Egyptian Journal of Medical Microbiology Volume 29 / No.1 / January 2020 1-9 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Relation between Interleukin -4 (590C/T) Gene Polymorphism and Hepatocellular Carcinoma Risk in HBV and HCV Patients

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Key words:
Risk, carcinoma, hepatocellular carcinoma, interleukin-4, gene, polymorphism

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ABSTRACT

Background: *Interleukin-4 (IL-4) is an important modulator in the immune response of macrophages, B and T cells to stand in front of infections and malignancy.* ***Objectives:*** *This study aimed to assess the association between IL-4 gene 590C\T polymorphism and risk of hepatocellular carcinoma (HCC) on top of viral hepatitis.* ***Methodology:*** *This study was conducted on 220 patients and 60 apparently healthy individuals. One hundred and twenty patients with HCV infection (group 1) classified as sixty patients with liver cirrhosis and sixty with HCC, one hundred patients with HBV infection (group 2) classified as fifty with liver cirrhosis and fifty with HCC. Virus status of the patients was confirmed by measuring HBsAg, HCV antibodies and real time PCR. Liver cirrhosis was assessed by laboratory investigations, abdomino-pelvic ultrasound and CHILD score. Patients with HCC were diagnosed by triphasic CT, alphafeto-protein level (AFP) and biopsy. The studied groups were genotyped for IL-4 590C/T gene polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.* ***Results:*** *IL-4 590C/T gene analysis detected significant variation between studied groups, regarding genotype and allele frequencies (p=0.025 and p=0.002 respectively). There were higher frequencies of CC genotype and C allele in HCC and cirrhotic hepatitis C patients than controls. C allele had higher prevalence in HCC than cirrhosis in HBV patients. CT+CC genotype carriers had an elevated HCC risk odd ratio (OR): 4.6 [95% CI: 1.5 –14] and OR 3.6 [95% CI: 1.1 –11.6], in HCV and HBV patients in contrast to controls. C allele was associated with increased cirrhotic and HCC risk in HCV infected patients with OR= 4 [95% CI: 1.8 – 8.8] and OR= 2.3 [95% CI: 1.1 – 5.2] versus control group. In HBV patients C allele showed higher HCC risk with OR= 4.2 [95% CI: 1.8 – 9.5] when compared to controls.* ***Conclusion:*** *IL-4 590C/T gene polymorphism may have a role in occurrence of HCC on top of liver cirrhosis.*

INTRODUCTION

Etiology of hepatocellular carcinoma (HCC) differs from the molecular and clinical points of view. Hepatitis B virus (HBV) is the commonest etiology in eastern countries, while hepatitis C virus (HCV) is the most common in the western countries [1]. A great challenge present in finding the correlation between the etiology, HCC occurrence and its molecular features [2].

Chronic infection with HBV leads to HCC occurrence, with a risk reaching 25 to 37-fold that of healthy. HCC caused by HBV infection may occur on both cirrhotic and healthy liver [3]. One of the main mechanisms of carcinogenesis related to HBV infection is chronic inflammation, [4]. Incorporation of HBV into human genome might induce, or to be associated with, genetic instability [5].

Most HCC due to HCV infection occurs on top of cirrhosis. Some studies have suggested that HCV proteins have directed oncogenic abilities. HCV have been found to interact on signaling pathways such as the Wnt/B-catenine, TGFB, NFKB or P53 pathway by regulating transcription, translation or by a post-translational mechanism. In addition, NS3, NS4B, NS5 and HCV core protein have been shown transformation of fibroblasts in vitro by many authors [6,7].

Interleukin-4 (IL-4), a cytokine produced mostly by stimulated T helper 2 (Th2) cells, plays important

role in immune and inflammatory process [8]. It is important in humoral and cell-mediated immunity, it is a modulator in the immune response of B cells, T cells, and macrophages to stand in front of infections and malignancy [9].

The human IL-4 gene presents on chromosome 5q31 and consists of 25 kbps. More than 50 allelic variant polymorphisms for IL-4 gene have been found [10]. The most commonly reported variation within the IL-4 gene promoter region is the polymorphism -590C\T and it has multiple functions in both cancer and allergic diseases [11].

IL-4 gene polymorphisms have an important role in the response to HBV vaccine and risk of HBV in pathogenesis of HBV infection. However, role of these polymorphisms in HCV related liver disease and the development of HCC have not been completely clarified [12].

In order to search for possible target for HCC prevention, the aim of this study was to assess the association between IL-4 gene 590C\T polymorphism and risk of HCC in patients with HBV and HCV related cirrhosis.

METHODOLOGY

Study population and selection of patients:

This study was conducted during the period from December 2016 to November 2018 at Menoufia University Hospitals. The study was approved by the local ethics committee of the Menoufia University and informed consents were obtained from all the participants.

Two hundred and twenty Egyptian patients were included in this work. They were selected from Inpatients and Outpatient's Clinic of Tropical Medicine Department, as well as Clinical Oncology Department Menoufia University Hospital.

Our patients were classified into two groups: *group 1*: involved one hundred and twenty patients with HCV infection classified as sixty patients with liver cirrhosis and sixty patients with HCC, *group 2:* involved one hundred patients with HBV infection classified as fifty patients with liver cirrhosis and fifty patients with HCC. *Group 3*: sixty apparently healthy participants of matched age and sex were included as a control group.

All Laboratory investigations and genetic analysis were carried out in Clinical Pathology, Medical Microbiology and Immunology, Medical Biochemistry & Molecular Biology Departments, Faculty of Medicine, Menoufia University.

Virus status of the patients was assessed by presence of HBsAg, HCV antibodies in patient's sera and real time PCR. Liver cirrhosis was assessed by clinical evaluation; laboratory investigations, abdomino-pelvic ultrasound and CHILD score. Patients with HCC were diagnosed by triphasic CT showed features of HCC together with alphafeto-protein level (AFP) and biopsy.

Patients with autoimmune hepatitis, haemochromatosis, Wilson's disease, significant alcohol intake or non-alcoholic steatohepatitis (NASH) were excluded. In addition, patients with malignancy elsewhere were excluded.

Laboratory investigations:

HBsAg, HCVAbs and AFP were detected in separated sera by enzyme-linked fluorescent immunoassay method (ELFA) using mini VIDAS systems (bioMérieux, Marcy l'Etoile, France). For positive cases, HCV-RNA and HBV- DNA were confirmed by real time PCR. ALT level was determined by kinetic UV optimized method (IFCC) using LTEC Kit, England. Albumin and total bilirubin levels were determined by colorimetric method using kit supplied by Spectrum Diagnostics, Germany. Prothrombin time was determined using a BIOMED-LlQUIPLASTIN diagnostic kit, Germany, the international normalized ratio (INR) was estimated utilizing the tables provided by the manufacturer.

DNA amplification and Genetic polymorphism analysis:

2 ml of venous blood were withdrawn from all the studied subjects and evacuated into ethylene diamine tetra acetic acid (EDTA) tubes under the standard precaution and immediately kept at -20°C for molecular testing of polymorphism by PCR-RFLP. Later the Genomic DNA (gDNA) was extracted by Qiagen Genomic DNA Purification Kit (USA), following the instructions of manufacturer. Using Thermo Scientific Nanodrop apparatus, the DNA concentration was determined. The isolated DNA was stored at -20°C until analysis of IL-4 -590C/T gene polymorphism was performed.

IL-4 -590C/T gene polymorphism analysis:

To analyse polymorphic sites, PCR amplification was conducted in 50 µl reaction mixture containing 1 µL of gDNA, 1 µL of every primer (Forward primer: F:5'-AACACCTAAACTTGGGAGGA-3' and Reverse primer: R:5'-CTGTCATGGAAAAGCTGATCT-3', 25 µL of Taq polymerase (Promega, Madison WI, USA) and 22 µL of nuclease-free water. Initial denaturation at 95°C for 5 minutes was done. Then 35 cycles of 30 s at 94°C, 45 s at 56°C, and 50 s at 72°C, and as a final extension 7 min at 72 °C. We considered negative control in all PCR runs to detect any possible contamination. PCR output was added to 2.5 µL 10 X TBE Buffer and 10 U of the Fast Digest® PsyI restriction enzyme (New England BioLabs, Hitchin, UK) and incubated for 30 minutes at 37°C. The digested outcome has been loaded on a 2.0% agarose gel previously stained by ethidium bromide and was visualized using ultraviolet light. The C allele had PsyI enzyme cleavage site and digested to 177 and 17 bp fragments; the T allele had no cleavage site for PsyI

enzyme and only produced 195 bp fragments. Samples yielding 195 bp fragment were considered as genotype homozygote T/T, those with 177 and 17 bp fragments were considered as genotype homozygote C/C, while the presence of 195, 177 and 17 were considered as genotype heterozygote C/T **(13) (Figure 1).**

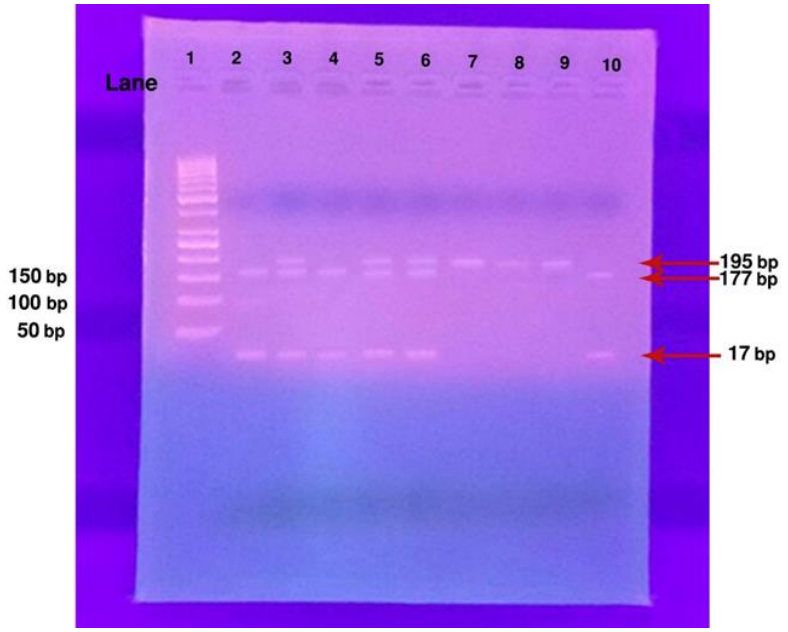

Fig. 1: Agarose gel electrophoresis showing PCR- RFLP analysis of IL 4 gene after addition of Psy I restriction enzyme: Lane1: 50-bp DNA ladder, Lanes 2, 4 & 10 (C/C) band (177 and 17 bp), lanes 3, 5 & 6 (C/T) band (195, 177 and 17 bp), lanes 7,8& 9 (T/T) band 195 bp.

Statistical analysis:

The data were collected and statistically analyzed by using SPSS ((version 17; SPSS Inc., Chicago, IL, USA). The Quantitative data were presented as mean and standard deviation (X ± SD). Qualitative data were presented as number (No) and percentage and analyzed using Chi square test. Normally and abnormally distributed quantitative data between two groups were determined by using T-test and man-witteny test respectively. Normally and abnormally distributed quantitative data between three groups were assessed by ANOVA test and Kruskal-Wallis test respectively.) and logistic regression analysis used to calculate the 95% confidence intervals (CI). A P value was considered significant at ≤ 0.05.

RESULTS

Among the 280 participants included in the study (220 patients and 60 healthy cases as controls, there was no significant difference between the studied groups: regarding age and gender as summarized in (table1).

There were not any significant differences between HCV and HBV patients regarding child score, or tumor size in HCC patients. For measured laboratory parameters, there were high significant variations between all studies groups ($p<0.001^{*}$) with significant elevation of ALT, bilirubin, INR and AFP levels and significant decrease in albumin level in both cirrhotic and HCC patients (either HCV or HBV related) as compared to controls. Additionally, HCC patients showed higher AFP level than cirrhotic patients in both HCV and HBV groups ($p1<0.001$ and $p2<0.001$ respectively) (table1).

Table 1: Comparison between the different studied groups according to different parameters

	Control (n = 60)	HCV infected patients (n = 120)		HBV infected patients (n = 100)		Test of Sig.	P
		Cirrhosis (n=60)	HCC (n=60)	Cirrhosis (n=50)	HCC (n=50)		
Age (years)	56 ± 7.2	55.9 ± 4.7	58.4 ± 5.2	56.7 ± 5.3	57.8 ± 5.9	F=2.209	0.068
Gender							
Male	30 (50%)	28 (46.7%)	32 (53.3%)	30 (60%)	28 (56%)	χ^2= 2.347	0.672
Female	30 (50%)	32 (53.3%)	28 (46.7%)	20 (40%)	22 (44%)		
Jaundice	–	38 (63.3%)	40 (66.7%)	32 (64%)	34 (68%)	χ^2=0.348	0.951
Encephalopathy	–	32(53.3%)	38 (63.3%)	18 (36%)	34 (68%)	χ^2=12.46*	0.006*
Ascites							
No	–	8 (13.3%)	2 (3.3%)	6 (12%)	0 (0%)		
Mild	–	14 (23.3%)	10 (16.7%)	8 (16%)	4 (8%)	χ^2= 17.870*	0.031*
Moderate	–	20 (33.3%)	26 (43.3%)	16 (32%)	24 (48%)		
Marked	–	18 (30%)	22 (36.7%)	20 (40%)	22 (44%)		
Child score							
A	–	12 (20%)	8 (13.3%)	8 (16%)	4 (8%)		
B	–	17(28.3%)	17 (28.3%)	10 (20%)	16 (32%)	χ^2= 5.020	0.541
C	–	31 (51.7%)	35 (58.3%)	32 (64%)	30 (60%)		
Anorxia	–	18 (30%)	24 (40%)	12 (24%)	18 (36%)	χ^2=3.617	0.306
Tumor size	–	–	6.1 ± 3.3	–	5.6 ± 3.1	t=0.889	0.376
ALT (U/L)	22 (21 – 33)	45# (24 – 154)	65# (26 – 213)	45# (31 – 215)	87# (32 – 276)	H=160.65*	<0.001*
Bilirubin	1 (0.7 – 1)	3.2# (1.6– 8.9)	2.8# (1.5– 8.5)	3# (1.6 – 8.9)	3# (1.5 – 12.5)	H=144.48*	<0.001*
Albumin	4.4 ± 0.4	2.8# ± 0.7	2.5# ± 0.5	2.7# ± 0.7	2.4# ± 0.4	F=142.68*	<0.001*
INR	1 ± 0.03	1.7# ± 0.32	1.6# ± 0.27	1.8# ± 0.32	1.6# ± 0.25	F=85.735*	<0.001*
AFP	3 (1.7 – 5.1)	18# (4 – 107)	144.5# (49–1290)	16# (4 – 107)	276# (45–3400)	H=212.54*	<0.001*
	p_1<0.001*, p_2<0.001*, p_3=0.748, p_4=0.382						

χ^2: **Chi square test** **F**: **ANOVA test** **U: Mann Whitney test** H: **Kruskal Wallis test**
p: p value for comparing between different groups
*: Statistically significant at p ≤ 0.05
#: Significant with control group
p1: p value for comparing between cirrhosis and HCC in top of HCV
p2: p value for comparing between cirrhosis and HCC in top of HBV
p3: p value for comparing between HCV and HBV infection with cirrhosis
p4: p value for comparing between HCV and HBV infection with HCC

Genotype polymorphisms and the allele frequency among the studied groups

Genetic association of the study population:

According to Hardy-Weinberg equilibrium (HWE), IL4 590C/T Polymorphism distribution was (χ^2= 1.710 and p= 0.191) and (χ^2= 2.778 and p= 0.095) in patients and controls respectively. All were agreed with HWE.

IL-4 590C/T Polymorphism:

Results of IL-4 590C/T gene analysis detected significant variation between the studied groups, as regard the genotypes and alleles frequencies (p = 0.029 and p = 0.003 respectively). There were higher frequency of CC genotype and C allele than TT genotype and T allele in all patients sub groups (cirrhotic or HCC) than healthy controls. While there were no significant differences between cirrhotic and HCC patients or HCV and HBV patients regarding genotypes and alleles distributions (p_1=0.245, p_2=0.922, p_3=0.747, p_4=0.922) (table 2).

Table (2): Comparison between the different studied groups according to IL4 CT polymorphism

	Control (n = 60)	HCV infected patients (n = 120)		HBV infected patients (n = 100)		χ^2	P
		Cirrhosis (n=60)	HCC (n=60)	Cirrhosis (n=50)	HCC (n=50)		
IL4 CT polymorphism		#	#	#	#		
TT	28(46.7%)	22(36.7%)	16(26.7%)	20(40%)	15(30%)		
CT	29(48.3%)	23(38.3%)	26(43.3%)	18(36%)	19(38%)	17.139*	0.029*
CC	3(5%)	15(25%)	18(30%)	12(24%)	16(32%)		
		p_1=0.496, p_2=0.519, p_3=0.940, p_4=0.847					
Allele		#	#	#	#		
T	85(70.8%)	67(55.8%)	58(48.3%)	58(58%)	49(49%)	15.695*	0.003*
C	35(29.2%)	53(44.2%)	62(51.7%)	42(42%)	51(51%)		
		p_1=0.245, p_2=0.922, p_3=0.747, p_4=0.922					

χ^2: **Chi square test**
p: p value for comparing between different groups
#: Significant with control group
p_1: p value for comparing between cirrhosis and HCC **in top of HCV**
p_2: p value for comparing between cirrhosis and HCC **in top of HBV**
p_3: p value for comparing between HCV and HBV infection with cirrhosis
p_4: p value for comparing between HCV and HBV infection with HCC
*: Statistically significant at $p \leq 0.05$

Risk of Association of IL-4 590C/T polymorphism with cirrhosis and HCC

As listed in (table3), we revealed that CC vs. TT genotype carriers showed positive correlation with cirrhosis & HCC risk in HCV patients as compared to controls with OR= 6.364 [95% CI: 1.6 – 24.8] and OR= 10.50 [95% CI: 2.7 – 41.2] respectively, Similarly in HBV patients CC genotypes demonstrated elevated risk for cirrhosis and HCC with OR 5.600 [95% CI: 1.4 – 22.5] and OR= 9.956 [95% CI: 2.5 – 39.7] respectively. In addition C allele was associated with increased cirrhotic and HCC risk in HCV infected patients with OR= 1.921 [95% CI: 1.1 – 3.3] and OR= 2.391 [95% CI: 1.4 – 4] versus control group. In HBV patients C allele showed higher cirrhotic and HCC risk with OR= 1.759 [95% CI: 1 – 3.1] and OR= 5.385 [95% CI: 2.9 – 10.1] when compared to controls as well as higher risk in HCC patients comparing to cirrhotic patients OR= 3.062 [95% CI: 1.6 – 5.8].

Table (3): Estimates of cirrhosis, HCC risk in cases HCV and HBV patients regarding to genotype

Polymorphism	Control (n= 60)	HCV infected patients (n = 120)					HBV infected patients (n = 100)				
		Cirrhosis (n=60)	OR_1 (95%CI)	HCC (n=60)	OR_1 (95%CI)	OR_2 (95%CI)	Cirrhosis (n=50)	OR_1 (95%CI)	HCC (n=50)	OR_1 (95%CI)	OR_2 (95%CI)
IL-4											
TT	28	22		16			20		15		
CT	29	23	1.009 (0.5 – 2.2)	26	1.569 (0.7 – 3.5)	1.554 (0.7 – 3.7)	18	0.869 (0.4 – 2)	19	1.223 (0.5 – 2.9)	1.407 (0.6 – 3.6)
CC	3	15	6.364* (1.6 – 24.8)	18	10.50* (2.7 – 41.2)	1.650 (0.6 – 4.2)	12	5.600* (1.4 – 22.5)	16	9.956* (2.5 – 39.7)	1.778 (0.7 – 4.8)
Allele											
T	85	67		58			58		23		
C	35	53	1.921* (1.1 – 3.3)	62	2.391* (1.4 – 4)	1.351 (0.8 – 2.2)	**42**	1.759* (1 – 3.1)	51	5.385* (2.9 – 10.1)	3.062* (1.6 – 5.8)

OR: Odds ratio
CI: Confidence interval
OR_1: HCC and cirrhosis vs control
OR_2: HCC vs cirrhosis

Association of studied genes and different parameters

In table 4, results of association of IL-4 590C/T polymorphism with different clinical parameters in cirrhotic and HCC patients showed that**,** there were not any significant variations in IL-4 590C genotype and allele frequency in relation with AFP level in cirrhotic or HCC patients and tumor size in HCC patients either HCV or HBV related. In terms of child score, this analysis reported significant associations with both IL-4 590C/T genotypes and alleles distributions in all patients' subgroups with C allele entailed highest level of child score C in comparing to T allele in cirrhotic patients [(HCV related: 62.3 %, $p_2=0.003^*$) (HBV: related 66.7%, $p_2<0.011^*$)] and HCC patients[(HCV related: 72.6 %, $p_2=0.001^*$) (HBV related: 66.7%, $p_2<0.001^*$)].

Table 4: Relation between IL-4 polymorphism and different parameters in each group

		IL-4 polymorphism				
		TT	CT	CC	T	C
AFP	Top of HCV					
	Cirrhosis	22(6 – 107)	16(5 – 107)	20(4 – 46)	20(5 – 107)	16(4 – 107)
			$p_1=0.394$		$P_2=0.152$	
	HCC	82(49 – 765)	279(49 – 1290)	98(54 – 1290)	99(49 – 1290)	190(49 – 1290)
			$p_1=0.157$		$P_2=0.501$	
	Top of HBV					
	Cirrhosis	31.5(11 – 107)	11(4 – 107)	16(5 – 107)	20(4 – 107)	13.5(4 – 107)
			$p_1=0.080$		$P_2=0.076$	
	HCC	600(55 – 2654)	234(54 – 3400)	765(45 – 2000)	276(54 – 3400)	276(45 – 3400)
			$p_1=0.688$		$P_2=0.804$	
Child score	Top of HCV					
	Cirrhosis					
	A	9(40.9%)	4(17.4%)	2(13.3%)	22(32.8%)	8(15.1%)
	B	10(45.55)	4(17.4%)	4(26.7%)	24(35.8%)	12(22.6%)
	C	3(13.6%)	15(65.2%)	9(60%)	21(31.3%)	33(62.3%)
			$p_1=0.004^*$		$P_2=0.003^*$	
	HCC					
	A	8(50%)	0(0%)	2(11.1%)	16(27.6%)	4(6.5%)
	B	5(31.3%)	9(34.6%)	2(11.1%)	19(32.8%)	13(21%)
	C	3(18.8%)	17(65.4%)	14(77.8%)	23(39.7%)	45(72.6%)
			$p_1<0.001^*$		$P_2<0.001^*$	
	Top of HBV					
	Cirrhosis					
	A	10(50%)	4(22.2%)	1(8.3%)	24(41.4%)	6(14.3%)
	B	2(10%)	6(33.3%)	1(8.3%)	10(17.2%)	8(19%)
	C	8(40%)	8(44.4%)	10(83.3%)	24(41.4%)	28(66.7%)
			$p_1=0.033^*$		$P_2=0.011^*$	
	HCC					
	A	7(46.7%)	1(5.3%)	0(0%)	15(30.6%)	1(2%)
	B	3(20%)	6(31.6%)	5(31.3%)	12(24.5%)	16(31.4%)
	C	5(33.3%)	12(63.2%)	11(68.8%)	22(44.9%)	34(66.7%)
			$p_1=0.008^*$		$P_2<0.001^*$	
Tumor size	Top of HCV					
	HCC	5.9 ± 3.7	6.7 ± 3.2	5.4 ± 3	6.3 ± 3.4	6 ± 3.1
			$p_1=0.477$		$P_2=0.607$	
	Top of HBV					
	HCC	4.4 ± 2.2	6.3 ± 3.4	5.8 ± 3.2	5.1 ± 2.8	5.98 ± 3.2
			$p_1=0.196$		$P_2=0.158$	

Qualitative data were described using number and percent and was compared using Chi square test, Monte Carlo test, or Fisher Exact test. Abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test or Kruskal Wallis test

p_1: p value for comparing between overall IL-4 genotypes

p_2: p value for comparing between T and C alleles ; *: Statistically significant at $p \leq 0.05$

DISCUSSION

One of the most prevalent tumor and cancer-related deaths all over the world is hepatocellular carcinoma. Hepatitis B and C viruses are important causes to HCC [14]. Hepatocellular carcinoma arises mainly in tissues with long-standing inflammation and fibrosis, which induces progression of the tumor and increases resistance to treatment [15]. The pathogeneses of HCC is associated with many predisposing factors, together with presence of genetic predisposition [16].

Polymorphisms in cytokine gene affect inflammatory-related pathways, and increase susceptibility to different types of cancer. As the normal function of the immune system relies on a genetically determined balance between Th1 and Th2 lymphocytes, the role of IL-4 is of major significance, as a crucial moderator of this balance [17].

IL4-590C/T polymorphism in the pro-motor region of IL-4 gene affects its secretion [18,19]. Interleukin-4 (IL-4) is considered a potent modifier of antitumor immune responses having tumor-promoting and tumor-inhibiting functions as well, since it owns immunosuppressive and anti-angiogenic properties [20].

In management of HCC both curative and preventive measures could be considered. To date, only anti-viral, anti-inflammatory and iron depletion therapies are likely to exhibit clinically meaningful effect as HCC prevention.(21)

The current study aimed to detect the association between IL-4 gene 590C/T polymorphism and risk of HCC in patients with HBV and HCV related cirrhosis.

Among the included participants, serum AFP level was significantly different between control group and patients with either liver cirrhosis or HCC where, AFP was significantly elevated in patients with liver cirrhosis and much more elevated in patients with HCC indicating its possible role in diagnosis and follow up in those patients.

However, there was no significant difference between HCV, HBV infected patients regarding serum AFP, and this can be explained by the fact that AFP is related to hepatocyte pathology regardless of the causative pathogen.

In this work, we revealed an elevated frequency of CC genotype and C allele than TT genotype and T allele in HCC in HCV and HBV groups and cirrhotic HCV patients when compared with healthy controls. CT+CC genotype and C allele carriers had an elevated HCC risk in both HCV and HBV patients. Moreover C allele was associated with increased risk of liver cirrhosis in HBV patient as well.

There were not any associations of IL-4 590C/T genotypes, alleles, and haplotypes with the overall chronic hepatitis B, liver cirrhosis and HCC patients in the study done **by Lu et al.** [22] on Chinese Population. However, in subgroup analysis, they observed that CC genotypes were significantly associated with chronic hepatitis B (CHB) in males as compared with the other genotypes. While in females, parallel pattern of an increased CHB risk with CC genotypes was perceived but not enough to be statistically significant. Also the study conducted in North China by Gao et al.[23] to assess the relationship between some cytokine polymorphism and chronic hepatitis they showed that, CC and CT genotypes frequencies had significant elevation in HBV infected patients with abnormal liver enzyme levels which consistent with our finding.

On the other hand, Wu et al.[24] found significant correlation between the IL-4 590C/T polymorphism (TT+CT vs CC) and HCV infection as well as HCC risk in Asian populations, although no significant relation was found in Caucasian populations. Also Zheng et al.[13] found an association between T allele and risk of hepatic diseases like cirrhosis in Caucasian populations affected with HBV and HCV.

Regarding to our results T allele may be the wild allele in IL-4 590 C/T polymorphism in this group of Egyptian population and the variant one is the C allele, which may be responsible for diverse liver diseases. In the current study, the IL-4 590T allele frequencies among healthy controls were 76.7% and the C allele frequencies represented 23.3% which were related to those of Chinese Population (82.6% and 17.4% respectively)[22]. In contrast, Caucasians reported 13.7% for T allele and 86.3%for C allele in the control subjects[13].

In preceding two studies, researchers detected a reduced in HCC risk associated with CC genotype of IL-4 590 in China and a non-significant result in non-Asians in USA. [17,25].

On studying the association of IL-4 590C/T Polymorphism with other types of cancer, Jia et al.[20] demonstrated that IL-4 590C/T Polymorphism was significantly related to cancer risk. The CT/TT genotype had a lower risk of gastric cancer or breast cancer than the CC genotype and this was in agreement with us. In addition, the CT genotype was declared to be associated with elevated risk of prostate cancer. Subsequent subgroup analysis demonstrated that, cancer risk was higher in both Asian and Caucasian. These conflicting results might be attributed to variations in ethnicity, sample size, natural history of viral hepatitis in China compared to Egypt and even in study design.

CONCLUSION

The results of this research explored that, IL-4 590C/T Polymorphism in this group of Egyptian population has an elevated frequency of CC genotype and C allele in both HCV and HBV related cirrhotic patients as well as HCC patients. CT+CC genotype and

C allele carriers have an elevated HCC risk in HCV and HBV patients and high risk of liver cirrhosis in patients with chronic HCV. IL-4 590C/T gene polymorphism may have a role in progression of liver cirrhosis and hazard relationship for HCC development.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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