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The relationship between hepatitis C - related hepatocellular carcinoma and *IL-23 receptor* gene polymorphism

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Abstract:

Aims: This study evaluated the relationship between *developing interleukin-23 receptor (IL- 23R) gene polymorphism and hepatitis C-related hepatocellular carcinoma (HCC)* in Egyptian patients.

Patients & Methods: The current study included 250 subjects. They were divided into three groups: one hundred patients with hepatitis C virus (HCV) infection without HCC, one hundred patients with HCV and HCC, and fifty healthy non-hepatic volunteers as a control group. *IL- 23R* gene polymorphism (rs10889677) was genotyped by restriction fragment length polymorphism-polymerase chain reaction (RFLP- PCR).

Results: There was a significant difference between the studied groups regarding the frequency of genotypes and alleles (P =0.006 and < 0.001, respectively). Additionally, under the recessive inheritance model, IL-23R polymorphism is significantly associated with HCC development (P=0.001). Moreover, a significant protective effect of the rs10889677 C allele in HCC susceptibility was detected (OR = 0.15, 95% CI = 0.05–0.39, P < 0.001). AC and CC genotypes also had a significant protective effect (OR = 0.16, 95% CI = 0.05–0.46, P < 0.001).

Conclusions: there is a possible relationship between IL- 23R (rs10889677) polymorphism and HCC risk in Egyptian individuals where AC and CC genotypes and C alleles are protective.

Keywords: HCC; HCV; *IL-23R;* gene polymorphism.

Introduction

Egypt is considered to have the highest hepatitis C virus (HCV) infection prevalence worldwide. The Demographic Health Survey (DHS) of 2015 showed a seroprevalence of 10% among the age group between 15 and 59 years [1]. The most common HCV genotype among the Egyptian population is genotype 4, representing 2-3% of world genotypes [2]. Hepatocellular carcinoma (HCC) is a highly heterogeneous tumor complicating chronic HCV infection and is mainly

initiated by many genetic changes [3]. HCC is the world's third and sixth most prevalent cause of cancer death in men and women, respectively [4].

HCC usually develops on top of liver cirrhosis which results from several factors, including alcohol abuse, infection, especially HCV and HBV, and nonalcoholic liver disease [5]. Besides, several genetic and environmental variables contribute to this condition. For example, several polymorphisms in cytokine genes were reported to associate with an increased vulnerability to infection with HCV or an increased tendency to HCC progression [6,7].

The interleukin-23 receptor (IL- 23R) shares the same site as the IL-12R. It triggers memory T helper (Th) 17 cell-mediated inflammatory activity [8], which is essential in the inflammatory and immunological surveillance of HCV and HBV carcinogenesis [9, 10]. Many studies have investigated the role of *IL-23R* gene polymorphism in different disorders, such as inflammatory bowel disease [11], rheumatoid arthritis [12], and stomach cancer [13]. However, there is currently no solid evidence relating the *IL-23R* gene polymorphism to HCV-related HCC development. As a result, our current research focused on this variation in the gene and how it leads to HCC in patients with HCV in Egypt.

Materials and Methods

This case-control study was carried out at the Department of Internal Medicine, Zagazig University, in collaboration with the Departments of Medical Biochemistry& Molecular Biology and Medical Oncology, Zagazig University, from January 2021 to January 2022. The study was performed after receiving approval from the Institutional Review Board of the Faculty of Medicine, Zagazig University. Patients involved in the study gave written consent.

This study included 250 subjects: one hundred having HCV and newly diagnosed with HCC and one hundred having HCV without HCC. Another 50 healthy subjects without a history of liver disease were included as a control group.

All patients with liver disease other than HCV were excluded from the study. Also, subjects with HIV or other malignancies were excluded from the study.

HCV infection was diagnosed by antibody positivity by enzyme-linked immunosorbent assay (ELISA) (Anaheim, CA 92801, USA). HCV RNA was detected by real-time polymerase chain reaction (COBAS amplifier, TaqMan 48, Roche Mannheim, Germany). History taking and general examination were performed for all participants. Then, the patients were subjected to the following investigations: total serum bilirubin, total protein, serum albumin, alanine transaminase (ALT), aspartate transaminase (AST), α-fetoprotein (AFP), prothrombin time (PT), and platelet count. HCC was diagnosed by ultrasonography and typical radiological criteria in triphasic magnetic resonance imaging (MRI) and triphasic computerized tomography (CT).

Ten millimeters of venous blood was withdrawn, and genomic DNA was isolated from ethylene diamine tetra-acetic acid (EDTA) collected blood using a genomic DNA Mini kit (Geneaid, Taiwan). Extracted DNA was checked for quality and quantity using Nanodrop spectrophotometry (ND 1000-NanoDrop®). The samples were stored at - 20°C till further analysis.

The restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method was used to investigate the polymorphism in the *IL-23R* gene (rs10889677). We used forward primer 5′-ATCGTGAATGAGGAGTTGCC -3′ and reverse primer 5′-TGTGCCTGTATGTGTGACCA -3′. The PCR cycling conditions were 5 minutes at 94°C followed by 35 cycles of 1 minute at 94°C, 1 minute at 65°C, and 2 minutes at 72°C, with a final step at 72°C for 20 minutes. A 10-μL PCR product was digested at 37°C for 4 hours in a 15-μL reaction containing 5 U of MnlI (New England Biolabs, Beverly, MA). Digested products were separated on a 2.5% agarose gel stained with ethidium bromide. The detected genotypes were AA (286, 185bp), AC (286, 225, 185, 61bp), and CC (225, 185, 61bp) [14].

Statistical analysis:

The sample size was calculated using Epi Info program 6 (Atlanta, Georgia, USA). The statistical package SPSS Version 20 inc. (Chicago, USA) was used to analyze the collected data. Quantitative data were represented as mean± standard deviation. The Chi-square test ($\chi 2$) was used to compare proportions as appropriate. T-test was used to detect the difference between two groups and one-way ANOVA for multigroup comparisons. A *P* Value < 0.05 was considered statistically significant at a 95% confidence interval.

Results

There were no significant differences in age, sex, or mean age at 1st HCV diagnosis between the examined groups. Compared to the control group, the patient groups demonstrated substantial elevations in total bilirubin, ALT, AST, ALP, GGT, and AFP. In contrast, they showed significant declines in protein and serum albumin. Comparing HCV cases with and without HCC, there were substantial differences in protein, albumin, ALT, and AFP, as well as highly significant differences in AST and GGT. In contrast, no significant difference in total bilirubin or PT was detected (Table 1).

*Table 1: **The demographic and laboratory characteristics of the diseased and control groups.***

Parameters	Control group (n=50)	HCV patients without HCC (n=100)	HCV patients with HCC (n=100)	*P*
Age (years)	56.65±5.25	55.62±7.31	57.68±7.42	0.130

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Sex				
Male	28 (56%)	55 (55%)	57 (57%)	0.960
Female	22 (44%)	45 (45%)	43 (43%)	
Age at first HCV diagnosis (years)		36.26±5.46	37.49±6.70	0.164
Total Bilirubin (mg/dL)	0.82±0.21	1.03±0.31[a]	1.13±0.47[a]	**<0.001****
Total Protein (g/dL)	7.21±0.82	6.80±1.34[a]	6.49±1.44[ab]	**0.001***
Albumin (g/dL)	4.02±0.41	2.68±0.93[a]	2.27±0.82[ab]	**<0.001****
ALT (U/L)	21.94±13.60	68.39±17.20[a]	76.53±19.06[ab]	**<0.001****
AST (U/L)	17.61±9.58	66.44±14.32[a]	83.74±23.38[ab]	**<0.001****
ALP (U/L)	91.88±28.63	193.85±56.86[a]	203.12±69.32[a]	**<0.001****
GGT (U/L)	22.87±7.00	46.66±16.79[a]	56.86±17.72[ab]	**<0.001****
AFP (ug/L)	4.74±0.41	8.65±2.47[a]	146.45±130.80[ab]	**<0.001****
PT (seconds)	12.60±1.61	12.58±2.54	13.20±1.80[a]	0.04*

*[a] Against controls; [b] HCV without HCC against HCV with HCC; *: significant P<0.05; **: highly significant P<0.001*

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The frequency of genotypes of IL-23R (rs10889677) gene polymorphism in the studied groups is presented in figure 1. Our results revealed a significant difference in the frequency of genotypes among the three studied groups (P=0.006). However, using Chi for trend, there was no significant difference in genotypes between HCV without the HCC group and the control group (P=0.369). At the same time, there were substantial differences in genotypes between HCV with the HCC group and the control group and HCV without the HCC group (P=0.001 and 0.01, respectively) (Table 2).

Regarding allele frequency, our results revealed a significant difference between the studied groups (P<0.001). Using Chi for trend, there was no significant difference in allele frequency between HCV without the HCC group and the control group (P=0.145). However, there were substantial differences in allele frequency between HCV with the HCC group and both the control group and HCV without the HCC group (P<0.001 and 0.001, respectively) (Table 2).

Under the recessive inheritance model, IL-23R polymorphism was significantly associated with HCC development (P=0.001), with a considerable difference between HCV individuals with HCC and HCV patients without HCC (P = 0.009). Also, a highly significant difference was detected between HCV individuals with HCC and control subjects (P<0.001) (Table 2).

Table 2: ***Different genotypes and allelic frequencies of the IL23R (rs10889677 A>C) gene polymorphism.***

Genotype	Control group (n=50)	HCV patients without HCC (n=100)	HCV patients with HCC (n=100)	*P*	Chi for trend	OR (95% CI)	*P*
Allele frequency							
A	83 (83%)	178 (89%)	194 (97%)	<0.001**	0.145$	0.60 (0.30–1.19) @	0.07
C	17 (17%)	22 (11%)	6 (3%)		<0.001@**	0.15 (0.05-0.39) #	<0.001**
					0.001#*		

Genotypes							
AA	36 (72%)	82 (82%)	94(94%)	0.006*	0.369$	Reference	
AC	11 (22%)	14 (14%)	6 (6%)		<0.001@**	0.55 (0.23–1.34) @	0.103
CC	3 (6%)	4 (4%)	0 (0%)		0.01#*	0.58 (0.12–2.75) @	0.257
						0.20 (0.07-0.60) #	0.001*
						------------------#	-----
Recessive model							
AA	36 (36%)	82 (82%)	94 (94%)		0.158$	Reference	
AC + CC	14 (28%)	18 (18%)	6 (6%)	0.001	<0.001@**	0.56 (0.25-1.25) @	0.08
					0.009#*	0.16 (0.05-0.46) #	<0.001**

*$: HCV patients without HCC against control group; @ HCV with HCC group against control group; #: HCV with HCC group against HCV without HCC; OR: Odds Ratio; *: significant; **: highly significant*

A significant protective effect of the rs10889677 C allele in HCC susceptibility was detected (OR = 0.15, 95% CI = 0.05–0.39, P < 0.001). AC and CC genotypes also had a significant protective effect (OR = 0.16, 95% CI = 0.05–0.46, P < 0.001).

Discussion

HCC is the most conflicting complication of HCV [15,16]. Many treatment modalities are present at the time but with poor prognosis, especially with an aggressive form of HCC [17]. Therefore, there was an urgent need to study the association between genetic variations and the occurrence of HCC [18]. *IL-23R* polymorphism has been studied extensively with autoimmune and inflammatory diseases such as rheumatoid arthritis [12]. Besides, *IL-23R* gene polymorphisms were documented to be closely associated with the development of cancers such as stomach cancer [13]. However, other studies stated that IL-23 is a protective cytokine [19]. So, we studied the correlation between *IL-23R* single nucleotide polymorphism (SNP) rs10889677 and the occurrence of HCV-related HCC in Egyptian patients.

Our study revealed that *IL-23R* gene polymorphism (rs10889677) was significantly associated with HCC risk development. This finding is similar to that found by Amer et al. (2017) and Pan

and Wang (2019) [21]. In our study, AC and CC genotypes and C alleles seem protective against HCC development. Similarly, a significant association between the A allele and ankylosing spondylitis susceptibility was detected [22].

The impact of IL-23 in carcinogenesis needs to be better understood, most probably tissue-specific. Wendling showed that IL-23 is a carcinogenic cytokine [23], contrary to other studies that stated that IL-23 is a protective cytokine [19]. IL-23 is an innate immune-suppressive cytokine in several carcinogenesis models in rats. It was demonstrated that IL-23 and IL-23R play a crucial role in T helper 17 (Th-17) cell-mediated immunity, tumor-promoting proinflammatory operation, failure of CD8+T immune surveillance, and pathogenesis of cancers [24, 25]. Hori et al. and Kim et al. stated that the IL-23R signaling pathway in T cell regulatory state (Tregs) also enhances Tregs' immunosuppressive effect, leading to impaired immune response and promoting the invasion of the immune system by cancer [26,27]. From the previous links between the two kinds of T cells, IL-23R most probably play an essential role in cancer development and progression. In some experimental studies, genetic deletion or antibody-mediated elimination of IL-23R leads to increased settling of cytotoxic T cells into the tissue rendering an increased tendency to protect against carcinogenesis development [17]. Because IL-23 promotes and activates inflammatory processes and tumorigenesis, blocking IL-23 may benefit cancer development prevention and targeted cancer therapy [14].

Limitations:

The risk of HCC is affected by several factors other than genetic factors. Furthermore, screening for associations between genetic variants and HCC risk requires a large sample size and long-term follow-up. Additional research is needed, including a large population of different races and ethnicities using more polymorphic sites to translate these findings into clinical application.

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In conclusion, this study revealed that *IL-23R rs10889677* AC and CC genotypes and C allele possibly protect Egyptian HCV-infected individuals against developing HCC.

Footnotes.

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Declaration of competing interest

There are no conflicts of interest related to this study.

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