IMPACT OF IL28B POLYMORPHISM ON TREATMENT INDUCED VIRAL CLEARANCE IN HCV INFECTED EGYPTIAN PATIENTS

Manar Obada¹, Ashraf El-Fert¹, Wesam Morad², Nermin Ehsan³, Omkolsoum Alhadad⁴, Hala El-Said⁴

DEPARTMENTS OF: CLINICAL BIOCHEMISTRY¹, ENVIRONMENTAL HEALTH OF LIVER², PATHOLOGY³, HEPATOLOGY⁴, NATIONAL LIVER INSTITUTE-MENOUFIA UNIVERSITY.

Received 5/9/2014– Accepted 24/11/2014

ABSTRACT

Background and objective: Interleukin (IL) 28B single nucleotide polymorphisms (SNP) was recently recognized as predictor of SVR in HCV infected patients treated by combination therapy of pegylated-interferon (Peg-IFN) and ribavirin (RBV). The aim of the current study was to assess IL 28B polymorphism SNP (rs12979860) as a predictor of response to combined Peg-INF/RBV therapy in Egyptian chronic HCV infected patients.

Design and methods: The study was conducted on 247 HCV infected patients and 100 apparently healthy control subjects. All patients were treated with PEG-IFN-α/ribavirin; and they were classified according to their response to treatment. Genotyping of IL28B rs12979860 was performed on peripheral blood DNA using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay.

Results: The overall frequency of IL28B genotypes was 24.7%, 50.2% and 25.1% for genotypes CC, CT and TT respectively, while the response rate was 82%, 38.7% and 43.8% for genotypes CC, CT and TT respectively, moreover, genotype CC had increased probability to HCV clearance than both genotypes CT and TT with OR 7.71 (95%CI: 3.71-15.79).

Conclusion: Genotyping of IL28B at SNP rs12979860 could be used as a guide to tailor treatment in Egyptian patients infected with HCV for better outcome.
**Key wards:** Hepatitis C virus, Pegylated-interferon, ribavirin, sustained virological response, IL28B

**INTRODUCTION**

Hepatitis C virus (HCV) infection has become a worldwide health problem \((\text{Lauer and Walker, 2001})\). About 170 million people are affected with hepatitis C virus (HCV) throughout the world \((\text{Lavanchy, 2009})\). Spontaneous clearance of hepatitis C virus (HCV) occurs in 30% of patients with acute infections; the remaining patients develop chronic infections and have predispositions to cirrhosis and hepatocellular carcinoma \((\text{Wilson et al., 2006})\).

The primary goal of any HCV therapy is to achieve a sustained virological response (SVR), in which HCV RNA remains undetectable at 24 weeks after therapy ends. The current standard therapy for genotype 4 HCV is based on a combination of pegylated interferon (Peg-IFN) and ribavirin (RBV). Many host and viral factors particularly those associated with racial descent, the genotype of HCV and variation in certain host genes; influence the treatment response to the antiviral combination therapy \((\text{McHutchison et al., 2009; Wu et al., 2012})\).

Moreover, side effects from the therapy such as influenza-like symptoms, psychiatric symptoms and hematological abnormalities, could result in dose reduction or even the premature discontinuation of the treatment \((\text{McHutchison et al., 2009})\). So, it is necessary to predict an individual’s response before or at an early stage of the treatment, to increase treatment success rate and avoid potential adverse events in patients who do not benefit from the treatment and also to reduce the cost of therapy \((\text{Shirakawa et al., 2008})\).

Previous studies reported that treatment with Peg-IFN and RBV results in a lower SVR rate in patients with HCV genotype 1 (HCV1) than in patients with HCV genotypes 2 and 3. SVR is achieved in about 75% of patients infected with HCV genotype 2 or 3 \((\text{Fried et al., 2002; Lagging et al., 2011})\), compared to 50% of patients with HCV genotype 1 and genotype 4 (mainly found in Egypt where HCV-4 represents more than 90% of all HCV cases) \((\text{Antaki et al., 2010})\).
Recent genome-wide association studies have shown that single nucleotide polymorphism (SNPs) located close to coding region of the interleukin 28B gene on chromosome 19 (rs12979860) is strongly associated with spontaneous clearance of HCV and treatment response to standard therapy in HCV-infected patients (Thomas et al., 2009; Tanaka et al., 2009).

The CC genotype of rs12979860 was considered to be associated with a better treatment response (Jia et al., 2012) as it was reported to have SVR rates of about 70%, a two to three fold increase, over patients who carry one of the poor response genotypes (e.g. C/T, T/T at rs12979860) (Chen et al., 2012). This association was extended to spontaneous clearance of HCV (Berger and Kim, 2012).

As the majority of studies focused on genotypes 1, 2 and 3, there is few data so far, regarding the role of IL28B polymorphism in HCV-4 patients with respect to response to either antiviral therapy or fibrosis progression (Khattab et al., 2011). The present study aimed to assess IL 28B polymorphism SNP (rs12979860) as a predictor of response to combined Peg/INF-RBV therapy and determine its impact on treatment-induced viral clearance in Egyptian chronic HCV infected patients.

**MATERIAL AND METHODS**

This study included 247 consecutive chronic HCV infected adult patients who were recruited from the interferon clinic at National Liver Institute (NLI), Menoufia University, in the period from September 2010 to June 2011. In addition, 100 apparently healthy subjects of matched age and gender served as a control group. All patients underwent antiviral treatment and follow up protocol according to the standard clinical practice for 18 months. The study was approved by the Institution’s ethical committee, and a written informed consent was taken from all participants enrolled in the study. Patients had to meet the following conditions:

**Inclusion Criteria**: 1. Treatment naïve patients. 2. Positive for anti-HCV antibody, had a repeatedly positive HCV RNA (by PCR), higher than 1000 IU/ml. 3. Persistently elevated serum alanine aminotransferase (ALT) levels and histological features of chronic HCV infection in liver biopsy done within 3 month before initiation of therapy. 4. Age: not less than 18 years. 5. ANA titer less than 1/60, normal TSH and kidney functions.
Exclusion Criteria: 1. Evidence of decompensated liver disease. 2. Seropositive for HIV and HBsAg. 4. Evidence of other etiology of liver disease. 3. Moderate to severe anemia (Hb<10g/dl), neutropenia (neutrophil count<2000/mm^3). 4. Thrombocytopenia (PLT<75000/mm^3). 5. Significant history of cardiovascular and neuropsychiatric diseases. 6. Previous treatment with IFN.

All patients received combined pegylated interferon & ribavirin therapy: Peg-IFN2α at a dose of 180 mcg once weekly plus ribavirin. The dose of ribavirin was adjusted according to body weight (less than 75 kg: 1000 mg per day, 75 kg or more: 1200 mg per day). Patients received treatment for 48 weeks and were followed up for 6 months after end of therapy.

Early virological response (EVR) was defined as > 2 log decrease in HCV RNA at treatment week 12; non-responders were defined as patients with an insufficient virologic response at 12 weeks (a detectable HCV level and/or a decrease of less than 2 log drop) or at 24 weeks (a detectable HCV RNA level) were considered as treatment failure and was discontinued the therapy; sustained virological response (SVR) was defined as undetectable levels of HCV RNA 24 weeks after the completion of therapy (72 weeks after the initiation of treatment); relapsers were defined as patients with detectable HCV RNA levels during the follow up evaluation of patients who had achieved absence of detectable HCV RNA at the end of the course of treatment (Ghany et al., 2009).

End Points: sustained virological response was considered the primary end point. Non-response and/or relapse were considered the secondary end point.

Laboratory Investigations

Base line blood samples were obtained before commencing treatment and were analyzed for blood chemistry, HCV RNA and hematology, using fully automated auto analyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA), COBAS® TaqMan® HCV assay (Roche Molecular Diagnostics, CA, USA) with lower limit of quantitation of 15 IU/ml, and Sysmex K-21 (Sysmex Corporation, Kobe, Japan), respectively. Periodic blood samples were taken from patients only for determination of transaminases, complete blood count and HCV RNA on weeks (12, 24, and 48) during treatment & at 6 months after stopping therapy.
The histopathological assessment of necro-inflammatory grade and fibrosis stage was scored using the modified Ishak scoring system before commencing treatment (Ishak et al., 1995).

**Molecular testing**

Genomic DNA was extracted from EDTA-treated peripheral blood using Gene JET Whole Blood Genomic DNA Purification Mini Kit, (Thermo Fisher Scientific, MA, USA). *IL28B* (rs12979860) genotyping was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay as previously reported (Sharafi et al., 2012). An amplification product 241bp was obtained using standard PCR assay with the following primers (rs12-Forward 5'-GCGGAAGGAGCAGTTGCGCT-3', bst-Reverse 5'-GGGGCTTTTGGCTGGGGAGTG-3',) (Primers and probe were purchased from Metabion, Martinsried, Germany). Reaction mix was constituted of 15pmol of each of forward and reverse primers; 12.5µl of 2x Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, MA, USA) and 100ng of the genomic DNA and the total volume of reaction was topped up to 25µl with DNase-free water. The PCR was performed on Applied Biosystems Veriti™ thermocycler (Thermo Fisher Scientific Inc., Life Technologies™, CA, USA) according to cycling conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for .0 s, with a final extension of 72°C for 5 min.

After confirmation of successful PCR amplification using 1.5% added to digestion mixture of 3ul buffer (R) [10mM Tris-HCl (pH 8.5), 10mM MgCl₂, 100mM KCl, 0.1mg/ml BSA], 10 units of Bsh1236I (Thermo Fisher Scientific, MA, USA), and the volume was topped up with H₂O to 30ul and incubated at 37°C overnight. The restriction digests were electrophoresed on 3% Agarose gels (Axygen, CA, USA) for 3 hours at 60V and photographed using InGenius gel documentation system (Syngene, Cambridge, UK). *IL28B* CC genotype was identified by the presence of 2 fragments of 196bp and 45bp

Statistical analysis
Data analysis was performed using SPSS software for Windows (version 16.00, SPSS, Inc., Chicago, IL, USA). The observed alleles frequencies were compared with expected values calculated from Hardy–Weinberg equilibrium theory; the data were expressed as mean ± SD or proportions. Chi-square test or Fisher’ exact test was used for comparison of categorical variables. The Student’s t-test and ANOVA was used in the case of continuous data. All the significant variables from univariate analysis as predictors of SVR undergo stepwise multivariate logistic regression analysis. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Demographic, clinical, biochemical, histological data and response to treatment

Table (1) shows the characteristics of the studied HCV infected patients according to response to treatment with PEG-IFN/RBV; the study recruited 247 Egyptian chronic HCV infected patients with mean age of 41.60±9.42 years, and 68.8% of them were males. Overall, 119/247 (48.2%) patients achieved SVR, whereas 128 (51.8%) did not. No significant differences were observed regarding gender, ALT, AST, AFP levels, Hb concentration and platelets count between the SVR and non-SVR groups (p>0.05). Meanwhile, age and BMI were significantly lower in patients who achieved SVR (p<0.05). Regarding viral load (VL) we divided the patients into two groups below and more than 600.000 IU/ml (Grandi et al., 2013), patients with SVR had a significantly lower VL (p<0.01). Concerning the stage of liver fibrosis, significant differences were found between the two groups F1-F2 (mild/moderate) and F3-F4 (severe) (p<0.05) as patients with stages F1-F2 attained SVR more than those with stages F3-F4. Also, patients with no diabetes mellitus were significantly associated with a better response to treatment (SVR) compared to diabetic patients (p<0.01). Moreover, patients with viral load <600.000 IU/ml, mild/moderate fibrosis stage and without diabetes achieved SVR with an OR, 95% CI of (2.6, 1.3-3.6), (2.0, 1.2-3.3) and (4.1, 1.9-8.7) respectively compared to those with viral load >600.000IU/ml, sever fibrosis and diabetics respectively.
**Frequency of IL28B alleles and genotypes**

The CT polymorphism produced 3 fragments of 241, 196, and 45bp* (*the 45bp fragment was inconspicuous on gels). IL28B TT genotype didn’t digest by Bsh1236I and yielded 241bp fragment (Figure 1).

**Figure (1)** Agarose gel electrophoresis of PCR-RFLP fragments for *IL28B* genotypes analysis. The CC genotype was identified by the presence of 196bp fragment. The TT genotype didn’t digest by Bsh1236I and yielded 241bp fragment. The CT polymorphism showed of 241, and 196bp fragments. The samples were electrophoresed against a ready-to-use, Gene Ruler™ Low Range DNA Ladder 25 to 700bp (Thermo Fisher Scientific, CA, USA), landmark bands are 100bp & 300bp respectively.

*IL28B* alleles were normally distributed in healthy controls according to Hardy Weinberg equilibrium equation. The various *IL28B* genotypes showed no significant differences between HCV infected patients and healthy controls \((p>0.05)\) (Figure 2). The frequency of the C allele in all patients was 49.8% however, it was 62.2% in patients with SVR versus 38.3% in patients who did not respond to treatment showing statistical significant difference between the two groups \((p<0.05)\); moreover, the C allele was associated with
increased susceptibility to achieve SVR compared to T allele with OR 2.65, 95% CI: 1.84-3.814. Statistical analysis of genotypes IL28B revealed a significant difference between patients with SVR compared to patients who did not respond to treatment \((p<0.05)\) where 82% (50/61) of patients with CC genotype achieved SVR compared to patients with CT and TT genotypes who showed 38.7% (48/124), 33.9% (21/62) SVR respectively. Also, genotype CC was associated with achieving SVR when compared to other genotypes (TT+CT) with OR 7.71, 95%CI: 3.71-15.79. (Table 2 & Figure 3)

**Association between IL28B alleles and genotypes and treatment outcome**

Comparing IL28B different genotypes regarding the three treatment outcome (SVR, relapse or non-response), showed that 202 patients attained EVR, and 45 patients were non-responder at week 12. Then another 40 patients did not respond at week 24 and discontinued the therapy (a total of 85 (34.4%) were non-responders); whereas, 119 patients achieved SVR and 43(17.4%) patients relapsed. The CC genotype was associated with high response rates to treatment and low relapse rates along the whole study, as there were 61 patients carried genotype CC; 93.4% (57/61) of them were early responders, 82% (50/61) achieved SVR, 11.5% (7/61) were non-responders and 6.6% (4/61) relapsed. Moreover, the patients with SVR were significantly higher for carriers of the CC genotype in comparison to non-responders and relapsers (42.0% vs. 8.2% vs. 9.3% respectively) \((p<0.01)\) (Table 3). The CC genotype showed no significant difference between relapsers and non-responders \((p>0.05)\).

Also, the C allele showed highly significant increase in patients achieved SVR compared to each of non-responder and relapers. Also, the C allele was more frequent in patients with SVR when compared to T allele (62.2% vs. 37.8%) (Table 3).

No significant difference was detected between various IL28B genotypes (CC, CT, TT) regarding age, gender, ALT, AST, AFP levels, HB concentration, platelets count, BMI, basal viral load, presence of diabetes mellitus and stage of fibrosis \((p>0.05)\) (data not shown).

**Multivariate analysis of predictors of SVR**

Multivariate analysis of the factors, which were significant in the univariate analysis, revealed that the IL28B genotype CC was the only significant factor predicting SVR with OR 6.49; 95% CI: 2.30-18.29.
**Table (I):** Demographic, biochemical, histological data of HCV infected patients according to response to treatment

<table>
<thead>
<tr>
<th>Variables</th>
<th>HCV patients (No. 247)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SVR (No. 119)</td>
<td>Non-SVR (No. 128)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>p-value*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.4 ±10.1</td>
<td>44.8 ±8.2</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>24.2±2.5</td>
<td>27.4±2.2</td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>39.2±23.2</td>
<td>43.0±26.3</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>40.75±21.78</td>
<td>43.0±26.9</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb gm/dl</td>
<td>13.1±1.7</td>
<td>13.2±1.5</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (10³/mm³)</td>
<td>231.7±66.0</td>
<td>220.9±58.9</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td>11.7±5.9</td>
<td>10.6±5.7</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>85 (71.4)</td>
<td>85 (66.4)</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral load (IU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 600.000</td>
<td>59 (49.6)</td>
<td>41 (32.1)</td>
<td>&lt; 0.01</td>
<td>2.6</td>
<td>(1.3-3.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 600.000</td>
<td>60 (50.4)</td>
<td>87 (67.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1-F2</td>
<td>76 (63.9)</td>
<td>60 (46.9)</td>
<td>&lt; 0.01</td>
<td>2.0</td>
<td>(1.2-3.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3-F4</td>
<td>43 (36.1)</td>
<td>68 (53.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>109 (91.6)</td>
<td>93 (72.7)</td>
<td>&lt; 0.01</td>
<td>4.1</td>
<td>(1.9-8.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>10 (8.4)</td>
<td>35 (27.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA test; † Chi-square test; OR: odds Ratio. CI: confidence interval.

SVR: sustained virological response, BMI: body mass index, ALT: alanine transferase, AST: aspartate amino transferase, F1-F2: minimal to mild fibrosis, F3-F4: sever fibrosis.
**Figure (2):** Comparison between HCV infected patients and healthy control regarding IL28B genotype (p>0.05: non-significant).

**Table (2)** Frequencies of IL28B alleles and genotypes in all patients and their association with response to treatment.

<table>
<thead>
<tr>
<th>HCV patients (No. 247) No. (%)</th>
<th>IL28B (rs12979860)</th>
<th>SVR (No. 119) No. (%)</th>
<th>Non-SVR (No. 128) No. (%)</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>246 (49.8) C allele</td>
<td>148 (62.2)</td>
<td>98 (38.3)</td>
<td></td>
<td>2.65 (1.84-3.81)</td>
</tr>
<tr>
<td>248 (50.2) T allele</td>
<td>90 (37.8)</td>
<td>158 (61.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61 (24.7) CC</td>
<td>50 (42)</td>
<td>11 (8.6)</td>
<td></td>
<td>7.71 (3.71-15.8)</td>
</tr>
<tr>
<td>186 (75.3) CT+TT</td>
<td>69 (58.0)</td>
<td>117 (91.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SVR: sustained virological response, p<0.05

**Figure (3):** Association of IL28B genotypes with response to treatment.
Table (3): Distribution and comparison of IL28B alleles and genotypes in HCV infected patients regarding treatment outcome.

<table>
<thead>
<tr>
<th>Total HCV patients (No. 247)</th>
<th>IL28B (rs12979860)</th>
<th>SVR (No.119)</th>
<th>Non-SVR (No. 128)</th>
<th>X^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C allele</td>
<td>148</td>
<td>62</td>
<td>28.85</td>
</tr>
<tr>
<td></td>
<td>T allele</td>
<td>90</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>246</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>248</td>
<td>CC</td>
<td>50</td>
<td>7</td>
<td>38.94</td>
</tr>
<tr>
<td>61</td>
<td>CT</td>
<td>48</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>TT</td>
<td>21</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>CC</td>
<td>50</td>
<td>7</td>
<td>37.06</td>
</tr>
<tr>
<td>186</td>
<td>CT+TT</td>
<td>69</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>SVR: sustained virological response</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Interferon-α and ribavirin are likely to retain pivotal roles in the management of chronic HCV infection. In the current study, 48.2% of patients achieved SVR, 17.4% relapsed and 34.4% did not respond to treatment. These results reflected the poor response of Egyptian patients to standard Peg-Interferon/RBV treatment which may be due to the different genotype pattern in our population and HCV genotype 4 prevalent in Egypt. Our findings were comparable with previous study which reported that African HCV infected patients still had the poorest response to treatment with peg IFN and ribavirin (Thompson et al., 2010).

Analysis of baseline characteristics of patients revealed the high probability of achieving SVR was accompanied with younger age of patients, low virological load, low BMI, moderate stage of fibrosis and absence of diabetes mellitus. These results were in agreement with Romero-Gomez et al., (2003), who stated that high viral load, older age, advanced fibrosis or cirrhosis and insulin resistance are predictors of poor treatment response.

IL28B is expressed by peripheral blood mononuclear cells, dendritic cells and hepatocytes; upon infection with viruses or
stimulation with double stranded RNA, IL28B in turn activates signal transduction through JAK/STAT pathway and exerts antiviral activity and has an impact on natural clearance of HCV (Uze and Monneron, 2007).

The present study revealed that the frequency of the favorable C allele was higher in patients with SVR compared to patients with non-SVR (62.2% vs. 38.3%), moreover the odds ratio of patients carried C allele was 2.65 (95%CI: 1.84-3.814) hence more likely to have HCV clearance than those carrying the T allele (p<0.05).

The current study showed that the overall frequency of IL28B genotypes was 24.7% (61/247), 50.2% (124/247) and 25.1% (62/247) for genotypes CC, CT and TT respectively, while the response rate was 82% (50/61), 38.7% (48/124) and 33.9% (21/62) for genotypes CC, CT and TT respectively (p<0.01). Also, we noticed that among 17.4% relapsed patients; 9.3% of patients were of CC genotypes, 65.1% were of CT genotypes and 25.6% were of TT genotypes.

These results were in consistence with those reported by Asselah et al., (2012) where the frequencies of IL28B genotypes were 26.8%, 52.4% and 20.8% for CC, CT and TT respectively and the response rate was 81.8%, 46.5% and 29.4% for genotypes CC, CT and TT respectively in HCV-4 infected patients. Also, Thomas et al., (2009) stated that the CC genotype at IL28B SNP rs12979860 is associated with an approximately three times higher clearance rate versus C/T, T/T genotypes. Similarly, Ge et al., (2009) found that Asian patients have the highest C allele frequency at rs12979860, followed by European patients and African patients. The SVR rates across different population groups displayed a striking concordance with C allele frequency at rs12979860. This finding partly explains the differential treatment response in patients of different racial descent. The noticed low prevalence rate of CC genotype among patients included in our study could be attributed to the general low prevalence of CC genotype in Egyptians as it was also low in the control group; 27% (27/100) (p>0.05).

In the current study, patients carried the CC genotype had increased probability to HCV clearance than those with genotypes CT and TT with OR 7.71 (95%CI: 3.71-15.79). In agreement with this finding, previous studies performed by Pineda et al. (2010) & Stattermayer et al. (2011) found that the C/C genotype at rs12979860 was associated with SVR in patients infected with HCV.
genotype 4, suggesting that *IL28B* genotype could be a predictor of treatment-induced clearance in HCV infections.

Moreover, the CC genotype showed higher virological response rates throughout the whole study (at weeks 12, 24, 48) and lower rates of post-treatment relapse compared to other genotypes (93.4% EVR, 82% SVR, 11.5% non-responder and 6.6% relapers for CC vs 79% EVR, 38.7% SVR, 38.7% non-responders and 22.6% relapers for CT vs 75.8% EVR, 33.9% SVR, 48.4% non-responders and 17.7% relapers for TT).

These results agree with previous studies revealing that PEG-IFN/RBV therapy is more effective at blocking the production of HCV in patients with the *IL28B* CC genotype and that the rate of viral drop is increased in patients with the CC genotype compared to those with CT or TT genotypes, and CC genotype correlates well with higher rates of SVR (*Thompson et al., 2010; Lindh et al., 2011; Statttemayer et al., 2011*).

The current study revealed that CC genotype showed no significant difference between relapsers and non-responders (*p* > 0.05), denoting that this favorable genotype is affecting SVR status only and could not be used to differentiate between non-responders and relapers; *Grandi et al., (2013)* agreed with these results.

Our results, as well as those of previous studies (*Suppiah et al., 2009; Tanaka et al., 2009; Grandi et al., 2013*), showed that patients with *IL28B* CC genotype have a high chance of SVR and might possibly need only the standard Peg-IFN and ribavirin treatment, particularly if accompanied with other positive predictors: low viral load, young age, absence of diabetes mellitus and low BMI.

However, patients with genotypes CT and TT who had low response and high relapse rates (non-SVR rate was: 61.3% (76/124) for CT and was 66.1% (41/62) for TT genotypes, respectively might need more care and could be subjected to triple therapy or direct acting antiviral agents (DAAs) plus Peg-IFN and ribavirin especially if accompanied with other negative predictors: old age, previous non-response, high viral load, obesity, insulin resistance, as effective therapies should be established on the baseline characteristics of the patients and should follow a response-directed method.

Multivariate analysis of significant factors affecting response rate showed that *IL28B* CC genotype was the only significant factor predicting SVR independent of other factors, such as age, stage of
liver fibrosis, BMI and VL with OR 6.49; 95% CI: 2.30-18.2; confirming the results of the study done by Ge et al., (2009) who concluded that \textit{IL28B} polymorphism had a strong impact on SVR of HCV, and can provide useful pretreatment stratification of patients for HCV treatment.

In consistent with our findings, Bronowicki et al., (2012) reported that 100% of treatment-naive HCV patients with \textit{IL28B} CC achieved SVR, even with 12 weeks of a protease inhibitor (telaprevir) and standard combination therapy. Therefore, \textit{IL28B} genotyping may continue to be of importance in the shortening of treatment duration. Although, Stättermayer et al., (2011) found that SVR rates were similar between those carried CC genotype and those with the T allele (80.5% vs. 74.4%, \(p>0.05\)), they stated that \textit{IL28B} genotype may be included in treatment algorithms to adapt therapy among patients who have failed to achieve rapid virological response (RVR) and reported that a lack of RVR in patients with unfavorable \textit{IL28B} genotypes should not be considered as an unethical stopping rule; nevertheless, premature therapy termination should be considered in cases with severe treatment side effects, poor motivation or severe co-morbidities.

**CONCLUSIONS**

\textit{IL28B} polymorphism genotyping is a good pretreatment predictor of response to combined PEG-IFN/RBV treatment in Egyptian patients with HCV related chronic liver disease. Analysis of IL28B genotype at SNP rs12979860 could be used as a guide to customize optimum personalized treatment strategy for HCV infected Egyptian patients. Further studies for other predictors of treatment response, independent of \textit{IL28B} genotype, might provide more informed clinical decision making for better outcome.

**ACKNOWLEDGEMENT & FINANCIAL DISCLOSURE**

This research was fully funded by a National Grant from Science and Technology development Fund (STDF), Egypt.

None of the contributing authors have any conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.
REFERENCES


**IMPACT OF IL28B POLYMORPHISM ON TREATMENT**

---


الملخص العربي

تأثير النمط الجيني للانتترلوكين 28 ب على الاستجابة للعلاج في مرضى الالتهاب الكبدى الفيروسي سي المصريين

منار عبادة، أشرف الفرت، وسام مراد، نرمين إحسان، أم كلثوم الحداد، هالة السعيد

قسم: الكيمياء الحيوية الإكلينيكية، الصحة العامة، الباثولوجي، طب الكبد، معهد الكبد، جامعة المنوفية

يعتبر النمط الجيني للانتترلوكين 28 ب من العوامل المؤثرة للإستجابة للعلاج بعقار الأنتريفرون والريبيافيرين في مرضى الالتهاب الكبدى الفيروسي سي. تهدف هذه الدراسة إلى تحديد دور النمط الجيني للانتترلوكين 28 ب كمؤشر للإستجابة للعلاج بعقار الأنتريفرون والريبيافيرين في مرضى المصريين المصابين بالالتهاب الكبدى الفيروسي سي المزمن.

اجريت هذه الدراسة على 247 مريض بالالتهاب الكبدى الفيروسي سي الذين يعالجون بعقار الأنتريفرون والريبيافيرين وقد تم تقسيمهم حسب الاستجابة للعلاج بالإضافة إلى 100 شخص من الأصحاء كمجموعة ضابطة. وقد تم اخذ التاريخ المرضي لهم والكشف الطبي وعمل عينة كبدية قبل العلاج لتحديد نسبة تليف الكبد، أيضاً تم عمل تحاليل وظائف الكبد وصورة الدم كاملة وتحليل الفيروسات الكبدية وقد تكررت هذه التحاليل عند الأسبوع 12، 42، 84 من العلاج وداخلياً بعد 6 شهور من توقف العلاج. وتتم تحليل معرفة النمط الجيني للانتترلوكين 28 ب. وقد أثبت النتائج أن معظم المرضى المستجيبين للعلاج بعقار الأنتريفرون والريبيافيرين حتى بعد توقف العلاج لمدة 6 شهور هم من يحملون النوع TT، CT، CC الجنيني وان المرضى الذين يحملون النوع الجنيني CC والمرضى الذين يحملون النوع الجنيني TT كان تحسين استجابتهم اقل مقارنة بهم ودراسة العوامل الأخرى المؤثرة في العلاج وجد أن عمر المرضى ومرض السكر ودرجة تليف الكبد ونسبة الفيروس سي في الدم يؤثران في استجابة المريض للعلاج. وتوصى هذه الدراسة بإجراء تحليل النمط الجيني للانتترلوكين 28 ب للمصابين بالالتهاب الكبدى الفيروسي سي المزمن قبل بدء العلاج للوصول لأفضل النتائج.