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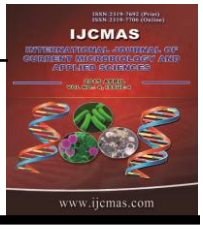

Original Research Article

Interleukin 28B rs12979860 polymorphism and High serum Gamma-glutamyl transpeptidase activity Predict Non - Virological response to Interferon-alpha/Ribavirin Combined Therapy in Chronic hepatitis C genotype 4 Egyptian patients

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Keywords

HCV, IL28B single nucleotide polymorphism, GGT, Real-Time Polymerase Chain, SOC treatment

A B S T R A C T

Egypt has the highest hepatitis C virus (HCV) prevalence in the world, Both host and viral factors are considered important in prediction of virological response to combined pegylated interferon-Alpha and oral ribavirin ,standard of care (SOC) treatment of HCV infection. This study was conducted to assess IL28B single nucleotide polymorphism (SNP rs 12979860) and other host factors as predictors of response. Eighty eight chronic hepatitis C virus (genotype-4) Egyptian patients treated with SOC for 24 weeks were included and thirty apparently healthy subjects as controls. Genotyping of the IL28B rs12979860 was done by Real-Time Polymerase Chain (RT-PCR). Some biochemical and virological investigations were made on venous blood samples. IL-28 rs12799860 genotypes in HCV patients were as follows; CC genotypes in 26.1 % (n = 23), CT in 58.0 % (n = 51) and TT in 15.9 % (n = 14). The response rate was 91.3 % in CC genotype versus 56.7 % in CT genotype and 50.0 % in TT genotypes which was statistically significant (p=0.007). These data suggest that rs12979860 polymorphism of IL 28 gene and high serum *gamma-glutamyl transpeptidase* activity (GGT) are the most important host factors and strong predictors for virological response in HCV-genotype4 Egyptian patients.

Introduction

Egypt has the highest hepatitis C virus (HCV) prevalence in the world, it has been reported that 14.7% of Egyptian population is chronically infected with HCV and 90% of them are genotype-4 (El-Zanaty et al, 2011) and (Guerra et al, 2012).

Up to now, the standard of care (SOC) treatment of chronic HCV infection consists of (pegylated) interferon-Alpha and oral ribavirin. (Hendy et al, 2011). The treatment has 2 goals; the first is to achieve sustained eradication of HCV (ie, sustained virologic response SVR), which is defined as the

persistent absence of detectable HCV RNA in serum of patients 6 months or more after completing antiviral treatment. The second goal is to prevent progression to cirrhosis, hepatocellular carcinoma (HCC) and decompensated liver disease requiring liver transplantation (M.R. Pinzone, et al 2014).

Moreover, responsiveness to hepatitis C virus therapy depends not only on viral factors but also on host factors .Viral factors, those that are related to HCV virus, include viral genotype and viral load (Asselah et al, 2010). While, host factors include sex, age, obesity, insulin resistance, type 2 diabetes mellitus (T2DM), ethnicity (race), degree of liver fibrosis and genetics as IL28 polymorphisms (Hendy et al, 2011). Many viral and host factors have been investigated as predictors for achieving viral clearance; However, knowledge of factors predicting sensitivity to combined antiviral treatment is still limited and restricted in populations infected with HCV-4.

Interleukin-28B (IL-28B), named also as interferon λ-3, has been shown to be involved in the control of HCV infection, IL-28B has a role in the regulation of intracellular IFN stimulated gene (ISG) expression (Nikolaus et al, 2014). It is expressed by peripheral blood mononuclear cells; dendritic cells upon infection with viruses. IL-28B exhibits antiviral activity, having an impact on natural clearance of HCV, and the genetic polymorphism of the encoding IL-28B gene may determine the clearance of HCV. (Li S et al, 2011).

IL-28B gene polymorphism currently seems to be one of the strongest predictors of SVR. Genome-wide association studies (GWAS) reported the association between rs12979860 and Sustained Virological Response (SVR) in patients with genotype 1 and treated with PEG-IFN-α2a or PEG-IFN-α2b. (Thompson et al, 2010). Few data are present for this association in patients with genotype 4. There is no doughty that the high treatment cost presents a high economic burden in developing country like Egypt, that necessitates a more research on predictors of (SOC) treatment response.

Accordingly, The present study aimed to assess IL28B single nucleotide polymorphism (rs 12979860) and other host factors as predictors of response to pegylated interferon plus ribavirin given as a SOC treatment to Egyptian cohort with chronic hepatitis C virus genotype 4 .

Materials and Methods

The study was carried out after the approval of the Ethical Committee of Medical Research Institute. A signed formal consent was obtained from all subjects enrolled in the study. This study included one hundred and eighty subjects divided as, thirty apparently healthy subjects were taken as controls to be compared with the patients group for the genetic study and eighty eight CHC infected patients who were positive HCV antibody and HCV RNA (HCV-Ribonucleic acid).They were treated with 180µg Peg–IFN α-2a or 1.5µg/kg Peg–IFN α-2b once a week in combination with RBV (800-1400 mg/day) for 24 weeks. Those patients were selected from the outpatient clinic of the Medical Research Institute, Alexandria University who were eligible and fulfilling the criteria for treatment with combined Peg–IFN α and RBV and followed up for 24 weeks of treatment. Reverse transcriptase polymerase chain reaction (RT-PCR) for HCV-RNA was done at 0, 12, and 24 weeks of the treatment. According to the response to the standard of care (SOC) therapy, patients were divided into the following groups:

Group 1: Responders: those are patients

whose HCV-RNA by Polymerase chain reaction (PCR) was negative after 24 weeks of therapy.

Group 2: Non-responders: those are patients whose HCV-RNA by PCR was still positive after 24 weeks of therapy. None of the subjects was positive for hepatitis B surface antigen (HBs Ag) or positive for Anti-Nuclear Antibody (ANA).

All studied subjects were subjected to thorough clinical examination including anthropometric measurements with calculation of body mass index (BMI=body weight in kilograms divided by the square of height in meter kg/m2) [(198)] and Abdominal ultrasonography. Liver biopsies were done to assess the stage of liver fibrosis. Following twelve hours fasting venous blood samples were withdrawn from each subject and were taken into the following tubes, First: Tripotassium citrate tube for prothrombin time and activity, Second: K3-EDTA (tri-potassium ethylene diamine tetraacetic acid) for complete blood count and DNA extraction, the later was done daily and stored at (-80^{o}C) before IL-28B genotyping, The last: A plain tube without anticoagulant where sera were immediately separated and divided into aliquots which were utilized in routine biochemical investigations; including Liver function tests, fasting blood sugar (FBS) and post prandial sugar (PPS).

Which were all done on chemistry autoanalzer(OLYMPUS AU400).Also, Fasting serum insulin was measured using a two site, solid phase chemiluminescent enzyme immunometric assay (CLIA) by the Immulite 1000 Automated Analyzer (Diagnostic Products Corporation) and Insulin resistance (IR) was then investigated by the homeostasis model for the assessment of IR (HOMA-IR)=fasting insulin(µU/ml)× fasting glucose (mmol/L)/22.5. Virological investigations including viral load by real time PCR) and Quantitativ HCV_RNA(ribonucleic acid) levels analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) at 0, 12, 24 weeks of starting treatment were done using Applied Biosystems(API 7000).

Single nucleotide polymorphisms (SNPs) detection and genotyping of the IL28B rs1297980:

The rs 12979860 SNP in the region of IL28B-Gene was analyzed by (RT-PCR) using Applied Biosystems (API 7000).Freshly withdrawn whole blood samples on K3-EDTA were used for genomic DNA Extraction from peripheral blood leukocytes that was carried out using QIAamp® DNA blood Mini kit (Qiagen Hilden, Germany) according to the manufacturer's instructions. The purity and concentration of stored extracted genomic DNA were determined using the Thermo Scientific Nano Drop™ 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware USA). (Sambrook, et al 2006)

Genotyping of single nucleotide polymorphisms (SNPs) of the IL28B rs12979860 was carried out by the allelic discrimination Real-Time Polymerase Chain Reaction SNP genotyping technology using dual labeled fluorogenic TaqMan® minor groove binder (MGB) probes (Callegaro, et al 2006), with Sequence-specific forward and reverse primers pre-designed to amplify the sequence containing the SNP (Applied Biosystems-Life Technologies, Carlsbad, California, USA) according to the manufacturer's instructions. (NB: their sequences are not revealed by the company)

In a 20 µl total volume; 5 µl total DNA; 0.5 µl IL28B Rs60(rs12979860) forward primer; 0.5 µl IL28B Rs60 reverse primer;

0.5 µl IL28B Rs60 probe T allele; 0.5 µl IL28B Rs60 Probe C allele ; 10 µl Universal TaqMan PCR master mix(2X) and 3 µl nuclease free water.

The thermal cycling conditions of the StepOne real-time PCR system (*Applied Biosystems-Life Technologies, Carlsbad*, California, USA) were programmed as follows: 30 seconds at 60^0 C, 10 minutes at 95^0C, followed by 40 cycles consisting of 15 seconds denaturation at 95^0C and 1 minute annealing/extension at 60^0C and 30 seconds at 60^0C.

Data analysis was done using StepOne v2.2.1 Sequence Detection System (SDS) Software (Applied Biosystems-Life Technologies, Carlsbad, California, USA), which used the three different types of fluorescence measurements made during the tube read i.e. FAM, VIC and ROX (the Passive Reference dye used) to calculate Reporter normalized (Rn) ratios for each reporter dye in each reaction tube. This normalization was necessary to correct for fluorescent fluctuations due to changes in concentration or volume. Normalization was accomplished by dividing the emission intensity of the reporter dye (FAM or VIC) by the emission intensity of the Passive Reference (ROX dye) to obtain a ratio defined as the Rn (Reporter normalized) Accordingly, the substantial increase in Rn ratio of each reporter dye in each reaction tube was then correlated to the specific allele(s) present in this sample and accordingly its genotype could be interpreted, where increase in VIC dye indicated homozygosity for A allele, FAM dye indicated homozygosity for C allele, and both dyes indicated heterozygosity for AC.

Statistical analysis: Statistical calculations were performed using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 20 for Microsoft Windows. Mean ± standard error (X ± SE), frequencies (number of cases) and relative frequencies (percentages) were used where appropriate. Mann–Whitney U test and Kruskal–Wallis test were used to analyze continuous variables where appropriate. We used the Spearman rank correlations to evaluate the relationship between pairs of markers. For comparing categorical data, Chi square ($\chi 2$) test was performed. Fisher exact test was used instead when the expected frequency is less than 5. A probability value (*p* value) less than 0.05 was considered statistically significant. Hardy-Weinberg Equilibrium: G. H. Hardy and W. Weinberg noted that with some well-defined assumptions, the population allele frequencies could be used to calculate the equilibrium-expected genotypic proportions.

Results and Discussion

The response to Peg-IFN-α/RBV was examined in all patients after 24 weeks of treatment by qualitative RT-PCR. The results showed that 64% of the patients responded to the treatment (responder group, n= 57) while 36% of the patients did not respond to the standard of care treatment (non-responder, n= 31). Statistical analysis of baseline demographic and biochemical characteristics of non-responders (NR) and responders (R) HCV Patients are shown in (Table 1). The male/female ratio in HCV patients responder group was (59.6%)/(40.4%) which was higher than that in non-responder patients group (71%)/(29%), but did not show statistical significance, P=0.292). The mean body mass index (BMI) of HCV responder patients is 27.7 ± 0.2 Kg/m^2 which was significantly lower than that in non-responder group, *p* = 0.001 (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. In HCV responder patients, the serum activity level of GGT was significantly lower than that in apparently non-responder group, $p = 0.0001$ (Table 1). The mean concentration level of serum albumin in HCV responder patients was significantly higher than that in apparently non-responded group, $p = 0.043$ (Table 1). The concentration levels of total and direct bilirubin in HCV responder patients were significantly lower than those in non-responded group,($p = 0.005$, 0.0001, respectively) (Table 1). Also, the mean fasting serum insulin level in HCV responder patients group was significantly lower when compared to that in non-responded group, $p= 0.048$ (Table 1). Moreover, in responder patient group, the mean levels of thyroid stimulating hormone (TSH) and alpha-feto protein (AFP); 2.07 ± 0.15 µIU/ml and 5.36 ± 0.92 mg/ml, respectively, were significantly lower than that in non-responder group, $p = 0.018$, $p = 0.004$ respectively (Table 1). In addition, the mean level of HDL-cholesterol in HCV responder patients group (54.9 mg/dL ± 2.7) was significantly elevated when compared to that in HCV non-responder patients group (46.6 mg/dL ± 2.3), $p = 0.021$ (Table 1). Furthermore, in HCV responder patients group, the mean value of international normalized ratio (INR) was significantly lower than that in non-responder patients group, $p = 0.0001$ (Table 1).

Pretreatment liver biopsy samples were assessed for all HCV patients. The data showed that the majority of HCV patients were staging F1 (46.6 %) with total count 41 cases divided to 9 non responder (NR) (29.03% of non-responder) and 32 responder (R) (56.14% of responder) however, the advanced stages of fibrosis (F3 +F4) were more frequently found in non responder patients than responders (Table 2). Grading of histological Activity is shown in (Table 2) with significantly higher degree of activity in non-responders compared to non-responders.

On the other hand, univariate analysis identified GGT (OR: 0.219, CI 95%:0.068 – 0.710, $p = 0.006$), body mass index (OR: 0.152, CI 95%: 0.029 – 0.804, $p = 0.021$), HOMA-IR (OR: 0.215, CI 95%: 0.084 – 0.547, p = 0.001) and liver fibrosis stage (OR: 0.170, CI 95%: 0.059 – 0.491, $p = 0.001$) as independent predictors for virlogical response, (Table 3).

IL-28 rs12799860 Genotype Distribution

The IL-28 rs12799860 genotypes (CC, CT and TT) distribution in apparently healthy subjects and in HCV patients is shown in (Table 4). The frequencies of the IL-28 rs12799860 genotypes in healthy subject were as follows; CC 43.3 % (n = 13), CT 53.3 % (n = 16) and TT 3.3 % (n = 1), while In HCV patients (n = 88), the frequencies of the IL-28 rs12799860 different genotype were as follows; CC 26.1 % (n = 23), CT 58.0 % (n = 51) and TT 15.9 % (n = 14). It should be noted that 91.3 % of HCV patients with CC alleles (21/23), 56.7 % of HCV patients with heterozygous CT (29/51) and 50.0 % HCV patients with recessive alleles TT (7/14) were responders, respectively. The distribution of these different IL-28 rs12799860 genotypes among all studied groups are in agreement with Hardy-Weinberg equilibrium,($p > 0.05$), except for non-responders HCV patients is not consistent with Hardy-Weinberg equilibrium,($p = 0.011$), (results not shown). It should be noted that the response rate to the standard of care (SOC) therapy varied according to IL28rs12979860 SNP genotypes, as it was 91.3 % in CC genotype

(21/23) versus 56.7 % (29/51) in heterozygous CT and 50.0 % (7/14) in TT genotypes which when compared was statistically significant (p=0.007) (Table 4).When we studied the association between the virological response and IL28rs12979860 SNP genotypes, we 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) (

Table5), 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 suggest possible use of rs12979860 of IL 28B gene polymorphism as a predictor of response to SOC treatment of HCV-genotype 4.

According to IL28B rs12979860 genotypes, we further divided HCV patients (n=88) into two subgroups; those with CC genotype were 23 (21 responders and 2 non-responders) and those with non CC genotypes (CT+TT) were 65 (29 responders and 36 non-responders). When these two subgroups were compared as regards fibrosis stage, it was found that high degree of fibrosis (F3+F4) was detected in 76.6% of HCV patients (both responder and non-responders) with non-CC genotypes. While the lower degree of fibrosis (F1+F2) was detected in almost 100% of responders with CC genotype and in 85.5% of non-responders with CC genotype . Also, the results revealed that almost 89% of non-responder HCV patient's with non CC genotype has high serum GGT activity. (

Table6). Otherwise there were no statistically significant differences between the two subgroups concerning other measurements.

As previously mentioned, the main factors influencing the efficacy of HCV antiviral treatments are divided into two categories; viral and host-related.

The viral category includes the HCV genotype and baseline viral load (Zhu et al, 2013). The later was found to be an important determinant of treatment response, the lower the viral load the better the chance of eradicating the hepatitis C virus. In contrast, the data of the present study, despite the lack of statistical significance, showed that responders had a higher baseline viral load than non-responders. Domagalski et al, 2013 , have reported that the baseline viral load in responder was higher than that in non-responders HCV patients. These results may point to the role of other viral/host factors in predicting overall virological response.

On the other hand, host factors that have been studied as predictors of response to

HCV therapy with Peg-IFN-α/RBV included age, sex, race, fasting glucose level, insulin resistance, stage of fibrosis, GGT level and body mass-index (BMI) (Jacobson , et al 2007)& (Yu, et al 2011).

As regards patient age, it is generally believed that younger individuals (usually < 40 years of age) respond better to IFN-α treatment than older persons (287,288). However, the results of the present study showed that mean average age of responders was not significantly different from that of non-responders.

The data of the present study revealed a significant elevation in the mean GGT activity level in non-responder HCV patients when compared to that in responder HCV patients, Table (1).Similarly, (Brjalin et al, 2013) , have reported that the mean GGT level significantly lower in the SVR group than in the non-SVR group. Low GGT levels were found to be an independent predictor of both RVR and SVR in various cohorts of HCV-monoinfected patients. (James, et al 2013) & (Weich, et al 2011). This in agreement with the data of the present study that revealed GGT as an independent predictor of virological response. Table (2)

It has been suggested that obesity, (BMI greater than 30 kg/m2), is a risk factor for non-response to antiviral therapy independent of genotype and cirrhosis in patients with chronic hepatitis C. Obese patients had approximately an 80% lower chance of a sustained response to therapy compared with normal or overweight patients(Delgado-Borrego, et al 2010) .

In accordance with these findings, we found that the mean value BMI of non-responder HCV patients was significantly higher than that in responder HCV patients. Also our data showed that obesity (BMI ≥ 30 kg/m2) is one of the independent predictors of virological response.Table (2). 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.

Several studies have reported association between insulin resistance (IR) and chronic HCV infection especially with genotype 1 and 4 (Moucari, et al 2008) & (Petta et al, 2008). In consistence with these observation, the current study reports that almost 50% of the HCVpatients have IR (HOMA-IR ≥ 2). Also, the mean concentration level of fasting insulin was significantly higher in non-responder when compared to that in responder HCV patients. In the present study, the mean level of HOMA-IR in nonresponder HCV patients was higher than that in responder HCV patients without reaching a significant level. Similarly, Del Campo et al, 2013 have reported similar results where no significant difference in HOMA-IR level was observed between SVR and Non SVR.

However, Deltenre et al , 2011 in their a meta-analysis study, have reported that on considering only studies using the same cutoff value of 2 to define IR, a 22% decrease in SVR rates was still found. They also stated that this cutoff (2) is a robust negative predictive factor for SVR. In these aspects, the results of the present study revealed a 21.5% decrease in response rate.

Chronic hepatitis C infection progresses to fibrosis and in 20%–30% of cases leads toliver cirrhosis with the risk of hepatocellular carcinoma (HCC) (4%). Gamail et al, 2013 have reported that stage of fibrosis in patients with HCV genotype 4 is one of the most important predictors of EVR, as the higher the stage of fibrosis, the lower the achievement of a complete EVR.

Their results were also reported by Torres et al, 2010, who demonstrated, that one of the independent factors associated with a complete EVR was the non-cirrhotic status of the patients at baseline.

It has been demonstrated that the presence of liver steatosis and significant fibrosis, also significantly impairs the chance for SVR, with rates of 15–40% according to the degree of steatosis and fibrosis (Guedj , et al 2012) & (Westin , et al 2007). The data of the present study are in agreement with and confirm the previously mentioned observation and it also found that elevated stages of fibrosis reduce the chance for virological response with rate of 17%. Moreover, the data of the presented study found that the fibrosis stage is one of the strong independent predictors of sustained virrological response to therapy in chronic hepatitis C genotype 4 Egyptian patients. (Table 2).

Currently, candidate gene approaches had been implemented to discover the host factors associated with the HCV treatment response (Mosbruger, et al 2010). Several favorable genotypes significantly predicting higher sustained virological response rates including IL-28 CCrs12979860 irrespective of the race have been reported(Fellay et al, 2009) .The data of the present study revealed that The frequency of the IL-28-rs12979860 genotypes showed that more than half of the HCV patients were CT (58 %), followed by CC (26 %) then TT (16 %). The same frequency order of IL-28B rs12979860 genotype was previously reported (Khairy, et al 2013).

Also, the data of the present study revealed that the frequency of IL-28-rs12979860 CC genotype in responder patients (91.3 %) was higher than in non-responder patients (8.7 %). Other studies have reported similar findings (Khairy, et al 2013) & (Olfat, et al 2012).

Meanwhile, the data of the present study revealed a significant association between CC genotype and response to Peg-IFN-α/RBV. The response rates were almost 91 %, 57 % and 50 % for genotypes CC, CT and TT, respectively. This in agreement with previous studies, where the high rates of response were observed in patients with CC genotype (Asselah, et al 2012) & (De Nicola, et al 2012).

The exact biological pathways underlining the IL28B gene SNP association with treatment response and viral clearance remain unknown. Host innate immune mechanisms including IFN-λ, a direct product of the IL28B gene, control viral infection (Marcello, et al 2006). Initial binding of IFN-λs to its receptor is followed by several events that end with the induction of interferon-stimulating genes (ISGs) (Robek, et al 2005). It is speculated that this mechanism suppresses viral infection. Moreover, IFN-λ has been shown to block HCV replication in human hepatocytes in vitro (Robek, et al 2005). The role of IL-28B variants in relation to INF-λ3 expression could be explained by a number of possibilities. The lower intrahepatic expression of IFNstimulated genes in IL28B CC carriers than patients with CT/TT variants might facilitate the antiviral activity of IFN-based therapy (Thompson et al, 2010). Furthermore, IL28B variants might cause an abnormal expression of endogenous IFN- λ3 (non-, weak-, or hyper-functioning variants) and this migsxzht alter the host response to antiviral therapy by negative feedback (Balagopal, et al 2010).

Though Agu'ndez et al. 2012, have found that these genetic traits were not related with the levels of intrahepatic IL28B gene expression but the baseline expression of interferon stimulated genes (ISGs) was

found to be significantly higher in patients carrying the minor rs12979860 T allele.

Table.1 Statistical Analysis of Baseline Demographic and Biochemical Characteristics of Non Responders and Responders HCV Patients

Parameter	HCV Patients (n = 88)		p^*
	Non Responders (n= 31)	Responders (n = 57)	
Age (yrs)	44 ± 1.7	43.2 ± 1.4	0.705
Sex M/F	22(71%)/9(29%)	34(59.6%)/23(40.4%)	$0.292^{\chi 2}$
Body Mass Index (Kg/m^2)	28.7 ± 0.4	27.1 ± 0.2*	0.001^a
< 30 Kg/m^2	25 (80.6 %)	55 (96.5 %)	0.021^{*F}
≥ 30 Kg/m^2	6 (19.4 %)	2 (3.5 %)	
ALT (IU/L)	51.3 ± 6.1	50.1 ± 4.2	0.529^a
AST (IU/L)	48.5 ± 4.2	47.4 ± 4.4	0.196^a
GGT (IU/L)	73.8 ± 8.1	53.4 ± 10.4	0.0001^a
Albumin (g/dL)	3.97 ± 0.08	4.17 ± 0.05	0.043
Total Bilirubin (mg/dL)	0.93 ± 0.1	0.70 ± 0.05	0.005^a
Direct Bilirubin (mg/dL)	0.36 ± 0.05	0.20 ± 0.02	0.0001^a
Fasting Blood Sugar (mmol)	6.68 ± 0.81	5.66 ± 0.28	0.389^a
Fasting Insulin (µIU/ml)	12.41 ± 2.17	8.56 ± 0.61	0.048^a
Postprandial Blood Sugar (mg/dL)	136.7 ± 12.6	128.9 ± 5.7	0.286^a
TSH (µIU/ml)	2.71 ± 0.2	2.07 ± 0.15	0.018^a
HOMA-IR	4.8 ± 1.8	2.34 ± 0.34	0.110^a
White Blood Cells (WBCs) x 10^3	4.68 ± 0.23	4.91 ± 0.22	0.875^a
Hemoglobin (mg/dL)	12.7 ± 0.3	12.6 ± 0.17	0.780^a
Platelets(x 10^3µl)	189.61 ± 10.9	183.12 ± 6.97	0.653^a
International Normalized Ratio (INR)	1.11 ± 0.02	1.03 ± 0.01	0.0001^a
Prothrombin concentration %	90.1± 1.9	96.8 ± 0.7	0.0001^a
Alpha-Feto protein(mg/ml)	14.00 ± 4.48	5.36 ± 0.92*	0.004^a
Baseline Viral Load($\log_{10}$ IU/ml)	986598 ± 142855	1695561 ± 355799*	0.564^a

M/F: male to female ratio, ALT: alanine amino transferase, AST: aspartate aminotransferase, TSH: thyroid stimulating hormone, GGT: gamma glutamyl transferase, HOMA-IR: homeostasis model for the assessment of IR.

Student "t" test applied for all except for a Mann Whitny U test was applied, "F" FE: Fisher Exact test, , c2: Chi square test

p* value was considered significant at $p < 0.05$

Table.2 Baseline Clinico-pathological characteristics of HCV Patients as a whole and in the two subgroups Non-responders and Responders HCV patients

	HCV Patients (n = 88)	Non responders (n = 31)	Responder (n = 57)
Fibrosis stage			
F_1	41 (46.59 %)	9 (29.03%)	32 (56.14 %)
F_2	26 (29.55 %)	8 (25.81%)	18 (31.58 %)
p		$0.419^{\chi 2}$	
F_3	20 (22.72 %)	13 (41.94%)	7 (12.28 %)
F_4	1 (1.14 %)	1 (3.22%)	----------
p		1.000^{F}	
p		0.005^{*MC}	
Histological Activity			
A_1	23 (26.14%)	8 (25.81 %)	24 (42.11 %)
A_2	42 (47.72%)	9 (29.03 %)	33 (57.89 %)
A_3	13 (14.77%)	13 (41.94 %)	0
A_4	1 (1.14%)	1 (3.22 %)	0
p		$<0.001^{*MC}$	
Ultra Sound			
Fatty liver	33 (37.5%)	10 (32.3 %)	23 (40.4%)
Non Fatty liver	55 (62.5%)	21(67.7 %)	34 (59.6%)
p		$0.454^{\chi 2}$	

χ^2: Chi square test MC: Monte Carlo test FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table.3 Logistic Regression of Some Factors Associated with Response in Patients with HCV

Factor	OR	CI 95%	*p*
AFP (mg/ml)	0.302	0.081 – 1.120	0.065
GGT (IU/L)	0.219	0.068 – 0.710	0.006*
Albumin (mg/dL)	2.758	0.721 – 10.55	0.106
BMI (> 30 Kg/m^2)	0.152	0.029 – 0.804	0.021*
HOMA-IR	0.215	0.084 - 0.547	0.001*
Virus load, log_{10} IU/mL	1.538	0.721 – 10.55	0.106
Fibrosis (F1+F2 *vs.* F3 + F4)	0.170	0.029 – 0.804	0.021*
Fatty Liver	0.704	0.084 - 0.547	0.001*

AFP: alfa-fetoprotein, GGT: gamma glutamyl transferase OR = Odds Ratio, CI = Confidence Interval * p value was considered significant at $p < 0.05$

Table.4 Comparison of IL-28 rs12799860 SNP genotype frequencies among studied subjects

IL-28 rs12799860 SNP Genotype	Controls (n=30)	Non-responders (n=31)	Responders (n=57)	Exact P-value
CC (Homo-wild)	13 (43.3%)	2 (08.7%)	21 (91.3%)	p = 0.007*
CT (Hetero)	16 (53.3%)	22 (43.1 %)	29 (56.7%)	
TT (Homo-mutant)	1 (3.3%)	7 (50.0%)	7 (50.0%)	
Total	30	31	57	88

. *: Statistically significant at $p \leq 0.05$

Table.5 Comparison of rs12979860 SNP allele frequencies among studied subjects

rs12979860 SNP allele	Controls (n=30)	Non-responders (n=31)	Responders (n=57)	Total	Exact P-value	Odds ratio (95% CI)
Wild-Allele C	42	26	71	97	p = 0.007*	0.737 (0.233-0.822)
Mutant-Allele T	18	36	43	79		
Total	60	62	114	176		

OR = Odds Ratio, CI = Confidence Interval
* *p* value was considered significant at $p < 0.05$

Table.6 Relation of IL-28B rs12979860 Genotypes to the stage of fibrosis and serum GGT activity n Non- Responders and Responders HCV Patients

	Non Responders (n= 31)		Responders (n = 57)	
	CC (2)	**CT/TT (29)**	**CC (21)**	**CT/TT (36)**
Stage of Fibrosis				
F1 + F2	2 (100 %)	12 (41.4 %)	18 (85.7 %)	32 (88.9 %)
F3 + F4	0 (0.0 %)	17(58.6 %)	3 (14.3 %)	4 (11.1 %)
P	0.510^{FE}		0.701^{FE}	
Serum GGT Activity (IU/L)				
≥ 23.7 (cut off value**)**	2 (100 %)	26 (89.7 %)	9 (42.9 %)	25 (69.4 %)
< 23.7 (cut off value**)**	0 (00.0 %)	3 (10.3 %)	12 (57.1 %)	11 (30.6 %)
P	1.000^{FE}		$0.048^{*\chi 2}$	

χ^2: Chi square test FE: Fisher Exact test *: Statistically significant at $p \leq 0.05$

It has been reported that baseline GGT was associated with different host factors including interleukin (IL-28B) rs12979860 CT and TT genotypes and numerous markers of liver disease injury and severity (Everhart, et al 2013). A new finding concerned the associations with IL28B rs12979860 genotype and GGT activity with treatment outcome was observed by Agundez, et al 2012 who found that GGT was strongly associated with the T allele, whose presence reduces the likelihood of response to therapy. Of greater significance, patients with at least one copy of the T allele had poorer virological response with increasing GGT. However, it is not clear why GGT activity appears to potentiate the effect of the rs12979860 genotype. Interestingly, the results of the present study confirm the previous finding as it showed that that almost 89% of non-responder HCV patient's non CC genotype (CT+TT) has high serum GGT activity and about 89.7 % of them have serum GGT activity higher than 23.7 IU/L.(Table 6) This increases the predictive value of IL28B rs12979860 genotype found in the present study.

The association between IL28B polymorphisms and liver fibrosis progression is controversial (Agu'ndez et al.,2012) & (Gomez et al., 2011). In this study, The association between IL28B polymorphisms and liver fibrosis progression was studied and the results showed that high frequencies of elevated stages of fibrosis were observed in non-responders HCV patients with non-CC IL28B polymorphism.(Table 6) . In a study made on 629 Italian patients, IL28B rs12979860 TT-genotype was found to be an independent predictor of a higher Ishak staging (Falleti, et al 2011) . Also, Fabris et al 2012, analyzed retrospectively in a longitudinal study the fibrosis progression in Hepatitis C genotyp 1–4 patients. Over a period of 10 years Non-CC carriers with a serum cholesterol ≤175 mg/dL had fibrosis progression more frequently. Nevertheless, to determine the role of IL28B in fibrosis progression for clinical decision making further studies are needed.

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