



FULL LENGTH ARTICLE

Correlation between IL28B/TLR4 genetic variants and HCC development with/without DAAs treatment in chronic HCV patients

Ghada M. Salum ^a, Reham M. Dawood ^{a,*}, Mai Abd el-Meguid ^a,
Noha E. Ibrahim ^a, Ashraf O. Abdel Aziz ^b, Mostafa K. El Awady ^a

^a Department of Microbial Biotechnology, Genetic Engineering Division, National Research Centre, 33 EL Bohouth St. (former El Tahrir St.), Dokki, Giza, P.O. 12622, Egypt

^b Department of Tropical Medicine, Faculty of Medicine, Cairo University, Giza, P.O. 12622, Egypt

Received 4 April 2019; received in revised form 13 May 2019; accepted 20 May 2019

Available online 27 May 2019

KEYWORDS

DAAs;
DCV;
HCV;
HCC;
IL28B;
SOF;
TLR4

Abstract In Egypt, Sofosbuvir (SOF) in combination with Dataclasvir (DCV) is the broadly used DAAs with excellent therapeutic profile. This study is designed to explore the relation between IL28B/TLR4 genetic variants and each of the followings; HCC development post SOF/DCV treatment, progression to HCC in naïve patients and SOF/DCV therapy outcome. A total of 493 blood samples were collected (controls ($n = 70$); HCV patients treated with SOF/DCV ($n = 252$) of whom 65 patients developed HCC, 187 patients didn't develop HCC (125 responders, 62 relapsers); naïve HCV patients ($n = 171$) had early ($n = 48$), late liver fibrosis ($n = 21$) and HCC ($n = 102$)). Both SNPs were genotyped using a TaqMan 5' allelic discrimination assay. At IL28B rs12979860 SNP, the C allele was significantly correlating with the response rate more than T allele (OR 1.9, 95% CI 1.29–2.9, $p = 0.004$), while at TLR4 rs4986791 SNP, no association was found (OR 6.5, 95% 0.57–75.28, $p = 0.09$). Both SNPs couldn't detect the probability for HCC emergence after treatment. In naïve patients, the protective alleles were detected in their lowest frequency in HCC patients ($p = 0.1$, for rs12979860 and, $p = 0.001$ for rs4986791). SOF/DCV combination improved SVR rates in HCV genotype 4a infected patients regardless of IL28B genotype, with the best rates in those lacking the T allele.

Abbreviations: SOF, Sofosbuvir; DCV, Dataclasvir; DAAs, Direct acting antiviral agents; HCV, hepatitis C virus; HCC, Hepatocellular carcinoma; IFN λ , Type III IFNs; SNP, single nucleotide polymorphism; SVR, sustained virological response; PAMPs/DAMPs, pathogen/damage associated molecular patterns; TLRs, toll like receptors; JAK/STAT, Janus kinase/signal transducers and activators of transcription; ISGs, interferon-stimulated genes.

* Corresponding author. Microbial Biotechnology Department, Genetic Engineering Division, National Research Center, EL Bohouth St. (former El Tahrir St.), Dokki, Giza, P.O. 12622, Egypt.

E-mail address: rmhaemd@hotmail.com (R.M. Dawood).

Peer review under responsibility of Chongqing Medical University.

<https://doi.org/10.1016/j.gendis.2019.05.004>

2352-3042/Copyright © 2019, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Direct acting antiviral agents (DAAs) have ultimately simplified the management of hepatitis C virus (HCV) infection to most health care providers.¹ In Egypt, Sofosbuvir (SOF) in combination with Dataclasvir (DCV) is the broadly used DAAs with excellent therapeutic profile.^{2,3} SOF is a pyrimidine nucleotide analogue that interrupts viral replication.⁴ Meanwhile, reports on the impact of DAAs therapy on the augmented appearance of Hepatocellular carcinoma (HCC) are conflicted.^{5–7} Deeper understanding of the determinants of DAAs therapy outcome is a must.

Type III IFNs (IFN λ) is a family of four genes; the most prominent one is IFN λ 3 (IL28B) which is a prime chemical courier of immune reactions.⁸ A single nucleotide polymorphism (SNP) in the promoter region of the IL28B gene, the rs12979860, was highlighted as the strongest baseline predictor for interferon-based therapy outcomes in HCV genotype 4 patients, where sustained virological response (SVR) rates seem to be IL28B dependent.^{9–13} Nonetheless, the relevance of IL28B polymorphism to SOF/DCV regimen is not yet clear.

HCC can be stimulated by pathogen recognition receptors (PRRs), which are the molecules involved in the pathogen/damage associated molecular patterns (PAMPs/DAMPs) recognition.¹⁴ Among PRRs, toll like receptors (TLRs), especially TLR4 are the most important PRRs¹⁵ which have been evaluated regarding their roles in the pathogenesis of HCC.¹⁶ Upon binding to lipopolysaccharide, TLR4 launches an intricate complex with myeloid differentiation factor-2, ending with the provocation of adaptor proteins to the intracellular Toll/IL-1 receptor domains.^{17–19}

TLR4 gene polymorphisms may be associated with at least twelve types of cancer especially HCC.^{20–26} The most intensively investigated TLR4 gene polymorphism is (rs4986791). Previous studies have already demonstrated that liver fibrosis and HCC risks do not coincide in carriers of the TLR4 minor allele.⁵²

With scanty and discordant information about the association of HCC with DAAs therapy, we hypothesized that both IL28B and TLR4 polymorphisms (rs12979860 and rs4986791, respectively) may predict the poor response to DAAs and/or the probability for progression to HCC among HCV-chronically infected patients. To investigate this hypothesis, we tested both SNPs on two groups of HCV-chronically infected patients (genotype-4); the first group was treated with SOF/DCV who progressed to HCC after DAAs treatment (HCC post SOF/DCV) or did not progress to HCC (either responders or relapsers), the second group was treatment-naïve having different degrees of liver fibrosis.

Patients

Ethical statement

All eligible individuals agreed to voluntary participation and signed an informed consent form. The study was approved by the research ethics committee of National Research Center, Cairo, Egypt, in compliance with Helsinki Declaration 1975 revised in 2008 which contains regulatory norms and guidelines for research involving human beings.

Study population

A total of 493 subjects were included in this study and were categorized as follows:

- Healthy controls ($n = 70$): the enrolled 70 healthy subjects had no history of HBV infection (negative HBsAg), HCV infection (negative for HCV Ab & RNA) or Schistosoma infection.
- Patients treated with SOF/DCV ($n = 252$): All patients were treated with combination of Sofosbuvir and Dataclasvir for 12 or 24 weeks at Endemic Medicine Department and Kasr Al-Aini Viral Hepatitis Center, Faculty of Medicine, Cairo University between January 2017 and March 2018. Of 252 patients, SVR was achieved in one hundred and twenty five patients (50%) (responders). Definition of SVR was undetectable serum HCV-RNA 24 weeks after cessation of treatment. Other sixty two patients (25%) were persistent to infection; they have quantifiable serum HCV RNA levels at the end of the treatment (relapsers). Sixty five patients (25%) developed HCC during or within 6-mo surveillance period following SOF/DCV treatment confirmed via imaging and histopathological examination (HCC post SOF/DCV).
- Naïve HCV-chronically infected patients ($n = 171$): the untreated patients were classified according to the METAVIR histological liver biopsy grading into early fibrosis (EF (F0–F1), $n = 48$), late fibrosis (LF (F2–F4), $n = 21$) and HCC ($n = 102$).

All HCV patients were clinically investigated at the Department of Tropical Medicine, Kasr El Ainy Hospital, Cairo University. All patients were also evaluated by clinical and laboratory parameters, including biochemical (alanine aminotransferase (ALT), aspartate aminotransferase (AST), Albumin, Bilirubin Total and platelets count (Plt)) and serological test (anti-HCV) and histopathology of liver biopsy. The HCV-RNA levels in this study were measured using the Artus HCV RT-PCR Quantification Kit (Qiagen) on Real-Time PCR system according to the manufacturer's protocol. The diagnosis of HCC was made after reviewing images

generated with several imaging modalities. Patients having other cancers were excluded.

Methods

Extraction of peripheral blood DNA

Peripheral blood on EDTA was withdrawn from all subjects and genomic DNA was extracted using genomic DNA extraction kits (Qiagen, Milan Italy). Plasma were separated before DNA extraction and utilized for testing; HBVsAg, auto-immune antibodies and antibodies for Schistosoma. Purified genomic DNA samples were quantified using by ultraviolet absorbance at 260 nm using a Thermo Scientific NanoDrop™ Spectrophotometer. The DNA was stored at -20°C .

Genotyping of the IL28B rs12979860 C/T and TLR4 rs4986791 C/T polymorphisms

The SNPs on IL28B (rs12979860) and TLR4 (rs4986791) were identified using a real-time PCR protocol based on the pre-validated TaqMan MGB™ probe for allelic discrimination assay (Applied Biosystems). Briefly, 1.25 μL of a 40X combined primer and probe mix (ABI/Life Technologies, USA) was added to 12.5 μL of 2X TaqMan® Universal PCR master mix (ABI/Life technologies, USA) in a 25 μL final volume of DNase/RNase-free water (Invitrogen/Life Technologies, USA) and template. The cycle conditions were: 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min. The last two steps were repeated 40 times. The PCR run was performed on Rotor Gene real-time PCR system (Qiagen, Santa Clarita, CA). Allelic discrimination plots were produced in

Statistical Package for The Social Sciences (SPSS version 16.0; SPSS, Chicago, IL).

Statistical analysis

Data were analyzed using SPSS 16.0. Data were presented as mean $\pm$ standard deviation. Categorical variables were compared with the χ^2 or Fisher exact tests, each when appropriate, and the effect of differences was established by calculating the odds ratio with the 95% confidence interval (95%CI). According to variable distribution, 1-way ANOVA or nonparametric Kruskal–Wallis test was used for multi-group comparisons. The nonparametric Mann–Whitney U test was used to compare median values between two groups for quantitative data. A difference between groups was considered to be significant if $P < 0.05$.

Results

Baseline patient's features

The clinical features of all SOF/DCV treated patients (HCC post SOF/DCV, responders and relapsers) were shown in Table 1. Clinical features revealed no significant differences between all patients groups in several parameters such as: sex, AST, ALT, TSH and FT4 (Table 1, $p > 0.05$ for all parameters). Older age and late fibrosis stages (F2–F4) were significantly found in almost all HCC post treatment and relapsers patients (Table 1, $p < 0.01$ for all parameters). Furthermore, relapsers showed decreased albumin levels, total bilirubin, prothrombin time and increased platelet counts (Table 1, $p < 0.01$ for all parameters) when compared with Responders. Upon comparing to responders

Table 1 Baseline patient's (treated with SOF/DCV) characteristics.

	HCC post SOF/DCV N = 65	Responder to SOF/DCV N = 125	Relapsers to SOF/DCV N = 62	P value
Age (years)	59.14 $\pm$ 5.44 a	43.4 $\pm$ 9.77 b	62 $\pm$ 6.48 a	<0.01**
sex				0.2
	F (%)	65 (51.8%)	16 (25.8%)	
	M (%)	60 (48.2%)	46 (74.2%)	
Fibrosis				<0.01**
	F0–F2	29.7%	—	
	F3–F4	70.3%	100%	
^a Albumin (g/dL)	3.50 $\pm$ 0.61 a	4.09 $\pm$ 0.47 b	3.57 $\pm$ 0.39 ab	<0.01**
^a Bilirubin Total (mg/dL)	1.42 $\pm$ 0.94 a	1.01 $\pm$ 0.27 b	0.95 $\pm$ 0.42 ab	<0.01**
^a AST (U/L)	50.44 $\pm$ 21.16 a	48.48 $\pm$ 12.97 a	46.5 $\pm$ 14 a	0.74
^a ALT (U/L)	39.96 $\pm$ 16.8 a	42.93 $\pm$ 5.88 a	42.5 $\pm$ 9.98 a	0.3
^a INR	1.26 $\pm$ 0.20 b	1.06 $\pm$ 0.16 a	1.08 $\pm$ 0.1 a	<0.01**
^a PLT (cmm3)	124 $\pm$ 60 b	197 $\pm$ 80 a	252 $\pm$ 76 a	<0.01**
^a AFP (ng/mL)	6461 $\pm$ 33952 b	866 $\pm$ 5878 a	849 $\pm$ 1548 a	<0.01**
^a Child–Pugh score	5.98 $\pm$ 1.252 b	5.15 $\pm$ 0.390 a	5.08 $\pm$ 0.20 a	<0.01**
^a TSH (mU/L)	3.60 $\pm$ 1.18 a	3.73 $\pm$ 1.33 a	2.93 $\pm$ 1.91 a	0.430
^a FT4 (pmol/L)	1.45 $\pm$ 0.47 a	1.61 $\pm$ 0.61 a	1.60 $\pm$ 0.5 a	0.21

ALT points to serum alanine aminotransferase, AST points to serum aspartate aminotransferase; INR points to international normalized ratio of prothrombin time; PLT points to platelets count; AFP points to alpha fetoprotein; TSH points to thyroid stimulating hormone; FT4 points to free thyroxine.

"N" points to the sample size.

Identical letters in rows mean no significant difference at the level of 0.05.

Different letters in rows mean significant difference at the level of 0.05.

^a Data are dissected as mean and standard error of mean.

and relapsers groups, HCC post SOF/DCV patients showed decreased albumin levels, prothrombin concentration, platelet counts and elevated total bilirubin, AFP and ratio of prothrombin time (Table 1, $p < 0.01$ for all parameters).

The clinical features of chronic HCV patients (Early Fibrosis (EF), Late Fibrosis (LF) and HCC) were shown in Table 2. Clinical features revealed no significant differences between all patients groups in two parameters; AFP and FT4 (Table 2, $p > 0.05$ for all parameters). Older age and high levels of ALT & AST were significantly found in HCC. Furthermore, HCC patients showed decreased albumin levels, platelet counts, prothrombin time and increased total bilirubin when compared with early and late fibrosis patients (Table 2, $p < 0.01$ for all parameters).

Distribution of IL28B rs12979860 and TLR4 rs4986791 genotypes in Egyptian population

To estimate the frequencies of the IL28B and TLR4 genotypes in Egyptian population, the SNPs IL28B rs12979860 and TLR4 rs4986791 were genotyped using a TaqMan 5' allelic discrimination assay on DNA from 70 to 30 healthy controls, respectively (Table 3). Overall, the genotype distribution at the rs12979860-C/T locus was as follows: 31 (44%) individuals were CC homozygous, 30 (43%) were heterozygous CT and 9 (13%) were T/T homozygotes. At the TLR4 rs4986791 -C/T, the distribution was CC 28 (93.3%) and 2 (6.7%) were CT, while the TT homozygote was completely absent in our samples (Table 3).

Table 2 Baseline patient's characteristics.

	Early Fibrosis (F0–F1) N = 48	Late Fibrosis (F2–F4) N = 21	HCC N = 102	P value
Age (years)	42.04 ± 9.33	46.78 ± 10.67	59.6 ± 5.66	<0.01**
^a Albumin (g/dL)	4.26 ± 0.41	3.70 ± 0.41	3.32 ± 0.55	<0.01**
^a Bilirubin Total (mg/dL)	0.96 ± 0.26	1.13 ± 0.41	1.28 ± 0.72	<0.01**
^a AST (U/L)	45.44 ± 9.16	48.34 ± 15.16	55.08 ± 19.97	<0.01**
^a ALT (U/L)	42.03 ± 5.8	42.03 ± 5.49	46.93 ± 18.88	0.046*
^a INR	1.06 ± 0.13	1.19 ± 0.13	1.03 ± 0.18	<0.01**
^a PLT (cmm3)	205 ± 64	165 ± 125	128 ± 62	<0.01**
^a AFP (ng/mL)	6461 ± 33952 b	6461 ± 33952	3027 ± 21195	0.194
^a TSH (mU/L)	3.86 ± 1.14	3.29 ± 1.58	3.53 ± 1.23	0.030*
^a FT4 (pmol/L)	1.45 ± 0.47	1.45 ± 0.47	1.43 ± 0.48	0.177

^a Data are dissected as mean and standard error of mean.

* $p \leq 0.05$; ** $p \leq 0.01$.

ALT points to serum alanine aminotransferase; AST points to serum aspartate aminotransferase; INR points to international normalized ratio of prothrombin time; PLT points to platelets count; AFP points to alpha fetoprotein; TSH points to thyroid stimulating hormone; FT4 points to free thyroxine.

"N" points to the sample size.

Identical letters in rows mean no significant difference at the level of 0.05.

Different letters in rows mean significant difference at the level of 0.05.

Table 3 Distribution of IL28B rs12979860 and TLR4 rs4986791 genotypes in Healthy Subjects and Chronic HCV -Infected patients treated with SOF/DCV(Responder and Relapsers).

	Controls	Responders	Relapsers	P value
IL28 SNP rs12979860 genotype Number	N = 70	N = 125	N = 62	
CC	31 (44%)	40 (32%)	11 (18%)	0.003**
CT	30 (43%)	66 (53%)	31 (50%)	
TT	9 (13%)	19 (15%)	20 (32%)	
Alleles	92 (66%)	146 (59%)	53 (43%)	0.001***
C				
T	48 (34%)	104 (41%)	71 (57%)	
TLR4 SNP rs4986791 genotype Number	N = 30	N = 125	N = 10	
CC	28 (93%)	123 (98%)	9 (90%)	0.144
CT	2 (7%)	2 (2%)	1 (10%)	
Alleles	58 (97%)	248 (99%)	19 (95%)	
C				0.149
T	2 (3%)	2 (1%)	1 (5%)	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

"N" points to the sample size.

Distribution of IL28B rs12979860 and TLR4 rs4986791 genotypes in responders and relapsers to SOF and DCV treatment

To gain perspectives on the impact of IL28B rs12979860 C/T and TLR4 rs4986791 C/T polymorphisms on the response to SOF/DCV therapy in the Egyptian population, we genotyped both SNPs in HCV patients who attained SVR after SOF/DCV treatment (responders) and those who have persistent infection (relapsers). When we compared the IL28B typing records derived from 125 responders and 62 relapsers with 70 healthy controls, the results revealed that the frequency of the unfavorable TT genotype was markedly diminished from 32% in relapsers to 15% in responders and to 13% in healthy controls (Table 3, $p = 0.003$). On the other hand, we compared TLR4 rs4986791 C/T genotypes data between 125 responders, 10 relapsers and 30 controls. Unfortunately, the results revealed the complete absence of the protective TT genotype in all samples, while the frequency of the CC

genotype was slightly decreased from 98% in responders to 93% in healthy controls to 90% in relapsers. (Table 3, $p = 0.144$). Upon comparing responders to relapsers, IL28B rs12979860 and TLR4 rs4986791 C alleles were more frequent in responders, while IL28B rs12979860 and TLR4 rs4986791 T alleles were more frequent in relapsers (Table 6, OR 1.9, 95% CI 1.29–2.9, $p = 0.004$ for IL28B rs12979860; OR 6.5, 95% CI 0.57–75.28, $p = 0.2$ for TLR4 rs4986791).

IL28B rs12979860 C/T and TLR4 rs4986791 C/T Polymorphisms couldn't predict the probability for HCC emergence following SOF and DCV treatment

We also stratified the patients on the basis of the occurrence of clinically evident HCC within the 6-mo surveillance period following SOF/DCV treatment (HCC post SOF/DCV treatment patients) or not (No HCC post SOF/DCV treatment patients either responders or relapsers). The distribution of the two polymorphisms was very close in both

Table 4 Distribution of IL28B rs12979860 and TLR4 rs4986791 genotypes in 65 patients HCC post SOF/DCV and 187 patients didn't progress to HCC post SOF/DCV.

	HCC post SOF/DCV	No HCC post SOF/DCV	P value
IL28 SNP rs12979860 genotype Number	N = 65	N = 187	
CC	20 (31%)	51 (27%)	0.457
CT	36 (55%)	97 (26%)	
TT	9 (6%)	39 (20%)	
Alleles C	76 (58%)	199 (53%)	0.31
T	54 (42%)	175 (47%)	
TLR4 SNP rs4986791 genotype Number	N = 65	N = 135	
CC	62 (95%)	132 (98%)	0.353
CT	3 (5%)	3 (2%)	
Alleles C	127 (98%)	267 (99%)	0.356
T	3 (2%)	3 (1%)	

$p > 0.05$ is not significant.

"N" points to the sample size.

Table 5 Distribution of IL28B rs12979860 and TLR4 rs4986791 genotypes in Healthy Subjects and Genotype 4-Infected Patients with Early, Late Liver Fibrosis and Hepatocellular Carcinoma.

	Controls	Early Fibrosis (F0–F1)	Late Fibrosis (F2–F4)	HCC	P value
IL28 SNP rs12979860 genotype Number	N = 70	N = 48	N = 21	N = 102	
CC	31 (44%)	14 (29%)	10 (48%)	42 (41%)	0.05*
CT	30 (43%)	29 (60%)	7 (33%)	93 (91%)	
TT	9 (13%)	5 (11%)	4 (19%)	28 (28%)	
Alleles C	92 (66%)	57 (59%)	27 (64%)	108 (53%)	0.1
T	48 (32%)	39 (41%)	15 (36%)	96 (47%)	
TLR4 SNP rs4986791 genotype Number	N = 30	N = 48	N = 21	N = 102	
CC	28 (93%)	48 (100%)	17 (81%)	101 (99%)	0.001***
CT	2 (7%)	0	4 (19%)	1 (1%)	
Alleles C	58 (97%)	96 (100%)	38 (90%)	203 (99%)	0.001***
T	2 (3%)	0	4 (10%)	1 (1%)	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

"N" points to the sample size.

Table 6 Chi-squared and Odd Ratio Testing of IL28B rs12979860 and TLR4 rs4986791 alleles (C vs, T) Distribution in Different Outcomes of HCV Infection.

	Chi Square (χ^2)	Odds ratio	95% CI for odds ratio Lower -Upper	P value
IL28 SNP rs12979860				
Responders vs. Relapsers	8.2	1.9	1.2–2.9	0.004**
HCC post SOF/DCV vs. No HCC post SOF/DCV	1.074	1.24	0.83–1.85	0.3
(Naïve)F0–F1 vs. HCC	1.09	1.29	0.78–1.95	0.4
(Naïve)F2–F4 vs. HCC	1.5	1.5	0.81–2.96	0.2
TLR4 SNP rs4986791				
Responder vs. Relapsers	2.97	6.5	0.566–75.28	0.09
HCC post SOF/DCV vs. No HCC post SOF/DCV	0.85	0.48	0.1–2.4	0.4
(Naïve)F0–F1 vs. HCC	0.47	—	—	0.5
(Naïve)F2–F4 vs. HCC	14.28	0.047	0.005–0.43	0.0001***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

The value of χ^2 refers to the difference between expected and observed values for allele counts.

groups and no significant differences were found; the C/C genotype at IL28 rs12979860 represents 31% from HCC post SOF/DCV and 27% from No HCC post SOF/DCV patients (Table 4). Among alleles, C allele represents around 58% from both groups and T allele represents around 42% from both groups (Table 6, OR 1.24, 95% CI 0.83–1.85, $p = 0.3$). While the CC genotype at TLR4 rs4986791 represents 95%, 98% from HCC post SOF/DCV and No HCC post SOF/DCV patients, respectively. C/T genotype represents 5%, 2% from HCC and No HCC patients, respectively. (Table 6, OR 0.48, 95% CI 0.095–2.4, $p = 0.4$). Taken together, rs12979860 genotype could predict the response in SOF/DCV treated patients but couldn't predict the probability for HCC emergence within the 6-mo surveillance period following SOF/DCV treatment.

Distribution of IL28 rs12979860 and TLR4 rs4986791 genotypes in healthy controls and chronic HCV patients with EF, LF and HCC

To rule whether the two studied polymorphisms (IL28 rs12979860 and TLR4 rs4986791) could discriminate between the EF, LF and HCC patients or not, the distribution of the two polymorphisms was compared. The CC genotype at IL28 rs12979860 represents 29%, 48% and 41% from EF, LF and HCC patients, respectively. CT genotype represents 60%, 33% and 91% from EF, LF and HCC patients, respectively. TT genotype rises from 11%, 19% at EF and LF patients, respectively to 28% at HCC group (Table 5; $p = 0.05$). Among alleles, the frequency of IL28B rs12979860 C allele was significantly higher in EF patients than in HCC patients (Table 6, OR 1.23, 95% CI 0.78–1.95, $p = 0.4$), whereas the frequency of T allele was higher in HCC than in LF (Table 6, OR 1.5, 95% CI 0.81–2.96, $p = 0.2$).

On the other hand, the CC genotype at TLR4 rs4986791 represents 100%, 81% and 97% from EF, LF and HCC patients, respectively. The minor protective TT genotype was completely absent in all subjects, whereas CT genotype was absent at the EF group and represents 19%, 3% from LF and HCC patients, respectively (Table 5; $p = 0.001$). The frequency of the protective T allele was higher in LF than in HCC (Table 6, OR 0.047, 95% CI 0.005–0.43, $p = 0.001$).

Discussion

Following the DAAs therapy, treatment failure is robustly accompanied with the emergence of viral variants with resistance associated substitutions particularly in NS5A and NS5B genes. Accordingly, these substitutions may molder the efficacy of DAAs therapy.^{27–29} To this end, it might be useful to stand on predictors of response to treatment.

The allelic frequencies of the IL-28B rs12979860 C/T polymorphism not only deduced the probability to eradicate the HCV infection spontaneously^{30,31} and the sustained response to pegylated-IFN and ribavirin therapy in patients with HCV chronic hepatitis (except in patients infected with genotype-5),^{32–34} but also discriminated between patients with liver cirrhosis of viral origin and patients with cirrhosis of other origins.³⁵ Once again herein, IL28B SNP rs12979860 shows a significant difference between responders and relapsers to SOF/DCV treatment. Carriage of the unfavorable TT genotype occurred more frequently in relapsers than in responders. The meta-analysis assessed by Bota et al., (2013) was in agreement with current finding where the patients were infected with genotype 1 and treated with triple therapy (pegIFN + ribavirin + telaprevir or boceprevir).³⁶

The present data revealed that IL28B SNP rs12979860 genetic variation is a significant contributor to HCC development in treatment naïve patients with early and/or late stage of fibrosis. Previous studies proposed that the patient with IL28B non-responder genotype or previous PEG-IFN/RBV treatment failure, should be retreated with DAAs-based regimens in order to provide the patient with an optimal chance for achieving an SVR.⁹ Upon binding to its receptors, IFN λ 3 activates Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling, evokes interferon-stimulated genes (ISGs) and ends up with creating a terrible antiviral state.¹⁰ Our previous studies confirmed that the genetic variants dramatically orchestrate the mRNA expression of ISGs which are the master players of innate immunity against HCV burden.^{37–40} The study by Poordad et al, (2012) highlighted the $A > 1$ log₁₀ decline in HCV RNA at the fourth week of therapy is the premier predictor of a SVR, regardless of both polymorphisms in IL-28B (rs12979860 and rs8099917).⁴¹

On the contrary, no significant association was detected either in allele or genotype frequencies for the TLR4 genetic polymorphism (rs4986791) upon comparing the responders to SOF/DCV treatment with relapsers. This finding is in accordance with previous studies which weakened the effect of host genetics against the higher potency offered by DAAs.^{9,42}

The immune–genetic profile of the lethal HCC cancer is yet to be completely understood.^{43,44} Viral, liver pathogenesis and host factors are the dominant risk factors monitoring HCC development.^{16,45,46} Among the latter, genetic polymorphisms have been related to the risk of developing HCC.⁴⁷ Other finding of current study concerns the absence of a strict association between IL-28B rs12979860 C/T and TLR4 rs4986791 C/T polymorphisms and the occurrence of HCC following SOF/DCV treatment. Earlier studies with interferon-based HCV therapy never elaborated this phenomenon due to a number of reasons. One, is that DAAs lack immune-stimulating and antitumor effects.^{1,6,48} Second, is that HCC may be present but undiagnosed at the time of antiviral treatment.^{49,50}

We have found a lower proportion of the protective allele at TLR4 rs4986791 among relapsers and HCC patients than in the corresponding responders and/or non HCC groups, but differences were only significant at the naïve patients, a fact that may be clarified partially by the absence of the protective TT genotype in the Egyptian population that may border our power to disclose its merit. In accord with this finding, Brazilian study negated any frequency or association between TLR4 rs4986791 SNP and susceptibility to HCV burden in its population.⁵¹ However, many reports elaborated the engagement of endogenous ligands (like damage-associated molecular patterns) by TLR4 which triggers downstream signaling, evokes specific transcription factors and finally provokes the expression of interferons among other inflammatory cytokines during HCV infection.^{19,52}

Conclusion

Again, the combination of SOF/DCV improved SVR rates in the Egyptian population regardless of IL28 genotype, with the best rates in those without the T allele. Current study may be limited by its retrospective design and the low frequency of TLR4 (rs 4986791) polymorphic T allele in Egyptian population. To our knowledge this is the first Egyptian study to report the correlation between both IL28B and TLR4 polymorphisms (rs12979860 and rs4986791, respectively) and the response to SOF/DCV which may help health care providers particularly in our resource-constrained country.

Author contributions

Study concept and design: Mostafa El Awady and Reham Dawood. Acquisition of laboratory data: Ghada Salum, Mai Abdel Meguid, Reham Dawood. Analysis and interpretation of data: Reham Dawood, Ghada Salum, Mai Abd el-Meguid, and Noha E Ibrahim. literature search: Reham Dawood, Ghada Salum. Drafting of the article: Ghada Salum. Critical revision of the article for important intellectual content:

Mostafa El Awady, Reham Dawood. Sample collection and clinical evaluation: Ashraf O Abdel Aziz. Statistical analysis: Ghada Salum, Noha E Ibrahim. All authors read and approved the final manuscript.

Author agreement/declaration

All authors have seen and approved the final version of the manuscript being submitted. All authors confirm that this work is original and all data, tables, etc. used in the manuscript are prepared originally by authors, and has not been published elsewhere nor is it currently under consideration elsewhere.

Conflicts of interest

All authors have none to declare.

Role of the funding source

The funding source (i.e. STDF) has been only responsible for the financial aspects of the project and has no functional role in aspects like (study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication)

Acknowledgments

We would like to acknowledge the Science and Technology Development Fund in Egypt (STDF) (Grant No. 15002) to Reham DAWOOD for financially supporting the current research.

References

1. Butt AS, Sharif F, Abid S. Impact of direct acting antivirals on occurrence and recurrence of hepatocellular carcinoma: biologically plausible or an epiphenomenon? *World J Hepatol.* 2018;10(2):267–276. <https://doi.org/10.4254/wjh.v10.i2.267>.
2. Omran D, Alboraie M, Zayed RA, et al. Towards hepatitis C virus elimination: Egyptian experience, achievements and limitations. *World J Gastroenterol.* 2018;24(38):4330–4340. <https://doi.org/10.3748/wjg.v24.i38.4330>.
3. Ahmed OA, Elsebaey MA, Fouad MHA, et al. Outcomes and predictors of treatment response with sofosbuvir plus daclatasvir with or without ribavirin in Egyptian patients with genotype 4 hepatitis C virus infection. *Infect Drug Resist.* 2018; 11:441–445. <https://doi.org/10.2147/IDR.S160593>.
4. Liu CH, Huang YJ, Yang SS, et al. Generic sofosbuvir-based interferon-free direct acting antiviral agents for patients with chronic hepatitis C virus infection: a real-world multicenter observational study. *Sci Rep.* 2018. <https://doi.org/10.1038/s41598-018-32060-7>.
5. Ravi S, Axley P, Jones DA, et al. Unusually high rates of hepatocellular carcinoma after treatment with direct-acting antiviral therapy for hepatitis C related cirrhosis. *Gastroenterology.* 2017;152(4):911–912. <https://doi.org/10.1053/j.gastro.2016.12.021>.
6. Brown RS. The possible association between DAA treatment for HCV infection and HCC recurrence. *Gastroenterol Hepatol.* 2016;12(12):776–779. <https://doi.org/10.1097/MOG.000000000000177>.

7. Kassas M El, Funk | AL, Salaheldin | M, et al. Increased recurrence rates of hepatocellular carcinoma after DAA therapy in a hepatitis C-infected Egyptian cohort: a comparative analysis. *J Viral Hepat.* 2018;25. <https://doi.org/10.1111/jvh.12854>.
8. Prokunina-Olsson L, Muchmore B, Tang W, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet.* 2013;45(2):164–171. <https://doi.org/10.1038/ng.2521>.
9. Ahlenstiel G, Booth DR, George J. Review Will IL28B polymorphisms remain relevant to direct-acting antiviral treatment paradigms?. 2012;1170:1163–1170. <https://doi.org/10.3851/IMP2427>.
10. Asselah T, De Muynck S, Broet P, et al. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. *J Hepatol.* 2012;56(3):527–532. <https://doi.org/10.1016/j.jhep.2011.09.008> \ rs0168-8278(11)00718-5 [pii].
11. El Awady MK, Bader el Din NG, Tabll A, et al. IL28B polymorphism and cytomegalovirus predict response to treatment in Egyptian HCV type 4 patients. *World J Gastroenterol.* 2013;19(2):290–298. <https://doi.org/10.3748/wjg.v19.i2.290>.
12. Conteddu V, Sansonno D, Russi S, Pavone F, Dammacco F. Therapy of chronic hepatitis C virus infection in the era of direct-acting and host-targeting antiviral agents. *J Infect.* 2014;68(1):1–20. <https://doi.org/10.1016/j.jinf.2013.08.019>.
13. Abdelwahab SF, Zakaria Z, Allam WR, et al. Interleukin 28B.rs12979860 genotype does not affect hepatitis C viral load in Egyptians with genotype 4 chronic infection. *Arch Virol.* 2015;160(11):2833–2837. <https://doi.org/10.1007/s00705-015-2555-3>.
14. Wang W, Pan Q, Fuhler GM, Smits R, Peppelenbosch MP. Action and function of Wnt/ β -catenin signaling in the progression from chronic hepatitis C to hepatocellular carcinoma. *J Gastroenterol.* 2017;52(4):419–431. <https://doi.org/10.1007/s00535-016-1299-5>.
15. Cariani E, Roli L, Missale G, Villa E, Ferrari C, Trenti T. Interleukin 28B polymorphisms as predictors of sustained virological response in chronic hepatitis C: systematic review and meta-analysis. *Pharmacogenomics J.* 2016;16(10):18–29. <https://doi.org/10.1038/tpj.2015.28>.
16. Sepehri Z, Kiani Z, Kohan F, Alavian SM, Ghavami S, PT CR. *Life Sci.* 2017. <https://doi.org/10.1016/j.lfs.2017.04.025>.
17. Delale T, Paquin A, Asselin-paturel C, et al. MyD88-Dependent and -Independent Murine Cytomegalovirus Sensing for IFN- α Release and Initiation of Immune Responses In Vivo. *J Immunol.* 2017. <https://doi.org/10.4049/jimmunol.175.10.6723>.
18. De Re V, De Zorzi M, Caggiari L, et al. HCV-related liver and lymphoproliferative diseases: association with polymorphisms of IL28B and TLR2. *Oncotarget.* 2016;7(25). www.impactjournals.com/oncotarget. Accessed April 12, 2018.
19. Bhattacharyya S, Kelley K, Melichian DS, et al. Toll-like receptor 4 signaling augments transforming growth factor- β responses: a novel mechanism for maintaining and amplifying fibrosis in scleroderma. *Am J Pathol.* 2013;182(1):192–205. <https://doi.org/10.1016/j.ajpath.2012.09.007>.
20. Garza-Gonzalez E, Bosques-Padilla FJ, Mendoza-Ibarra SI, Flores-Gutierrez JP, Maldonado-Garza HJ, Perez-Perez GI. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer.* 2007;7:3–7. <https://doi.org/10.1186/1471-2407-7-70>.
21. Hold GL, Rabkin CS, Chow WH, et al. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. *Gastroenterology.* 2007;132:905–912. <https://doi.org/10.1053/j.gastro.2006.12.026>.
22. Ashton KA, Proietto A, Otton G, et al. Toll-Like Receptor (TLR) and Nucleosome-binding Oligomerization Domain (NOD) gene polymorphisms and endometrial cancer risk. *BMC Cancer.* 2010;10. <https://doi.org/10.1186/1471-2407-10-382>.
23. Srivastava K, Srivastava A, Kumar A, Mittal B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. *Liver Int.* 2010;30(7):1067–1072. <https://doi.org/10.1111/j.1478-3231.2010.02268.x>.
24. Hart K, Landvik NE, Lind H, Skaug V, Haugen A, Zienoldddy S. A combination of functional polymorphisms in the CASP8, MMP1, IL10 and SEPS1 genes affects risk of non-small cell lung cancer. *Lung Cancer.* 2011;71(2):123–129. <https://doi.org/10.1016/j.lungcan.2010.04.016>.
25. Huang XJ, Lü X, Lei YF, et al. Cellular immunogenicity of a multi-epitope peptide vaccine candidate based on hepatitis C virus NS5A, NS4B and core proteins in HHD-2 mice. *J Virol Methods.* 2013;189(1):47–52. <https://doi.org/10.1016/j.jviro.2013.01.003>.
26. Zhang K, Zhou B, Wang Y, Rao L, Zhang L. The TLR4 gene polymorphisms and susceptibility to cancer: a systematic review and meta-analysis. *Eur J Cancer.* 2013;49(4):946–954. <https://doi.org/10.1016/j.ejca.2012.09.022>.
27. Petta S, Craxi A. Therapeutic algorithms for chronic hepatitis C in the DAA era during the current economic crisis: whom to treat? How to treat? When to treat? *BMC Infect Dis.* 2012;12(Suppl 2):S3. <https://doi.org/10.1186/1471-2334-12-S2-S3>.
28. Pawlotsky JM. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. *Gastroenterology.* 2016;151(1):70–86. <https://doi.org/10.1053/j.gastro.2016.04.003>.
29. Spengler U. Direct antiviral agents (DAAs) - a new age in the treatment of hepatitis C virus infection. *Pharmacol Ther.* 2018;183:118–126. <https://doi.org/10.1016/j.pharmthera.2017.10.009>.
30. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature.* 2009. <https://doi.org/10.1038/nature08463>.
31. Machicote A, Flichmann D, Arana E, et al. IL28B SNPs rs12979860 and rs8099917 are associated with inflammatory response in Argentine chronic HCV patients. *Int J Clin Med.* 2018;09(02):79–91. <https://doi.org/10.4236/ijcm.2018.92009>.
32. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature.* 2009;461. <https://doi.org/10.1038/nature08309>.
33. Antaki N, Bibert S, Kebbewar K, et al. IL28B polymorphisms do not predict response to therapy in chronic hepatitis C with HCV genotype 5. *Gut.* 2012. <https://doi.org/10.1136/gutjnl-2012-302019>.
34. De Souza SL, Vidal LL, Ramos J, et al. Hepatitis C virus-infected responders and relapsers to treatment show similar genetic profiles of IL28B and IL10 single nucleotide polymorphisms. *BioMed Res Int.* 2018;2018. <https://doi.org/10.1155/2018/2931486>.
35. Fabris C, Falletti E, Cussigh A, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol.* 2011;54(4):716–722. <https://doi.org/10.1016/j.jhep.2010.07.019>.
36. Bota S, Sporea I, Roxana, et al. Role of Interleukin-28B Polymorphism as a Predictor of Sustained Virological Response in Patients with Chronic Hepatitis C Treated with Triple Therapy: A Systematic Review and Meta-Analysis. *Clin Drug Investig.* 2013. <https://doi.org/10.1007/s40261-013-0074-0>.
37. Ibrahim MK, Salum GM, Bader El Din NG, et al. Transcriptional dysregulation of upstream signaling of IFN pathway in chronic HCV type 4 induced liver fibrosis. *PLoS One.* 2016;11(5). <https://doi.org/10.1371/journal.pone.0154512>.

38. Salum GM, Bader El Din NG, Ibrahim MK, et al. Vascular endothelial growth factor expression in hepatitis C virus-induced liver fibrosis: a potential biomarker. *J Interf Cytokine Res.* 2017;37(7):310–316. <https://doi.org/10.1089/jir.2016.0127>.
39. Salum GM, Bader El Din NG, Ibrahim MK, Dawood RM, Farouk S, El Awady MK. Correlation between TGF- β 1 and c-MET expression in HCV genotype 4-induced liver fibrosis. *J Interf Cytokine Res.* 2018;38(12):552–558. <https://doi.org/10.1089/jir.2018.0096>.
40. Dawood RM, el-Meguid MA, Ibrahim MK, et al. Dysregulation of fibrosis related genes in HCV induced liver disease. *Gene.* 2018; 664:58–69. <https://doi.org/10.1016/j.gene.2018.04.032>.
41. Poordad F, Bronowicki J, Gordon SC, et al. Factors that predict response of patients with hepatitis C virus infection to Boceprevir. *Gastroenterology.* 2012;143(3). <https://doi.org/10.1053/j.gastro.2012.05.011>, 608–618.e5.
42. Jacobson IM, Catlett I, Marcellin P, et al. 1369 telaprevir substantially improved svr rates across all IL28B genotypes in the advance trial. *J Hepatol.* 2011;54:S542–S543. [https://doi.org/10.1016/S0168-8278\(11\)61371-8](https://doi.org/10.1016/S0168-8278(11)61371-8).
43. Alavian SM, Haghbin H. Relative importance of hepatitis B and C viruses in hepatocellular carcinoma in EMRO countries and the Middle East: a systematic review. *Hepat Mon.* 2016;16(3), e35106. <https://doi.org/10.5812/hepatmon.35106>.
44. Altekruse SF, McGlynn KA, Dickie LA, Kleiner DE. Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992–2008 - altekruse - 2012 - hepatology - wiley online library. *Hepatology.* 2012;55(2):476–482. <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21953588&retmode=ref&cmd=prlinks%5Cnpapers3://publication/doi/10.1002/hep.24710>.
45. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. *J Gastroenterol Hepatol.* 1997;12(9–10):S294–S308. <https://doi.org/10.1111/j.1440-1746.1997.tb00513.x>.
46. Yang T, Zhang J, Lu J-H, Yang G-S, Wu M-C, Yu W-F. Risk factors influencing postoperative outcomes of major hepatic resection of hepatocellular carcinoma for patients with underlying liver diseases. *World J Surg.* 2011;35(9):2073–2082. <https://doi.org/10.1007/s00268-011-1161-0>.
47. Agúndez JA, García-Martin E, Devesa MJ, et al. Polymorphism of the TLR4 gene reduces the risk of hepatitis C virus-induced hepatocellular carcinoma. *Oncology.* 2012;82(1):35–40. <https://doi.org/10.1159/000335606>.
48. De Re V, Gragnani L, Fognani E, et al. Impact of immunogenetic IL28B polymorphism on natural outcome of HCV infection. *Bio-Med Res Int.* 2014;2014. <https://doi.org/10.1155/2014/710642>.
49. Feldmann G, Nischalke HD, Nattermann J, et al. Induction of interleukin-6 by hepatitis C virus core protein in hepatitis C-associated mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. *Clin Cancer Res.* 2006;12(15):4491–4498. <https://doi.org/10.1158/1078-0432.CCR-06-0154>.
50. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol.* 2018;68(1):25–32. <https://doi.org/10.1016/j.jhep.2017.08.030>.
51. Pires-Neto O de S, de Sá KSG, Santana BB, et al. Lack of association between polymorphisms of the TLR4 gene and infection with the hepatitis B and C viruses. *Mediat Inflamm.* 2015;2015:150673. <https://doi.org/10.1155/2015/150673>.
52. Minmin S, Xiaoqian X, Hao C, et al. Single Nucleotide Polymorphisms of Toll-like Receptor 4 Decrease the Risk of Development of Hepatocellular Carcinoma. *PLoS one.* 2011. <https://doi.org/10.1371/journal.pone.0019466>.