



ORIGINAL RESEARCH ARTICLE

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IL-28B single nucleotide polymorphism as a predictor of hepatocellular carcinoma after treatment of chronic hepatitis C patients with direct acting antivirals

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Abstract

Background: Hepatitis C virus (HCV) infection is considered one of the main causes of chronic liver diseases. HCV is responsible for 25% of HCC cases worldwide. DAAs represent an important step for HCV eradication. The aim was to study the role of IL-28B single nucleotide polymorphism in the prediction of HCC in patients with HCV-related cirrhosis after DAAs.

Results: This study was done at Mansoura Specialized Hospital: 50 cases HCC after DAAs, 50 cases without HCC after DAAs, and 100 controls. SNPs of the IL-28B gene were genotyped. There was an insignificant difference between HCC patients and the cirrhotic group as regards genotypes (p value = 0.26) and alleles (p value = 0.77). A significant association in SNP of IL-28B between healthy individuals and the cirrhotic group was detected. C C genotype (28%) and C T (64%) genotype were more prevalent in the healthy group than in the cirrhotic group 20% and 52% respectively while T T genotype was more prevalent in cirrhotic patients (28%) than controls (8%). C allele was protective against cirrhosis with 60% distribution in healthy individuals and 46% in the cirrhotic group. T allele was more prevalent in cirrhotic (54%) than the normal group (40%)

Conclusion: Although IL-28B SNP had a role in HCV-related cirrhosis progression, it did not predict the probability for HCC development following DAAs.

Keywords: HCV, HCC, SNPs

Background

Hepatitis C virus (HCV) infection is considered one of the main causes of chronic liver diseases around the world. There are about 71 million chronically infected persons [1]. HCV is responsible for 27% of cases of cirrhosis and 25% of HCC cases worldwide [2]. Hepatocellular carcinoma (HCC) is the primary liver cancer derived from the hepatocytes [3]. DAAs represent uprising

in HCV eradication [4]. The incidence of HCC is reduced in chronic hepatitis C genotype 4 patients with liver cirrhosis (F4) and advanced hepatic fibrosis (F3) who achieved SVR following DAAs treatment to 2.9% as compared with 5.3% in untreated patients [5]. Especially in absence of HCC risk factors, the recurrence incidence is decreased [6], also most recent articles found a reduced incidence, and the debate is near the end [7]. Unexpected high rate of HCC recurrence has been reported about 28.8% despite complete response to treatment [8]. IL-28B is a cytokine responsible for transcription of subsets of genes that play a crucial role in antiviral immune response [9]. Although the role of IL-

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28B SNP in HCC remains controversial, single nucleotide polymorphism of IL-28B can predict the response to DAAs with favorable and unfavorable genotypes [10].

Objectives

The objective is to study the role of IL-28B single nucleotide polymorphism in the prediction of HCC in patients with HCV-related cirrhosis after DAAs.

Methods

Study design

The present study was a cross-sectional study.

Settings

The study was conducted at Mansoura Specialized Medical Hospital.

Participants

This study included 50 cirrhotic patients due to HCV with a history of DAA therapy, 50 cirrhotic patients due to HCV with history of DAA therapy who developed HCC, 100 normal individuals neither infected by HCV nor had HCC as a control group, and all of them were from Mansoura Specialized Medical Hospital.

Variables

Inclusion criteria

Inclusion criteria include adult patients 18–65 years at Specialized Medical Hospital, Mansoura University inpatient and outpatient departments. They received DAAs therapy (sofosbuvir 400 mg once daily, daclatasvir 60 mg once daily, ribavirin 800–1200 mg) and SVR after 3 months of therapy or after 6 months of sofosbuvir and daclatasvir in patients who did not tolerate ribavirin.

Exclusion criteria

1. Co-infection with HBV or HIV.
2. Previous HCV treatment with interferon-based therapy.
3. Other etiologies of cirrhosis: autoimmune hepatitis, NASH, alcoholic, and other causes.
4. Known drug abuse.
5. Pregnant females.
6. Organ failure: heart failure, respiratory failure, renal failure.

Data sources/measurement

All selected patients were subjected to history taking and physical examination.

Laboratory and radiological investigations

These include complete blood count (CBC), serum albumin, total, direct bilirubin, INR, AST, ALT, creatinine, alpha fetoprotein, and IL28B gene polymorphism PCR.

Also, abdominal ultrasound, triphasic CT: HCC cases were diagnosed based on the arterial phase enhancement and washout during the portal venous phase. One case underwent liver biopsy after CT and MRI had been done without definite diagnosis of HCC and revealed undifferentiated carcinoma.

Bias

N/A

Study size

The study size is determined by the statistician.

Quantitative variables

N/A

Statistical methods

The collected data were coded and fed into the SPSS system (Statistical Package for Social Sciences) ver. 22

Results

Table 1 shows that groups are matched according to age and sex with male predominance in both cirrhotic and HCC (p value = 0.135, p value = 0.06), respectively

Table 2 shows significant increase in hepatic decompensation in the HCC group as regards encephalopathy, ascites, jaundice, and hematemesis (p value = 0.008, p value = 0.04, p value = 0.001, and p value = 0.023, respectively)

Table 3 shows significant lower serum albumin, Hgb, and platelets in HCC group with p value = 0.02, p value = 0.03, and $p < 0.001$, respectively, and a significant higher AST, total, direct bilirubin, and INR in the HCC group with p value = 0.001, $p = 0.005$, $p = 0.02$, and $p = 0.009$, respectively.

Table 4 shows a significant increase in Child B and C among the HCC group (p value < 0.001)

Table 5 shows the classification of HCC according to the number of lesions and BCLC, duration between end of treatment and HCC and associated PVT, and nodal and extrahepatic spread.

$p1$ difference between the control group and cirrhotic group, $p2$ difference between the control group and HCC, $p3$ difference between the cirrhotic and HCC cases

Table 6 shows an insignificant distribution in genotypes and alleles of IL-28B SNP between the HCC group and cirrhotic group and a significant distribution in genotypes and alleles between the control and cirrhotic groups.

Table 1 Demographic characteristics of the studied groups

	Control group N=100	Cirrhosis group N=50	HCC group N=50	Test of significance
Age/years				$t=1.51$
Mean±SD	57.92±7.40	55.24±5.42	57.08±6.74	$p=0.135$
Sex, n (%)				
Male	58 (58.0)	33 (66.0)	37 (74.0)	$\chi^2=5.70$
Female	42 (42.0)	17 (34.0)	13 (26.0)	$p=0.06$

*Statistically significant (if $p < 0.05$)

Table 7 shows that age and AFP are significant (p value = 0.002, p value = 0.009) respectively as predictors of HCC.

Table 8 shows a significant association between genotype and PVTT (P value = 0.02) and an insignificant association between genotype and other criteria of HCC cases

Participants

This study included 50 cirrhotic patients due to HCV with a history of DAA therapy, 50 cirrhotic patients due to HCV with a history of DAA therapy who developed HCC, and 100 normal individuals neither infected by HCV nor had HCC as a control group.

Descriptive data, outcome data, and main results

Descriptive data, outcome data, and main results are shown in Tables 1, 2, 3, 4, 5, 6, 7, and 8.

Other analyses

N/A

Key results

IL-28B SNP could not predict HCC development after DAAs.

IL-28 SNP had a significant role in the development of HCV-related cirrhosis.

AFP and age are strong predictors of HCC.

HCC leads to rapid deterioration of liver functions.

Close follow-up by US and AFP is recommended post DAAs specially in advanced fibrosis and cirrhosis.

Discussion

This work has great importance as prediction of post DAAs HCC is the headache association of such revolution in the treatment of HCV-related liver disease, reaffirming the role of IL-28B SNP in progression to and of cirrhosis is expected, and the results were surprising.

The present study includes 50 HCC patients post DAAs, 50 cirrhotic patients post DAAs without HCC, and 100 normal as a control.

In our study, the distribution of the polymorphisms between the HCC group and cirrhotic group was close in both, and no significant differences were found. In the HCC group, C C genotype, T T genotype, and C T genotype were 14%, 18%, and 68% while they represented 20%, 28%, and 52% respectively in cirrhotic group (p value = 0.26). As regards alleles, there were no significant differences for C and T alleles between the HCC group (48%, 52%) and cirrhotic group (46%, 54%) (p value = 0.77).

These results denote a lack of association between SNP of IL-28B and HCC post DAAs. Therefore, SNP of IL-28B cannot predict the emergence of HCC after DAAs.

Salum et al. [11] reported similar results of no significance was found in SNP of IL-28B in prediction of HCC post DAAs. They found C C, T T, and C T genotypes

Table 2 clinical presentation of the studied groups

	Cirrhosis group N=50(%)	HCC group N=50(%)	Test of significance
Hepatic encephalopathy	8 (16.0)	20 (40.0)	$\chi^2=7.14$ $p=0.008^*$
Ascites	17 (34.0)	27 (54.0)	$\chi^2=4.06$ $p=0.04^*$
Jaundice	11 (22.0)	26 (52.0)	$\chi^2=9.65$ $p=0.001^*$
Hematemesis	26 (52.0)	37 (74.0)	$\chi^2=5.19$ $p=0.023^*$
DM	20 (40.0)	18 (36.0)	$\chi^2=0.170$ $p=0.680$

*Statistically significant (if $p < 0.05$)

Table 3 Laboratory results of the studied groups

	Cirrhosis group N=50	HCC group N=50	Test of significance
INR	1.49±0.37	1.29±0.34	t=2.68 p=0.009*
Albumin (g/dl)	3.12±0.49	2.83±0.77	t=2.29 p=0.024*
AST (IU/ml)	55.70±37.29	95.0±73.97	z=3.21 p=0.001*
ALT (IU/ml)	46.50±24.52	60.22±41.63	z=1.44 p=0.149
Platelet/cm (× 10 ³)	106.22±42.06	88.02±39.54	z=2.23 p=0.001*
WBCs/cm	5.74±2.61	6.44±2.96	z=1.18 p=0.235
Hgb (g/dl)	9.11±0.97	9.71±1.73	t=2.12 p=0.036*
Creatinine (mg/dl)	1.11±0.37	1.18±0.49	t=0.851 p=0.397
Total bilirubin (mg/dl)	2.76±2.7	5.09±5.27	z=2.82 p=0.005*
Direct bilirubin (mg/dl)	1.67±2.06	3.15±3.6	z=2.19 p=0.028*

*Statistically significant (if p< 0.05)

were 31%, 6%, and 55% in HCC group while in cirrhotic group were 27%, 20%, and 26% (p value = 0.457). For alleles, they found in the HCC group C allele was 58% and T allele was 42%, while in the cirrhotic group, C allele was 53% and T alleles was 47% (p value = 0.31) which also refer to lack of significance between SNP of IL-28 and HCC post DAAs.

On the opposite side, Simili et al. [12] reported a significant association between SNP of IL-28 and HCC post DAAs. T T genotype was unfavorable and associated with a higher risk of HCC post DAAs (p value = 0.024).

This conflict with the previous results is found as this study included a small number of HCC cases after DAAs of only 11 patients. Six of them had a history of HCC and were treated at least 6 months before DAAs, and 5 of them were without recurrence while a number of patients without recurrence in our study was 50 cases and in Salum et al. [11] was 65 patients.

Table 4 Child–Pugh score classification distribution among studied groups

	Cirrhosis group N=50 (%)	HCC group N=50 (%)	Test of significance
Child score			
A	34 (68.0)	6 (12.0)	$\chi^2=33.21$ P< 0.001*
B	13 (26.0)	31 (62.0)	
C	3 (6.0)	13 (26.0)	

*Statistically significant (if p< 0.05)

HCV Genotype 4 is the most common genotype in Egypt while genotype 1a is the most common in Europe. Genotype distribution is important in predicting disease progression, response to therapy, progression of fibrosis, and a higher risk of HCC [13].

In our study, a significant association between SNP of IL-28B in healthy individuals and the cirrhotic group was detected (p value = 0.004). A significant increase was observed in frequencies of IL-28B C C genotype in the healthy population (28%) than the cirrhotic group (20%) and in C T (64%) genotype in the healthy group than

Table 5 Characterization of HCC cases

Character	HCC group (N=50)
PVT	11 (22%)
Extrahepatic, LN spread	7 (14%)
Median period between EOT and HCC	18 m ± 6 m
Number of focal lesions	
Single	23 (46%)
2–3	12 (24%)
> 3	15 (30%)
BCLC	
Very early, early (0,A)	12 (24%)
Intermediate (B)	16 (32%)
Advanced (C)	4 (8%)
Terminal (D)	18 (36%)

Table 6 Genotypes and alleles distribution among studied groups

Polymorphism	Control group N=100 (%)	Cirrhosis group N=50 (%)	HCC group N=50 (%)	Test of significance	
TT	8 (8.0)	14 (28.0)	9 (18.0)	$\chi^2=13.12$	p1=0.004*
CC	28 (28.0)	10 (20.0)	7 (14.0)	$p=0.01^*$	p2=0.054
CT	64 (64.0)	26 (52.0)	34 (68.0)		p3=0.26
HWE	0.008*	0.741	0.126		
Allele	n=200	N=100	N=100		p1=0.02*
T	80 (40)	54 (54.0)	52 (52.0)	$\chi^2=6.87$	p2=0.051
C	120 (60)	46 (46.0)	48 (48.0)	$p=0.03^*$	p3=0.77

*Statistically significant (if $p < 0.05$)

the cirrhotic group (52%). On the other hand, T T genotype was more prevalent in cirrhotic patients (28%) than controls (8%).

As regards alleles, C allele appeared to be protective against cirrhosis with 60% distribution in healthy individuals and 46% in the cirrhotic group. T allele was more prevalent in cirrhotic (54%) than the normal group (40%) (p value =0.02)

A significant increase ($P < 0.0005$) was observed in frequencies of IL-28B C C genotypes in the healthy population than in the cirrhotic group (48%, 13%) respectively [14].

Attallah et al. [15] found that IL-28B T T genotype is more prevalent in patients with advanced fibrosis and cirrhosis among HCV genotype 4 Egyptian patients (p value < 0.05).

Also, Fuente et al. [16] found TT genotype in IL28B polymorphism was highly prevalent in HCV cirrhotic patients but it did not directly influence hepatocarcinogenesis.

These similar results suggest the role of SNP IL-28 in HCV disease progression and liver cirrhosis.

Limitations

Transient elastography was not done as it is very expensive for our patients. Also, the relatively small number of patients was due to the difficulty in acceptance by patients to be included in a research study in addition to the high expense of the kits.

Table 7 Predictors of hepatocellular carcinoma among studied cases

	β	P value	AOR (95% CI)
Age/years	0.167	0.002	1.182 (1.06–1.32)
Albumin (g/dl)	- 0.542	0.363	0.582 (0.181–1.87)
AST (IU/ml)	0.006	0.370	1.006 (0.993–1.02)
AFP (ng/ml)	0.181	0.009	1.198 (1.05–1.37)
Hb (g/dl)	0.295	0.393	1.343 (0.683–2.64)
Total bilirubin (mg/dl)	- 1.093	0.314	0.335 (0.04–2.82)
Direct bilirubin (mg/dl)	1.337	0.349	3.809 (0.233–62.38)

*Statistically significant (if $p < 0.05$)

Interpretation

Our results should be interpreted with caution because of several limitations. We recruited 200 samples in this study; the sample size of each group was relatively small which may restrict its detailed subgroup analysis by the clinical index. All participants were all from Mansoura Specialized Medical Hospital which may not stand for all the Egyptian population.

Generalizability

The fundamental experiments should be further conducted to validate our results and explore the possible mechanism.

Table 8 Association between genotype and demographic and laboratory findings among studied HCC cases

	CC N=7	TT N=9	CT N=34	Test of significance
DM	3	6	9	$P=0.245$
Sex				
Male	5	10	22	$P=0.73$
Female	2	2	9	
AF				
> 400	4	2	14	$P=0.356$
≤400	3	7	20	
PVT	2	3	6	$P=0.02^*$
Child				$P=0.162$
A	3	2	10	
B	2	2	17	
C	2	5	7	
Size				$P=0.144$
≤5	5	3	23	
> 5	2	6	11	
Number of lesions				
Single	4	2	10	$P=0.468$
≤3	2	3	15	
> 3	1	4	9	

Conclusion

Although IL-28B SNP had a role in HCV-related cirrhosis progression, it did not predict the probability for HCC development following DAAs. Further studies are recommended.

Abbreviations

HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; SNPs: Single nucleotide polymorphisms; AFP: Alpha fetoprotein; DAAs: Direct-acting antivirals; SVR: Sustained virologic response; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; PCR: Polymerase chain reaction; ALT: Alanine aminotransferase; AST: Aspartate transaminase; INR: International normalized ratio; CBC: Complete blood count; SPSS: Statistical Package for the Social Sciences; AUC: Area under the curve; CT: Computed tomography; AOR: Adjusted odds ratio; HWE: Hardy-Weinberg Equilibrium; JAK: Janus kinase; STAT: Signal transducer and activator of transcription proteins

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Study design

The present study was cross-sectional in nature, and patients were selected from Mansoura Specialized Medical Hospital, Faculty of Medicine, Mansoura University.

Authors' contributions

The authors have read and approved the manuscript. NAFA (CA): idea of the study, design, and publishing. ASHA: literature search, clinical follow-up, and statistics. RAEB: laboratory studies. IAEE: manuscript review, editing, and data collection

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate:

The study protocol was investigated and approved by the Medical Ethics Research Team, Faculty of Medicine, Mansoura University (code number MS. 18. 11.364). Every case, after guaranteeing privacy, has given informed written consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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