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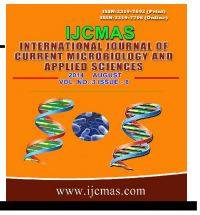

Original Research Article

Role of interleukin 28B gene polymorphism in prediction of response to standard of care therapy in Chronic Hepatitis C patients in Sharkia Governrate, Egypt

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Keywords

HCV, IL28 B, polymorphism Chronic Hepatitis C

A B S T R A C T

To detect of the role of IL28B rs12979860 C/T single nucleotide polymorphism (SNP) in the prediction of response to antiviral therapy in chronic hepatitis C patients in Sharkia Governorate, Egypt. Egypt has high prevalence of HCV (predominantly genotype 4) all over the world. Combined PEG-IFN and ribavirin is still the only standard of care treatment in Egypt in spite of its side effects, high costs and low sustained virological response rates. Hence, this provides a compelling reason for the identification of predictors of disease response to treatment. Ninety two chronic hepatitis C virus (HCV) patients, who were submitted to combined pegylated interferon alpha (PEG-IFN) and ribavirin therapy, were enrolled in this study. SNP for rs12979860 was done by realtime PCR technique. Response rate was recorded and compared among patients with different genotypes. The CC genotype of rs12979860 was identified in 26 patients (28.9 %), the CT genotype in 54 cases (60%) and the TT genotype in 10 cases (11.1%). The response rate in CC genotype was 76.9 % (20/26), 40.6 % (22/54) for CT and 40% (4/10) for TT genotypes which was statistically significant (p=0.002). These data suggest that IL28B rs12979860 C/T SNP is an important predictive biomarker for sustained virological response (SVR) in patients with HCV genotype 4.

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. It is considered a major cause of chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma, as well as, one of the leading indications for liver transplant (Shepard et al., 2005) Egypt has high prevalence of HCV infection, with 14.7%

positive HCV antibody and 9.8% positive HCV RNA among people (15-59 years old) (El Zanaty, 2009).

Combined pegylated interferon alpha (PEG-IFN) and ribavirin therapy are still the standard of care therapy (SOC) for chronic hepatitis C (CHC) infection in Egypt. The rate of sustained virological response (SVR) is around 50% (Kamal , 2011) and it is of major interest for both patient care and economic approach to predict failure of response. Several independent genome-wide association studies (GWAS) reported that single nucleotide polymorphisms (SNPs) near the IL28B (IFN-λ3) locus displayed an association with treatment response, mainly in HCV genotype 1 (Rauch et al., 2010**).** Few data are so far available regarding the role of IL28B polymorphism in HCV-4 patients with respect to response to combined antiviral therapy (Khattab et al., 2011)

This study is a case-control study, aimed at assessing the role of IL28B rs12979860 C/T single SNP in the prediction of response to antiviral therapy in chronic HCV patients in Sharkia Governorate, Egypt.

Materials and Methods

This study was conducted in Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. Ninety two patients with CHC who received SOC were recruited from the follow up clinic of the Viral Hepatitis Treatment Center at Al-Ahrar Hospital (VHTC), Sharkia Governrate, Egypt, to be enrolled in this study in the period from October 2013 to January 2014. Patients included 71 (77.2%) males and 21 (22.8%) females with their ages ranged from 22 to 59 years old (47.28 ± 8.32). This work was approved by the ethical committee of Faculty of Medicine, Zagazig University. Informed written consent was obtained from each patient before inclusion in this study. Patients were divided into two groups according to their response to therapy; the first group was the non responder group (case group) which included 46 chronic HCV patients who failed to respond to combined therapy with persistent HCV RNA detection at any time after 12 weeks of treatment and up to 72 weeks after initiation of therapy. The second group was the responder group (control group) which included 46 chronic HCV patients with SVR who had undetected HCV RNA in their serum from week 12 and up to week 72 after initiation of therapy.

All patients were submitted to SOC of pegylated interferon-alpha 2a 180 mcg per week or pegylated interferon-alpha 2b 1.5 mcg per Kg in combination with ribavirin 600-1400 mg per day according to their body weight for 48 weeks. Retrospective collection of patients' data was done using the data recorded in their files as well as via patients' interview to fill the missed data. This data included demographic (age – sex), clinical (body mass index), baseline biochemical (liver function tests, insulin resistance, thyroid stimulating hormone level and alpha fetoprotein level), virological (viral load by real time PCR) and histopathologic characteristics (stage of fibrosis).

IL28B rs12989760 SNP detection and genotyping

Two ml blood were withdrawn under complete aseptic measures from every patient and were dispensed in a sterile vaccutainer tube containing ethylene-diamine tetra acetic acid (EDTA). DNA extraction was done using PureLink® Genomic DNA (Invitrogen, Life technologies, USA) according to the

manufacturer's instructions. DNA quantitation and purity assessment was done using Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA).

IL28B genotyping was done using Taqman Universal Master Mix II (Applied Biosystems,USA) that is supplied in a 2✕concentration and Custom Taqman IL28B SNP (rs12979860) Genotyping Assay (Applied Biosystems, USA) which consists of two tubes each one contains two primers for amplifying the polymorphic sequence of interest and two TaqMan® MGB probes with 2 different reporter dyes for distinguishing between the two alleles (primers and probes sequences were not shown in the kit).

Real-time PCR was performed using 7500 real time PCR system (Applied Biosystems, USA) with the following cycling conditions**;** polymerase activation step for 10 minutes at 95°C and two step-cycling of 40 cycles that includes denaturation step for 15 seconds at 95°C and combined annealing and extension for 90 seconds at 60°C.

Statistical analysis

Statistical package for social sciences (SPSS) program version 16 for windows and Epi info computer program were used for data analysis. Quantitative variables were summarized using mean ± SD. Independent t-test was done to compare two normally distributed variables and Chi square (χ^2) test for categorical qualitative variables. p value was significant at ≤ 0.05 level.

Results and Discussion

The characteristics of the patients with chronic HCV infection (before therapy) are shown in Tables 1 and 2. CC genotype was detected in 26 cases with a percentage 28.9% of the total determined cases (90 cases). Of the 26 cases with CC genotype, 20 patients were responders (76.9%) and only 6 cases were non responders (23.1%). The CT genotype was detected in 54 cases with a percentage 60% of total determined cases (90 cases). Of the 54 cases with CT genotype, 32 cases were non responders (59.3 %) and 22 were responders (40.7 %). The TT genotype, on the other hand, was detected in 10 cases with a percentage 11.1% of total determined cases (90 cases). Among those with TT genotype (10 cases), 6 cases were non responders (60 %) and 4 patients were responders (40%). Two undetermined cases with a percentage 2.17% of total number of cases (92 cases) were detected. Both belonged to the non responders. The response rate in CC genotype was 76.9 % (20/26) versus 40.6 % (22/54) for CT and 40% (4/10) for TT genotypes which when compared was statistically significant (p=0.002) (Table 3).

According to IL28B gene polymorphism, patients were further divided into two groups; the favorable genotype (CC), this included 26 patients, and the unfavorable genotypes (CT+TT) which included 64 patients (Table 4). We found that no significant differences were present between different genotypes regarding the sex of patients (p= 0.065), their viral load (p= 0.35), the fibrosis stage (p=0.186), the body mass index (p=0.907), or being diabetic or not (p= 0.705). On the other hand, a significant difference (p= 0.025) was present between both groups regarding the activity stage where 92.3% of those having the favorable genotype were stage A1 or A2 and 7.6% were stage A3, while all patients with the unfavorable genotypes were in stage A1 or A2. In addition, a highly significant difference (p= 0.002) was present concerning the level of ALT between both groups where higher level was found in the favorable (CC) genotype (mean 79.46 ± 30.88) compared to the unfavorable

genotypes (CT+TT) (mean 48.67 ± 19.33).

IL28B encodes IFN-λ3 which is involved in viral infection control, including HCV. It is also closely related to both *IL28A* and *IL29* genes that all encode type Ⅲ IFNs at chromosomal region 19q13. The three cytokine genes are induced by viral infection and have antiviral activity (Derbala et al., 2012). The current study was designed to clarify the effect of rs12979860 located nearest to interleukin 28B (*IL28B)* on HCV outcome after combined PEG-IFN and ribavirin therapy.

The study involved 46 responder and 46 non responder HCV patients to SOC therapy. A highly significant difference (p=0.001) was detected concerning the level of AFP between both groups where the mean ± SD in the responder group was 7.06 ± 8.25 compared to 17.94 ± 12.65 in the non responders. This comes in accordance with Khairy et al., 2013 who reported that the baseline AFP was an important predictor of antiviral therapy response in chronic HCV patients and that higher serum AFP level was the strongest predictor of failure to achieve SVR in their studied patients. This comes also in agreement with previous studies including HCV genotype 4 (Gad et al., 2009) & (Males et al., 2007) and genotype 1(Akuta et al., 2007) & (Chen et al., 2007) which highlighted the same findings. Another significant difference (p=0.03) regarding platelet count was found between both groups and this comes in keeping with that reported by Kanda et al., 2013 who found that, in treatment-naive patients, the SVR rate of the pretreatment platelet count < 130000/ μL group was significantly lower than that of the pretreatment platelet count ≥ 130000/μL group.

Previous studies had reported that the high IL-28B cytokine producing C/C genotype enhanced the spontaneous resolution of HCV infection and that patients who harbored the C allele at rs12979860 were more prone to respond to treatment and clear HCV two to three fold greater than patients who did not possess this genetic polymorphism. Moreover, the frequency of IL-28B C/C genotype was found to be lower in HCV patients than in controls, which suggest a primary role for IL-28B in the resolution and even the protection against chronic HCV infection (Thomas et al., 2009) & (Par et al., 2011)**.** In the current study, all cases were subjected for testing IL28B gene polymorphism rs12979860 using real time PCR. The CC genotype was detected in 26 cases forming 28.9% of the total determined cases (90 cases). On the other hand, the CT and TT genotypes were detected in 54 (60%) and 10 (11.1%) of the total determined cases (90 cases), respectively. This comes in keeping with De Nicola et al., 2012 who found that of 112 treated patients (75 of Egyptian descent) 23% were genotype CC, 63% CT and 14 % were TT, also with Khairy et al., 2013 who found that almost half (56%) of HCV4 Egyptian patients were CT followed by CC (25 %) then TT which had the least expression (19 %).

When IL28B gene polymorphism rs12979860 was compared with SVR to SOC therapy in the current study, it was found that the response rate was higher for the CC genotype (76.9 %) compared to 40.7 % for CT and 40% for TT genotypes which was statistically significant (p= 0.002). This finding is supported by Asselah et al., (2010) who showed a better treatment response rate of the C Allele of the IL28B gene SNP rs12979860 where the response rates were 81.8%, 46.5% and 29.4% for genotypes CC, CT and TT respectively, also De Nicola et al. (2012) found that 88% of

SVR patients were CC compared to 37% for CT/TT ($p < 0.0001$). Similar findings were also reported by Jia et al. (2012) where SVR rate was 72.7 % for the CC, 41.6% and 34.4% for CT and TT, respectively.

The exact mechanism and explanation behind the association between genetic variations in the IL28B gene and combined therapy outcome is not clear but spontaneous clearance of HCV was related to these variations (Derbala et al., 2012). The lower intrahepatic expression of IFN-stimulated genes in IL28B CC carriers than patients with CT/TT variants might facilitate the antiviral activity of IFN-based therapy (Thompson 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. In this way, it leaves a narrower margin of response when HCV acutely infects the liver or when interferon-based therapy is instituted in chronically infected patients. These findings may explain why the spontaneous clearance of HCV virus is more frequent in subjects with the rs12979860 C/C genotype and that this genotype is a predictor of SVR.

In the current study, there was no significant relation between IL28B gene polymorphism and viral load ($p= 0.3$). This comes in keeping with previous studies (Derbala et al., 2012) &(Shi et al., 2012) .On the other hand, this comes in contrast with Ge et al. (2009) who reported viral load with the CC patients, also with Hendy et al., 2011 who revealed higher viral load among their CC patients and explained this as hypothetically, patients harboring allele C could display lower activity of their endogenous INF- α allowing higher viral replication while keeping an enhanced susceptibility to exogenous INF- α therapy. This controversy between the current study results and the other studies could be explained by that the exact mechanism underlying this genetic association with viral loads is unclear. However, the polymorphism has no association with the baseline viral loads (which might influence the treatment response) as higher or lower, and this implies that the association of the polymorphism with viral clearance and viral loads may be unrelated.

No significant relation ($p= 0.06$) between IL28B gene polymorphism and gender was detected in the current study. The same result was recorded in previous studies (Derbala et al.,2012) , (Hendy et al., 2011) & (Montes-Cano et al., 2010) .On the other hand, this comes in contrast with Rao et al. (2012) who reported a significant genetic polymorphism among women who responded to treatment. This could be explained by the difference in tested SNPs as they reported this gender related-difference with rs8099917 TT genotype.

Liver fibrosis in patients with chronic hepatitis C is believed to result from immune-mediated phenomena rather than the direct consequence of a cytopathic effect of HCV replication (Barreiro et al., 2011). The association between IL28B polymorphisms and liver fibrosis progression is controversial (Agu'ndez et al., 2012) & (Gomez et al., 2011). In this study, no significant relation ($p=0.186$) between liver fibrosis and IL28B gene polymorphism was found which comes in agreement with that recorded previously (Derbala et al., 2012), (Asselah et al., 2010) &(Marabita et al.,2011).On the other hand, Barreiro et al., 2011 reported that IL28B CC carriers might experience a more rapid progression of HCV-related liver fibrosis. Falleti et al.

(2011) have found, in addition, that the unfavorable rs12979860 T/T gene pattern was associated with worse liver fibrosis in a group of 629 HCV patients which comes in contrast with the current study. These differences could be explained by the different nature of the study carried by Barreiro et al. (2011) which was conducted on 304 HIV-HCV co infected patients. The other study carried by Falleti et al., 2011 was done on a relatively large sample size of 629 patients with 200 of them having cirrhosis.

In the current study, a significant relation between IL28B gene polymorphism rs12979860 and both activity (p= 0.02) and ALT level (p= 0.002) was found. These findings come in keeping with those reported previously by Thompson et al. (2012) where the SNP rs12979860 was the only SNP associated with baseline ALT level (p=4.2x10-9) and also the only SNP associated with METAVIR A2-3 activity (p=1.8 X 10-8) with no other genetic loci were associated with ALT level or A2-3 activity. In addition**,** Agu'ndez et al. (2012) found that rs12979860 CC genotype is associated with higher serum ALT than the remaining genotypes but they didn't confirm the suggested relation between the IL28B gene polymorphism and the histological necroinflammatory activity. This could be attributed to that most patients included in their study were infected with HCV genotype 1 and all their patients were white with most of them were Spaniards in contrast to our study which was done on Egyptian patients mostly with genotype 4a as reported by WHO (2014).

Table.1 Epidemiological, biochemical and virologic characteristics of the studied groups

Character	**Responders (n=46) Mean ± SD**	**Non responders (n=46) Mean ± SD**	**Test**	***P***
Age (Years)	47 ± 8.79	47.56± 7.91	t=0.3240	0.740
BMI	26.45 ± 2.81	25.73 ± 3.13	t=1.156	0.25
Hb (g/dl)	14.18 ± 0.99	14.43 ± 0.99	t=1.128	0.22
Serum albumin (g/dl)	4.1 ± 0.49	4.11 ± 0.47	t=0.710	0.47
TLC ($x10^3$/µl)	6.3 ± 1.2	5.6 ± 1.2	t=1.516	0.14
TSH (µIU/ml)	1.8 ± 0.99	1.72 ± 1.02	t=0.66	0.51
AFP (mg/ml)	7.06 ± 8.25	17.94 ± 12.65	t=-4.62	0.001**
AST (U/L)	59.8 ± 26.2	58.17 ± 19.6	t=0.341	0.73
ALT (U/L)	59.08 ± 29.84	55.52 ± 23.5	t=0.63	0.52
Platelet count ($x10^3$/ µl)	213.52 ± 30.15	154.52 ± 31.5	t=9.174	0.03**
Viral load	823282.76 ± 904438.74	1262480.56 ± 1310764.674	t=-1.870	0.07
Presence of diabetes	8/38	8/38	χ^2=0.39	0.53

Hb; hemoglobin content, **TLC**; total leukocytic count, **TSH**; thyroid stimulating hormone, **AFP**; alpha fetoprotein, **AST**; aspartate aminotransaminase, **ALT**; alanine aminotransaminase. **highly significant

Table.2 Histopathological (fibrosis) and activity stages of both groups

	Responders (64) n (%)	**Non responders (64) n (%)**	χ^2	***P***
Stage of fibrosis				
F1	26 (56.5)	12 (26.1)		
F2	18 (39.1)	28 (60.9)	1.985	0.739
F3	2 (4.3)	6 (13)		
Stage of activity				
A1	30 (65.2)	22 (47.8)		
A2	16 (34.8)	22 (47.8)		
A3	---- (0.0)	2 (4.3)	4.17	0.12

Table.3 IL28 B (rs12979860) gene polymorphism and genotypes in both groups

Genotypes	**Responders (n=46)**	**Non responders (n=46)**	**Total (n=92)**	χ^2	***P***
CC	20	6	26		
CT	22	32	54		
TT	4	6	10	9.74	0.002**
Undetermined	0	2	2		
C	62	44	80		
T	30	44	64		

**highly significant

Table.4 Relation between IL28B rs12979860 genotypes and different characters of studied patients

Character	**Favorable genotype (n=26)n (%)**	**Unfavorable genotypes (n=64)n(%)**	χ^2	***P***
Sex				
Male	17 (65.4)	54 (84.4)		
Female	9 (34.6)	10 (15.6)	5.54	0.065
Biochemical & Virological				
ALT				
< 40 U/L	1 (3.8)	23 (35.9)		
≥ 40 U/L	25 (96.2)	41 (64.1)	9.7	0.002**
Viral load				
< 600.000	11 (42.3)	34 (53.1)		
≥ 600.000	15 (57.7)	30 (46.9)	0.865	0.35
Fibrosis stage				
F1+F2	22 (84.6)	60 (93.7)		
F3	4 (15.3)	4 (6.2)	1.905	0.186
Activity stage				
A1+A2	24 (92.3)	64 (100)		
A3	2 (7.6)	---(0.0)	5.03	0.025*
BMI				
< 30	21 (80.7)	51 (79.6)		
≥ 30	5 (19.2)	13 (20.3)	0.014	0.907
Presence of diabetes				
Yes	22 (84.6)	52 (81.25)		
No	4 (15.3)	12 (18.75)	0.143	0.705

*significant **highly significant

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