THE ASSOCIATION BETWEEN HEPATIC ISG EXPRESSION, GENETIC VARIATION IN INTERLEUKIN 28B, AND THE RESPONSE TO INTERFERON THERAPY AMONG PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

Hanan Abdel-Haleem Marzouk¹; Hanan Abd El- Hafez¹; Abd El Rahman Zekri²; Medhat Hassan El Sahar³; Mohamed Ibrahim Elfar³
¹Department of Endemic Medicine and Hepatology, Faculty of Medicine, Cairo University, Cairo, Egypt.
²Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt
³Department of Gastroenterology and Hepatology, El Agouza Police authority Hospital, Giza, Egypt.
³farco77@hotmail.com

ABSTRACT

Background

Chronic HCV is a worldwide health problem. HCV infection can activate the endogenous interferon (IFN) system in the liver leading to induction of several IFN-stimulated genes (ISG) in the liver with a strong association between allelic variants of the IL28B genotype and the response to interferon therapy.

Objectives

The aim of this study was to determine the ability of ISG polymorphism and IL28B to predict patient response to IFN therapy; this will guide patient selection for INF treatment.

Methods

We conducted a case-control study on 50 patients with chronic HCV and 20 healthy controls. HCV patients were categorized into responders and non-responders. IL28B genotypes and the intrahepatic ISG expression were investigated and analyzed in relation to the response to treatment.

Results

IL28B CC genotype was the dominant genotype in HCV group (48%). IL28B genotype CC was strongly associated with ISG down-regulation while CT and TT genotypes were significantly correlated with ISG up-regulation (r=0.42, P<0.001). Patients who responded to the treatment were likely to have IL28B CC genotype (OR=1.551, P=0.013), F1-F2 grades of fibrosis (OR=1.11, P=0.028), and lower grade of steatosis (OR=1.21, P=0.036).

Conclusions

Our study shows a strong association between IL28B genotype and ISGs expression in HCV patients. Besides the clinical and viral factors, IL28B genotype and ISGs expression levels are significant predictors of the response to INF/ribavirin therapy; patients with IL28B CC-genotype and low ISGs expression levels were likely to respond while those with IL28B CT/TT genotypes and high ISGs expression levels were not likely to respond.

Keywords: Hepatitis C, Gene expression, Interferon, Interleukin, Genetic Polymorphism.
I. INTRODUCTION

Hepatitis C virus (HCV) infects 70 million people worldwide and is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1]. Egypt has the highest prevalence of infection in the world [2]. Egypt also has the largest number of patients with genotype 4 HCV, more than 90% of those infected or approximately six million people [3]. Chronic HCV infection in the (15-59) age group is near 10% and the rate increase with age to reach more than 25% in 50-60 year-olds with near 4% among 15-19 year-olds which constitutes a major concern [4].

Treatment with pegylated interferon-alpha (peg-IFN-α) and ribavirin (RBV) results in a sustained viral response (SVR) in approximately 50% of patients infected with HCV of genotype 1 and 80% of those with HCV genotypes 2 or 3 [5].

Various well-described side effects as a flu-like syndrome, hematologic abnormalities, and adverse neuropsychiatric events often necessitate dose reduction, and about 10% to 14% of patients require premature withdrawal from INF-based therapy to avoid these side effects in patients who are not likely to respond to the treatment [6] which is also beneficial to reduce the substantial cost of PEG-IFN-α/RBV treatment. It is essential to predict patients response during early treatment or even before initiating the treatment. Research into the predictors of patient response to INF therapy showed that several viral factors, such as genotype 1, high baseline viral load, viral kinetics during treatment, and amino acid pattern in the interferon signaling pathway [8].

Infection with HCV can activate the endogenous IFN system in the liver. However, despite a strong induction of hundreds of IFN-stimulated genes (ISG) in the liver, the activation of the endogenous IFN system in CHC is ineffective in clearing the infection and even impedes the response to therapy, most likely by inducing a refractory state of the IFN signaling pathway [8].

The causal factors and the mechanism underlying this preactivation of the IFN system in some patients with CHC are not well understood. However, studies show that the cleavage of mitochondrial antiviral signaling protein by HCV NS3-4A protease correlates with reduced activation of the endogenous IFN system [9].

Recently, several groups reported a strong association between allelic variants of the IL28B gene encoding IFN-λ3 and response to treatment. Failure to respond to treatment was associated with the minor alleles of rs12979860 (T) [10], rs8099917 (G) [11] and rs12980275 (G) [12]. The association was significant in HCV genotypes 1 and 4 but not in HCV genotypes 2 and 3 [11].

Recently, several genome-wide association studies have revealed that single nucleotide polymorphisms (SNPs) in the 19q13 region, in close proximity to three genes (IL28A, IL28B, and IL29) encoding cytokines of the IFN-1 (i.e. type III IFN) family, predict spontaneous clearance of HCV infection [11] as well as SVR following peg-IFN/ RBV therapy among patients infected with HCV genotype 1 [13].

Three of these SNPs are reportedly highly predictive of favorable treatment response among HCV genotype 1 infected patients: rs12979860 [10], rs12980275 (14) and rs8099917 [11] with a strong linkage disequilibrium noted between rs12979860 and rs8099917 [11].

Since genome-wide association studies have hypothesized that allelic variants in IL28B might be responsible for the difference of endogenous IFN activation and response to treatment, we performed this study to investigate the association between IL28B polymorphisms and hepatic ISG expression and to investigate the role of ISG expression levels and IL28B genotypes as predictors of INF/RBV treatment outcome.

II. METHODS

We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines when reporting this manuscript [14]. The study was approved by the ethics committee of the National Cancer Institute, Cairo University.

2.1 Study Design, Setting, and duration
We conducted a case-control study at the Gastroenterology and Hepatology Department of El Agouza Police Authority Hospital, Giza, Egypt. The study was conducted on patients with chronic HCV who were receiving peg-IFN-α in combination with RBV during the period from October 2011 to April 2013.

2.2 Eligibility Criteria of the study population

Study subjects were selected according to the following criteria: (1) male and female patients aged between 18 to 60 years; (2) patients with chronic HCV infection confirmed by seropositive anti-HCV antibody detection using the 3rd generation ELISA and HCV-RNA seropositive with histopathological criteria of chronic HCV liver disease, (3) HCV patients with elevated ALT levels with a level above the upper limit of the normal for at least two occasions within a period of 6 months.

We excluded patients in any of the following conditions: (1) patients with evidence of hepatocellular carcinoma, (2) patients with decompensated liver disease including those with abnormal blood picture in the form of anaemia, leukopenia, or thrombocytopenia, (3) patients with abnormal creatinine level or thyroid functions, (4) patients with a poorly controlled coexisting medical conditions or psychiatric disorders, (5) patients with other causes of chronic liver disease as autoimmune liver disease, (6) patients with hemoglobinopathies, (7) patients with HBsAg or HIV positive, and (8) patients with a previous treatment with IFN-based therapy.

2.3 Study groups

The study included two main groups: (1) Cases: HCV patients receiving antiviral treatment with peg-IFN-α and RBV; (2) Controls: Healthy individuals who are preparing for liver donation. By the end of the treatment period, HCV patients were classified according to the response to treatment into responders (SVR group) and non-responders (NR group).

2.4 Recruitment of the control group

The control group consisted of healthy individuals who are liver donors preparing for living liver donation, therefore, ensuring that patients in the control group have been extensively examined for all liver-related diseases and medications. We used the medical records of the participants to confirm that they did not have any associated comorbid conditions and to ensure that participants have no history of any relevant liver diseases.

2.5 Drugs used in this study

HCV patients were treated with the following regimen: peg-IFN-α 2b (Pegintron 1.5 µg/kg), SC injection once a week with Ribavirin: (Rebetol 200 mg), and the weight-based dose of ribavirin considering 15 mg per kg daily.

2.6 Baseline Assessment of the study participants

All participants underwent a full clinical assessment and history taking. For all participants, the following laboratory tests were done:

**HCV viral markers:** Anti-HCV antibody, HCV-RNA by PCR, HBsAg, HBe Ab, and HIV Ab

**Immune markers:** ANA, AMA, ASMA

**Thyroid functions:** FT3, FT4, TSH

**Complete blood picture**

**Liver biochemical profile:** serum transaminases, serum total, and direct bilirubin, serum albumin, INR, AFP estimation, and serum alkaline phosphatase

**Renal function tests:** serum urea and creatinine

2.7 Extraction of genome DNA

DNA samples from patients and controls were genotyped for the IL28B rs8099917, rs12979860 and rs12980275 polymorphism with TaqMan SNP genotyping assays (Applied Biosystems Inc, Foster City, CA), using the ABI 7500 Fast real-time thermocycler, according to manufacturers recommended protocols. TaqMan probes and
primers had been designed and synthesized by Applied Biosystems Inc. Automated allele calling was performed using SDS software from Applied Biosystems Inc. Positive and negative controls were used in each genotyping assay.

2.8 Preparation of liver tissue samples
Liver biopsy samples were taken from all patients before treatment and the control group (As a workup for subjects preparing as donors for living donor liver transplantation). The biopsy samples were divided into two parts: the first part was immersed in formalin for histological assessment and the second was cut at 5µm paraffin sections and immersed in Eppendorf tube for RNA isolation. Liver tissue RNA was isolated using the RNeasy Mini kit (QIAGEN) according to the manufacturer’s instructions. Histopathological examination of a core needle liver biopsy from patients and controls group according to Ishak scoring system.

2.9 Hierarchical clustering and pathway analysis of gene chip data
Gene chip data analysis was performed using BRB-Array Tools the data were log-transferred, normalized, centered, and applied to the average linkage hierarchical clustering with centered correlation.

For gene chip analysis, we selected 37 representative ISGs. Hepatic gene expression profiling was obtained from 50 CH-C patients before the initiation of IFN and RBV combination therapy and the 100 most up-regulated genes were selected. We evaluated hepatic gene expression in 50 patients before treatment and from 20 control groups. Pathway analysis was performed using Meta Core (GeneGo, St. Joseph, MI, USA).

2.10 Statistical analysis
Categorical data were summarized as frequencies and percentages. While continuous data were initially tested for normality using the Kolmogorov Smirnov test. Continuous data were presented as mean and standard deviation. For comparison of categorical and continuous variables, we used the Chi-square test and student t-test, respectively. For multiple group comparison means, Student’s t-test was used. To study the relationship between two variable Pearson’s correlation tests (correlation coefficient r) were calculated. An alpha level below 0.05 was considered for statistical significance. All analyses were done using the Statistical Package for Social Science (SPSS software version 18 for Windows).

III. RESULTS

3.1 Characteristics of the study population
Our study included 50 subjects (responders: n=32 and non-responders: n=18). The mean age of the study population was 39.7 years for the responders and 40.3 years for the non-responders. The demographic characteristics of the study groups are shown in Table 1.

3.2 The distribution of IL28B genotypes in HCV patients
The genotyping of IL2B in the HCV groups showed CC genotype in 24 patients (48%), CT genotype in 18 patients (36%), and TT genotype in 8 patients (16%) while the genotypes of the control group were CC genotype in 12 patients (60%), CT genotype in 8 patients (40%), and none with TT genotype (0%). CC and TT genotypes were significantly more frequent in the patient group than the control group (P<0.05).

3.3 Factors Correlated with the Response to Treatment
The response to treatment was negatively correlated with the stage of fibrosis, steatosis, viral load quantification by PCR, and the level of serum total bilirubin. On the other hand, the response to treatment was not significantly correlated with patient age, gender, other pre-treatment associated diseases, or the side effects occurred during the treatment (Table 1 and Table 2).

3.4 Association between the response to treatment and the categories of IL28B genotypes and ISGs expression
There was a statistically significant association between IL28B genotypes and the treatment response; patients with CC genotype had a higher response to the treatment compared to those with CT and TT genotypes (53.1% vs. 34.4% and 12.5%, respectively).
There was a significant negative correlation between ISG expression and the response to treatment; patients with response to the treatment had significantly less ISGs expression compared with those in the non-response group (Table 3).

3.5 Correlation between IL28B and grade of steatosis

In terms of the correlation between the IL28B genotype and grade of steatosis in the liver biopsy, CC genotype was associated with a lower grade of steatosis compared to CT and TT genotypes in HCV patients as well as healthy controls. In addition, there was a higher grade of steatosis in HCV patients compared to the control group.

3.6 Correlation between ISG expression, grade of fibrosis, and response to treatment

There was a significant positive correlation between ISG expression and the grade of fibrosis in the liver biopsy (r=0.3, P=0.046). Patients with a low grade of fibrosis and ISG downregulations had a better response to the treatment and vice versa.

3.7 Correlation between IL28B genotypes and ISG expression

Interestingly, the IL28B genotypes and ISG expression were significantly correlated (r=0.42, P<0.001); ISG downregulations was associated with C/C genotype while ISG upregulation was related to C/T and T/T genotypes.

3.8 Predictors of the response to treatment

Results of the logistic regression analysis showed that smoking, grade of fibrosis, total bilirubin, steatosis, viral load by PCR, IL28B genotyping, and ISG expression were significant predictors to predict the response to the interferon and ribavirin therapy in patients with chronic HCV infection (Table 4).

IV. DISCUSSION

4.1 Summary of the main findings

We aimed to investigate the association between IL28B polymorphisms and hepatic ISG expression and compare the abilities of ISG levels and IL28B genotype to predict treatment outcome, so we studied 50 patients with HCV related chronic liver disease and 20 patients who were served as a control group who underwent laboratory investigations and liver core biopsy and identification of genotype, grade of fibrosis, steatosis, and interferon stimulating genes expression and they had been furtherly studied according to the response to the treatment with interferon and ribavirin therapy.

Our study showed that gender, grade of fibrosis, and grade of steatosis were different among HCV patients who responded to the ribavirin/interferon regimen compared to those who did not respond. Moreover, IL28B genotype and ISG expression were correlated with the response to treatment and might be used as predictors of response to the treatment.

4.2 Predictors of response to IFN therapy

Clinical factors that might affect the response to PEG-IFN-α/RBV therapy are age, gender, pre-treatment HCV RNA levels, higher pre-treatment AST levels, liver fibrosis status, and insulin resistance [15].

We also found that the grade of fibrosis and steatosis were negatively correlated with SVR rate. Duarte-Rojo provided an explanation for this observation; the effect of non-CC genotypes on increased ISG might favor intrahepatic lipid deposition by suppressing lipoprotein lipase and limiting the conversion of very low-density lipoprotein to low-density lipoprotein [16].

We found that patients who achieved SVR had significantly lower pre-treatment viral load (195842.8 IU/ml) compared with those the non-responders group (444791.0 IU/ml). This observation was noticed previously by Doss et al.[17] who reported that baseline HCV RNA ≥800,000 IU/mL as a factor associated with worse treatment outcome.

In our study multivariate analysis showed that hepatic ISG (<3.5), fibrosis stages (F1-F2), lower steatosis percentage, and lower viral load are significant predictors of achieving SVR in HCV patients.
These results are consistent with those of Rauch et al.[11] who found that the predictive ability of the IL28B genotype was stronger than the other factors associated with the response such as the pre-treatment viral load, ethnicity, fibrosis stage, age, and metabolic factors [11,18].

4.3 The role of ISG expression in predicting the response to treatment

In addition to viral factors, hepatic gene expression before and during IFN treatment has been examined to determine host factors associated with the response to treatment [19,20]. In this study, we investigated three ISG (Mx1, IFI44, and IFIT1) out of the 15 genes validated by RTD-PCR. The expression levels of these three ISG were higher than the expression levels of the other ISGs. We found that the proportion of patients with NR to treatment was significantly higher in the upregulated ISG group. On the other hand, the proportion of patients achieving SVR was significantly higher in the downregulated ISG (P=0.004). These findings are consistent with previous reports showing a higher response to treatment in patients with downregulated ISG [19,20].

Upregulated ISG prior to the treatment might be related to the poor induction of ISGs and the impaired eradication of HCV during treatment [19]. Analysis of hepatic gene expression demonstrated that the up-regulation of ISGs in the liver before treatment might be related to poor treatment response [19,21]. To reveal the underlying mechanism of treatment resistance, two reports compared gene expression profiling in the liver before and during therapy and showed that patients with upregulated ISGs prior to the treatment failed to further induce ISGs following the administration of IFN and could not eliminate HCV [19,20].

Honda et al.[22] analyzed gene expression profiles of 91 patients who received pegylated-interferon and ribavirin combination therapy. They found a high proportion of patients with no response to treatment in the up-regulated ISGs group compared to the downregulated ISGs group (P=0.002). Their multivariate regression analysis showed that ISGs of <3.5 and fibrosis stages of F1 and F2 could significantly predict the response to treatment.

Petersen et al.[23] studied the associated between the ISGs expression and the response to pegylated-interferon/ribavirin treatment in 53 HIV/HCV coinfected patients. They found that ISG induction was more frequent in patients achieving the SVR but not in the non-responder group. They concluded that the failure to respond to IFN-based therapy was associated with diminished ISG response to therapy.

Abe et al.[24] studied the expression levels of 16 genes that promote antiviral state and 4 suppressor genes. They found that the expression levels of both an antiviral MxA and a suppressor SOCS1 were independent predictors for non-response. Also, the rs12979860 genotype may be associated with response to combination therapy through an inverse relationship between antiviral and suppressor ISGs in the liver.

4.4 The association between IL28B genotype, ISGs expression, and the response to treatment

In our study, IL28B genotype was strongly associated with intrahepatic ISGs expression. IL28B genotypes of CT and TT were associated with higher ISG levels compared with the CC genotype which has lower ISG expression (P<0.001). Moreover, patients with CT and TT genotypes (those with high ISGs expression) were associated with poor response rates (CT 34.4% and TT 12.5%).

Our findings are consistent with the previous studies reporting a strong associated between ISGs expression levels and IL28B genotype in HCV patients [11,22,25,26]. Moreover, few reports suggest that the relationship between IL28B genotype and some ISGs expression exist in the normal liver even before HCV infection.

Previous studies reported that patients with non-CC genotypes (those with higher ISG expression levels in our study) were previously associated with lower rates of spontaneous clearance. In contrast, the CC genotype (which has low ISG expression in our study) has been associated with high rates of spontaneous clearance [11].

Raglow et al.[27] studied 64 normal liver specimens and 95 HCV infected liver specimens. They found that some ISGs were differentially expressed in normal liver specimens by IL28B genotypes; ISG15, HTATIP2, LGALS3BP, IRF2, and BCL2 were associated with C allele while IFNa, β, γ, λ3 and CD80 were associated with T allele. These findings suggest that the expression of some ISGs is associated with IL28B genotype in normal liver tissue prior to HCV infection.
McGilvray et al.[25] showed that ISGs expression levels vary according to IL28B genotype and are stronger predictors of response to IFN-based therapy among patients with chronic HCV infection.

4.5 Strength points and Limitations

The strength points of our study are: (1) the study is conducted in Egyptian population with the highest HCV prevalence in the world, (2) HCV genotype 4 is the most common in Egyptian population and few reports exist about the IL28B and ISGs association in this genotype, (3) we included a third group of healthy controls to provide a control group for the comparisons of the responders vs. non-responders. The limitations of our study are: (1) the relatively small sample size, and (2) the analysis of 3 out of the 15 ISGs.

4.6 Author’s conclusions

Our study shows a strong association between IL28B genotype and ISGs expression in HCV patients. Besides the clinical and viral factors, IL28B genotype and ISGs expression levels are significant predictors of the response to INF/ribavirin therapy; patients with IL28B CC-genotype and low ISGs expression levels were likely to respond while those with IL28B CT/TT genotypes and high ISGs expression levels were not likely to respond.

Acknowledgment: none

Conflict of interest: None to declare

Funding Source

The expenses of this study were covered by funds from (1) the Cancer Biology Department, National Cancer Institute, Cairo University, Egypt and (2) the Gastroenterology and Clinical Pathology Departments, Police Authority Hospital, Egypt.

REFERENCES


Table 1 shows the demographic characteristics of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>(Group 1) Responders</th>
<th>(Group 2) Non-responders</th>
<th>x²/t*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32.0</td>
<td>17.0</td>
<td>1.814</td>
<td>0.178</td>
</tr>
<tr>
<td>Female</td>
<td>0.0</td>
<td>1.0</td>
<td>0.147</td>
<td>0.884</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.9 (10.1)</td>
<td>40.3 (10.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilharziasis</td>
<td>15</td>
<td>5</td>
<td>1.120</td>
<td>0.571</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the histological findings of the patients and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Response (Group 1) No= 32</th>
<th>Non response (Group 2) No=18</th>
<th>Control Group No==20</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological activity index (HAI)</td>
<td>Mean 6.0 SD 2.1</td>
<td>Mean 6.4 SD 2.1</td>
<td>Mean 4.2 SD 2.1</td>
<td>0.211</td>
</tr>
<tr>
<td>Grade of fibrosis</td>
<td>1.7 SD 1.1</td>
<td>2.3 SD 1.1</td>
<td>0.8 SD 0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Grade of steatosis</td>
<td>5.4 SD 2.3</td>
<td>12.6 SD 5.0</td>
<td>3.9 SD 1.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3 shows the association between the response to treatment and the categories of IL28B genotypes and ISGs expression.

<table>
<thead>
<tr>
<th></th>
<th>Response</th>
<th>Non response</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISGs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>17 (70.83%)</td>
<td>7 (29.17%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Non CC</td>
<td>15 (57.69%)</td>
<td>11 (42.31%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 shows the results of the logistic regression of factors affecting response.

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% C.I. Lower</th>
<th>95% C.I. Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>3.8121</td>
<td>0.031</td>
<td>1.312</td>
<td>0.584</td>
<td>2.356</td>
</tr>
<tr>
<td>Albumin g</td>
<td>4.275</td>
<td>0.556</td>
<td>0.570</td>
<td>0.257</td>
<td>0.998</td>
</tr>
<tr>
<td>Total Bilirubin mg/dl</td>
<td>11.065</td>
<td>0.038</td>
<td>1.475</td>
<td>0.664</td>
<td>2.581</td>
</tr>
<tr>
<td>INR ratio</td>
<td>0.715</td>
<td>0.093</td>
<td>0.095</td>
<td>0.043</td>
<td>0.166</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>4.275</td>
<td>0.556</td>
<td>0.570</td>
<td>0.257</td>
<td>0.998</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>0.715</td>
<td>0.093</td>
<td>0.095</td>
<td>0.043</td>
<td>0.166</td>
</tr>
<tr>
<td>HGB g/dl</td>
<td>0.715</td>
<td>0.093</td>
<td>0.095</td>
<td>0.043</td>
<td>0.166</td>
</tr>
<tr>
<td>WBC (10³/µL)</td>
<td>3.385</td>
<td>0.440</td>
<td>0.451</td>
<td>0.203</td>
<td>0.789</td>
</tr>
<tr>
<td>Plt (10³/µL)</td>
<td>0.790</td>
<td>0.103</td>
<td>0.105</td>
<td>0.047</td>
<td>0.184</td>
</tr>
<tr>
<td>ESR (mm/hr.)</td>
<td>4.725</td>
<td>0.614</td>
<td>0.630</td>
<td>0.284</td>
<td>1.103</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.795</td>
<td>0.103</td>
<td>0.106</td>
<td>0.048</td>
<td>0.186</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>0.790</td>
<td>0.103</td>
<td>0.105</td>
<td>0.047</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>AFP(ng/mL)</td>
<td>4.485</td>
<td>0.583</td>
<td>0.598</td>
<td>0.269</td>
<td>1.047</td>
</tr>
<tr>
<td>Interferon µgm</td>
<td>3.745</td>
<td>0.487</td>
<td>0.499</td>
<td>0.225</td>
<td>0.873</td>
</tr>
<tr>
<td>PCR IU/ml</td>
<td>10.670</td>
<td>0.037</td>
<td>1.116</td>
<td>0.502</td>
<td>1.953</td>
</tr>
<tr>
<td>Histological activity index (HAI)</td>
<td>5.220</td>
<td>0.679</td>
<td>0.696</td>
<td>0.313</td>
<td>1.218</td>
</tr>
<tr>
<td>Grade of fibrosis</td>
<td>9.880</td>
<td>0.028</td>
<td>1.117</td>
<td>0.503</td>
<td>1.955</td>
</tr>
<tr>
<td>Steatosis (%)</td>
<td>14.120</td>
<td>0.036</td>
<td>1.216</td>
<td>0.547</td>
<td>2.128</td>
</tr>
<tr>
<td><strong>IL28B Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C/C</strong></td>
<td>11.635</td>
<td>0.013</td>
<td>1.551</td>
<td>0.698</td>
<td>2.714</td>
</tr>
<tr>
<td><strong>C/T</strong></td>
<td>0.960</td>
<td>0.125</td>
<td>0.128</td>
<td>0.058</td>
<td>0.224</td>
</tr>
<tr>
<td><strong>T/T</strong></td>
<td>5.770</td>
<td>0.750</td>
<td>0.769</td>
<td>0.346</td>
<td>1.346</td>
</tr>
<tr>
<td>ISGs</td>
<td>0.970</td>
<td>0.126</td>
<td>0.129</td>
<td>0.058</td>
<td>0.226</td>
</tr>
</tbody>
</table>

**Figure 1** shows the distribution of IL28B genotypes in the patients and control groups;

**Figure 2** shows the grade of steatosis in different IL28B genotype subgroups.
Figure 3 shows the distribution of: (A) Gender, (B) Grade of Fibrosis, (C) Steatosis, (D) Viral load by PCR, among the response and non-response groups.

Figure 4 shows the distribution of IL28B genotypes and ISG expression levels in the response and non-response groups.

Figure 5 shows the ISG expression levels among the IL28B genotypes.