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Original Research Article

Impact of IL28B Gene Polymorphism and HOMA-IR on Response to Pegylated-interferon and Ribavirin Therapy on Chronic HCV-4 Patients

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Background: **HCV has been identified as the cause of metabolic syndrome, a complex that includes dyslipidemia, diabetes and insulin resistance (IR). Insulin Resistance (IR) index, is a negative predictor of response to interferon-based therapies in patients with HCV. IL28B variations are strongly associated with on-treatment viral kinetics and approximately 2-fold increased sustained virologic response (SVR) rates in HCV genotype 1 and 4 patients, so IL28B gene polymorphism is also considered as a good predictor of treatment response.** ***Aim:*** **To determine the correlation between interleukin 28B genotype and insulin resistance in patients with chronic hepatitis C virus genotype 4 and response to Pegylated interferon and ribavirin treatment.** ***Methods:*** **The study included HCV Patients (n=50) and control patients (n=50). IR was estimated using Homeostasis model assessment (HOMA-IR) = (Serum Insulin (IU/ml)*Serum Glucose (mmol/L) /22.5), viral load was determined by real-time PCR, also SNP assay was done using IL28B polymorphism real time.** ***Results:*** **IL-28 rs12799860 genotypes in HCV patients were as follows; CC genotypes in 58 % (n = 29), CT in 36 % (n = 18) and TT in 6 % (n = 3). The response rate was 86.2 % in CC genotype versus 27.8 % in CT genotype and 2% in TT genotypes which was statistically significant (P=0.001). The correlation between HOMA and response among cases was significantly negative. HOMA-IR >2 was associated with poorer SVR response (39.3% vs. 58.7%; P = 0.007).** ***Conclusion:*** **Results show that IL28B and IR are independent variables associated with SVR for P+R treated patients.**

Keywords: IL28B, HOMA, HCV, Gene, Polymorphism, Insulin resistance, Ribavirin, Therapy.
Abbreviations: HCV: Hepatitis C Virus, **IR:** Insulin Resistance, **SVR:** Sustained Virological Response, **PEG-INF:** Pegylated interferon, **IL28B:** Interleukin 28 B, **HOMA:** Homeostasis model assessment.

INTRODUCTION

There are 160 million people infected with chronic HCV which corresponds to 2.35% of global prevalence (Lavanchy., 2011). Egypt has the highest prevalence of HCV genotype-4, which is responsible for about 90% of infections and considered the major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, and liver transplantation in the country (Nouroz et al., 2015). In consideration of the apparent limitations of current HCV therapy, especially high failure rate, prediction of treatment outcomes prior to the initiation of treatment and understanding of the molecular mechanism of HCV resistance are two important goals in HCV research. Patients would benefit if the prediction of the likelihood of a treatment response prior to initiating therapy, or at least soon after starting therapy, could be made. In fact, numerous host and viral factors have been used to predict treatment response to interferon-based therapy (Li et al., 2014). HCV has been identified as the cause of metabolic syndrome, a complex that includes dyslipidemia, diabetes and insulin resistance (IR). IR is a key feature of this syndrome and a variety of potential molecular pathways by which HCV may contribute to IR have been suggested. Patients infected with HCV have significantly higher IR than healthy controls matched for age, sex and body mass index (Mohamed et al., 2011). Patients who have chronic hepatitis C infection sustained virological response (SVR) rate

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is variable extremely high following standard care therapy with Peginterferon + ribavirin (P+R). Viral genotype, viral load, fibrosis stage and metabolic disturbances including obesity, insulin resistance and steatosis have been shown to be the main factors influencing SVR (Campo et al., 2012).

The genome-wide association studies (GWAS) have identified genetic variations near the IL28B gene which are strongly associated with spontaneous and treatment-induced clearance of hepatitis C virus (HCV) infection. Protective IL28B variations are strongly associated with on-treatment viral kinetics and approximately 2-fold increased sustained virologic response (SVR) rates in HCV genotype 1 and 4 patients. Spontaneous clearance of HCV occurs in only 15–50% of all HCV-infected individuals, while the majority of patients develop a chronic infection. It was found that IL28B rs12979860 is strongly associated with the chance to clear HCV spontaneously in populations of African, with an approximately three times higher clearance rate in individuals with the rs12979860 genotype C/C versus C/T, T/T (lange and zeuzem, 2011).

Hepatic steatosis has been described in 31-72% of chronic hepatitis C virus (HCV) liver biopsies. Steatosis has been related to disease progression and suggested as a predictor of treatment response in chronic HCV (Antúnez et al., 2004). The main aim of the present study was to determine the correlation between interleukin 28B genotype with insulin resistance in patients with chronic hepatitis C virus genotype 4 and response to Pegylated interferon and ribavirin treatment.

PATIENTS AND METHODS

Consecutive treatment-naïve patients with chronic hepatitis C type 4 attending outpatient clinic of National Hepatology and Tropical medicine research institute since February 2014 were recruited to receive treatment with P+R until February 2015. All patients received Peginterferon alfa-2a (180 g/kg bodyweight) and weight-related ribavirin. No patient had been previously treated with direct antiviral drugs. Patients with a type 2 diabetes mellitus, or showing fasting glucose >7 mmol/L, were also excluded.

Inclusion criteria: adult patients of both sexes (18-60 years old), diagnosis previous 6 months with positive HCV RNA in serum (by RT-PCR assay). The control group consisted of adults who proved negative for HCV RNA. Patients didn't receive any hepatitis treatment before the time of sampling. The presence of HBV infection or co-infection was excluded by serum ELISA for anti-HBc and HBsAg. In addition to investigations needed to fulfill the selection criteria, all individuals included in this study were subjected to the following:

An overnight (12 h) fasting blood sample was taken for routine clinical chemistry analyses. These included ALT, AST, ALP, Glucose, WBCs, alb, HB and TSH. All patients had positive anti-HCV, increased ALT and positive HCV RNA in serum. All patients were negative for HBsAg. Fasting samples of serum obtained after centrifugation were stored in aliquots at -80°C until assayed. Serum insulin levels, HBsAg, anti-HBc were tested were measured by ELIZA according to the manufacturer's instructions. The insulin resistance index was calculated using fasting values of plasma glucose and insulin, according to the HOMA model formula:

Insulin resistance or HOMA-IR = Fasting serum insulin (IU/L) × fasting serum glucose (mmol/L) ÷ 22.5.

Biochemical analysis

Venous blood samples were taken in the morning after 12-h overnight fast. Serum glucose, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), total bilirubin levels (Bil), and cholesterol (Chol) were measured by using standard clinical laboratory methods. Serum insulin levels and a-fetoprotein levels were estimated by serological techniques (Axyam System, Abbott Laboratories). Platelet count (Plt) and Prothrombin Time (PT) measurements were performed for all patients; normal PT was 12 seconds.

Insulin resistance (IR) was calculated on the basis of fasting levels of serum glucose and serum insulin, according to the homeostasis model assessment (HOMA) method. Calculation for the HOMA model followed a standard protocol; insulin resistance (HOMA-IR) = fasting glucose (mg/dL) × fasting insulin (μIU/mL)/22.5. The HOMA-IR index has seen widespread use, with various cut-off values for insulin resistance.

IL28B genotyping

DNA from patients was extracted from venous blood using standard methods. Genotyping of the rs12979860 was performed using a TaqMan 5′ allelic discrimination assay (Applied Biosystems). The probes were labelled with the fluorescent dyes VIC and FAM respectively. As the Taq polymerase extends the primer and synthesizes the nascent strand, the 5' to 3' exonuclease activity of the Taq polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thus relieving the quenching effect and allowing fluorescence of the fluorophore. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Genotyping of each sample was automatically attributed for allelic discrimination.

Statistical analysis

Data was analyzed using SPSS win statistical package version 15 (SPSS Inc., Chicago, IL). Chi-square test (Fisher's exact test) was used to examine relationships between qualitative variables. For quantitative data, comparison between two groups was undertaken using Mann-Whitney test and comparison between 3 groups with ANOVA test or Kruskal-Wallis test followed by a Post-Hoc "Scheffe test". Spearman-rho method was used to test the correlation between numerical variables. Multivariate analysis was performed using multiple linear regression model using forward method for the significant factors affecting fibrosis on univariate analysis. Multivariate analysis (logistic regression) was performed to find the predictors for HCC development All tests were two-tailed; a P-value <0.05 was considered significant.

RESULTS

The response to Peg-IFN-α/RBV was examined in all patients after 48 weeks of treatment by qualitative RT-PCR. The results showed that 64% of the patients responded to the treatment (responder group, n= 32) while 36% of the patients did not respond to the standard of care treatment (non-responder, n= 18). Statistical analysis of baseline demographic and biochemical characteristics of non-responders (NR) and responders (R) HCV Patients are shown in (Table 1).

The mean body mass index (BMI) of HCV responder patients is 21.03 Kg/m2 which was significantly lower than that of non-responder group (30.44 Kg/m2), P-value = 0.000 (Table 1). Moreover, the data of the current study demonstrated that obese patients as judged by their BMI had approximately an 85% lower chance of developing a response to therapy compared with normal or overweight patients.

The IL-28 rs12799860 genotypes (CC, CT and TT) distribution in apparently healthy subjects and in HCV patients were shown in (Figure 1). The frequencies of the IL-28 rs12799860 genotypes in healthy subject (n = 50) were as follows; CC 68 % (n = 34), CT 30 % (n = 15) and TT 2 % (n = 1), while in HCV patients (n = 50), the frequencies of the IL-28 rs12799860 different genotype were as follows; CC 58 % (n = 29), CT 36 % (n = 18) and TT 3 % (n = 2). It should be noted that 86.2 % of HCV patients with CC alleles (25/29), 27.7 % of HCV patients with heterozygous CT (5/18) and 33.3 % HCV patients with had alleles TT (1/3) were responders, respectively.

The distribution of these different IL-28 rs12799860 genotypes among all studied groups showed that the response rate to the standard of care therapy varied according to IL28rs12979860 SNP genotypes, (P-value = 0.001). When we studied the association between the virological response and IL28rs12979860 SNP genotypes, there was statistically significant association between the virological response and CC genotype where CC genotype has better chance for SVR than non-CC genotypes (TT/CT).

There was statistical significant difference between obese and non-obese as regard to T.Bil value (P-value = 0.005). There was no statistical difference between obese and non-obese for post-treated group as regard to AST, ALT, D.Bil, Glucose, AFP, TSH and BMI values (P-value = 0.118, P-value = 0.452, P-value = 0.256, P-value = 0.603, P-value = 0.908, P-value = 0.139 and P-value = 0.122 respectively). There was significant negative correlation between HOMA and response to Peg-INF α/ ribavirin therapy as P-value = 0.001 (Figure 2).

The ROC was plotted in accordance with the same model to establish specificity and sensitivity of HOMA IR values (Fig 3). The best cut-off value for this model was 0.4125 that provided a sensitivity of 81.3%, a specificity of 77.8%, for predicting SVR.

DISCUSSION

Hepatitis C virus (HCV) infection is one of the important causes of chronic liver disease worldwide. Currently, there are 7 major HCV genotypes, which have different geographical distributions and susceptibilities to interferon-*α* treatment. In Egypt, more than 90% of cases belong to HCV genotype 4. The combination of Pegylated interferon and ribavirin is still the most effective therapy in HCV-4, and the success rate of current therapy chronic hepatitis C is related to a variety of host and viral factors that can be used as response predictors (Khattab et al., 2016). HCV treatment responses vary with the genotype, with the highest SVR rates observed for genotype 2 and the lowest rates observed for genotypes 1 and 4 (David and Thomas., 2012).

Although HCV-4 was initially branded as a 'difficult-to-treat' genotype, recent data, especially from Egypt, where HCV-4 represents >90% of HCV infections, have suggested SVR rates between 43 and 70%, that is, intermediate between those reported for HCV-1 and HCV-2 or HCV-3. Major factors influencing response are of Egyptian origin, absence of advanced fibrosis and insulin resistance (HOMA-IR). As multivariate analyses have shown that polymorphisms near *IL28B* are as predictors of response in HCV-4 is obvious (Antaki et al., 2013). According to GWAS, SNP rs12979860 IL28B C/C genotype was strongly related with superior chances of SVR, and it was observed that ancestry had affected the results (Cavalcante et al., 2015).

Assessment of *IL28B* (rs12979680) polymorphism in our study revealed a 2–3 fold greater chance of SVR in patients carrying the C/C genotype than in patients with the T* genotype, which comes in accordance with numerous lines of evidence that have solidified the association between the IL28B C/C genotype and SVR in patients with chronic HCV. It is postulated that this SNP has effects on the binding of different transcription factors resulting in reduced expression of IL28B, and IFN- stimulated genes (ISGs) expression which may modulate the response to IFN. It was suggested that patients with CC genotype had low basal levels of hepatic ISG expression, and therefore when stimulated with IFN showed greater up-regulation of ISGs and hence better treatment response (Rizk et al., 2016).

Gomaa et al.,(2015) studied the association between the virological response and IL28rs12979860 SNP genotypes, they found statistically significant association between the virological response and CC genotype where CC genotype have better chance for SVR than non-CC genotypes (TT/CT) resulting in an odds ratio of 0.118 C.I. (0.026 – 0.546), P = 0.002 (results not displayed). These data suggest that CC genotype is a good predictor of SVR in chronic hepatitis C genotype 4 Egyptian patients.

There was also a statistically significant difference in the allelic frequencies of rs12979860 SNP of IL28 gene between responders and non-responders HCV patients (P = 0.007), where the responders had a higher frequency of the wild allele “C” than non-responders (62.3% versus 41.9%), and a lower frequency of the mutant allele “T” than non-responders (37.7% versus 58.1%), resulting in an overall odds ratio of 0.737 C.I(0.233-0.822). The frequency of C allele was higher in all groups except for non-responder group where the frequency of T allele was higher. The results of the present study showed that the C allele of rs12979860was significantly associated with virological response. This finding suggests the possibility of using rs12979860 of IL 28B gene polymorphism as a predictor of response to the treatment of HCV-genotype 4.

In the context of PEG-IFN/RBV therapy for CHC, *IL28B* genotypes are strongly associated with treatment efficacy in patients infected with HCV genotype 1 or 4, with some effects on other HCV genotypes. *IL28B* genotyping is also useful for pretreatment prediction of the outcome of DAA plus PEG-IFN/RBV therapy, especially in treatment-naïve patients (Matsuura et al., 2014).

As found by Navaneethan et al., (2009) that HCV infection is associated with insulin resistance as demonstrated in multiple studies. Also, chronic HCV infection is shown to increase the risk of developing diabetes mellitus by up to 11 times in epidemiological studies. Insulin resistance in chronic HCV infection is important in determining treatment outcome as significant correlation is demonstrated between insulin resistance and extent of liver fibrosis. The HOMA index, a measure of insulin resistance, was independently associated with SVR and in this study, there was a significant negative correlation between HOMA and response among cases. HOMA-IR >2 was associated with poorer SVR response (39.3% vs. 58.7%; P = 0.007).

HCV infection is associated with insulin resistance as demonstrated in multiple studies. Also, chronic HCV infection is shown to increase the risk of developing diabetes mellitus by up to 11 times in epidemiological studies.

Table 1: Laboratory findings among responders and non-responders

	Responders N=32 (64%)	Non-Responders N=18 (36%)	P-value
AST Range Mean±SD	20-90 36.28 ± 15.41	30-120 65 ± 25.47	0.001
ALT Range Mean±SD	17-42 31.75 ± 7.24	20-70 44.28 ± 10.68	0.001
AFP Range Mean±SD	4-20 8.61 ± 3.12	5-18 10.64 ± 4.43	0.005
TSH Range Mean±SD	0.8-7 3.4 ± 1.46	1-8 4.356 ± 1.04	0.039
HOMA Range Mean±SD	3-6.7 3.97 ± 0.65	3.5-7.7 5.48 ± 1.31	0.001
Fibro Range Mean±SD	1-6 3.34 ± 1.00	2-4 3.11 ± 0.58	0.373
BMI Range Mean ± SD	≥25 21.03 ± 5.66	≥25 30.44 ± 7.25	0.001
HAI Range Mean±SD	4-9 5.88 ± 1.16	4-8 6.06 ± 1.30	0.615

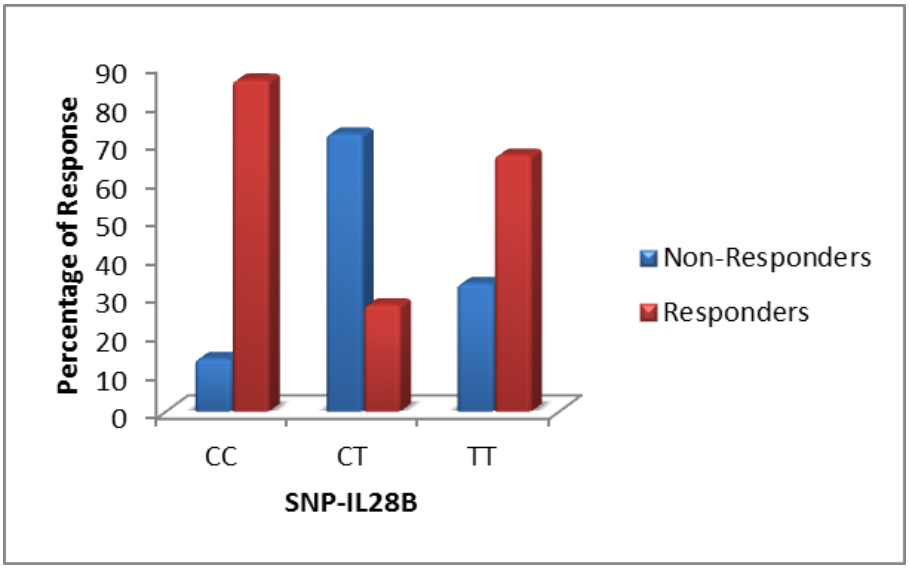

Figure 1: IL28B allelic discrimination.

Table 2: Comparison between obese and non-obese regarding o the laboratory findings

	Obese N=21	Non-obese N=29	P-value
AST Range Mean±SD	 29-120 50.33±27.334	 20-105 44±21.161	0.118
ALT Range Mean±SD	 20-60 37.38±9.468	 17-70 35.45±11.233	0.452
T.Bil Range Mean±SD	 0.5-2.8 1.210±0.7190	 0.5-45 4.245±10.4424	0.005
D.Bil Range Mean±SD	 0.1-0.9 0.324±0.2998	 0.1-1 3.3±1.145	0.256
Glucose Range Mean±SD	 66-170 101.10±21.686	 80-181 103.14±19.635	0.603
AFP Range Mean±SD	 4-20 9.11±3.821	 4-18 9.51±3.731	0.908
TSH Range Mean±SD	 0.8-8 3.576±1.9733	 1.1-6 3.866±1.2545	0.139
BMI Range Mean±SD	 16-40 22.48±6.728	 15-42 25.83±8.164	0.122

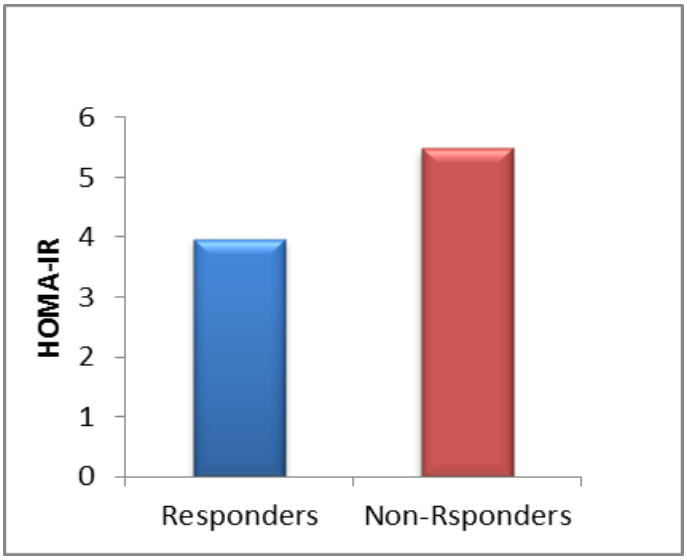

Figure 2: The correlation between HOMA and response to Peg-INF/ ribavirin therapy.

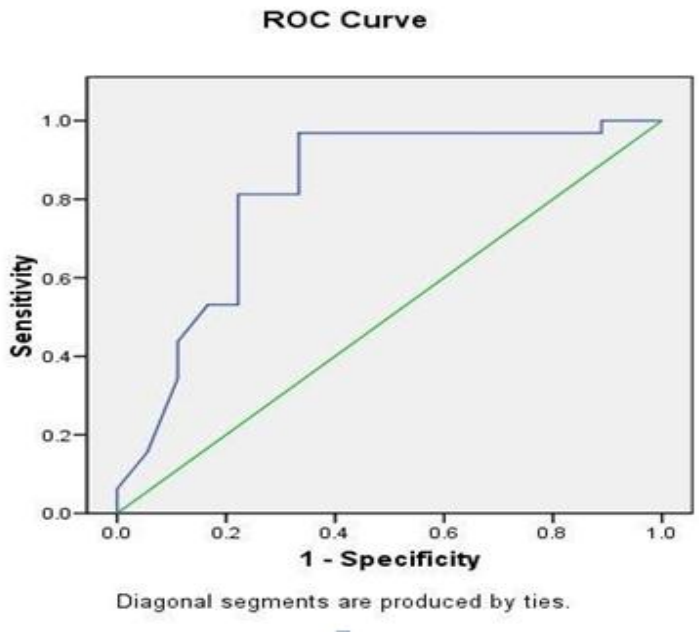

Figure 3: The ROC curve of HOMA -IR

Insulin resistance in chronic HCV infection is important in determining treatment outcome as significant correlation is demonstrated between insulin resistance and extent of liver fibrosis. The HOMA index, a measure of insulin resistance, was independently associated with SVR (Hammerstad et al., 2015).

Several prior studies using surrogate measures of IR (mainly HOMA-IR) report IR as a predictor of nonresponse to anti-HCV therapy. In a study of 159 Spanish patients, individuals with SVR had lower baseline HOMA-IR scores compared with those with non-SVR (2.4 vs. 3.8). In addition, after adjusting for genotype and liver fibrosis scores, the odds of non-sustained response to anti-HCV therapy were ; 1.8 times higher with increasing HOMA-IR scores (Brandman et al., 2012).

Obesity is a metabolic condition and is not simply a function of having high body weight. Patients who have CHC and are obese are more likely to be insulin-resistant and to have more advanced hepatic steatosis/steatohepatitis and fibrosis. These conditions are independent predictors of nonresponse to combination therapy with Peginterferon alpha and ribavirin, and obese patients are therefore more likely to be non-responders to combination therapy (Charlton et al., 2006).

There was significance negative correlation between BMI and response observed in our results also, Rozeik et al., (2015) found that Obese patients as judged by their BMI, independent of genotype and cirrhosis, had approximately an 80% lower chance of a sustained response to therapy compared with normal or overweight patients. The BMI, which describes relative weight for height, correlates with total body fat content, whereas weight on its own may not. Also, Imran et al (2013) showed that it has been shown that a body mass index (BMI) > 25 kg/m2 was linked with fibrosis. Approximately, 30% of HCV patients are obese and they respond poorly to interferon therapy. The poor treatment response in these patients is mostly attributed to altered metabolism due to cytokine production by adipocytes. Moreover, there is also a poor absorption of interferon in obese patients.

CONCLUSION

Finally, our findings showed that polymorphism in rs12979860 of IL-28B was related to the outcome of combined treatment response of HCV and may be useful to be considered as a pre-treatment predictor. Response to treatment tends to be more in CC genotyping patients than the others (CT/TT)..

COMPETING INTERESTS

Authors have declared that no competing interests exist. The authors alone are responsible for the content and writing of the paper. The authors did not receive any funds from any source

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