Interleukin 28B Gene Polymorphism as a Predictor of Early Virological Response to Pegylated Interferon Plus Ribavirin in Treatment of Egyptian Chronic Hepatitis C Virus Patients

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Abstract*:* Polymorphisms in the region of the interleukin (*IL*) *28B* gene have been associated with pegylated-interferon (PEG-IFN) and ribavirin treatment response mainly in genotype 1 HCV infections. However, there are few data on HCV genotype 4 (HCV-4) infections. We evaluated the association of *IL28B* polymorphism with early virological response to treatment. This study included 100 Egyptian HCV-4 patients .Free DNA extracted from all the 100patient's serum samples was analyzed by restriction enzymes of the SNP rs12979860 of *IL28B*. Genetic and bio-clinical features from patients having early virological response (50 EVR patients) and from those who did not respond to treatment (50 NR patients) were compared. Our data showed that most patients included in the study have CT genotype of the *IL28B* gene SNP rs12979860. The responders versus non responders were CT: 44% Vs 52% CC: 28% Vs 16% & TT 28% Vs 32%. No statistical significant was found between responder and the non response in CT and TT genotype while there is statistical significant difference in CC genotype which is higher in responder than non responder p value < 0.05. **Conclusion**: Patients with a CC genotype are much more likely to achieve an SVR compared with those with genotype TT or CT in patients infected with HCV-4 . The degree of fibrosis and base line viral load with analysis of *IL28B* genotype might be used to guide treatment for these patients.
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1.Introduction

Hepatitis C virus (HCV) infection is a major health problem throughout the world ***(Hnatyszyn, 2005).*** HCV infection is considered the most common etiology of chronic liver disease in Egypt, where the prevalence of antibodies to HCV (anti- HCV) is approximately 10-fold greater than in the United States and Europe (***Strickland et al., 2002).***

Egypt has the highest worldwide prevalence of HCV (10-20%). More than 90% of HCV isolates from Egyptian patients are of the genotype 4 variant ***(Kamal & Nasser, 2008).***

At least 70% of patients who contract HCV develop chronic hepatitis C, with 20-50% of these patients eventually progressing to cirrhosis and 5-7% developing hepatocellular carcinoma in 10-20 years. ***(Fattovich et al., 1997).***

HCV is classified into eleven major genotypes (designated 1-11), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1, 2, 3, etc.) based on the genomic sequence heterogeneity.

The variability is distributed throughout the genome. ***(Simmonds, 1999).***

Genotype is perhaps the strongest predictor of response. Patients infected with HCV genotype 2 or 3 are much more likely to achieve SVR to the current standard of care than patients infected with HCV genotype 1.Pretreatment predictors of response are useful when assessing the benefits and risks of therapy with patients, but they may not allow for an individualized determination of which patient will achieve viral eradication. The ability to respond to interferon-based therapy may be genetically determined. Recent genome-wide association studies have identified genetic determinants of treatment response in HCV - infected patients treated with pegylated interferon and ribavirin ***(McCarthy et al., 2010).***

The primary goal of antiviral therapy has always been to achieve viral eradication as a means of delaying progression to end-stage liver disease and preventing the development of hepatocellular carcinoma ***(Hoofnagle et al., 1997).***

A number of factors can affect response to treatment of HCV, and therefore help predict the likelihood of achieving SVR ***(Kau et al., 2008).*** Patient -related factors include ethnicity, sex, body mass index ***(Bressler et al., 2003)*** and the presence of metabolic syndrome ***(Romero -Gomez et al.,2005)*** .Disease/ viral-related factors include HCV genotype, baseline

viral load, and the degree of liver fibrosis ***(Zeuzem , 2004).***

Interferon (IFN) - α based regimen, particularly pegylated interferon and ribavirin combination therapy, are the current standard care for chronic hepatitis C virus (HCV) infection (***Hoofnagle, 2003).***

Treatment with pegylated interferon alpha *(PEG – IF N* α) and ribavirin (RBV) leads to sustained viral response in 40 – 80% of patients **(*Manns et al., 2001).***

Rapid virological response (RVR) and early virologic response (EVR) defined as undetectable HCV RNA at weeks 4 and 12 respectively have been shown as important tools for determination of duration of therapy, In chronic hepatitis C genotype 4 and undetectable HCV RNA at weeks 4 and 12, treatment with PEGIFN alpha-2b and ribavirin for 24 weeks and 36 weeks, could be sufficient ***(Ferrencie et al., 2008).***

The sustained virologic response (SVR) to PEG-IFN α and ribavirin combination therapy ranges from about 40 to 50 percent with genotype 1 (including 1a and 1b) to as high as70 to 80 percent with genotypes 2, 3 and 4 however, treatment is expensive and universally associated with adverse side effects ***(Fried et al., 2002).***

Due to the side effect profile associated with the standard of care regimen, assessing predictors of response prior to treatment initiation allows the treating physician to select those patients most likely to respond and achieve SVR, and thus provides critical information about overall management ***(Ghany et al., 2009).***

Determination of ***IL28B*** genotype before starting antiviral therapy may thus serve as a counseling tool to help patients decide whether to undergo treatment now or wait for future therapeutic options to become available. Treatment-week 12 response, or early virologic response (EVR), has emerged as an important determinant of SVR and duration of therapy. EVR can either be complete (cEVR, defined as no detectable virus at treatment week 12) or partial (pEVR, defined as a ≥ 2log decline in HCV RNA compared with baseline) ***(Brown , 2007).***

A single nucleotide polymorphism (SNP) near the IL28B gene predicts response to hepatitis C treatment with interferon and ribavirin ***(Thompson et al., 2010).*** The SNP was identified in a genome-wide association study (GWAS) and is to date the best example of a successful GWAS hit that is clinically relevant. ***(Maxmen, 2009).***

Although the *IL28B* polymorphism is a strong determinant of response to therapy, other factors clearly play a role because HCV genotype 1 – infected African American patients with a CC genotype have a lower response to treatment with the standard of care than do HCV genotype 1-infected white individuals with the same genotype ***(Thompson, 2010).***

Because of its significant impact on the treatment outcome, a genetic testing for the genotype of SNP of *IL28B* before deciding on treatment strategies has been proposed ***(Balagopal , 2010).*** However, several other SNPs of *IL28B* were also found to be highly associated with SVR, like rs8099917, rs12980275, and others ***(Suppiah , 2009).***

IL-28 is a cytokine that comes in two isoforms, IL-28A and IL-28B, and plays a role in immune defense against viruses, including the induction of an "antiviral state" by turning on Mx proteins, 2',5'-oligoadenylate synthetase as well as ISGF3G (Interferon Stimulated Gene Factor 3) ***(Kempuraj , 2004).***

IL-28 genes are located near IL-29 on chromosome 19 in humans. The two isoforms of IL-28 (IL-28A and IL-28B) are 96% homologous, although differences in the functions between the two forms remain unclear. The receptor for IL-28 is composed of a uniqueIL-28 Receptor Alpha chain which pairs with the IL-10Receptor Beta chain, leading many to classify IL-28 as an IL-10-like family member. ***(Sheppard, 2003).***

IL28B is a gene related to the interferon system. A genetic region near the IL28B gene is referred to as an IL28B genotype. There are three variations of IL28B genotypes: CC, CT or TT. These variations have been associated with a person's response to treatment for hepatitis C with pegylated-interferon and ribavirin. Studies have shown that people with the CC variation respond better to treatment with pegylated-interferon and ribavirin than those with the CT or TT variations. The CC variation is more frequent in Caucasians compared to African Americans (39 percent versus 16 percent), which may partially explain the lower response to treatment observed among African Americans in most clinical trials of pegylated-interferon and ribavirin ***(Ge , et al.,2009).***

SNPs rs12979860 and rs8099917, respectively located 3 and 8 kb upstream of IL28B, were the variants most strongly associated with treatment response in these studies. Among treatment naive G1-infected subjects of European ancestry who were enrolled in the IDEAL study, approximately 69% of those who carried 2 C alleles (C/C) at rs12979860 achieved SVR compared with 33% of those with the C/T genotype and 27% with genotype T/T.***(Thompson ,2010).*** Consistent findings were reported for rs8099917 among Japanese, Australian, and European populations. ***(Tanaka , 2009).*** These studies demonstrated that carriage of 2 IL28B favorable alleles strongly, but not fully, predicted SVR, while carriage of 1 or 2 unfavorable alleles did not completely predict failure to respond to treatment.

2.Patients and Methods

This study was conducted in cooperation between Tropical Medicine department Al Azhar University ,Tropical Medicine Research Institute (NHTMRI) and Medical Biochemistry Department International Islamic Center for Population Studies and Research Al Azhar University, at period from 10/2012-10/2013.

One hundred patients participated in the present study; were chosen according to the protocol of the National Hepatology and Tropical Medicine research Institute (NHTMRI) for assessment of Egyptians patients with chronic HCV infection, treated with either PegIFN-α-2a 180

µg/week subcutaneously, or PegIFN-α-2b 1.5 µg/kg once/week subcutaneously plus ribavirin 1000-1200 mg/day (for body weight <75 kg

or ≥75 kg, respectively), the patients treated for 12 weeks then quantitative PCR done for them to detect the early responders from non responders**.** The early responders subdivided into ,complete early virological response (cEVR, defined as no detectable virus at treatment week 12) or partial early virological response (pEVR, i.e. those with ≥ 2 log decline in HCV RNA compared with baseline after 12 weeks of treatment). Non responders at week 12 who gives either positive PCR or <2 log decline in HCV RNA compared with baseline. Actually we choose 50 patients responders at 12 injections, and 50 patients' non responders at 12 injections to compare results of IL28 B between patients with early virological response and non responders.

Patient's selection:

The patients enrolled in our study were taken from outpatients' clinics of NHTMRI. We conducted our study on 100 patients with chronic HCV infection who fulfilled the following inclusion criteria:

Inclusion criteria:

Patients with chronic HCV who are candidates for Interferon/ Ribavirin therapy according to protocol of (NHTMRI & National Committee).

Patient age is above 18 and below 60 years.

Positive anti-HCV and HCV RNA in serum (regardless of the viral load).

Compensated liver.

White blood cells > 4000/cc.

Hb > 11gm/dl.

Platelets >90000/cc.

Albumin > 3.5g/dl.

Fasting blood sugar < 115mg/dl.

If the patient is diabetic, glycosylated hemoglobin should be < 8.5%.

TSH within normal.

Serum Creatinine within normal.

ANA <1:160.

Prothrombin time < 2 seconds above the upper limit of normal.

Exclusion criteria:

- Any cause of liver disease other than chronic HCV based on the patient history, laboratory or liver biopsy findings as:

i. Autoimmune hepatitis.
ii. Hemochromatosis.
iii. Wilson's disease.
iv. Alpha 1-antitrypsin deficiency.
v. Alcoholic liver disease.
vi. Drug induced liver disease.

- Decompensated liver disease (more than grade A with Child-Pugh score).
- Hepatic tumors excluded by αFP and abdominal ultrasonography.
- CT abdominal scan was done if αFP was higher than 100ng/dl.
- Hbs Ag +ve patients.
- Pregnancy or breast feeding.
- Serious systemic disease (e.g. advanced ischemic heart disease).
- Severe pre-existing psychiatric condition.
- Poorly controlled diabetes (Hb A1C >8.5%).

Sample size determination:

Blood samples were obtained by vein puncture, for serum sample blood collected and allowed to clot, serum separated by centrifugation at room temperature and kept frozen till the time of analysis - For prothrombin time and concentration, the collected blood was added in .11 mol/L trisodium citrate, then centrifugated and the test was performed within 4 hours of sample collection.

The following laboratory tests were done:

- Complete blood count.

- Liver profile (ALT, AST, ALP, serum albumin, serum bilirubin), glucose and renal profile all were measured by Clinical chemistry Hitachi analyzer .

- Prothrombin time and international normalization ratio (INR) measured by (Dia Med Gmbh, Switzerland kit) using coagulation analyzer.

- Thyroid and autoimmune profile by ELFA technique using (Enzyme Linked Fluorescent Assay) by Vidas, BioMerieux, France.

- Hepatitis viral markers;

* HBs-Ag and HCV-Ab. by ELISA, using third generation kits (DiaSorin, Italy).

* Quantitative HCV RNA by PCR, serum HCV RNA was detected by real time PCR assay (Cobas Amplicor HCV monitor, version 2.0, Roche Diagnostics).

Liver biopsy was done in all patients except in: Extrahepatic manifestations e.g.1-kidney manifestations: membranoproliferative glomerulonephritis (MPGN). 2- Skin lesions: Porphyria cutanea tarda.3- rheumatologic and autoimmune manifestations….etc.

The histopathological examination was performed in order to assess histologic scoring for fibrosis according to Ishak classification ***(Ishak et al., 1995)*** and necro-inflammatory activity ***according to Knodell et al. (1981).***

Methods of IL28B genotyping

Blood samples obtained from 100 patients who underwent treatment with peg interferon/ribavirin for 12 weeks,50 patients were responders at week 12 and the others 50 patients were non responders then serum separated from these samples sent for IL28B genotyping (rs12979860) for association with treatment response.

Genotyping for the IL-28B rs12979860 C/T polymorphism was performed by polymerase chain reaction based restriction fragment length polymorphism assay. Genomic DNA was extracted from whole blood samples by means of the QIAamp DNA blood mini kit (Qiagen, Milan, Italy) according to manufacturer's instruction. A 242 base pair (bp) product was obtained with the forward primer 5- GCTTATCGCATACGGCTAGG 3- and the reverse primer 5- AGGCTCAGGGTCAATCACAG 3- , newly designed with the aid of NCBI Primer-Blast Tool. PCR amplification was carried out in a total

volume of 10 ml containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, Twenty 0.01%, 0.2 mM deoxyribonucleotides, 2–4 pmol of each primer, 2.0 mM $MgCl_2$, 0.5 units hot-start Taq DNA polymerase (RighTaq, Euroclone, Milan, Italy). Samples containing 10 ng of genomic DNA were subjected to 40 cycles of denaturation (at 95° C for 30 s), annealing (at 62 ° C for 30 s), and elongation (at 72° C for 30 s) using a Techne TC-412 thermal cycler. In a total volume of 20 ml, 10 ml of the amplicons were digested with 1 unit of the BstU-I restriction endonuclease (New England Bioloabs, Hitchin, UK) at 60 ° C overnight.

The fragments digested were, respectively, 135 + 82 + 25 bp for the C allele and 160 + 82 bp for the T allele variant. The fragments were resolved by electrophoresis in 3.5% agarose gel after staining with ethidium bromide.

Statistical Analysis

Chi-square test was used for the analysis of qualitative data. Mean and Standard deviation were estimated for quantitative data. In all tests, P value was considered significant if less than 0.05.The clinical variables included age, gender, viral load of HCV-RNA, Metavir fibrosis stage and *IL28B* SNPs.

3.Results

The A total of 100 patients enrolled in our study were 59 males and 41 females. The mean age of all patients was divided into 2 groups: ≤ 40 years and > 40 years the mean body mass index (BMI) for all patients was ≤30 kg/m^2.

The end point was at week 12, and the 100 patients divided into 50 patients with early response and 50 patients' non responders.

Analysis of SNPs rs 12979860 in the *IL28B* region on chromosome 19 was performed as shown in methods. And the results were:

48 patients had C/T genotype mutation, 22 patients had CC and 30 patients had TT genotype mutation. The relation of these genotypes to response is compared and shown in the next tables.

Table (1) showed that there are no significant differences between responder and non responder as regarding liver function test *p* value > 0.05 so it is statistically non-significant.

Table (2) showed that 52% of responders aged ≤ 40 years, and 48% of them aged > 40 years also 38% of non responders aged ≤ 40 years, and 62% of them aged > 40 years. *P* value is > 0.05 so it is non significant. This indicate that in our study age distribution isn't correlated significantly with EVR.

This table (3) showed that 60% of responders are male patients and 40% of them are female patients, also 58% of non responders are male and 42% of them are female. *P* value is > 0.05 so it is statistically non-significant.

Table (4) showed the relation between base line viral load and early virological response in the 100 patients enrolled in our study, and the cut point of PCR was 0.4×106 IU/ml. 74% of responders have base line viral load< 0.4×106 IU/ml and 26% of them have base line viral load ≥ 0.4×106 IU/ml .Also 36% of non-responders have base line viral load < 0.4×106 IU/ml and 64% of them have base line viral load≥ 0.4×106 IU/ml *P* value < 0.05 so it is statistically significant and indicates that patients with low base line viral load shows early virological response than patients with high base line viral load.

Table (5) showed that 80% of responder patients have mild degree fibrosis F1-2, and 20% of them have moderate to severe degree of fibrosis F3-4 while 62% of non responder patients have mild degree fibrosis F1-2 and 38% of them have moderate to severe degree of fibrosis F3-4 , *P* value is < 0.05 so it is statistically significant. This means that patients with mild degree of fibrosis show early virological response than patients with moderate to severe degree of fibrosis.

Table (6) showed that 44% of responder patients have CT genotype,28% of them have CC genotype, and 28% of them have TT genotype. While 52% of non responders have also CT genotype, 16% of them have CC genotype, and 32% have TT genotype. This indicates that most patients enrolled in the study have CT mutation and the percentage of responders is near to that of non responders in CT and TT mutation with

P value is > 0.05 so it is statistically non significant. While CC mutation in responder is higher than non responder and is statistically significant *p* value is <0.05.

Table (1): Liver function test in responder and non responder groups.

Group / Liver function test	Group 1 Responder N = 50	Group 11 Non responder N = 50	*P* value
AST (U/1)	49.23 ± 6.6	51.4 ± 24.17	> 0.05
ALT (U/1)	58.25 ± 5.5	62.9 ± 38.4	> 0.05
Albumin (g/dl)	4.12 ± 4.5	4.05 ± .32	> 0.05
Bilirubin (mg/dl)	0.65 ± 0.22	.6 ± .24	> 0.05
Prothrombin (Sec)	12.04 ± 1.2	13.04 ± 1.04	> 0.05

Table (2): Age distribution and its relation to early virological responseamong the studied patients.

Age	Responder		Non- responder		Significance
	N	%	N	%	
≤ 40 years	26	52	19	38	
> 40 years	24	8	31	62	> 0.05
Total	50	100	50	100	

Table (3):Gender distribution and its relation to early virological response among the studied patients.

Gender	Responder		Non- responder		Significance
	N	%	N	%	
Male	30	60	29	58	
Female	20	40	21	42	> 0.05
Total	50	100	50	100	

Table (4): Baseline viral load distribution and its relation to early virological response among the studied patients

Base line viral load	Responder		Non- responder		Significance
	N	%	N	%	
<0.4×106 IU/ml	37	74	18	36	
≥ 0.4×106 IU/ml	13	26	32	64	< 0.05
Total	50	100	50	100	

Table (5): Degree of fibrosis and its relation to early virological respons

Degree of fibrosis	Responder		Non- responder		Significance
	N	%	N	%	
Mild F1-2	40	80	31	62	
Moderate to sever F3-4	10	20	19	38	<0.05
Total	50	100	50	100	

Table (6): SNPs of IL28B and its relation to early virological response among the studied patients

IL28B genotype	Responder		Non- responder		Significance
	N	%	N	%	
CT	22	44	26	52	> 0.05
TT	14	28	16	32	> 0.05
CC	14	28	8	16	<0.05
Total	50	100	50	100	

4. Discussion

Viral hepatitis C is a major cause of liver related morbidity and mortality and represent a major health problem in Egypt and worldwide ***(Alberti and Benvegnu, 2003)*** and there is a large underlying

reservoir of HCV caused liver disease **(Strickland, 2002).**

Among the six major HCV genotypes, genotypes 1–4 account for nearly 90% of HCV-infected cases in western countries ***(Payan et al., 2005).*** HCV genotype 4 (HCV-4) is the most prevalent genotype in the Middle East and sub-Saharan Africa, with a prevalence ranging from 73% to 90% in Egypt ***(Zekri et al., 2001).*** Genotypes are of considerable clinical importance, because they affect response to antiviral therapy.

In Egypt and the Middle East, high sustained virological response (SVR) rates (67–70%) have been reported in patients infected with HCV-4 and treated with PEG-IFN plus ribavirin for 48 weeks ***(Thakeb et al., 2003).*** Pegylated interferon (PEG-IFN) Alf in combination with ribavirin (RBV) is the standard of care for adults with chronic hepatitis C.

The variability in response to treatment, especially between patients of different racial groups, suggested that human genetic variability might explain differences in treatment response and led to investigations of the role of host genetics in achieving an SVR, Genome-wide association studies which examine the association between >500,000 SNPs and a disease of interest, have been exceptionally successful in finding SNPs associated with response to hepatitis C treatment. In 2009, 3groups reported that SNPs located near the gene for interleukin-28B *(IL28B)* were strongly associated with the likelihood of achieving SVR with peg interferon + ribavirin treatment. ***(Ge et al., 2009*)**.

These studies demonstrated that carriage of 2 IL28B favorable alleles strongly, but not fully, predicted SVR, while carriage of 1 or 2 unfavorable alleles did not completely predict failure to respond to treatment. It appears that rs12979860 is more predictive of SVR than rs8099917, especially among people of African ancestry in whom rs8099917 is less polymorphic than rs12979860. ***(Ge et al., 2009).***

One hundred patients participated in the present study and they were randomly recruited according to the protocol of the National Hepatology and Tropical Medicine Research Institute (NHTMRI) for assessment of Egyptian patients with chronic HCV infection for treatment with peg interferon/ ribavirin combination therapy. The study selected the patients who have positive serology for HCV Abs, HCV viremia, Child A and liver biopsy showing chronic hepatitis with significant fibrosis, (F1- F4) using METAVIR score. They were treated for 12 weeks with peg interferon/ ribavirin combination therapy. On treatment PCR was done at week 12.

In the current study, we found that most patients who achieved EVR had lower pretreatment base line viral load than those non responders, (74% versus 64% respectively), and this difference was statistically significant.

These results agree with those reported by ***(Lee and Abdo, 2003)*** who found that a low baseline serum viral load (<2 million copies/mL or 800.000 IU/mL) was associated with a significantly higher probability of achieving SVR following interferon-based therapy. SVR rates with interferon /ribavirin combination treatment increased 1.5-fold after 24 weeks of treatment in patients with viral load <2 million copies/mL, in comparison with higher viral loads. However, ***(Derbala et al., 2005)*** didn't find a similar relation between the response rate and the pretreatment viral load in spite of better response rate in patients with low pre-treatment vireamia (<500 × 103 copies/mL). In a more recent study, SVR rate was significantly higher in patients with a pre-treatment baseline viral load less than 200.000 IU/mL than those with pre-treatment baseline viral load greater than 200.000 IU/mL ***(Muhammad and Sheikh 2009)***.

As regards the relation between the degree of liver fibrosis and the response to therapy for chronic HCV infection, the previous studies have reported favorable treatment outcomes among patients with no or minimal fibrosis in comparison to those with advanced disease stages. ***(Poynard et al., 2003)*** reported that the degree of liver fibrosis was one of the most important factors that can independently predict SVR to treatment.

Our results showed also those patients with mild degree of fibrosis F0-1 had EVR rates than those with moderate – severe degree of fibrosis (80% versus 20 %, and showed significant statistical difference between the two stages of liver fibrosis.

Recent genome-wide associated studies have explored this issue and demonstrated strong evidence that single nucleotide polymorphisms

(SNPs) of Interluekin-28B *(IL28B)* were significantly correlated with SVR when patients were treated with PegIFN/RBV***(Ge et al., 2009).*** Notably, the frequency of advantageous C allele of rs12979860 of *IL28B* was reported highest in Asians and lowest in African-Americans. In addition, the prevalence rates of CC genotype of rs1297860 paralleled with the SVR in each population ***(Ge et al., 2009).*** Furthermore, these genotypes of *IL28B* also correlated with the spontaneous clearance of hepatitis C virus **,** and with viral responses during treatment (***Thompson, 2010).*** Because of its significant impact on the treatment outcome, a genetic testing for the genotype of SNP of *IL28B* before deciding on treatment strategies has been proposed ***(Balagopal , 2010).*** However, several other SNPs of *IL28B* were also found to be highly associated with SVR, like rs8099917, rs12980275, and others ***(Suppiah , 2009).***

In the present study SNPs of *IL28* rs12979860 were analyzed for all patients, and patients classified

into patients with CC, CT, TT genotypes. Results shows that 44% of responder patients have CT genotype, 28% of them have CC genotype, and 28% of them have TT genotype. While 52% of non responders have CT genotype, 16% of them have CC genotype, and 32% have TT genotype. P value of CC mutation is < 0.05 so it is statistically significant. This study showed that most patients have CT genotype either responders or non responders. These results agreed with the study done by ***(El Ray et al., 2012)*** where they observed that the proportions of rs12979860 CC among HCV treated patients were 26.8%; CT was 52.4%, and TT was 20.8%,this means that most patients have CT genotype. Also the study of El Ray et al.- which included 164 HCV-4 patients from different ethnic groups (Egyptian, European, and Sub-Saharan African) observed that the response rates were 81.8%, 46.5%, and 29.4% for genotype CC, CT, and TT, respectively. They showed that the CC genotype is significantly associated with a better response rate for patients with chronic HCV-4 infection.

In another study an interesting observation was the different impact of the CC genotype of rs12979860 on the SVR in patients with or without RVR. In patients with RVR, only baseline viral load but not the genotype of rs12979860 could predict the SVR. This is an interesting observation. The possible explanation is the CC genotype of rs12979860 led to the RVR with odds ratio of 10.52. As a result, the majority of patients with RVR were CC genotypes. Therefore, the CC genotype lost their predictive ability for SVR in this patient group with high prevalence of CC genotype. On the contrary, in patients without RVR, only the genotype of rs12979860 but not the baseline viral load could predict the SVR. This group of patients without RVR was with lower prevalence of CC genotype. Therefore, the CC genotype could continue its influence and therefore predict the SVR in this group of patients. This observation was quite similar to the recent report about genotype 2/3 HCV infected patients who received treatment that the genotype of rs12979860 is a single predictor for SVR in patients without RVR ***(Mangia et al., 2010).***

A strong association between IL28B polymorphism and SVR had been reported, the underlying mechanisms are still unclear. Recent in-vitro report had shown that IL28B could inhibit HCV replication in a dose- and time- dependent manner and through the JAK-STAT pathway ***(Zhang et al., 2010).*** Consequently, it had been found that IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C ***(Urban et al., 2010).*** Furthermore, serum IL-28A/B levels were significantly higher in patients with chronic hepatitis C with good allele of IL28B genotype (***Langhans et al., 2010).***

Based on our results described previously, we can suggest genotyping for all patients according their SNPs of *IL28B* before starting treatment for patients with HCV chronic infection. All these evidences indicate both direct anti-viral effect and immune-mediated effect of IL28B could be affected by these polymorphisms. However, detailed mechanistic understanding needs further investigation.

The limitation of this study was the small number of patients included in the analysis. The other limitation was that we analyzed the genotype of rs12979860 only although there are other SNPs of IL28B gene, especially rs12979869 and rs8077717 which can also affect treatment outcome of patients with chronic hepatitis C.

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