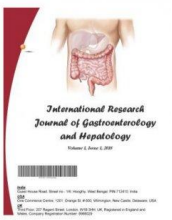

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Hepatocellular Carcinoma in Hepatitis C Virus Infected Patients and Interleukin-28B Gene Polymorphism: A Controversy Relationship

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is considered as one of the most common cancer worldwide for approximately 5.6% of all cancers, it is the third common cause of cancer death. It considers the most serious complication of hepatitis C virus infection, because the onset of the disease occurs slowly, many patients are unaware of their infection and at least 40% cases remain undetected.
Aim: This study was carried out to evaluate the association of IL28B gene polymorphism with HCC in HCV infected Egyptian patients in HCV genotype-4 infection.
Methods: This cross-sectiona, case control study was conducted on three groups. Group I (HCC cases among positive HCV antibody patients), Group II (positive HCV antibody patients with no

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hepatic focal lesion), and Group III (healthy individuals).Extracted DNA for each individual was examined for IL-28B rs8099917 SNP. The analysis was performed using taqman technique.
Results: The percentage of genotype IL-28B rs8099917 in the studied groups was statistically investigated which showed homo (TT) in (100%) in the control group and the polymorphism showed statistically significant different among the study groups (p value < 0.016). IL-28B rs8099917 allelic discrimination was reinvestigated again between HCC group and HCV group only but it showed no significant difference (p value = 0.814). The polymorphisms of IL-28B rs8099917 was statistically investigated regarding to clinical data of HCC group but it showed no significant difference.
Conclusion: IL-28B rs8099917 genotype showed a significant difference between HCV positive patients (either with HCC or without) compared with healthy people.

Keywords: Hepatocellular carcinoma; HCV infection; IL-28B rs8099917 allelic discrimination.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most leading cancer worldwide [1]. The incidence of HCC was reported as about 600,000 new cases each year which means it is the 5th most common cancer [2]. More than 80% of liver cancer cases and deaths occur in developing countries [3]. Different risk factors have been described to be associated with the development of HCC, such as chronic viral hepatitis B and chronic viral hepatitis C, alcohol abuse, and exposure to aflatoxins, although this can occur in people without any known risk factor [4]. Nearly, 5% of patients with chronic liver disease in Egypt was reported to develop HCC [5].The highest prevalence of HCV infection was reported in Egypt [6,7].

HCV infection in Egypt is considered as a national epidemic. That epidemic has been explained by extensive iatrogenic transmission during the parenteral-antischistosomal-therapy (PAT) mass-treatment campaigns [8,9]. Among the leading public health threats that converted into challenges in Egypt are HCV infection and its complications [10]. Several hospitals and community-based studies in Egypt have tried to evaluate the association's between viral hepatitis and HCC over a decade [11-14].

Hepatitis C virus (HCV) was examined in different aspects regarding to the genotype of IL28B such as treatment response of hepatitis C virus [15], clinical outcome [16,17], disease progression [18], associated mixed cryoglobulinemia [19], and detection of naïve HCV-4 patients [20]. Combined variables of HCV infection, hepatocellular carcinoma (HCC), and IL28B genotype were examined to study the relation of IL28B allele as a prognostic marker in the development of HCC among HCV patients in Japan and China [21-23].

2. PATIENTS AND METHODS

The current study was carried out as case control, cross sectional, descriptive study. The study was conducted to chronic hepatitis (C) patients attending the outpatient clinic and inpatient ward of the gastroenterology department at Suez Canal University Teaching Hospitals, Ismailia city, Egypt. The study was conducted between May 2015 and March 2016. The study included (110) subjects divided into three groups as a follow;

Group I: included 45 patients with hepatocellular carcinoma post HCV liver cirrhosis from both sexes defined by serum α-fetoprotein >400 ng/dl and by abdominal ultrasonography [24].

Group II: included 45 patients of chronic HCV patients with liver cirrhosis from both sexes and their clinical status based on child-Pugh score (Appendix 1) [25].

Group III: included 20 healthy control individuals negative in term of hepatitis virus, matching other groups for age and sex.

The inclusion criteria of patients included patients with positive anti-HCV antibody and age ranged between 18-60 years old while **the exclusion criteria included** any HCV positive antibody patient with coexistent of other chronic viral infection, suffering from malignancy or family history of malignancy.

3. STUDY METHODS

(A) Routine investigations

All patients included in the study were subjected to an Interview Questionnaire including age, gender, and duration of HCV infection, the received medications, presence of positive family

history of malignancies and presence of any other malignancy, Abdominal ultrasonographyto assess either(a) the presence of any hepatic echo pattern, (b) spleen size, (c) portal circulation, (d) portal tract thickening, (e) the presence of ascites or (f) any hepatic focal lesions,Laboratory Investigations:(a) complete blood count (CBC) analyzed using Sysmex xt-1800i cell counter (Sysmex, Germany), (b) liver biochemical profile such as: ALT, AST, serum bilirubin (total and direct), and serum albumin which all were analyzed using fully automated chemistry analyzer Cobas 6000; module *c511* (Roche, Germany), (c) prothrombin time and INR analyzed using automated coagulometer analyzer TECO Coatron A4 (TECO, Germany), (d) serum α-fetoprotein analyzed using automated analyzer Cobas 6000; module e601 (Roche, Germany).

(B) Detection of *IL28B* rs8099917 Polymorphism

Whole blood sample was collected into sterile vacuum tubes containing ethylene diamine tetra-acetate (EDTA) for DNA extraction using silica-based method by QIAamp® DNA Mini kit(Qiagen, Germany). Eluted DNA stored at (-20ºC) prior to genotyping of the rs8099917 SNP. The PCR mixture according to the kit protocol (Qiagen, Germany) composed of (5 ul) TaqMan® Universal Master Mix II, (0.5 ul) TaqMan® SNP Genotyping with TaqMan® MGB probes, (2.5 ul) DNAse free water, and (2 ul) template DNA. Analysis was performed usingStepOne™Real-Time PCR (Biosystem, USA). The Sequence Detection System (SDS) Software uses the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated which alleles are in each sample.

4. RESULTS

The current study examined the number of hepatic focal lesions using ultrasonography at the HCC group as shown at Table 1.

Complete blood count was investigated among the study groups as shown at Table (2) which showed statistically significant different at platelet count item (p value < 0.006). Post Hoc test was done for the platelet count between each 2 groups which showed statistically significant results between Group I and Group II and between Group I and Group III (p value < 0.013, p value < 0.005 respectively).

The liver function tests were investigated among all study groups as shown at Table (3) which showed statistically significant different in AST, ALT, Albumin, prothrombin time, INR and AFP. Post Hoc test was done for these studied laboratory tests between each two groups as shown in table (4). Child-Pugh Classification (class A, B, C) was statistically investigated between HCC group and HCV group which showed statistically significant difference (p value < 0.001) as shown in table (5). IL-28B rs8099917 allelic discrimination between the studied groups was statistically investigated which showed homo (TT) in (100%) of the control group and showed statistically significant different among the study groups (p value < 0.016) as shown in table (6). IL-28B rs8099917 allelic discrimination was reinvestigated again between HCC group and HCV group only but it showed no significant difference (p value = 0.814) as shown in table (7). The polymorphisms of IL-28B rs8099917 was statistically investigated regarding to clinical data of HCC group but it showed no significant difference as shown in table (8).

Table 1. Focal lesion number among HCC patients

	No.	N	%
Focal lesion number	Single	32	71.1
	Two	6	13.3
	Three	1	2.2
	Four or more	6	13.3

Table 2. Complete blood count among studied groups

	Group I HCC	Group II CLD	Group III (Control)	ANOVA	P value
	Mean ±S D				
Hb	11.13±2.10	11.42±1.76	12.98±1.48	2.748	0.074
TLC	6.71±4.49	6.85±3.15	5.80±1.27	0.367	0.601
Platelet count	113.9±54.7	180.5±115.9	235.4±31.2	5.751	0.006**

Table 3. Laboratory assay of liver function tests among studied groups

	Group I HCC	Group II CLD	Group III (Control)	ANOVA	P value
	Mean ±S D				
AST	94.7±20.9	101.6±24.02	32.4±13.6	3.978	0.028*
ALT	96.3±27.0	85.3±24.4	32.7±14.9	6.607	0.004**
T.bilirubin	4.11±2.2	5.19±2.4	0.73±0.26	1.974	0.139
D.bilirubin	2.68±1.4	4.19±2.91	0.43±0.21	1.935	0.144
Albumin	2.98±0.7	2.87±0.32	4.26±0.45	21.203	<0.001**
Prothrombin time(sec)	16.4±3.8	17.3±4.25	29.4±1.93	91.741	<0.001**
INR	1.40±0.39	1.55±0.36	1.14±0.05	10.209	<0.001**
AFP	987.5±401.6	14.3±15.1	4.61±1.7	7.425	0.001**

Table 4. Post Hoc test between each two groups of the study regarding to the significant laboratory results

		Mean difference	Std. error	P value
AST	Group I&II	17.5	21.6	0.493
	Group I&III	62.2	28.6	0.036*
	Group II&III	79.08	25.6	0.008**
ALT	Group I&II	22.49	13.4	0.117
	Group I&III	62.18	17.62	0.001**
	Group II&III	42.78	16.62	0.017*
Albumin	Group I&II	0.08	0.25	0.559
	Group I&III	1.08	0.21	<0.001**
	Group II&III	1.38	0.20	<0.001**
Prothrombin time(sec)	Group I&II	0.83	0.79	0.294
	Group I&III	12.96	1.01	<0.001**
	Group II&III	12.13	1.01	<0.001**
INR	Group I&II	0.141	0.09	0.074
	Group I&III	0.56	0.12	0.002**
	Group II&III	0.51	0.11	<0.001**
AFP	Group I&II	993.2	329.9	0.001**
	Group I&III	862.8	271.8	0.006*
	Group II&III	9.74	4.42	0.982

Table 5. Comparison between HCC and CLD groups regarding Child-Pugh classification

		HCC	CLD	χ^2	P value
		N (%)			
Child-Pugh class	A	9(20)	45(100)		
	B	19(42.2)	0	60.0	<0.001**
	C	17(37.8)	0		

Table 6. IL-28B rs8099917 allelic discrimination between the studied groups

	Group I HCC	Group II CLD	Group III Control	P value
	N (%)			
Hetero (GT)	13(52)	12(48)	0 (0)	0.016
Homo(TT)	32(49.2)	33(50.8)	20(100)	

Table 7. IL-28B rs8099917 allelic discrimination between the patients groups (HCC and HCV groups)

	Group I HCC	Group II CLD	χ^2	P value
	N (%)			
Hetero (GT)	13(52)	12(48)	0.055	0.814
Homo(TT)	32(49.2)	33(50.8)		

Table 8. Relation between IL-28B rs8099917 polymorphism and clinical data of HCC group

		Hetero (GT)=13	Homo(TT)=32	χ^2	P value
		N (%)			
Cachexia	No	7(30.4)	16(69.6)	0.055	
	Yes	6(27.3)	16(72.7)		0.815
Jaundice	No	9(32.1)	19(67.9)		
	Yes	4(23.5)	13(76.5)	0.382	0.537
Spleenomegaly	No	1(11.1)	8(88.9)		
	Yes	12(33.3)	24(66.7)	1.731	0.188
Ascites	No	7(28)	18(72)		
	Yes	6(30)	14(70)	0.022	0.883

4. DISCUSSION

The presented study had evaluated the relationship between IL-28B rs8099917 polymorphism and hepatocellular carcinoma in HCV infected patients.

One patients were collected from the outpatient clinic and the inpatient ward of the medicine department of Suez Canal university hospital, Ismailia, Egypt.

In the present study, the mean of age was higher in HCC group than CLD group and control group (56.33±3 years versus 44.77±8 years, respectively; $p<0.001$). This comes in agreement with a Japanese study performed by Joshita et al. [22] at Shinshu University Hospital to determine the association between the IL-28B rs8099917 SNP and HCC onset in Japanese patients with chronic HCV infection. The study results reported that the patients with HCC were significantly older than those without HCC ($p<0.0001$). Also, the results were consistent with Baghdady et al. [26], who reported that the mean age of the HCC patients was 58.70 ± 5.76 years. The previous findings referred that the higher the age, the more susceptibility to HCC. The average age at onset of HCV-related HCC is about the end of 5th decade and the beginning of 6th decade (58-64 years). The risk of HCC is age dependent and the age at onset has also prognostic influence. Some studies have reported that elderly patients had a poor outcome [27-29].

This study also revealed that there were higher frequencies of males compared to females in HCC and CLD groups (80% versus 20% in HCC group and 55.6% versus 44.4% in CLD group, respectively) , a study done by Rauch et al. [30] concluded that the male was associated with higher rates of chronicity in HCV patients. Many studies have indicated a greater proportion of males are HCV-infected in Egypt [31-35]. Also, Darbari et al. [36] reported that regardless of the geographic location, HCC occurs more frequently in men than in women, with the male: female ratios in various countries ranging from 2: 1 to 5: 1. Several previous studies identified HCC risk factors in patients with chronic liver injury, to know those at high risk. They include male gender, age and cirrhosis with portal hypertension [37-41].

The present study showed that there were no statistically significant differences between HCC and CLD groups regarding liver function tests (ALT, AST, albumin, PT and INR) ($p>0.05$). This may be due to the comparability of both groups regarding the medical history (abdominal pain, anorexia, bleeding and encephalopathy), the clinical data (cachexia and jaundice) and the ultrasound findings (portal vein dilatation and thrombosis), without statistically significant differences ($p>0.05$). However, these findings come in disagreement with the results of Joshita et al. [22] which showed that albumin levels were significantly lower and AST levels were significantly higher in HCC group as compared to liver cirrhosis patients.

In the other side, the results of the present study showed that there were significantly lower mean platelets levels in HCC patients compared to CLD patients. Similarly, the results obtained by Joshita et al. [22] showed that the platelets levels were significantly lower in HCC patients than in those without. The mean platelets count in HCC group was below average (113.9±54.7) and directed towards thrombocytopenic levels. This association between thrombocytopenia and HCC had been previously studied [42-44]. The study of Lu et al. [42] reported that thrombocytopenia is a surrogate for cirrhosis and a marker for the identification of patients at high-risk for HCC. Consistently, Carr et al. [43] confirmed the significant relationship between thrombocy-topenia and the tumor size in patients with HCC. In similar prospect, Kondo et al. [44] found that HCC patients with low platelets count had poor liver function, less anatomical resection and more frequent postoperative liver failure than other group of patients.

In the present study, there was a statistically significant higher mean alpha-fetoprotein (AFP) in HCC group than in CLD group (987.5±401.6 ng/mL versus 14.3±15.1 ng/mL, respectively; $p<0.001$). Current gold standard and most commonly used biomarker for patients at risk for HCC is AFP. Serum AFP levels of more than 400 ng/mL are considered diagnostic. Lower serum concentrations which are only transient in nature are more often present in benign liver disease [45]. High AFP serum levels have been found in 60–70% of patients with HCC [46]. Although hepatic resection has been considered the most efficient therapy for HCC, it is only suitable for 20% to 35% of patients because of poor hepatic reserve. Despite the hepatic resection of HCC, its recurrence remains common. Several studies have identified potential risk factors for HCC recurrence, including the presence of cirrhosis, high alpha-fetoprotein (AFP) levels, large tumor foci, and tumor multiplicity [37,47].

Regarding the analysis of IL-28B rs8099917 polymorphism, the present study revealed the presence of a statistical significant difference between the three studied groups with higher percentage of the homozygous TT genotype among the control group as compared to both of HCC and CLD groups. In line with our study, a Brazilian study performed by da Silva Conde et al. [48] reported the presence of a higher proportion of genotype TT of rs8099917 IL28B gene in the control group when compared to the CHC group. Our analysis of IL-28B rs8099917 polymorphism genotypes among the patient groups revealed that there was no significant difference between the HCC and CLD groups regarding homozygous TT and heterozygous GT genotypes ($p>0.05$). In similar study, Joshita et al. [22] stated that there was no significance difference between HCC and non-HCC groups ($p>0.05$) among patients with TT genotype and those with GT genotype. Also, there were another study done by Aziz et al. [49] reported that rs8099917 genotype TT was found in the majority of patients with chronic HCV genotype 3 (60%), while genotypes GT and GG were found in a limited number of patients (36.2% and 3.8% respectively). In contrary, a study performed by Asahina et al. [50] showed that the HCC incidence was significantly higher in rs8099917 non-TT (minor homozygote or heterozygote) patients than in rs8099917 TT (major homozygote) patients (20.8% versus 10.5%), ($p = 0.002$) and this difference is notable among patients with HCV genotype 1.

5. CONCLUSION

The present study concluded that IL-28B rs8099917 polymorphism analysis showed no significant difference between the HCC and CLD groups regarding homozygous and heterozygous genotypes ($p>0.05$). Multivariate analysis showed that male gender and older age were independent risk factors for hepatocellular carcinoma in HCV patients. However, the current and the collected previous data regarding IL28B rs12979860 polymorphism and hepato-carcinogenesis is still a controversy and further studies are required.

CONSENT

Before the initiation of the study, informed consent was given from all individuals selected for the study.

ETHICAL CONSIDERATION

This study was approved from local research ethics committee of faculty of medicine, Suez Canal University in February 2015. Before the initiation of the study, informed consent was given from all individuals selected for the study. The value and the aim of the work were explained to them. There was no probability of any mischief being inflicted on them; on the contrary, all would have the profit of the medical follow-up and the results of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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