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Interleukin-28B Polymorphism is a Pharmacogenetic Predictor during Sofosbuvir Plus Pegylated Interferon and Ribavirin Therapy for Chronic Hepatitis C Egyptian Patients

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Abstract

Background and aim: Interleukin-28B (IL-28B) polymorphism is a predictor of sustained virologic response (SVR), spontaneous clearance and personalizing therapy of hepatitis C virus (HCV). This study aimed to determine IL28B rs12979860 polymorphism among chronic hepatitis C (CHC) Egyptian patients as a step in personalized HCV therapy and pharmacogenomics.

Methods: CHC Egyptian patients were received sofosbuvir (SOF) plus pegylated interferon (PEG-IFN) and ribavirin (RBV) for 12 weeks. A total of 82 HCV infected Egyptian patients and 27 healthy individuals were included in the present study. CHC Patients were classified as achieving SVR if plasma HCV-RNA was undetectable (group A) and non-responders if plasma HCV-RNA was detectable (group B). IL28B genotypes were analyzed and their associations with SVR were selected.

Results: The end of treatment response (ETR) rate was 100%. However, SVR12 was 76.8% (group A) and 23.2% relapsed (group B). Among studied CHC patients, 50% were IL-28B TT, 40.2% CT, and 9.8% CC. while the percentage of their frequencies in the healthy people were 18.5%, 51.8%, and 29.6%, respectively. These results showed that the frequencies of TT genotypes were more prevalent in HCV patients. The genotype CC (n=8) achieved higher rates of SVR in group A (87.5%) than relapsed patients in group B (12.5%) and it had the least prevalence in group B compared with the frequencies of CT (21.2%) and TT (26.9%) genotypes. These results showed that CC genotype was associated with SVR.

Conclusions: It can be concluded that the individuals with IL28B TT genotype are more susceptible to HCV infection in Egyptian patients and relapse. Moreover, IL-28B CC is useful for pretreatment prediction of the outcome of HCV treatment. Hence, IL28B polymorphism could be considered as a pharmacogenetic predictor in personalized HCV therapy and pharmacogenomics during SOF plus PEG-IFN and RBV therapy for CHC Egyptian patients.

Introduction

In the late 1980s, HCV was discovered as non-A, non-B hepatitis [1]. HCV is a small particle, enveloped and positive sense single-stranded ribonucleic acid (+ssRNA) [2,3]. It belongs to the *Hepacivirus* genus within the Flaviviridae family [4,5].

Globally, there are 130-150 million people chronically infected with HCV [6]. In Africa, the highest prevalence has been reported in Egypt and Cameroon [7,8]. Egypt has the highest prevalence rate of HCV in the world [9,10].

HCV can lead to chronic hepatitis disease in millions of people and are the most common cause of liver cirrhosis and hepatocellular carcinoma [11]. Seven HCV genotypes and sixty seven subtypes have been identified [12]. HCV genotype 4 (HCV-G4) is the most common infection in Egypt, approximately 90% of all HCV patients [13-15].

Recently, many antiviral drugs have been developed. SOF was given in combination with RBV and take with or without Peg-IFN [6]. Moreover, Lawitz et al. [16] revealed that SOF plus Peg-IFN and RBV for just 12 weeks can achieve 89% and 96% SVR in treatment of HCV-G1 and HCV-G4 patients, respectively.

Many host genetic factors play an important role in the spontaneous clearance of HCV and the response of antiviral treatment in CHC patients. Genome wide association studies (GWAS) have identified a SNP near the IL28B gene on chromosomes 19 that encodes IFN-λ3 [17,18]. The SNP rs12979860 is located 3 kb upstream of the IL28B gene, which codes for IFN-λ3 and strongly predicts response to HCV treatment [19,20].

IL28B genotypes are strongly associated with treatment efficacy in patients infected with HCV-G1 and G4. Thus, IL28B genotyping is useful for pretreatment prediction of the outcome of direct acting antiviral (DAA) plus Peg-IFN/RBV therapy [21].

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HCV infections spontaneously clear in approximately 15–45% of infected individuals [22,23]. Currently, there is a strong association between the rate of spontaneous clearance of HCV and IFN-λ polymorphisms [24]. Furthermore, killer cell immunoglobulin-like receptor (KIR) genes with IFN-λ3 polymorphisms indirectly influence the activity of natural killer (NK) cells and can predict spontaneous resolution of acute HCV infection [25].

IL28B SNP polymorphism improves viral kinetics during PEG-IFN/RBV therapy [26]. However, Abdelwahab et al. [27] demonstrated that SNP rs12979860 polymorphism does not affect viral load among CHC Egyptian patients.

The CC genotype of SNP rs12979860 is the strongest genetic pretreatment predictor of SVR in HCV-G1 and G4 patients [21,28]. Moreover, high expression of IFN-λ has been recorded in patients with rs12979860 CC variant [29]. Recently, IL28B SNP, alpha fetoprotein level, and cytotoxic T-Lymphocytes antigen-4 (CTLA-4) SNP could be used in conjunction to predict treatment response in HCV-G4 infected Egyptian patients [30]. In addition, Esmail et al. [31] demonstrated that IL-28B SNP polymorphism is considered as a vital predictor of viral relapse.

HCV induces IFN-λ and interferon stimulated genes (ISGs) expression [32]. ISGs repress HCV replication [33]. Additionally, Marcello et al. [34] emerged that IFN-λ and IFN-α inhibit HCV replication. Hence, in the future, we can predict that pegylated recombinant interleukin 28 (PEG-rIL-28) may be used as a new antiviral drug in HCV treatment in combination with other antiviral agents.

Pharmacogenomics is the study of genetic variations that influence the response of individuals to drug treatment [35]. It can help in selection of the optimal drug, dose and avoid adverse drug reactions [36]. Clinically, many types of adverse drug reactions are related to polymorphic gene alleles [37].

Personalized medicine is a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease [38]. Besides, Pharmacogenomic information offers a personalized medicine approach to help clinicians and patients make better informed decisions to maximize response and minimize toxicity for the treatment of chronic HCV infection [39].

In Personalized medicine, molecular markers were used for detection of specific genetic traits for preventing and treating different pathologies [40]. In that way, Yanase et al. [41] demonstrated that some SNPs are associated with the metabolism of different drugs. IL28B genotyping was used in personalizing of HCV therapy as a pharmacogenetic predictor in CHC patients [42,43].

The Personalized medicine is our way and chance to improve health care system. Hence, the aim of this study is to determine SNP rs12979860 molecular marker genotypes distribution among CHC Egyptian patients as a step in personalized HCV therapy and pharmacogenomics during SOF plus PEG-IFN and RBV therapy.

Materials and Methods

Patients

The present study included 82 Egyptian patients with CHC (68 men and 14 women), mean age 51.5 ± 10.59 years. All CHC patients were collected from Sohag center of cardiac and digestive system, Sohag, Egypt. Seventy four were treatment-naïve and eight previously treated patients with Peg-IFN/RBV therapy for 12 weeks and non-response. In addition, 25.6% of patients had cirrhosis. Anti-HCV antibodies (anti-HCV Abs) were detected by using enzyme linked immunosorbent

assay (ELISA) technique according to the manufacturer's instructions (spectrum diagnostics reagents, Egypt, 3rd generation, cat. No. 1308001). All reactive anti-HCV Abs were confirmed by reverse transcription-PCR (RT-PCR) for HCV.

Control group

The individuals of control group were consisted of 27 healthy individuals (19 males and 8 females), mean age 33 ± 5.22 years, and were selected from blood bank unit in Sohag general hospital, Sohag, Egypt. They were seronegative for anti-HCV Abs, hepatitis B surfaces antigen (HBs Ag), hepatitis B core total antibodies (anti-HBc total) and anti-bilharzias. No abnormal laboratory findings with normal liver ultrasound.

Treatment regiments

CHC patients were received 12 weeks of triple combination therapy with SOF (400 mg once daily), Peg-INF (180 mcg/0.5 ml; fixed dose/week) plus RBV (1000 mg for ≤75 Kg or 1200 mg for >75 Kg).

Blood sampling

Two blood samples were obtained from each subject. The first sample was collected in serum-separating tubes. Blood was left at room temperature to clot, and then centrifuged at 4000-6000 rpm; serum was separated for biochemical analysis and RNA extraction. The second sample was collected in a clean and dry vacutainer tube with ethylene diamine tetra acetic acid (EDTA) anticoagulant for hematology analysis and IL28B SNP rs12979860 genotypes distribution study.

HCV RNA extraction

RNA was extracted from all serum samples (140 μL) by using of the QIAamp viral RNA extraction kit (Qiagen, Cat. No. 52904) according to manufacturer's instructions. Briefly, samples were lysed and the RNA was captured on the spin columns provided. The RNA was washed, eluted and then stored at -20°C until used in RT-PCR amplification.

Reverse transcription-PCR procedure

5`-UTR of HCV was amplified by using one step RT-PCR kit (Qiagen, Cat. No. 210210). The QIAGEN one step RT-PCR kit is designed to be used with primer pair selected from the highly conserved 5`-UTR of HCV genome [1]. The master mix typically contains all the components required for RT-PCR except the template RNA. The reactions were performed in 25 μl total volume and consist of the master mix plus template RNA. The 5`-UTR of HCV RNA amplification was performed by using of the Gene Amp PCR system 2400 (Biometra) programmed for 35 cycles. PCR products were visualized on 1.0% agarose using UV-trans-illuminator and photographed by a digital camera (FUJI 100) with orange filter [44].

DNA extraction

DNA extraction was done by using G-spin¯ total DNA extraction kit according to manufacturer's instructions (Spain, Cat. No. 17045). In brief, samples were lysed and the DNA was captured on the spin columns provided. The DNA was washed, eluted and then stored at -20°C until used in PCR amplification.

IL28B SNP rs12979860 genotyping detection

IL28B SNP rs12979860 genotyping was detected by multiplex tetra-primer PCR method (Control f: GCT CAG CGC CTC TTC CTC CT-3, Control R: TCC CAT ACA CCC GTT CCT GT-3, IL28B (T) F,

AGG AGC TCC CCG AAG GAG T-3, IL28B (C) R: TGC AAT TCA ACC CTG GTA CG-3) [45]. The reactions were performed in 50 μl total volume and consist of the master mix plus template DNA. The amplicons were separated by 2% agarose gel electrophoresis and stained with ethidium bromide (0.5 mg/L). Both homozygous (CC and TT) and heterozygous genotypes (CT) were detected on agarose gel.

Statistical analysis

Statistical analyses were performed by statistical package for social program (SPSS) software (version 20, SPSS, Inc, Chicago, IL, USA). The variables of CHC patients and the healthy individuals of control group were displayed as means ± standard deviation (SD).

Results

As shown in Table 1, the baseline characteristics of CHC patients (n=82) and healthy individuals of control group (n=27) were selected before therapy. All reactive anti-HCV Abs specimens were confirmed by direct detection of HCV RNA by RT-PCR (Figure 1).

End treatment response

All CHC Egyptian patients enrolled in this study were received 12 weeks of triple combination therapy. Followed by RT-PCR assay, HCV RNA is not detected in the blood specimens and the ETR rate was 100%.

Side effects during treatment period

Some side effects occurred during treatment period including thrombocytopenia (<150,000 cells/mm³), hemoglobin decreasing (≤12.5 g/dl for male and ≤11 g/dl for female), leucopenia (<4,000 cells/mm³), and ALT elevation in group A and B, as shown in Table 2, these side effects were increased and observed in relapsed patients than SVR individuals.

Sustained virologic response

After 12 weeks from termination of the triple combination therapy, all enrolled CHC Egyptian patients were observed by RT-PCR assay. Patients were classified as achieving SVR if plasma HCV-RNA was undetectable and non-responders if plasma HCV-RNA was detectable. Overall, sixty three patients (76.8%) were SVR12 to the treatment (group A) but nineteen patients (23.2%) were relapsed (group B). Furthermore, rates of SVR12 among previously treated and treatment-naïve patients were 87.5% and 75.7%, respectively (Figure 2).

IL28B SNP rs12989760 polymorphism

The SNP rs12979860 genotypes and their distributions were selected

in CHC patients and healthy people of control group (Figures 3a and 3b). The frequencies of CC, CT and TT genotypes were 9.8%, 40.2%, and 50% respectively in CHC patients, but for healthy control group, the frequencies were 29.6%, 51.8% and 18.5% for CC, CT, and TT respectively (Table 3). These results showed that the frequencies of TT variants were more prevalent in CHC patients compared with healthy individuals group.

IL28B variants and SVR

Among 82 CHC patients treated with triple combination therapy, 19 patients did not achieve a SVR12 while 63 patients achieved a SVR12. The relation between SVR of HCV and IL28B rs12989760 genotypes distribution was analyzed (Table 4). The genotype CC was achieved higher rates of SVR12 in group A (87.5%) than group B (12.5%), moreover it had a low prevalence in relapsed patients compared with the frequencies of CT and TT genotypes (21.2% and 26.9% respectively). These results emerged that CC genotypes were associated with SVR and influence the SVR rate in patients with CHC treated with triple therapy.

Discussion

As a result of GWAS, a SNP near the IL28B gene on chromosome 19 that encodes IFN-λ3 have been identified [17,18]. IFN-λ3 polymorphisms were associated with viral resolution and treatment outcomes among CHC patients [19,46].

Recently, pharmacogenomic is related to drug response and precision medicine [39]. Precision medicine allows doctors and researchers to predict treatment strategies for health care improvement. Moreover, IL28B genotyping was used as a pharmacogenetic predictor in personalizing of HCV therapy [42,43].

In the present study, IL-28B polymorphism among CHC Egyptian patients and healthy individuals were determined as a step in
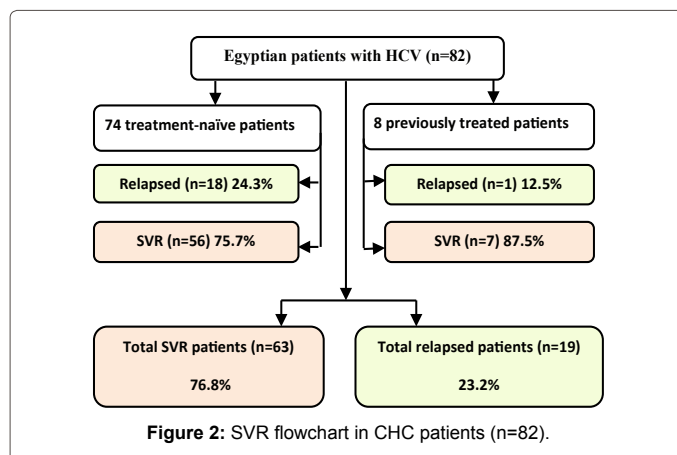

Figure 1: Ethidium bromide stained agarose gel electrophoresis of HCV 5'-UTR amplification by RT-PCR, M: 100 bp DNA ladder, lane 4: negative control, lane 7: positive control and Lanes 1, 2, 3, 5, 6 & 8: PCR-positive results (bands sizing 270 bp in each).

Variables	Control group (n=27)	CHC (n=82)
Age (year)	33 ± 5.22	51.5 ± 10.59
Gender (M/F)	19/8	68/14
Hb	14.31 ± 0.59	13.29 ± 1.08
WBC (×10³/μl)	7.84 ± 1.58	6.88 ± 2.10
Platelets (×10³/μl)	227 ± 30	190 ± 35
AST (U/L)	31.5 ± 7.9	47.5 ± 17.9
ALT (U/L)	28.9 ± 10.3	46.3 ± 22.4
Alb (mg/dl)	4.23 ± 0.15	3.86 ± 0.25
Creatinine (mg/dl)	0.87 ± 0.10	0.95 ± 0.13
Cirrhosis	0 (0%)	21 (25.6%)

M: Male; F: Female; Hb: Hemoglobin; WBC: White Blood Cells; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; Alb: Albumin

Table 1: Characteristics of the studied HCV patients and healthy controls.

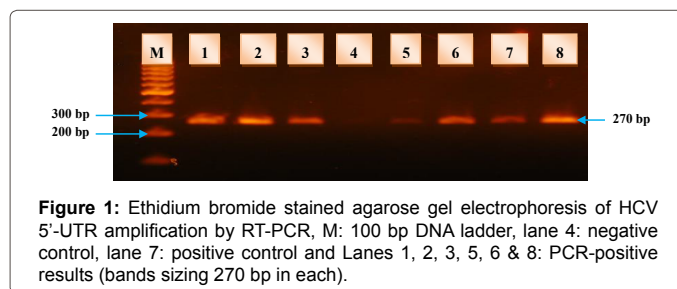**Figure 2:** SVR flowchart in CHC patients (n=82).

Figure 3a: Ethidium bromide stained agarose gel electrophoresis of SNP rs12979860 amplification by PCR, M: 100 bp DNA ladder, lane 1, 2, 3, 5, 6, 7, 9 and 11 the electrophoresis pattern of genotypes TT, Lanes 4, 8, 10 and 12 genotypes CT.

Figure 3b: PCR analysis of IL28B SNP rs12979860 (without control primers) and the products of PCR were separated by 2% agarose gel electrophoresis and stained with ethidium bromide (0.5 mg/L). M: 100 bp DNA ladder, lane 1 and 4: negative control, lanes 2, 3, 5, 8, and 9 the electrophoresis pattern of genotypes TT, Lanes 6 and 7 genotypes CC.

Side effect	Group A (n=63)	Group B (n=19)
Leucopenia	26.9% (n=17)	52.6% (n=10)
Hb decreasing	66.6% (n=42)	73.6% (n=14)
Thrombocytopenia	28.5% (n=18)	42.1% (n=8)
ALT elevation	26.9% (n=17)	31.5% (n=6)

Table 2: Hematologic abnormalities and ALT elevation.

Genotypes	Control group (n=27)	CHC (n=82)
CC	8 (29.6%)	8 (9.8%)
CT	14 (51.8%)	33 (40.2%)
TT	5 (18.5%)	41 (50%)
C allele (total)	30 (55.6%)	49 (29.9%)
T allele (total)	24 (44.4%)	115 (70.1%)

Table 3: IL28B rs12989760 polymorphism among whole studied groups.

CHC Patients	Group A (SVR)	Group B (Relapsed)
CC (n=8)	7 (87.5%)	1 (12.5%)
CT (n=33)	26 (78.8%)	7 (21.2%)
TT (n=41)	30 (73.2%)	11 (26.9%)

Table 4: IL28B rs12989760 polymorphism and SVR in CHC patients.

personalized HCV therapy and pharmacogenomics during SOF plus PEG-IFN and RBV therapy to give a right drug to the right patient and improve health care system.

SOF plus Peg-IFN and RBV for just 12 weeks leads to 89% and 96% SVR in treatment of HCV-G1 and HCV-G4 patients, respectively [16]. In our study all CHC Egyptian patients were treated with triple combination therapy and were followed by RT-PCR assay, HCV RNA is not detected in the blood specimens and the ETR rate was 100%. However, after 12 weeks from termination of the therapy, RT-PCR was observed, sixty three patients (76.8%) were SVR12 to the treatment (group A) but nineteen patients (23.2%) were relapsed (group B). Furthermore, rates of SVR among previously treated and treatment-naïve patients were 87.5% and 75.7%, respectively. Hence, SOF plus Peg-IFN and RBV for 12 weeks is effective in previously treated than treatment-naïve patients.

In the current study, some side effects occurred during treatment period in some cases, including thrombocytopenia, hemoglobin decreasing, leucopenia, and ALT elevation. In particular, these side effects were increased and observed in relapsed patients than SVR individuals. In that way, it was postulated by Torriani et al. [47] that RBV causes a hemolytic anemia and interferon can suppress bone marrow production of red blood cells, platelets and leukocytes. Additionally, ALT is the most specific marker of liver function and is used as an indirect marker of liver inflammation or injury [48].

The Prevalence of SNP rs12979860 genotypes CC, CT and TT genotypes in HCV patients and healthy persons were detected and the results showed that the frequency of TT genotype represents the highest widespread in CHC patients and the least prevalence in the healthy individuals. Hence, the TT genotype may be more effective in the progression of HCV infection and may increase the risk of HCC [49,50]

The relation between SVR of HCV and IL28B SNP rs12989760 polymorphisms were studied. The genotype CC achieved the highest rate of SVR12 compared with CT and TT genotypes. Moreover, CC genotype had the least prevalence in the relapsed patients compared with the frequencies of CT and TT variants. These results indicated that CC genotype was associated with SVR. In that way, Zeuzem et al. [51] recorded that the CC variant of rs12979860 was associated with increased likelihood of achieving SVR in comparison with non-CC variants. Consequently, the CC variant of SNP rs12979860 is the strongest genetic pretreatment predictor of SVR in HCV-G1 and G4 patients [21,28]

Conclusion

In sum, IL-28B CC is the predictive factor of response to treatment with SOF plus Peg-IFN and RBV combination therapy, and the individuals with IL28B TT genotype are more susceptible to HCV infection in Egyptian patients and relapse. In addition, this study provides that IL28B polymorphism could be considered as a pharmacogenetic predictor in personalized HCV therapy and pharmacogenomics during SOF plus PEG-IFN and RBV therapy for CHC Egyptian patients.

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