Study of Interleukin 28B rs12979860 and rs8099917 Polymorphisms and T-helper 1 Response in Hepatitis C Virus Patients

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HCV infection is a serious public health problem and a leading cause of chronic liver disease. It affects nearly 3% of the world's population with an associated high mortality. Egypt has the highest prevalence of HCV infection in the world (estimated at >10%). Peg-IFN-α and RBV are the most widely used therapy for HCV. Unfortunately, the rate of SVR is around 50%. In addition, it is expensive and associated with considerable adverse effects. Thus selection of patients with the highest probability of response is essential for clinical practice. It is suggested that some SNPs near IL- 28B gene could be important genetic predictors of treatment response among HCV. Our study aimed to study two different Interleukin 28B polymorphisms (rs12979860 and rs8099917) and T-helper 1 response in HCV infected patients. The current study was conducted on 60 chronic HCV infected patients and 20 healthy volunteers. Grouping of patients was done according to response to treatment into naïve, responder and non-responder HCV patients. Assessment of liver functions' tests, measuring HCV RNA levels using real time PCR, measurement of interferon-γ levels using ELISA and genotyping of IL-28 rs2979860 and rs8099917 SNPs using 5' nuclease assay were done. Concerning IL-28B rs12979860; TT genotype was highly expressed in non-responder HCV patients but statistically insignificant. While for IL-28B rs8099917, there was lack of association between its different genotypes and SVR. IFN-γ level was significantly increased among responder HCV patients carrying IL28B rs12979860 TT genotype and/or IL28B rs8099917 GG allele. There was statistically significant positive correlation between L28B rs8099917 GG genotype and HCV-RNA. In conclusion, IL-28B rs12979860 SNP could be used as an independent predictor for treatment response among HCV patients.

Hepatitis C virus (HCV) infection is a serious public health problem and a leading cause of chronic liver disease (Jiang *et al.,* 2015). It affects nearly 3% of the world's population with an associated high mortality that is expected to increase substantially in the next 20 years (Fawzi *et al.,* 2009). More than 80% of patients with acute HCV infection develop chronic hepatitis (CH) and are at risk of severe liver disease (as cirrhosis, end-stage liver disease and hepatocellular carcinoma "HCC") in their lifetimes (Wilkins *et al.,* 2015). Indeed, HCV infection is one of the leading reasons for liver transplantation worldwide.

Egypt has the highest prevalence of HCV infection in the world (estimated at >10%) and is approximately 10 folds greater than United States and Europe (Sievert *et al.,* 2011). It is estimated that overall prevalence of antibody to HCV in the Egyptian general population is around 15-20% and among the 15–59 years age group is 14.7% (Mohamoud *et al.,* 2013). Though an intra-familial spread of HCV in Egypt has been recently suggested, the very high prevalence of HCV infection has been traced back to the mass campaigns to treat schistosomiasis before 1980 with repeated intravenous injections of tartar emetic (potassium antimony tartarate) without following meticulous hygiene standards (Fawzi *et al.,* 2009).

The goal of therapy of chronic HCV infection is to reduce all-cause mortality and liver-associated complications (Wilkins *et al.,* 2015). In the early 2000s, the combination of pegylated interferon plus ribavirin (PEG-IFN/RBV) became the standard anti-HCV

treatment (Li *et al.,* 2015). It is widely used to treat chronic HCV infection (Milara *et al.,* 2015). Unfortunately this combination has a number of drawbacks and intolerable side effects that necessitate prematurely stopping treatment in >15% of patients, and dose reductions in another 20-40%. Moreover, this drug regimen is very expensive (Ho *et al.,* 2011). Thus selection of patients with the highest probability of response is essential for clinical practice.

Interleukin-28B (IL-28B), also known as IFN-λ3, is a member of a highly homologous type III IFN family which consists of three members: IL29 (IFN-λ1), IL-28A (IFN-λ2), and IL-28B (IFN-λ3) (Gad *et al.,* 2009). All of these are located on chromosome 19 (Kotenko *et al.,* 2003). Recent reports have shown direct antiviral activity and immune-mediated effects of IL-28B as it can inhibit HCV replication through the JAK-STAT pathway in time-and dose-dependent manner (Zhang *et al.,* 2011). Additionally, an association between IL-28B genotypes and intra-hepatic expression of interferon-stimulated genes was observed in chronically infected HCV patients (Urban *et al.,* 2010).

Genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNP) near IL-28B gene. These IL-28B genotypes greatly influence response to IFN-based therapy (Tanaka *et al.,* 2009). Some studies, in different populations, have reported that IL-28B SNPs may be prognostic markers for HCV treatment efficacy in HCV infection (Thompson *et al.,* 2010; Stättermayer *et al.,* 2011). It was found that IL-28B rs12979860 SNP is one of the few genetic predictors proven to be of clinical utility since it identifies patients with HCV genotype 1 who undergo spontaneous or treatment induced viral clearance (D'Ambrosio *et al.,* 2014).

Because of the robust frequency of IL-28B rs12979860 in different populations and its significant impact on treatment outcome, the determination of this SNP seemed sufficient for predicting therapy response. Also, the exact mechanisms by which IFN-λ3 polymorphisms affect immune function or exert specific antiviral effects in HCV-infected patients are still unclear (Kelly *et al.,* 2011).

HCV clearance has been associated with SNPs in regions of IL28B, which was discovered to be the best predictor of patient response to pegylated-IFN plus ribavirin for chronic HCV. These SNPs are different in the different population (Derbala *et al.,* 2013). The exact mechanisms by which IFN-λ3 polymorphisms affect immune function or exert specific antiviral effects in HCV-infected patients are still unclear (Kelly *et al.,* 2011). It is suggested that these SNPs influence the cellular immune responses. So the aim of the present research is to study two different IL-28B SNP (rs12979860 and rs8099917) and T-helper 1 response among HCV infected patients.

Subjects and Methods

Subjects

This study was conducted on 80 subjects; 60 HCV patients were diagnosed or followed up after treatment at the Microbiology department, Medical Research Institute, Alexandria University and 20 healthy volunteers. Subjects were divided into 4 groups; group 1(naïve HCV patients) included 20 HCV infected patients who did not receive any treatment, group 2 (responder HCV patients) included 20 HCV infected patients having sustained virological response (SVR) after 24weeks of treatment with Peg-IFN-α plus ribavirin, group 3 (non-responder HCV patients) included 20 HCV infected patients who did not respond to treatment or had a relapse after the end of the treatment and group 4 (control group) included 20 normal persons matched in age and sex with patients in the other groups. SVR was defined as undetectable HCVRNA 24 weeks after the end of the treatment. Relapse was defined as the reappearance of HCVRNA during follow-up in subjects with previous end of treatment response (ETR), which is undetectable serum HCVRNA at the end of therapy.

Inclusion criteria included HCV patients aged between 20 and 55 years old, untreated or receiving Peg-IFN-α plus ribavirin for 24 weeks. Chronic HCV infection was confirmed by positive serology for anti-HCV and active viral replication by the detection of HCV-RNA in the serum. Exclusion criteria included HBV infection, blood transfusion, renal, cardiac, neoplasic or immunological disorders. All participants were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study protocol, according to ethical guidelines of the Medical Research Institute, Alexandria University (Appendix 1, Informed Written Consent for Patient Participation in a Clinical Research, 2011).

Methods

All reagents used in the study were supplied by Sigma-Aldrich Chemical (St. Louis, MO, USA), E-Bioscience and Life Technology (St. Louis, MO, USA).

Liver functions' tests including serum albumin, ALT (SGPT) (RANDOX; alanine aminotransferase EC 2.6.2 IFCC kit), AST (SGOT) (RANDOX; aspartate aminotransferase EC 2.6.2 IFCC kit) and alkaline phosphatase were performed for all subjects under the study (Burtis *et al.,* 2006). Anti-HCV tests were performed using a commercial enzyme linked immune-sorbant assay (ELISA) kit (Axsym 3.0; Abbott Laboratories, Chicago, IL, USA).

I- Detection of HCV-RNA "viral load" by Real time PCR

RNA extraction was performed using QIAamp viral RNA mini kit spin protocol, Qiagen (Hilden, Germany). In brief, 140 µl serum were added to 560 µl of prepared buffer AVL containing carrier RNA, mixed by pulse-vortex for 15 sec and incubated at room temperature for 10 min., then briefly centrifuged to remove drops from the inside of the lid. Then 560 µl of ethanol (96-100%) were added to the sample, and mixed by pulse-vortex for 15 sec. the mixture was carefully applied to the QIAamp spin column (in a 2-ml collection tube), centrifuged at 6000 g (8000 rpm) for 1 min., washed with 500 µl of buffer AW1 and centrifuged at 8000 rpm for 1 min. The QIAamp spin column was carefully washed with 500 µl of buffer AW2 and centrifuged at full speed 14000 rpm for 3 min. Finally, QIAamp spin column was placed in a clean 1.5 ml microcentrifuge tube and eluted by 60 µl AVE were added equilibrated to room temperature for 1 min, then centrifuged at 8000 rpm for 1 min. The eluted RNA was stored at -20°C.

For PCR amplification, 10 µl of extracted RNA were added to 6 µl of HCV RG Master A and 9 µl of HCV RG Master B; to bring the reaction to a final volume of 25 µl. Then thermal profile was adjusted as follow: incubation at 50°C for 30 min to transcribe viral RNA to cDNA by RT. This was followed by AmpliTaq gold activation for 95° C for 10 min, followed by 45 cycles of three PCR-step amplification, denaturation for 95° C for 30 sec, followed by annealing at 50° C for 1 min and extension at 72° C for 30 sec, with end point fluorescence detection. The viral load was measured in international unit per ml. If there was no C.T the test was considered negative. A standard curve was drawn by plotting the different C.T of five quantitation external standard against different RNA concentrations.

II- Measurement of human IFN-γ

Interferon-γ levels were measured in all serum samples, using commercial BMS228 / BMS228TEN Human IFN gamma Platinum ELISA kit (ebioscience), according to manufacturers' instructions. Color intensity was measured by spectrophotometer at 450 nm. A standard curve was created by plotting the mean absorbance for each standard concentration on the ordinate against the human IFN-γ concentration on the abscissa. The best fit curve was drawn through the points of the graph. The concentration of circulating human IFN-γ was determined by finding the mean absorbance value on the ordinate and a horizontal line was extended to the standard curve. At the point of intersection, a vertical line was extended to abscissa to read the corresponding human IFN-γ concentration.

III- Assessment of IL-28 rs2979860 and rs8099917 SNP (TaqMan® SNP Genotyping Assays Protocol, Applied Biosystems, Copyright 2006, 2010)

SNPs were performed following the work of Derbala et al., 2013. Genomic DNA was extracted from EDTA whole blood samples using the PureLink® Genomic DNA Kits # K1820-01 (Invitrogen, Life Technologies) followed by assessment of DNA concentration and purity using a Nanodrop spectrophotometer. The extracted DNA was stored at -80ºC till used.

IL-28 polymorphisms; rs2979860 and rs8099917 were analyzed using 5' nuclease assay with a TaqMan MGB (minor groove binder) probe in a *StepOne*™ Real-Time PCR System (Applied Biosystems, Life Technologies). SNP Genotyping Assays contain VIC® dye-labeled probe, FAM™ dye-labeled probe, and two target-specific primers. TaqMan® probes incorporate MGB technology at the 3' end to deliver superior allelic discrimination. The MGB molecule binds to the DNA helix minor groove, improving hybridization based assays by stabilizing the MGB probe-template complex. This increased binding stability permits the use of probes as short as 13 bases for improved

mismatch discrimination and greater flexibility when designing assays for difficult or variable sequences. All MGB probes also include a non-fluorescent quencher (NFQ) that virtually eliminates the background fluorescence and provides excellent signal-to-noise ratio for superior assay sensitivity.

IL-28rs860 SNP and IL-28B.rs917 SNP primers and TaqMan MGB probes were provided by the assay on-demand ™ service by Applied Biosystems, Life Technologies. The 5′ nuclease assay was performed using 2 µl genomic DNA, 7 µl DNase-free water, 10 µl TaqMan Universal PCR Master Mix (2X) and 1 µl working stock of SNP genotyping assay (20X). The assay contain sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest, two TaqMan® MGB probes with NFQ (one VIC®-labeled probe to detect Allele 1 sequence, one FAM™-labelled probe to detect Allele 2 sequence). Negative controls (without DNA samples) were included in each run. Thermal cycling conditions were specified to be 10 min. at 95˚C followed by 40 PCR cycles each consists of 15 sec at 92˚ C and 1 min. at 60˚ C. Each reaction plate was loaded into *StepOne*™ Real-Time PCR System, then the run started.

When probes that have hybridized to the complementary sequence are cleaved, an increase in fluorescence signal occurs. Thus, the fluorescence signal generated by PCR amplification indicates which alleles are present in the sample (table 1).

Table 1. Correlation between fluorescence signals and sequences in a sample.

A substantial increase in…	Indicates…
VIC-dye fluorescence only	Homozygosity for Allele 1
FAM-dye fluorescence only	Homozygosity for Allele 2
Both VIC- and FAM-dye fluorescence	Allele 1-Allele 2 heterozygosity

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Monte Carlo correction. For normally distributed data, comparison between > two population were analyzed F-test (ANOVA) to be used. Correlations between two quantitative variables were assessed using Pearson coefficient. For abnormally distributed data, comparison between different groups were done using Kruskal Wallis test. Significance of the obtained results was judged at the 5% level (Kirkpatrick *et al.*, 2013).

Results

I- Subject's demographic data

Age and sex distributions among naïve, responder and non responder HCV patients and healthy control group didn't show any statistically significant differences.

II- Laboratory investigations

Results of liver functions tests (Albumin, ALT, AST and Alkaline phosphatase) are summarized in table 2. There was a statistically significant increase in AST and ALT values among non- responder HCV patients compared to the other studied groups (P=0.001). But there was no statistically significant differences between the studied groups as regard to serum albumin (P=0.519) and ALK (P=0.921) levels.

Results of HCV-RNA quantification were expressed as units/l and summarized in figure 1. There was a statistically significant increase in HCV-RNA among naïve HCV patients compared to other studied groups (P=0.001).

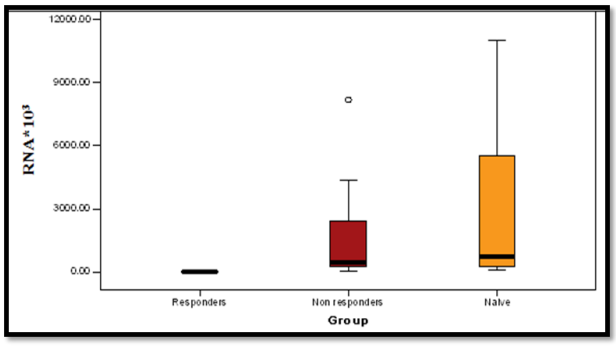

Figure 1. HCV-RNA in the studied HCV patients' groups.

Table 2. Comparison between the studied groups' liver functions

Lab investigations	Patients				*P value
	Naive	Responders	Non responders	Control	
Albumin (mg/dl) Mean±SD	4.42±0.64	4.55±1.44	4.05±0.55	4.11±0.47	NS
Alkaline phosphatase Mean±SD	72.3±95.25	77.9±41.72	80.9±42.74	64.5±13.13	NS
AST (U/L) Mean±SD	11.30±3.16	19±13.66	36.69±13.38	25.4±9.42	0.001
ALT (U/L) Mean±SD	11.9±2.47	22.1±18.82	39.41±13.65	14.4±7.35	0.001

* *P* > 0.05 is not significant (NS) by One way ANOVA

III- Immunological investigations

1) IL-28B variants among different groups:

IL-28B SNPs at rs917 and rs860 loci were identified using step One real-time PCR (figures 2-4). Concerning rs12979860, TT genotype was expressed in 30%, 40%, 70% and 40% of naïve, responder, non- responder HCV patients and healthy volunteers, respectively. CT genotype was expressed in 70%, 60%, 30% and 60% of naïve, responder, non- responder HCV patients and healthy volunteers, respectively. TT genotype was highly expressed in non- responder HCV patients but statistically insignificant (*P*=0.304) (figure 5).

While for rs8099917, TT genotype was expressed in 10% of non- responder HCV patients but not expressed in naïve, responder HCV patients or healthy volunteers at all. TG genotype was expressed in 30%, 30%, 30% and 10% of naïve, responder, non- responder HCV patients and healthy volunteers, respectively. GG genotype was expressed in 70%, 70%, 60% and 90% of naïve, responder, non- responder HCV patients and healthy volunteers, respectively. There was no statistically significant differences between the studied groups as regard to rs917 (*P*=0.563) (figure 6).

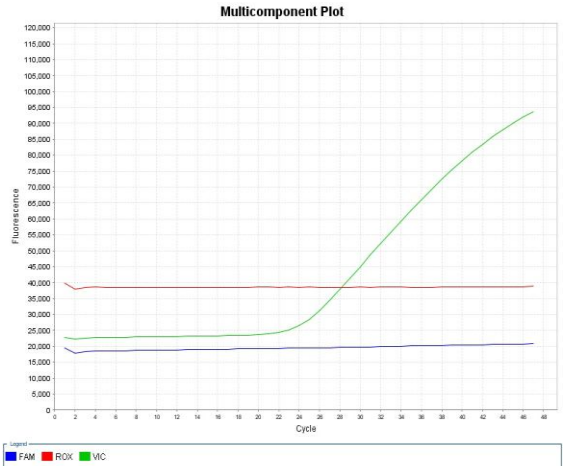

Figure 2. Real-time PCR picture displaying expression of rs860 CC or rs917 TT (VIC® dye is associated with the C allele of IL-28B rs860 or T allele of IL-28B rs917)

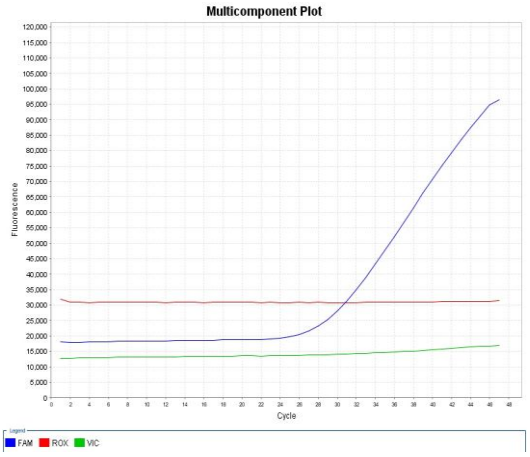

Figure 3. Real-time PCR picture displaying expression of rs860 TT or rs917 GG (FAM™ dye is associated with the T allele of IL-28B rs860 or G allele of IL-28B rs917)

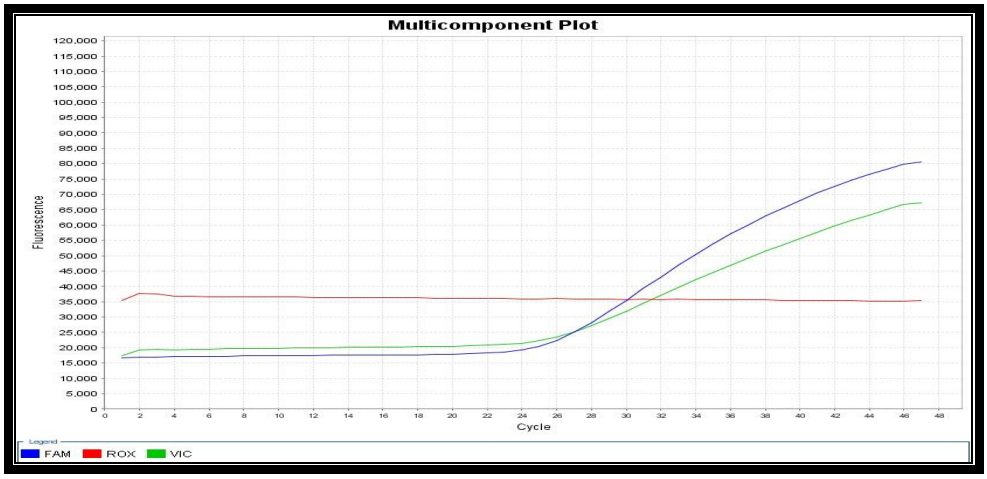

Figure 4. Real-time PCR picture displaying heterozygous expression of both alleles "rs860 CT or rs917 TG".

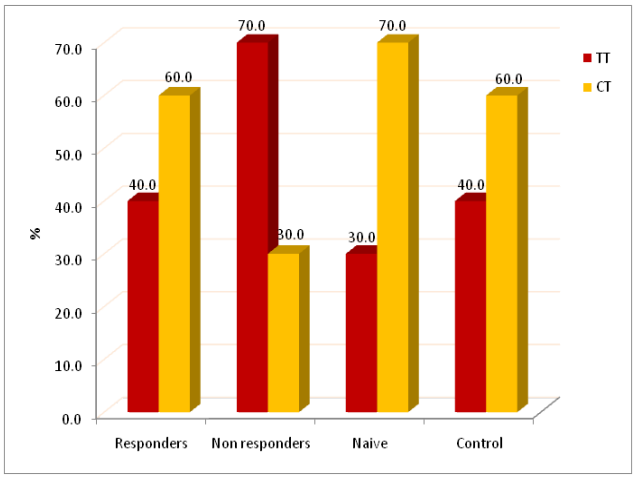

Figure 5. IL-28B (rs860) genotypes among studied groups of HCV patients and controls.

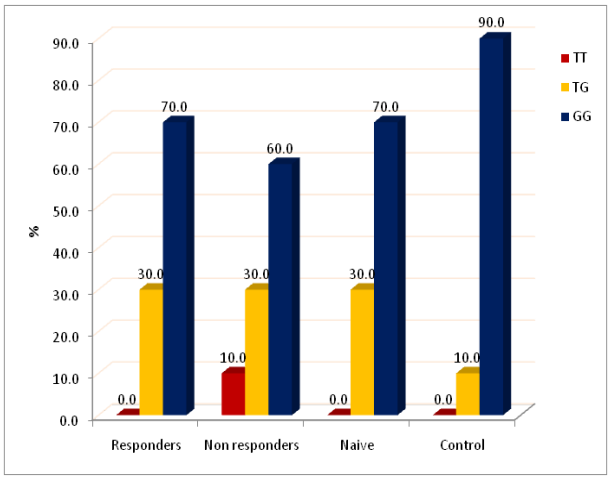

Figure 6. IL-28B (rs917) genotypes among different studied groups of HCV patients and controls.

The relation between IL-28B variants and HCV-RNA levels was summarized in figure 7. Analysis for rs917 revealed that mean of viral load was lower in patients with TT rs917 genotype (52×10^3), compared with patients with TG-GG rs917 genotypes (260×10^3 and 1585×10^3, respectively), and these differences were highly significant ($P < 0.050$). In addition, the mean of HCV-RNA among non-responder HCV patients carrying IL-28B.rs860 TT allele was markedly increased but statistically insignificant (figure 7).

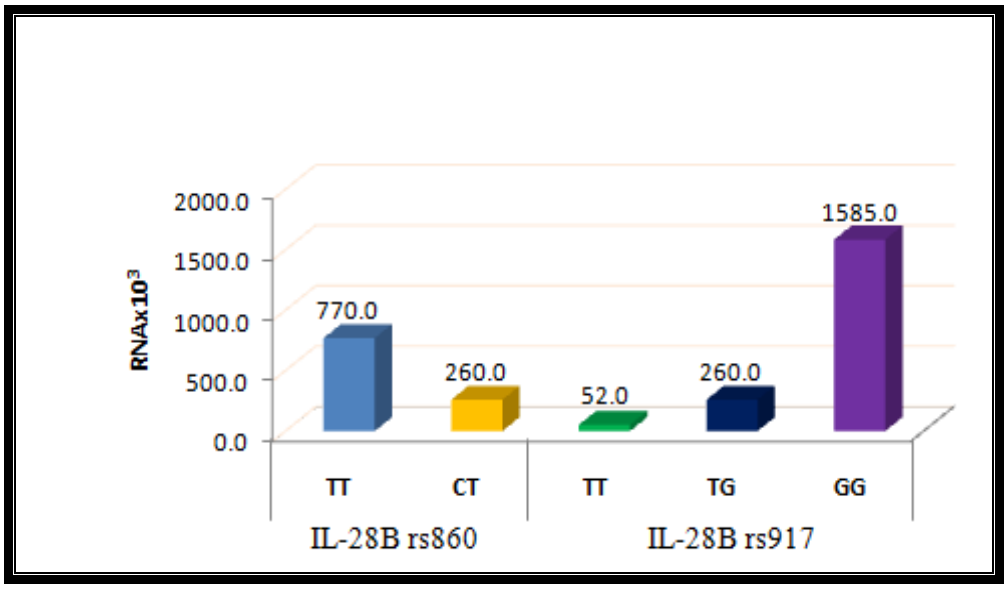

Figure 7. Relation between Il-28B rs860, rs917 SNPs and HCV-RNA among non-responder HCV patients

2) IFN-γ level:

IFN-γ levels were measured in all patients and controls, results were expressed as pg/ml and summarized in table 3. There was no statistically significant differences between the studied groups where *P*=0.326.

Table 3. Comparison between the studied groups of HCV patients and controls as regard IFN-γ levels.

IFN-γ (pg/ml)	Patients				*P* value
	Naive	Responders	Non responders	Control	
Mean±SD	244.3±172.5	219.3±49.2	227.8±75.2	163.4±58.7	NS

* *P* > 0.05 is not significant (NS) by One way ANOVA

There was a significant positive correlation between IFN-γ levels and AST in responders (*P*=0.004) and non responder (*P*=0.021) HCV patients as shown in figures 8. But There was a significantly negative correlation between HCV-RNA and IFN-γ levels in the non responder HCV patients (*P*=0.011) (figure 9 & table 4).

Additionally, IFN-γ level was significantly increased among responder HCV patients carrying IL28B rs12979860 TT genotype (*P*=0.037) and/or IL28B rs8099917 GG allele (*P*=0.023) (Figure 10).

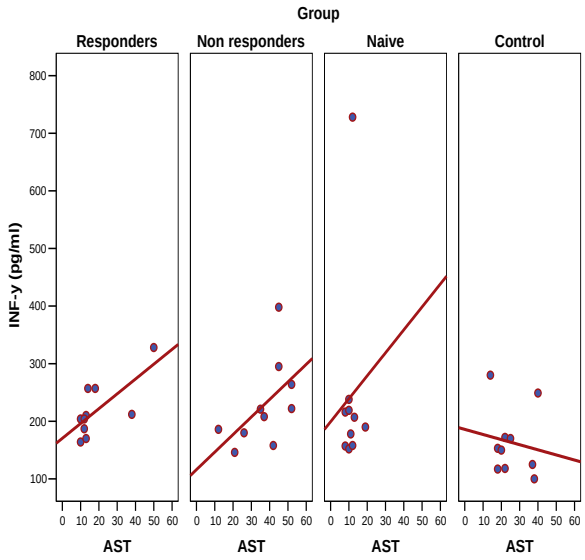

Figure 8. Relation between IFN-γ levels and AST in the studied groups.

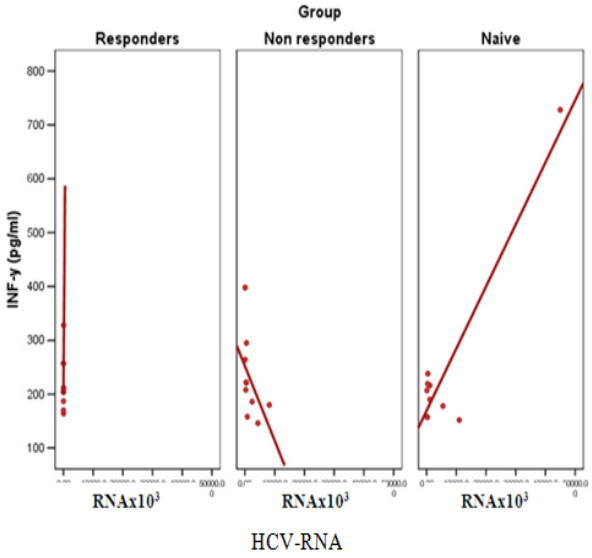

Figure 9. Correlation between IFN-γ and HCV-RNA in the studied HCV patients' groups.

Table 4. Relation between IFN-γ levels and HCV-RNA in the studied HCV patients' groups.

			HCV-RNA (*10^3)
IFN-γ	Naïve	r	0.14
		P	NS
	Responders	r	0.28
		P	NS
	Non responders	r	-0.758
		P	0.011*

r: Person cofficient, * *P* ≤ 0.05 (significant), NS: not significant

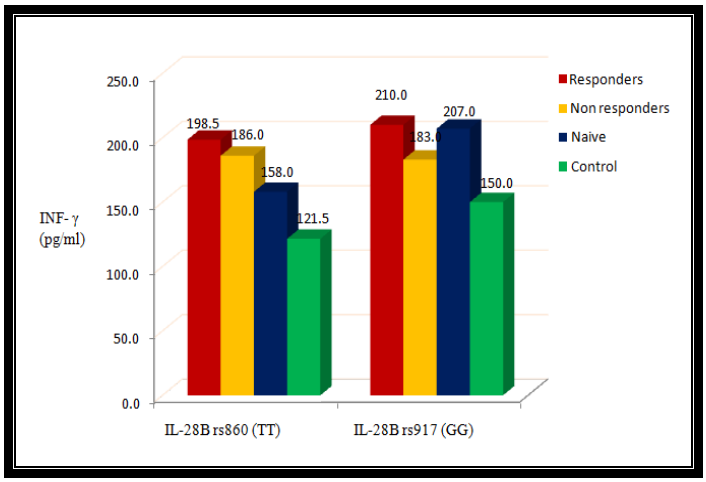

Figure 10. Comparison between studied groups regarding IL-28B alleles & IFN-γ levels.

Discussion

HCV is a small, hepatotropic, enveloped, single stranded RNA virus belongs to family flaviviridae and genus hepacivirus (Shrivastava *et al.,* 2015). Since its discovery in 1989, HCV has been revealed as a primary cause of chronic hepatitis, end-stage cirrhosis, and HCC. Worldwide, HCV is a global health burden with an estimated 200 million persons (approximately 3% of the global population) chronically infected with HCV, and 3-4 million persons are newly infected each year (Shrivastava *et al.,* 2015). Egypt has the highest prevalence of HCV in the world, estimated nationally at 14.7%, with 9% countrywide and up to 50% in certain rural areas (Kamal & Nasser, 2008).

Several independent GWAS have reported that SNPs in some cytokine genes may influence the production of the associated cytokines that affect the host immune response to Peg-IFN-α with ribavirin in HCV infected patients. In 2010, Thompson showed that C/C genotype of IL28B (rs12979860) is associated with a higher probability of rapid virologic response (P<0.0001), complete early virologic response (P<0.0001), and SVR (P <0.0001) compared to the C/T and T/T genotypes in Caucasians infected with HCV genotype 1. Another Japanese study (Akuta *et al.,* 2010) has shown that IL28B (rs8099917) polymorphism is a strong pre-treatment predictive factor in HCV patients receiving Peg-IFN + ribavirin + telaprevir.

The purpose of the present study was to study IL28B rs8099917/ rs12979860 SNP and T-helper 1 response in HCV infected patients. According to the present study's laboratory investigations' results, liver functions were markedly deteriorated in non-responder HCV patients compared to the other studied groups (P=0.001). Also, HCV-RNA levels were significantly increased among naïve HCV patients (P=0.001).

Genotyping of rs12979860 and rs8099917 near the IL-28B gene was performed by real time PCR using Taq-man probe assay. Concerning rs12979860, TT genotype was highly expressed in non-responder HCV patients but statistically insignificant (P=0.304).

In accordance with the present study's, Khairy *et al.,* (2013) have evaluated the predictive power of the rs12979860 IL28B SNP for treatment response in genotype 4 Egyptian patients. They concluded that absence of the C allele was significantly associated with failure of response. These results are in agreement with other studies (Derbala *et al.,* 2013; Milara *et al.,* 2015; Nadia *et al.,* 2015).

Nadia *et al.,* (2015) have found that the frequencies of rs12979860 CC type were higher among SVR groups. Similarly, Fateh *et al.,* (2015) have conducted study in Iran and reported that IL28B rs12979860 CC allele was significantly associated with response to pegIFN-α/RBV treatment.

Thus, the present data and previous researches firmly indicate that IL28B rs12979860 genotypes may be of a value in identifying subjects for whom the therapy might be successful, monitoring response to PEG-IFN/RBV therapy and predicting recurrence and prognosis of HCV infection.

While for rs8099917, the present study was in agreement with other studies which supported a lack of association of IL28B genotype with SVR in HCV infection (Kawaoka *et al.,* 2011; Sticchi *et al.,* 2013).

On contrary to these results, several studies conducted on different HCV genotypes in different populations all over the world and reported that rs8099917 TT genotype is significantly independent pre-treatment predictor of response to PEG-IFN/RBV in HCV infected patients than minor allele (TG/GG) carriers (Fathy *et al.,* 2015; Peng *et al.,* 2015).

Boglione *et al.*, (2015) have analyzed the influence of IL28B rs8099917/ rs12979860 on response to treatment with PEG-IFN. IL28B rs8099917/ rs12979860 TT/CC genotype was the most frequent in HCV-infected patients with SVR (P<0.001), while the TG/TC genotype was most frequent in non-SVR (P<0.001). IL28B rs8099917/rs12979860 TT/CC was a better predictive factor of SVR than rs12979860 CC alone (OR=9.829 vs. 2.663). In addition, TG/TC genotype is associated with non-SVR.

Moreover, in the current study, IFN-γ level was significantly increased among responder HCV patients carrying IL28B rs12979860 TT genotype (P=0.037) and/or IL28B rs8099917 GG allele (P=0.023). Pár *et al.*, (2013) have reported that IL28B rs12979860 CC polymorphism is associated with increased Th1 cytokine production of activated peripheral blood monocytes and lymphocytes in Hungarian HCV patients, which may play a role in interferon-induced rapid immune control and sustained virological response of pegylated interferon plus ribavirin treated patients.

On the other hand, there was negative significant correlation between HCV-RNA and IFN-γ levels in the non responder HCV patients (P=0.011). On contrary to these results, Domagalski *et al.* (2013) have found that the median baseline viral load for patients with IL-28B rs860 CC genotype was higher than that for patients with IL-28B rs860 CT-TT genotypes (P<0.001). Their analysis of IL-28B rs8099917 SNP revealed that baseline viral load was higher in patients with IL-28B rs917 TT genotype, compared with patients with IL-28B rs917 TG-GG genotypes, and these differences were highly significant (P<0.001). These contradicting results may be owing to the difference in populations, sample sizes and viral genotype.

According to our study findings, we can conclude that IL28B rs12979860 genotypes may be of a value in identifying subjects for whom the therapy might be successful, monitoring response to PEG-IFN/RBV therapy and predicting recurrence and prognosis of HCV infection, but there is lack of association between IL-28B rs8099917 SNP and SVR. In addition, there is an association between IL28B SNP and Th1 response. The results could verify that host genetics may be useful for the prediction of treatment outcomes and that IL28B SNP genotype is an important predictive biomarker for SVR in HCV infected patients. Further studies, based on a larger number of patients, are necessary to investigate the relation between IL-28B SNPs and HCV susceptibility, clearance and prognosis among HCV infected patients.

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