Influence of Interleukin 28 Gene Polymorphism on Response to Therapy of Chronic Hepatitis C Virus Infection in Children

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Abstract

Background: Investigating predictors of response to anti-HCV therapy may avoid the current expensive therapy associated with various side effects.

Aim of Work: To test for the hypothesis claiming that single nucleotide polymorphism (SNP) in the IL-28B gene might affect the response to antiviral treatment. The SNP rs12979860 C>T in the IL-28B gene was assayed, and its influence on response to HCV therapy was investigated in a cohort of HCV infected children.

Patients and Methods: Seventy three HCV infected children were treated with combined Peg-IFN α2b and ribavirin. Baseline viral load was determined, as well as viral load at 12 and 24 weeks after starting therapy. When viral load did not become negative or did not decrease by 2 log at 12 weeks/ or when it was still positive at 24 weeks, patients were considered non-responders and treatment was stopped for the remaining patients, who responded to treatment, HCV RNA was again assayed at the end of 48 weeks of therapy and 24 weeks after the end of therapy (sustained virological response: SVR). SNP analysis of IL-28B was done by real time polymerase chain reaction.

Results: SVR was achieved in 38.4%. There was a significant association between polymorphism in the IL-28B SNP rs12979860 and response to therapy in children, (p=0.016), where the CC genotype is more likely to respond to HCV therapy than CT and TT genotypes. Multivariate logistic regression analysis showed that IL28 gene SNP rs12979860 C>T and basal viral load were important independent factors predicting response to antiviral therapy in chronic hepatitis C children.

Conclusion: IL28B SNP rs12979860 C>T provides valuable information as regards the patient's liability to achieving SVR for the HCV therapy, which is increasingly relevant in the clinical practice. Multivariate logistic regression study showed that IL28 gene SNP rs12979860 and pretreatment viral load were crucial independent factors predicting response to HCV therapy in children.

Key Words: HCV – Interleukin 28B – RT-PCR – SNP rs12979860 C>T.

Introduction

AT least 170 million patients are infected with hepatitis C virus (HCV) according to the World Health Organization (WHO) report [1]. Egypt has the highest prevalence of HCV infection among other countries. It is estimated to be 25% in rural areas and 8% in urban [2,3]; with 8 to 10 million patients having anti-HCV antibodies and 5 to 7 million with active infections with positive HCV-RNA [4].

The pathogenesis of HCV infection remains unclear; however it seems to be directly related to interaction between host immune response and the potential of the virus to evade it.

Interleukins play a role in initiating and regulating the immune responses, consequently affecting susceptibility to HCV infection and the benefit of antiviral therapy.

Allelic variations in Interleukin 28B gene gained a great scientific interest. Several genome wide association studies detected a panel of SNPs on chromosome 19q13 that might be strongly associated with therapy-induced and or spontaneous clearance of HCV [5].

In hepatitis infection, the virus stimulates the innate immune system and consequently IFN-α production. Therefore PEG-IFN-α in combination with ribavirin is the most effective treatment for HCV eradication [6].

IL28B is a cytokine that shares the same intracellular pathways of IFN-α. IFN-α, either produced endogenously via the innate immunity in response to HCV or exogenously administered during treat-
Influence of Interleukin 28 Gene Polymorphisms

Intratumoral, up regulates the IL28B expression, consequently. IL28B amplifies the interferon-stimulated genes (ISG) expression following treatment by PEG-IFN-α. ISG are hundred genes that play roles in combating various viruses.

The IL28B polymorphism (rs) 12979860 C>T may influence the expression of IL28B and consequently the antiviral response [7]. The C/C genotype is associated with favorable outcomes, whereas the T/T genotype offers poor outcomes.

The current study aimed at testing the hypothesis that SNP of the IL-28B gene may influence response to antiviral treatment. The SNP rs12979860 C>T in IL-28B gene was assessed in the studied subjects and its impact on response to HCV therapy was analyzed.

Patients and Methods

The Ethical Committee of Faculty of Medicine, Cairo University approved the current study which was worked upon since 2010 to 2014 on 73 chronically infected children with HCV who were treated in the Pediatric Hepatology Unit in Cairo University Pediatric Hospital. Patients administered pegylated interferon (Peg-IFN) α2b and ribavirin as treatment for HCV infection.

Patients were investigated for their eligibility to join this study. According to the Helsinki Declaration [8]. Aim of the present study was explained to the included subjects and to their parents, who signed an informed consent before joining the present study and before any physical examination or laboratory investigations.

HCV infected children above 4 years, were included in the study. Patients were diagnosis based on virological, serological, and histological investigations. Pre and post-treatment histopathological examination of percutaneous needle liver biopsy was performed to the studied subjects. Children were not previously treated with interferon.

Exclusion criteria included: Patients with decompensated liver disease, anemia, (<10g/dL), neutropenia (<1500/mm^3), thrombocytopenia (<100,000/mm^3) or leucopenia (<3,000/mm^3) and high serum creatinine. Hepatitis B infection, Wilson’s disease, α1 antitrypsin deficiency, autoimmunity, uncontrolled thyroid disorder, poorly controlled diabetes mellitus, or psychiatric diseases were also considered exclusion criteria.

Five ml of blood in plain vacutainer were collected and fresh sera were separated and assayed for chemistry investigations, HCV antibodies as well as HCV-RNA titers. Another 2ml of blood were collected in EDTA vacutainer for the genotyping assay. DNA was extracted and stored at –20ºC till the investigations were done.

**Laboratory work up was done for all patients:**

Assessment of the patient’s viral load was done to the entire studied group by testing their sera for HCV RNA using quantitative real time PCR at baseline, 12, 24, 48 weeks after starting treatment and after 24 weeks after the end of treatment by Real time PCR.

The individuals whose HCV RNA was negative or decreased by >2 log at week 12 i.e. early virologic response (EVR) continued the antiviral therapy. If not, the patient was considered non-responder and therefore therapy was discontinued.

As for responders, HCV RNA was repeated again after 24 weeks of therapy and positive children were considered non-responder and therefore therapy was discontinued. As for responders at 24 weeks, continued treatment till 48 weeks, and at the end of treatment their end of treatment response (ETR) was assessed by repeating the HCV RNA. Sustained virologic response (SVR) was assessed in children who achieved ETR by repeating the HCV RNA after 24 weeks.

Routine laboratory investigations including, Complete blood picture (assayed on CELL-Dyn 3700), liver function tests including assay of direct and total bilirubin, albumin, AST, ALT, GGT and ALP were also investigated (on Hitachi 911*; Roche, Germany).

Genotyping of interleukin 28B gene polymorphism rs12979860 C>T, by real time PCR:

Extraction of DNA from whole blood using QIAamp DNA blood Mini kit-Qiagen. Amplification of DNA using Taq-Man SNP Genotyping Assays to define IL-28B gene polymorphism rs12979860 C>T.

Real-time PCR allelic discrimination assay:

Genotyping was done using Applied Biosystemstep oneTM Real-Time PCR System. Allelic discrimination tests of the C→T (dbSNP ID: rs12979860, were designed using Taq-Man SNP Genotyping Assay).

Reaction mixture PCR contained: 1.25 µl 20X working stock of SNP assay, 12.5 µl TaqMan universal PCR master mix (2X) and 5 of the child DNA which was completed to 25 µl by DNase-free water.
Activation of the enzyme at 95 °C for 10 minutes. Thermal cycling profile was: denaturation of the target DNA at 92 °C for 15 seconds, followed by 50 successive cycles of annealing and extension at 62 °C for 60 seconds. Sequence Detection System (SDS) Software was used to read and analyze the allelic discrimination plate using the VIC dye and Fam-dye Fig. (1).

Experiment: Untitled Applied Biosystems StepOneTM Instrument

Allelic Discrimination Plot

Allelic Discrimination Plot (SNP Assay: SNP Assay 1)

<table>
<thead>
<tr>
<th>Legend</th>
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<tbody>
<tr>
<td>Homozygous Allele 1 wild type/Allele 1 Wild type</td>
</tr>
<tr>
<td>Homozygous Allele 2 mutant type/Allele 2 mutant type</td>
</tr>
<tr>
<td>Homozygous Allele 1 wild type/Allele 2 mutant type</td>
</tr>
<tr>
<td>X Undetermined</td>
</tr>
</tbody>
</table>

Fig. (1): Allelic discrimination plot SNP rs12979860 done on the Applied Biosystem Step OneTM Real-Time PCR System.

Statistical methods:

The results of the current study were analyzed using the SPSS computer software package, version 15.0 (Chicago, IL, USA). Qualitative data were expressed as frequencies. Quantitative data were expressed as mean and SD. Differences between the two studied groups were compared by Student t-test when the data were normally distributed. Quantitative data were expressed in the form of median and interquartile range (IQR) where the differences between the two studied groups were compared by Mann Whitney test when data were skewed. Independent factors predicting response to interferon therapy in CHC patients were detected using multivariate logistic regression analysis. Differences were considered statistically significant at p≤0.05.

Results

Studied patients were divided into two different groups according to their response to therapy:

- **Group I: Responders**: This group included 28 patients, 16 males and 12 females, with mean age 11.9±2.9 years.

  Responders achieved SVR with normalization of liver function tests level and clearance of the virus, confirmed by negative HCV RNA-PCR, 24 weeks after cessation treatment.

- **Group II: Non-responders**: This group included 45 patients, 31 males and 14 females, with mean age 10.8±3 years.

  Demographic and lab data of the 2 groups are shown in (Table 1). GGT and Quantitative HCV RNA were significantly higher in non-responders (p=0.001, p=0.005 respectively) (Table 1).

Comparing the prevalence of SNP in IL28B genes in responders and non responders to HCV therapy, IL28B gene, SNP rs12979860, showed statistically significant difference (p=0.016), where the CC genotype is more likely to respond to HCV therapy than CT and TT genotypes (Table 2).

After subgrouping of the genotypes, rs12979860 in IL28B gene showed that patients with the CC genotype is 6.622 times more likely to respond to HCV therapy than CT and TT genotypes (Table 3).

Multivariate logistic regression to assess the influence of different factors on response to therapy showed that IL28 gene SNP rs12979860 and pretreatment viral load were independent factors predicting response to interferon therapy in chronic hepatitis C patients, (p=0.002), (p= 0.002) (Table 4).

Table (1): Baseline data in responders and non-responders to combined interferon/ribavirin therapy.

<table>
<thead>
<tr>
<th></th>
<th>Non Responders</th>
<th>Responders</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years): (mean±SD)</td>
<td>10.8±3.5</td>
<td>11.9±2.9</td>
<td>0.168</td>
</tr>
<tr>
<td>Gender: Males (n=47)</td>
<td>31 (68.9%)</td>
<td>16 (57.1%)</td>
<td>0.308</td>
</tr>
<tr>
<td>Females (n=26)</td>
<td>14 (31.1%)</td>
<td>12 (42.9%)</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L): [median]</td>
<td>53</td>
<td>57</td>
<td>0.551</td>
</tr>
<tr>
<td>[IQR range]</td>
<td>49.5</td>
<td>56.75</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml):</td>
<td>36.25</td>
<td>90</td>
<td>0.056</td>
</tr>
<tr>
<td>[Median]</td>
<td>76.5</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>[IQR range]</td>
<td>43.85</td>
<td>20.0</td>
<td>0.001</td>
</tr>
<tr>
<td>GGT (U/L): [Median]</td>
<td>418342</td>
<td>936.02</td>
<td>0.005</td>
</tr>
<tr>
<td>[IQR range]</td>
<td>3.170.500</td>
<td>473.592</td>
<td></td>
</tr>
</tbody>
</table>


Table (2): Genotype distribution of SNP rs12979860 in the two studied groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Non responders (n=45)</th>
<th>Responders (n=28)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL28B: rs12979860 C&gt;T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (6.7%)</td>
<td>9 (32.81%)</td>
<td>0.016</td>
</tr>
<tr>
<td>CT</td>
<td>28 (62.2%)</td>
<td>12 (42.9%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>14 (31.1%)</td>
<td>7 (25.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Prediction of response to interferon therapy according to genotypes of IL28B gene.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Non responders (n=45)</th>
<th>Responders (n=28)</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL28B: rs12979860 C&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (6.7%)</td>
<td>9 (32.1%)</td>
<td>6.622</td>
<td>(1.61-27)</td>
<td>0.004</td>
</tr>
<tr>
<td>CT and TT</td>
<td>42 (93.3%)</td>
<td>19 (67.9%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC and CT</td>
<td>31 (68.9%)</td>
<td>21 (75.0%)</td>
<td>1.355</td>
<td>(0.468-3.922)</td>
<td>0.575</td>
</tr>
<tr>
<td>TT</td>
<td>14 (31.1%)</td>
<td>7 (25.0%)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Table (4): Multivariate logistic analysis for predictors of response to IFN therapy.

<table>
<thead>
<tr>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL28B rs12979860 C&gt;T:</td>
<td>23.399 (3.140-174.34)</td>
<td>0.002</td>
</tr>
<tr>
<td>Base line quantitative PCR</td>
<td>3.671 (10.629-8.275)</td>
<td>0.002</td>
</tr>
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</table>

**Discussion**

HCV infection is a high-priority problem which needs to be addressed utilizing different approaches. Thus, studying the genes associated with HCV clearance is an important step which results in novel modalities for tailored patient treatment. The problem is partly related to the fact that the current treatment for HCV is costly, associated with various adverse effects, and results only in 50-70% SVR. Therefore, predicting the likelihood of response to HCV therapy before initiating treatment could be so beneficial [10].

The results of the present study revealed that 38.4% of HCV patients responded to Peg IFN α-2b plus ribavirin therapy (28 responders versus 45 non-responders). This finding is in agreement with Reis et al., [18] who observed SVR of 39.9%.

The main objective of the current study was to assess the role of the IL 28B SNP rs12979860 C>T in response to HCV therapy and clearance of HCV.

It was found that SNP rs12979860 in IL28B gene is associated with significant statistical difference between responders and non-responders to HCV therapy (p=0.016), where the CC genotype had greater percentage in responders, than TT genotypes (32.81% versus 25%) respectively.

As regard frequency of CC versus CT/TT genotypes of IL28B SNP rs 12979860, in the responders and non-responders, the wild type (CC) was (32.1% in responders, 6.7% in non-responders) while CT and TT genotypes were 67.9% in responders versus, 93.3% in non-responders (p=0.004). Based on the Odds ratio study, patients with the CC genotype have 6.62 folds higher chance to develop SVR than those with mutant types CT/TT.

A study stated that the protective effect of C allele is probably recessive, since no significant difference was found between the C/T and T/T genotypes in subjects of African, European, or combined ethnic groups for HCV clearance, and C/C genotype was always protective relative to C/T and/or T/T. These results highlight the protective effect of the C/C genotype on SVR after therapy of HCV [12].

The results of the current study are consistent with various studies that have shown that polymorphisms in the IL28B gene were assured to be related with therapy-induced viral clearance in HCV infected patients [13,14]. Also, Ge D., Fellay J., Suppiah V. and Thomas D.L. [5,7] have shown that CHC patients with C/C genotype have two-fold more chance of SVR than individuals carrying CT/TT genotypes.

In agreement with the results of the current study, Moreira et al., [15] observed that individuals with the CC genotype rs12979860 achieved higher SVR frequencies than patient with TT genotype in their study groups.

The relationship between IL28B gene polymorphisms and susceptibility or resistance to HCV infection and response to treatment is not obviously known. One explanation is that these variants
upstream of IL28B gene may influence IL28B protein production. Also SNP rs12979860 contains a CpG dinucleotide on a transcription factor site, that is crucial for DNA methylation. In turn methylated DNA may decrease the expression of IL28B and consequently leads to down-regulation of IFN-stimulated genes (ISGs) [16].

However, in contrast to the result of the current study, Duffy et al., [17] examined patients chronically infected with HCV where they found no significant association with therapy response in their cohort ($p=0.170$). This may be attributed to the small sample size [17].

Lastly, forward stepwise logistic regression was run to select the minimum combination of variables that maximally discriminate between responders and non-responders to HCV therapy. IL28 B gene SNP rs12979860 and basal viral load were used to construct a response-prediction model; These 2 independent variables significantly improved prediction of response to anti HCV therapy while all the remaining variables that were non-significant, did not contribute to prediction of response, and were accordingly excluded from the model.

The prediction model indicated that, a patient whose IL28 B gene SNP rs12979860 (CT or TT) is 23.4 times more prone not to respond to HCV therapy. The model also showed that, one unit increase in log basal viral load of HCV (e.g., from 10 to 100 or from 100 to 1000) is associated with an increase in the odds of non-response to HCV therapy by a factor of 3.7; controlling for other variables.

**Conclusion:**

In conclusion, genotyping of IL28B SNP provides highly valuable information regarding the patient’s vulnerability of achieving SVR for the treatment of HCV, which is increasingly relevant in clinical practice. Both the process of IL28B genotyping and the possibility of tailored therapy affect the pharmacoeconomics of hepatitis C therapy. IL28B SNP rs12979860 and basal viral load were used to construct a response-prediction model. These two independent variables improved significantly the prediction of HCV therapy response.

**References**


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