wagner M, Nasser S, *et al.*: Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. 2006 *Gastroenterology*; 130: 1086-1097.
  18. Camps J, Crisostomo S, Garcia-Granero M. *et al* Prediction of the response of chronic hepatitis C to interferon alfa: a statistical analysis of pre-treatment variables. *Gut* 1993. 34:1714–1717.
  19. Zeuzem S, Welsch C and Herrmann E. Pharmacokinetics of peg-interferons. *Semin. Liver Dis* 2003; 23(Suppl 1): 23-28.
  20. Fried MW, Shiffman ML, Reddy KR, *et al.* Combination of peginterferon alfa-2a plus ribavirin in patients with chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975–82.
  21. Serfaty L, Giral P, Loria A, *et al.* Factors predictive of the response to interferon in patients with chronic hepatitis C. *J Hepatol* 1994;21:12–17.
  22. Garcia-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: a lesson in detente. *Science* 2006 May 12;312(5775):879–82.
  23. Shiffman ML, Salvatore J, Hubbard S, *et al.*: Treatment of chronic hepatitis C virus genotype 1 with peg-interferon plus ribavirin. 2007 *Hepatology*; 46: 371-379.
  24. Ferenci P, Fried MW, Shiffman ML, *et al.* Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa 2a (40 KD)/ribavirin. *J Hepatol.* 2005;43:425-433.
  25. 24) Jensen DM, Morgan TR, Marcellin P, *et al.* Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006;43:954–60.
  26. Huang CF, Yang JF, Dai CY *et al.* Efficacy and safety of pegylated interferon combined with ribavirin for the treatment of older patients with chronic hepatitis C. *J. Infect.* 2010;Dis. 201(5), 751–759.
  27. Jacobson IM, Brown RS Jr, McCone J *et al.* Impact of weight-based ribavirin with peginterferon alfa-2b in African Americans with hepatitis C virus genotype 1. *Hepatology* 46(4), 982–990 (2007).
  28. Davis GL. Monitoring of viral levels during therapy of hepatitis C. *Hepatology* 2002;36:145–51.
  29. Poynard T, Ratziu V, Charlotte F, *et al.* Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *J Hepatol* 2001; 34: 730–9.

3/31/2015