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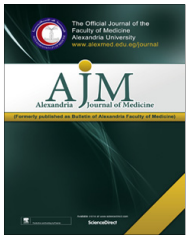

Role of IL-28B polymorphisms in virologic response to combined pegylated interferon and ribavirin therapy in genotype 4 chronic HCV infected patients with and without cirrhosis

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Abstract *Background:* Chronic hepatitis C virus (HCV) represents one of the common causes of chronic liver disease worldwide with Egypt having the highest prevalence, namely genotype 4. The rs12979860 CC genotype of the interleukin 28B (IL28B) polymorphisms is associated with high rates of sustained virological response to pegylated interferon and ribavirin in HCV genotype-1 patients. Data on other genotypes are more limited.

Objective: We aim to evaluate the predictive power of the rs12979860 IL28B single nucleotide polymorphisms for treatment response at 3 and 6 months in chronic HCV genotype 4 Egyptian patients in relation to other predictors.

Patients and methods: The study included 60 chronic HCV Egyptian patients receiving pegylated interferon and ribavirin therapy. Patients were classified into 2 groups; 30 patients with compensated cirrhosis, and 30 patients without cirrhosis. We analyzed selected pretreatment factors such as age, sex, HCV viral load, anti-schistosomal antibodies, insulin resistance, alpha fetoprotein, low and high density lipoproteins and single nucleotide polymorphisms of IL28B and tried to find out which of them influence sustained virological response.

Results: In univariate analysis, CC genotype showed a significant association with sustained virological response at 6 months among the cirrhotic patients (81.8% responders had the CC genotype, 58.3% had the CT/TT genotype) ($p = 0.009$). While in multivariate analysis, the presence

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of cirrhosis showed higher risk of failed response at 3 and 6 months ($p = 0.016$ and 0.020 respectively). Also, positive schistosoma serology was an important negative predictor of response at 3 and 6 months in both groups ($p = 0.003$ and 0.001 respectively).

Conclusion: In Egypt, where chronic HCV genotype 4 and schistosoma coinfection predominate, both schistosoma infection and cirrhosis are more potent than IL28B polymorphisms as strong baseline negative predictors of hepatitis C treatment response.

1. Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. The long-term impact of HCV infection is highly variable, from minimal changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma (HCC). The number of chronically infected persons worldwide is estimated to be about 160 million, but most of them are unaware of their infection.[1]

In fact, Egypt has the largest epidemic of HCV in the world with an overall serum positive prevalence of 14.7% as reported by the Egyptian demographic health survey, with >90% of cases having HCV genotype 4.[2,3] Hepatitis C virus (HCV) genotype 4 is the most frequent cause of chronic hepatitis C in the Middle East, North Africa, and sub-Saharan Africa.[4] It has been reported to be frequently associated with cirrhosis and a poor response to interferon (IFN).[5,6] Furthermore, epidemiological reports indicate that HCV genotype 4 is beginning to spread from its native African and Middle Eastern origins to countries of Southern Europe such as France, Italy and Spain and in some foci in the United States, particularly among intravenous drug users.[7–9]

Chronic hepatitis C proceeds toward cirrhosis over several decades. On average, 10 to 20% of patients develop cirrhosis over 20–30 years of infection.[1] Until 2011, treatment with pegylated interferon (PEG-IFN) and ribavirin (RBV) was the standard of care (SOC) for all HCV genotypes, with HCV clearance depending both on virus and host-related factors.[10]

It has been reported that treatment with conventional IFN is less effective in patients with genotypes 1 and 4 than in patients with genotypes 2 and 3.[11] European Association for the Study of the Liver recommended that liver disease severity should be assessed prior to therapy. Identifying patients with cirrhosis is of particular importance, as their prognosis, their likelihood of response and the duration of therapy are altered.[1]

Currently the treatment of chronic hepatitis is inclined toward individualized therapy, based on the knowledge of factors predicting response to treatment.[12] All treatment-naïve patients with compensated disease due to HCV should be considered for therapy. The goal of therapy is to eradicate HCV infection to prevent liver cirrhosis, HCC, and death. The endpoint of therapy is undetectable HCV RNA in a sensitive assay (<15 IU/ml) 12 and 24 weeks after the end of treatment (i.e. sustained virologic response SVR12 and SVR24). In patients with cirrhosis, HCV eradication reduces the rate of decompensation and will reduce, albeit not abolish the risk of HCC. In these patients screening for HCC should be continued.[1]

Several independent genome-wide association studies (GWAS) reported single nucleotide polymorphisms (SNPs) rs12979860, located 3 kilobases upstream of the IL28B gene, encodes a type III IFN (IFN-3) is associated with more than a 2-fold difference in the rate of SVR.[6] CC genotype of IL-28B is associated with a 2–3-fold increase in sustained virologic response (SVR) compared to CT or TT genotype.[13,14]

The mechanism by which SNPs influence the outcome of HCV infection and its treatment is not clear. It is suggested that regulation of the promoter region of IL28B in antiviral activity may also affect two other genes belonging to interferon (IFN) λ family encoded in this region.[15] There are a few data so far regarding the role of IL28B polymorphism in HCV-4 patients with respect to response to antiviral therapy or fibrosis progression.[16] The use of these genetic markers may help us to select patients who are more or less prone to respond to pegylated interferon plus ribavirin.

2. Aim of the work

This study assessed the predictive power of rs12979860 IL-28B variations on the response to PEG-IFN/RBV therapy in a group of Egyptian patients infected with chronic hepatitis C (CHC) genotype 4 with and without cirrhosis, in relation to other predictors of response.

3. Patients and methods

3.1. Ethical aspects

The study was approved by the local ethics committee of Alexandria University. All patients provided written informed consent to participate in this study.

3.2. Patients and clinical data

Chronic infection was defined as a detectable HCV RNA for at least 6 months.[17] The current study included 60 patients with chronic HCV infection classified according to liver biopsy and laboratory parameters into 2 groups: 30 patients with chronic HCV and compensated cirrhosis, and 30 chronic HCV infected patients without cirrhosis. All patients underwent treatment under a standard protocol with PEG-IFN and weight-based ribavirin (RBV) for 48 weeks. Patients were recruited from the Tropical department of Alexandria Main University Hospital.

Patients were subjected to thorough history taking, clinical examination and routine pre-treatment work up. The baseline HCV RNA load and subsequent viral concentrations in response to treatment were measured using a quantitative polymerase chain reaction (PCR) assay according to the available technique (Applied Biosystem, USA), with a detection

limit of 30 IU/ml. The HCV genotype was determined using the INNO-LiPA v2.0 (Innogenetics, Zwijndrecht, Belgium) HCV assay.

Histopathological examination of histological activity and degree of hepatic fibrosis, of ultrasound guided percutaneous liver biopsy, was performed according to the Metavir score.[18] The exclusion criteria included any cause of liver disease other than chronic HCV based on the patient history, laboratory or liver biopsy findings as: HBV/HIV coinfection, autoimmune hepatitis, hemochromatosis, Wilson's disease, Alpha 1-antitrypsin deficiency, alcoholic liver disease, drug induced liver disease, decompensated liver disease[19] (Total serum bilirubin >1.5 g/dL; INR >1.5; serum albumin <3.4; platelet count $<75{,}000$ mm) and evidence of hepatic decompensation (hepatic encephalopathy or ascites), hepatic tumors, pregnancy or breast feeding, advanced ischemic heart disease and uncontrolled diabetes (Hb A1C $>8.5\%$).

The outcome variable in this study was sustained virologic response, defined as non detectable HCV RNA in serum 24 weeks after the completion of HCV therapy (SVR24). Sustained virologic response is defined as non detectable HCV RNA in serum 12 weeks after the completion of HCV therapy (SVR12).[1] Patients with detectable plasma HCV RNA at week 24, were considered to be non responders.[20]

3.3. Determination of the IL28B genotype

Genomic DNA are isolated from 200 µl of blood using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol, and preserved at −70 °C for genetic determinations. SNPs near *IL28B* (marker *rs12979860*) were genotyped using TAQMAN genotyping assay (Applied Biosystems). Primer and probe sequences were: Forward Primer 5.-GCCTGTCGTGTACTGAACCA, Reverse Primer 5.-GCGCGGAGTGCAATTCAAC, Probe (C allele) 5.-VIC-TGGTTCGCGCCTTC, Probe (T allele) 5.-FAM-CTGGTT CACGCCTTC. Genotyping was performed in 25 µl reaction volume containing 10 ng DNA, 12.5.l TaqMan® Universal PCR Master Mix and 1.25.l (40×) Custom® SNP Genotyping Assays.

3.4. Statistical analysis

Data were analyzed using SPSS version 16.0, the 0.05 level was used as the cut off value for statistical significance. Counts and percentage were used for describing and summarizing qualitative data, the arithmetic mean ($\overline{X}$) and the standard deviation (SD) were used as measures of central tendency and dispersion respectively for normally distributed quantitative data. The association between two qualitative variables was done using Chi square test (χ^2) and Fisher Exact test (FET), and Monte Carlo Exact probability (MCP) was used when χ^2 is not valid ($>20\%$ of the expected cells have count less than 5), Mann–whitney Z test was done for comparing two independent quantitative non-normally distributed variables. A stepwise logistic regression model to extract the most significant factors contributing in the response of the patient in 3 and 6 months was done.

4. Results

4.1. Features of the study population

The study group consisted of 60 chronic HCV infected patients, out of whom 30 were cirrhotic. Clinical and demographic characteristics of the patients enrolled in this study are summarized in Table 1. All patients were infected with HCV genotype 4 and were on anti-viral therapy. These characteristics were comparable in the 2 groups with no statistical significance except for the mean age which was slightly higher among the HCV group as compared to the group of HCV combined with cirrhosis, this difference although very small showed to be significant. The AST showed a significantly higher mean among the chronic HCV and cirrhosis group as compared to the other group. Schistosomal serology showed significant association, denoting a more frequent schistosoma infection among chronic HCV cirrhotic patients. All other studied lab parameters showed some difference but yet not statistically significant. The genotype frequency of rs12979860 CT was the most common (56.7%), occurring more frequently in non-cirrhotic patients (60%), than in chronic HCV with the cirrhosis group. This was statistically non significant.

4.2. Outcome of antiviral therapy (Table 1)

Cirrhotic patients achieved significantly lower SVR12 and SVR24 rates than non cirrhotics (12/30 = 40% and 14/30 = 46.7%) versus (23/30 = 76.7% and 24/30 = 80%) ($p = 0.004$ and 0.007) respectively.

4.3. Baseline predictors of treatment outcome

4.3.1. SVR12

Higher BMI was significantly associated with failure of response among the non cirrhotic group, when compared to responders (30.7 ± 1.9 and 27.7 ± 2.8 respectively) ($p = 0.015$) while higher GGTP, INR, PT, AFP, insulin resistance index (IR) and lower serum albumin levels, as well as a higher rate of positive schistosomal serology, were significantly associated with failure of response in the cirrhotic group ($p < 0.05$) (Table 2).

In the logistic regression model, we evaluated the odds ratio (OR) and the 95% confidence interval (CI) of SVR12, depending on the most significant predictors of response after 3 months.

The OR revealed that cirrhotic patients had a 6.39 higher risk of non response than non cirrhotic patients ($p = 0.016$). Presence of schistosomal antibodies had nearly 12 times greater probability of achieving non response compared with negative schistosomal serology ($p = 0.003$). Also patients with higher BMI had 19-fold higher chance more than non obese (Normal and Overweight) to be non responders ($p = 0.001$) (Table 3).

4.3.2. SVR24

In the non cirrhotic group, higher mean of cholesterol and LDL levels, lower mean INR, PT and negative serology for schistosomiasis demonstrated a significant association with response.

Table 1 Demographic and biochemical features of patients included in this study.

Parameter	Group		Test of significance (p Value)
	Chronic HCV + cirrhosis (n = 30)	Chronic HCV (n = 30)	
Age (years)	45.3 ± 7.7	49 ± 7.7	Z = 2.01 (0.045)
Female (N/%) (17/28.3)	8 (26.7)	9 (30)	X^2 = 0.08
Male (N/%) (43/71.7)	22 (73.3)	21 (70)	p = 0.774
BMI (kg/m^2)	28 ± 3.1	28.4 ± 2.9	Z = 0.75 (0.451)
AST(IU/L)	53 ± 23.7	47.3 ± 38.6	Z = 2.20 (0.028)
Alb (g/dl)	3.4 ± 0.4	4.1 ± 0.53	Z = 1.19 (0.233)
GGTP (IU/L)	60.6 ± 47.3	72.8 ± 92.5	Z = 0.34 (0.734)
TLC count (×10^3/mm^3)	4.50 ± 1.43	4.89 ± 1.61	Z = 0.98 (0.328)
Hb (g/dl)	12.7 ± 1.3	12.7 ± 1.6	Z = 0.26 (0.796)
Platelets (×10^3/mm^3)	179.8 ± 63	183.5 ± 60.1	Z = 0.18 (0.859)
INR	1.1 ± 0.1	1.1 ± 0.0	Z = 1.61 (0.107)
PT	13.06 ± 1.37	12.29 ± 0.41	Z = 1.82 (0.069)
PCR-HCV (×10^6 IU/ml)	1.3 ± 1.4	1.8 ± 3.4	Z = 0.03 (0.976)
AFP(ng/ml)	9.1 ± 22.1	10.6 ± 22.2	Z = 0.26 (0.796)
Schistosomal serology (N/%)			
Negative (43/71.7)	17 (56.7)	26 (86.7)	X^2 = 6.65
Positive (17/28.3)	13 (43.3)	4 (13.3)	p = 0.010
Cholesterol (mg/dl)	149.4 ± 38.9	151.6 ± 30.6	Z = 0.40 (0.690)
HDL (mg/dl)	48.2 ± 18.2	53.9 ± 18.8	Z = 1.37 (0.171)
LDL (mg/dl)	78.9 ± 27.0	75.9 ± 29.8	Z = 0.53 (0.600)
IR	4.8 ± 10.2	2.4 ± 1.4	Z = 0.27 (0.784)
IL28B genotypes (N/%)			
CC (18/30)	11 (36.7)	7 (23.3)	MCp = 0.471
CT (34/56.7)	16 (53.3)	18 (60)	
TT (8/13.3)	3 (10)	5 (16.7)	
SVR12 (N/%)			
Responders (35/58.3)	12 (40)	23 (76.7)	X^2 = 8.5
Non responders (25/41.7)	18 (60)	7 (23.3)	p = 0.004
SVR24 (N/%)			
Responders (38/63.3)	14 (46.7)	24 (80)	X^2 = 7.18
Non responders (22/36.7)	16 (53.3)	6 (20)	p = 0.007

Note: BMI: body mass index, (calculated as the square of height in meters divided by weight in kilograms), AST: aspartate aminotransferase, Alb: albumin, GGTP: gamma-glutamyl transpeptidase, TLC: total leukocytic count, Hb: hemoglobin, PT: prothrombin time, AFP: alpha fetoprotein, HDL: high-density lipoprotein, LDL: low-density lipoprotein, IR: insulin resistance, SVR12: sustained virologic response 12 weeks after the completion of HCV, SVR24: sustained virologic response 24 weeks after the completion of HCV therapy. Data are presented as mean ± SD unless otherwise indicated.

In the cirrhotic group of patients, GGTP, INR, PT, AFP showed a higher mean among non responders than responders and this was shown to be significant. The higher mean of HDL, negative schistosomal serology and CC IL28 B genotype showed a significant association with SVR24. Nine responders (81.8%) had the CC genotype, and 5 (58.3%) had the CT/TT genotype (p = 0.009) (Table 4).

As for the OR and the 95% CI of SVR24 after 6 months, positive schistosomal serology is the strongest predictor of non response, being more potent (p = 0.001) than the presence of cirrhosis (p = 0.020) and IL28B genotype (Table 5).

5. Discussion

This study was undertaken to assess the predictive power of rs12979860 IL-28B variations on the response to PEG-IFN-./RBV therapy in a group of Egyptian patients infected with chronic HCV genotype 4 with and without compensated cirrhosis, in relation to other predictors of response.

There are many pretreatment factors such as age, sex, ethnicity, body mass index, insulin resistance, hepatic steatosis, and degree of liver fibrosis, HCV genotype, baseline viral load and viral kinetics during treatment which can influence the response to the therapy with Peg-IFN and ribavirin.[12]

Our findings showed that the higher mean age and the presence of negative schistosomal serology were statistically significant in the non-cirrhotic group (p = 0.045, p = 0.010 respectively), denoting a more frequent schistosoma infection among chronic HCV cirrhotic patients. Kamal et al. [21] reported that HCV/schistosomiasis coinfected patients have more rapid progression of hepatic fibrosis than those with HCV monoinfection.

Base line AST level was significantly lower in the non-cirrhotic group (p = 0.028), as reported by Al Ashgar et al.[22] who demonstrated that the AST reflects less severe histopathological parameters.

Recent genome-wide association studies (GWAS) have shown that human genetic variations (single-nucleotide polymorphisms, SNPs) around the gene for interleukin 28B

Table 2 Relationship between SVR12 and demographic and biochemical variables among both studied groups.

Parameter	Chronic HCV with cirrhosis		*p*-Value	Chronic HCV without cirrhosis		*p*-Value
	Responders (n = 12)	Non-responders (n = 18)		Responders (n = 23)	Non-responders (n = 7)	
Age (years)	43.1 ± 9.8	46.8 ± 5.8	0.106	48.4 ± 7.5	51.0 ± 8.5	0.446
BMI (kg/m^2)	27.2 ± 2.3	28.6 ± 3.6	0.363	27.7 ± 2.8	30.7 ± 1.9	0.015
Alb (g/dl)	4.2 ± 0.3	3.8 ± 0.4	0.018	4.1 ± 0.5	3.9 ± 0.7	0.507
GGTP (IU/L)	40.3 ± 29.0	74.2 ± 52.8	0.026	3.7 ± 96.9	67.0 ± 83.1	0.624
AST (IU/L)	51.9 ± 25.3	53.7 ± 23.3	0.932	51.2 ± 43.1	34.4 ± 11.6	0.508
TLC (×10^3/mm^3)	4.3 ± 1.1	4.6 ± 1.6	0.671	5.1 ± 1.5	4.2 ± 2.0	0.141
Hb (g/dl)	12.9 ± 1.5	12.5 ± 1.2	0.351	12.8 ± 1.7	12.3 ± 1.0	0.572
Platelets (×10^3/mm^3)	176.5 ± 54.7	181.9 ± 69.4	0.816	185.9 ± 65.5	175.6 ± 40.6	0.961
INR	1.0 ± 0.1	1.1 ± 0.1	0.029	1.0 ± 0.0	1.0 ± 0.0	0.499
PT	12.5 ± 0.8	13.5 ± 1.5	0.020	12.3 ± 0.4	12.2 ± 0.4	0.587
PCR-HCV (×10^6 IU/ml)	1722030.9 ± 1114642.4	1089318.8 ± 1480267.6	0.057	1112806.3 ± 1067043.9	4184974.9 ± 6605613.9	0.249
AFP (ng/ml) (mean ± SD)	4.1 ± 5.1	12.4 ± 28.1	0.028	8.0 ± 12.3	19.2 ± 41.4	0.731
Cholesterol (mg/dl)	152.3 ± 32.5	147.6 ± 43.4	0.687	154.3 ± 29.6	142.4 ± 34.4	0.364
HDL (mg/dl)	57.4 ± 23.1	42.0 ± 11.0	0.094	54.1 ± 20.8	53.3 ± 11.6	0.864
LDL (mg/dl)	73.8 ± 16.8	82.3 ± 32.1	0.485	79.2 ± 30.2	64.9 ± 28.0	0.229
IR	1.6 ± 0.7	6.9 ± 12.9	0.034	2.4 ± 1.4	2.3 ± 1.4	0.922
Male (N/%)	8 (36.4)	14 (63.6)	FETp = 0.678	15 (71.4)	6 (28.6)	FETp = 0.393
Female (N/%)	4 (50.0)	4 (50.0)		8 (88.9)	1 (11.1)	
Schistosomal serology (N/%)						
Negative	10 (58.8)	7 (41.2)	FET p = 0016.	20 (76.9)	6 (23.1)	FETp = 0.999
Positive	2 (15.4)	11 (84.6)		3 (75.0)	1 (25.0)	
IL28B (N/%)						
CC	6 (54.5)	5 (45.5)	MCP = 0.267	5 (71.4)	2 (28.6)	MCP = 0.591
CT	6 (37.5)	10 (62.5)		13 (72.2)	5 (27.8)	
TT	0 (0.0)	3 (100)		5 (100)	0 (0.0)	

Data are presented as mean ± SD unless otherwise indicated.

Table 3 Odds ratio of SVR12 depending on the patient group, BMI and schistosomal serology.

Factors affecting SVR12	p value	OR	95% CI
Cirrhotic group	0.016	6.39	1.41–28.87
BMI	0.001	19.07	3.15–115.66
Positive schistosomal serology	0.003	12.88	2.34–70.83
Model X^2 = 26.57 p = 0.000			

OR: odds ratio, CI: confidence interval.

(IL-28B) may explain differences in the results of the treatment of adults chronically infected with HCV and that they can be useful as therapy response markers.[17]

The IL28B polymorphism rs12979860 has a marked differential distribution between racial groups, being least frequent in African Americans, most frequent in Asians, and with an intermediate frequency in Hispanics and Caucasians.[23]

This global difference of allele frequency might explain the ethnic variations seen in the treatment response among these populations.[24]

The frequency of IL28 genotype in our genotype 4 Egyptian patients showed that almost half of them were of the CT genotype (56.7%) followed by CC (30%) while TT had the least expression (13%). The CC genotype occurred more frequently in cirrhotic patients compared to non cirrhotic patients (11/30 vs. 7/30), unlike CT and TT genotypes that were more frequent in chronic HCV patients compared to HCV with cirrhosis (18/30 and 5/30 vs. 16/30 and 3/30 respectively), but this was statistically non significant.

These results were matching with the study of Khairy et al.[23] who reported that the frequency of IL28 genotype in their 263 chronic HCV patients genotype 4 Egyptian patients was 56% for the CT genotype, followed by 25% for CC while TT had the least expression (19%). De Nicola group (2012)[25] which included 128 patients with genotype 4, 68% Egyptians, showed 63% CT, 14% TT, and 23% CC expression. Also, Asselah and colleagues[26] studied 164 patients with genotype 4 (43% Egyptians), and found the difference in distribution of IL 28 B genotype between Egyptians and Subsaharan Africans; in the Egyptian ethnicity the frequency was 55% CC, 11% TT and 34% CT, while in the in sub-Saharan group the TT genotype was the most predominant form (48%). El-Awady and colleagues during 2012 also in a study on genotype 4, found that the frequencies of genotypes were 48% CC, 14% TT, and 38% CT for their studied patients.[27]

This was inconsistent with previous results describing a higher incidence of TT or CT allele in cases of cirrhosis and faster fibrosis progression in HCV-infected liver transplant recipients and liver from donors with the TT genotype,[28,29] and that TT genotype occurred more frequently in patients with end stage liver disease,[23] as well as that of Ciesla et al.[15], who studied 64 Caucasian chronic HCV patients on IFN and

Table 4 Relationship between SVR24 and demographic and biochemical variables among both studied groups.

Parameter	Chronic HCV with cirrhosis		*p*-Value	Chronic HCV without cirrhosis		*p*-Value
	Responders ($n = 14$)	Non-responders ($n = 16$)		Responders ($n = 24$)	Non-responders ($n = 6$)	
Age (years)	44.1 ± 9.0	46.4 ± 6.5	0.337	49.3 ± 7.6	48.0 ± 8.7	0.815
BMI (kg/m^2)	28.2 ± 2.7	27.9 ± 3.6	0.506	28.8 ± 2.7	26.7 ± 3.3	0.186
Alb (g/dl)	4.1 ± 0.4	3.8 ± 0.4	0.132	4.1 ± 0.5	4.0 ± 0.6	0.406
GGTP (IU/L)	32.6 ± 17.6	85.2 ± 51.8	0.001	72.3 ± 102.2	71.7 ± 39.4	0.169
AST (IU/L)	53.1 ± 28.2	52.8 ± 19.9	0.901	50.0 ± 42.7	36.5 ± 8.2	0.917
TLC ($\times 10^3$/mm^3)	4.6 ± 1.8	4.4 ± 1.1	0.917	4.7 ± 1.7	5.6 ± 1.4	0.213
Hb (g/dl)	12.4 ± 1.2	12.9 ± .4	0.454	12.8 ± 1.7	12.4 ± 1.3	0.604
Platelets ($\times 10^3$/mm^3)	175.4 ± 52.2	183.6 ± 72.6	0.835	182.3 ± 58.8	188.3 ± 71.0	0.795
PT	12.4 ± 0.8	13.7 ± 1.5	0.001	12.2 ± 0.4	12.6 ± 0.5	0.036
INR	1.0 ± 0.1	1.1 ± 0.1	0.000	1.0 ± 0.0	1.1 ± 0.0	0.058
PCR-HCV ($\times 10^6$ IU/ml)	1801612.1 ± 1743753.6	940596.3 ± 763112.5	0.212	1977492.3 ± 3780153.0	1238258.8 ± 1115577.7	0.717
AFP (ng/ml)	3.3 ± 2.7	14.2 ± 29.7	0.010	5.6 ± 7.3	30.9 ± 44.7	0.062
Cholesterol (mg/dl)	158.1 ± 38.0	141.8 ± 39.2	0.339	158.1 ± 29.7	125.5 ± 18.7	0.022
HDL (mg/dl)	57.3 ± 21.8	40.2 ± 9.2	0.019	52.8 ± 20.6	58.3 ± 9.2	0.222
LDL (mg/dl)	75.9 ± 24.8	1.5 ± 29.4	0.708	81.2 ± 29.9	54.5 ± 19.3	0.038
IR	2.0 ± 1.4	7.2 ± 13.7	0.096	2.3 ± 1.4	2.5 ± 1.4	0.678
Male (*N*/%)	9 (40.9)	13 (59.1)	FETp = 0.417	16 (76.2)	5 (23.8)	FETp = 0.400
Female (*N*/%)	5 (62.5)	3 (37.5)		8 (88.9)	1 (11.1)	
Schistosomal serology (*N*/%)						
Negative	13 (76.5)	4 (23.5)	FETp = 0.000	23 (88.5)	3 (11.5)	FETp = 0.018
Positive	1 (7.7)	12 (92.3)		1 (25.0)	3 (75.0)	
IL28B (*N*/%)						
CC	9 (81.8)	2 (18.2)	MCP = 0.009	7 (100.0)	0 (0.0)	MCP = 0.372
CT	4 (25.0)	12 (75.0)		13 (72.2)	5 (27.8)	
TT	1 (33.3)	2 (66.7)		4 (80.0)	1 (20.0)	

Data are presented as mean ± SD unless otherwise indicated.

Table 5 Odds ratio of SVR24 depending on the patient group, type of polymorphism of IL28B and schistosomal serology.

Factors affecting SVR24	*p* value	OR	95% CI
Cirrhotic group	0.020	11.40	1.46–88.84
CT	0.043		
CC	0.384	2.99	0.25–35.24
TT	0.064	0.03	0.00–1.22
Positive schistosomal serology	0.001	132.25	7.34–2383.52
Model $X^2 = 46.43$	$p = 0.000$		

OR: odds ratio, CI: confidence interval.

Ribavirin therapy, where patients with the TT genotype had a more active state of necroinflammation in the histological analysis, and borderline significance of greater severity of fibrosis. Taking into consideration that the distribution of IL28B genotypes among both studied groups in the present study was not statistically significant, it was not possible to confirm the impact of the genotype on the progress of CHC.

Endpoint of therapy is the SVR, defined by undetectable HCV RNA 24 weeks after the end of therapy, as assessed by a sensitive molecular method with a lower limit of detection <15 IU/ml (SVR24). Long-term follow-up studies have shown that an SVR corresponds to a definitive cure of HCV infection in more than 99% of cases.[1] The validity of using undetectable HCV RNA at 12 weeks after the end of therapy (SVR12) has been accepted by regulators in the US and Europe, given that the concordance with SVR24 is 99%.[30]

Various studies from European and Middle Eastern countries showed that the SVR in genotype 4 for combination therapy, pegylated interferon and ribavirin, ranges between 43% and 70%.[31,32]

In the current study, SVR12 and SVR24 showed significant association with non cirrhotic patients ($p = 0.004$, $p = 0.007$ respectively) denoting higher response among the same group.

This was close to Aghemo et al. results,[33] where 53% of 409 HCV cirrhotic patients achieving an SVR compared to 75% of non cirrhotics, and Thompson et al. study,[34] showing lower SVR rates in IL28B CC patients with bridging fibrosis and cirrhosis.

Little is known about predictors of response within populations infected with genotype 4. In previous studies on genotype 4; age, pretreatment viral load, and stage of fibrosis were considered as good predictive factors.[35,36]

The gene expression and the role of IL28B gene SNP rs12979860 in response to treatment in genotype 4 were recently studied by limited research with CC genotype of higher response rate.[25,26]

Relationship between baseline parameters and virological response in the current study showed that, higher base line GGTP, INR, PT, AFP levels, higher IR index, lower albumin level and positive schistosomal serology antibodies are significantly associated with unfavorable outcomes after 3 months

(SVR12) in cirrhotic group, while lower BMI value was the only significant predictor of SVR12 in non cirrhotic group.

The role of insulin resistance in response to HCV therapy is controversial,[20] the correlation between insulin resistance and higher BMI and response to antiviral therapy observed in the present study is consistent with Ciesla et al. results.[15]

In this study, although CC patients are more prone than patients with the T allele to achieve an SVR12 in cirrhotic patients, this is not the case in HCV non cirrhotic patients, where CT/TT alleles are more prone to achieve SVR12, but this was statistically non significant.

Concerning rates of SVR24 in our study, cirrhotic patients (genotype CC) did achieve it more frequently as compared to genotype CT/TT, which was statistically significant ($p = 0.009$), while in the non cirrhotic group, this difference was statistically non significant.

This was in harmony with Khairy et al.[23] study, where the CC genotype was significantly correlated with SVR in comparison to CT and TT. The response rates were 50%, 47.4% and 25% for genotype CC, CT, and TT respectively. Absence of C allele (TT genotype) was associated with 75% failure of response; either early failure, e.g. non response (54.5%), or late failure, e.g. relapsers (20.5%). This is in agreement with previous studies reported on genotype 1[15,37,38] and studies conducted on genotype 4.[26,25]

On the other hand, in the study of Ciesla et al.,[15] 60% of patients with SVR had TT (8%) or CT (52%) genotypes, which are not known factors associated with higher IFN responsiveness. These values are similar to those described in previous reports, where analysis of the differences in the course of therapy in patients with the CT allele and a SVR revealed higher baseline platelet and neutrophil levels. A low baseline platelet count was significantly associated with the need for IFN dose reduction in the group without a SVR.[39]

In the present study, negative serology for schistosoma antibodies was a common predictor of SVR24 in both groups of patients. Besides, we found 2 independent prognostic factors for SVR24 in cirrhotic patients: Lower INR and PT level and higher cholesterol and LDL levels. As regards the non cirrhotic group, higher HDL level was a predictor of SVR24, while higher GGT, INR, PT and AFP levels were associated with failure of response. Base line viral load and base line AST levels were not predictors of SVR24 in both groups.

In the study of Abdel-Rahman et al.[40] the EVR, virological response at week 24, and SVR were significantly higher in patients with negative schistosomal serology. This finding may be attributed to the fact that coinfected patients with a down-regulated immune response to HCV leading to reduced IFN., interleukin (IL)-4 and IL-10 secreted by HCV-specific T cells.

In contrast to our study, the analysis presented by McCarthy et al.[38] showed that lower viral load before treatment predicted higher SVR. This phenomenon was not confirmed in our study, possibly due to the small sample size. This result has been affirmed by Correia et al.[41] emphasizing that the baseline HCV RNA is trivial for SVR.

In the study of Khairy et al.[23], lower base line AST but not ALT was an independent predictor of SVR in patients with chronic HCV genotype 4. This was in accordance with Al Ashgar et al. study[22] which demonstrated that the AST reflects less severe histopathological parameters in sustained responders.

Concerning the plasma levels of LDL, in vitro studies have shown that LDL may competitively inhibit the binding of HCV to the LDL receptor, which functions as one of the cellular receptors for HCV. This competitive blockade would hamper the infection of hepatocytes with HCV. Accordingly, higher levels of plasma LDL (as in the current results) have been shown to be an independent predictor of SVR, in studies specifically designed to appraise this issue.[20]

Higher serum AFP level was a strong negative predictor of SVR24 in the studied patients. Previous studies including HCV genotype 4[42,43] and genotype 1[44,45] highlighted the same findings. Abdoul et al.[46] examined the association between serum alpha-fetoprotein level and sustained SVR in 93 chronic hepatitis C patients and found that the SVR rate was much higher among patients with serum AFP levels below rather than above a median value of 5.7 ng/ml, denoting that serum AFP should be added to the list of factors predictive of treatment response in chronic hepatitis C.

The results of multivariate analysis in this study show that 3 factors only were considered to be significant negative predictors of response after 3 months (SVR12), which are presence of cirrhosis, high BMI and positive schistosomal serology. As regards SVR24 after 6 months, positive schistosomal serology is the strongest predictor of non response, being more potent than the presence of cirrhosis and IL28B genotype.

We can conclude that concomitant absence of cirrhosis and negative serology for bilharzial infections do seem to improve responses achieved with pegylated interferon plus ribavirin combination therapy for HCV genotype 4. In Egypt, where chronic HCV genotype 4 and schistosoma coinfection predominate, both schistosoma infection and cirrhosis are more potent than IL28B polymorphisms as strong baseline negative predictors of hepatitis C treatment response.

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Conflict of interest

None declared.

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