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# Association of IL-10 and TNF- $\alpha$ polymorphisms with risk and aggressiveness of hepatocellular carcinoma in patients with HCV-related cirrhosis

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## Abstract

**Background:** Hepatitis C virus (HCV) infection is a significant risk factor for cirrhosis and hepatocellular carcinoma (HCC) that carry a high mortality. The study aims to investigate the effect of tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-10 polymorphisms on risk and pattern of HCC in patients with HCV-related cirrhosis.

**Results:** The mean age of the HCC group was  $56.21 \pm 4.62$  years and  $54.27 \pm 7.63$  years for the cirrhotic group. The GG genotype of TNF- $\alpha$  and TT genotype of IL-10 showed a higher incidence of HCC in comparison to the cirrhotic group with  $P = 0.01$  and  $0.004$ . On the calculation of the aggressiveness index (Agi), the TT haplotype was significantly associated with more aggressive tumours in contrast to the other haplotypes with  $P < 0.001$ . There is a significant association of portal vein thrombosis, ascites and high Agi with the GG haplotype in contrast to the other haplotypes with  $P = 0.002$ ,  $0.029$  and  $< 0.001$ , respectively, as regards TNF- $\alpha$ . High Agi (C) was associated with the TT haplotype of IL-10 and GG haplotype of TNF- $\alpha$ .

**Conclusion:** Our data bring an essential association of IL-10 and TNF polymorphism with the occurrence of HCC in patients with HCV-related liver cirrhosis. The GG haplotype of TNF- $\alpha$  and TT/AT haplotype of IL-10 are associated with the more aggressive pattern of HCC, so those patients must be treated as early as possible.

**Keywords:** Interleukin, Tumour necrosis factor, Hepatocellular carcinoma

## Background

Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are global health problems as they are associated with high morbidity and mortality. It is estimated that about 200 million people are infected with HCV, among which 170 million are chronically infected [1].

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related deaths worldwide [2]. More than 600,000 people die

from HCC each year [3]. The aetiology is still unclear; however, several major risk factors of HCC have been shown to contribute to hepatocarcinogenesis. Chronic inflammation induced by the action of various inflammatory mediators has been recently identified as a cofactor in carcinogenesis [3]. Among these inflammatory mediators, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) plays an essential role and has been implicated in inflammation-associated tumours [4].

An "HCC Aggressiveness" scoring system was recently described, which incorporates four tumour-related parameters such as maximum tumour diameter (MTD), presence of portal vein thrombosis (PVT), number of lesions and serum alpha-fetoprotein (AFP) levels. The

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score was shown to predict survival in patients with HCC [5].

Aroucha et al. concluded that polymorphisms in TNF- $\alpha$  and interleukin (IL)-10 were associated with increased risk of HCC development in HCV chronically infected patients. The GG genotype of TNF- $\alpha$  and genotypes associated with low/intermediate levels of IL-10 were shown to be associated with increased risk of development of HCC. Moreover, the TT genotype of the IL-10 -819 was significantly correlated with advanced stages of HCC as well as with multiplicity of lesions. These variants were shown to be associated with more inflammation in the liver, mediated by Th1 cytokines and may increase the risk to have HCC and bring an adverse prognosis in these patients [6].

### Aim of the study

In this study, we tried to assess the association of TNF- $\alpha$  and IL-10 polymorphisms with the risk and aggressiveness of HCC in patients with HCV-related cirrhosis.

## Methods

### Patients

This is a case-control study that was conducted on 73 patients attending to early detection at HCC Clinic, Specialized Medical Hospital, Mansoura University, from June 2019 to February 2020 for follow-up of HCC and 85 patients with HCV-related cirrhosis without evidence of hepatic focal lesions proved by post-contrast triphasic computed tomography (CT) scan. Patients with cirrhosis related to other causes than HCV, patients with any tumour outside the liver and patients with ongoing organ failure were excluded from the study. All patients were proved to be HCV infected through the history of previous antiviral treatment for HCV or through testing for HCV Ab or HCV RNA. All patients were exposed to complete history taking with stress on history of smoking or ex-smoker, diabetes, hypertension, history of previous treatment for HCV or bilharziasis and family history of cancer. Complete clinical examination was done for all patients with stress on manifestations of hepatocellular failure. Baseline tumour characters, including MTD, the number of focal lesions and the presence of portal vein thrombosis, were collected from imaging reports done at Specialized Medical Hospital for the HCC group.

### Laboratory assessment

Complete blood count, liver function tests with Child-Pugh classification, HBsAg, HCV Ab, serum creatinine and AFP were done for all patients.

### IL-10 and TNF- $\alpha$ polymorphism determination

Peripheral blood was used to extract genomic DNA using the Wizard Genomic Blood DNA Isolation Kit (Promega, Madison, WI). We stored samples at  $-80^{\circ}\text{C}$  until single nucleotide polymorphism (SNP) genotyping by real-time polymerase chain reaction (PCR) was done. In IL-10 gene, we tested one substitution at position -819 C>T (rs1800871). In TNF- $\alpha$ , the substitution at position -308 G>A (rs1800629) was tested. We used TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA), according to the instructions of the manufacturer.

According to Ventura et al., the aggressiveness index (AgI) score was divided into three categories: a, score  $< 4$ ; b,  $4 < \text{score} \leq 7$ ; and c, score  $\geq 8$  (Table 1) [7].

Aggressiveness index was calculated for all patients with HCC. According to the result of genotype frequency distribution of IL-10 -819 (rs1800871) and TNF- $\alpha$  -308 (rs1800629), patients will be divided into groups and compared.

### Statistical analysis

Data were fed to the computer and analysed using IBM SPSS Corp. (released in 2013, IBM SPSS Statistics for Windows, version 22.0, Armonk, NY: IBM Corp). Qualitative data were described using the number and per cent. Quantitative data were described using median and interquartile range for non-parametric data and mean, the standard deviation for parametric data after testing normality using the Kolmogorov-Smirnov test. The significance of the obtained results was judged at the 0.05 level.

Data analysis was done using the chi-square test and Monte Carlo test for comparison of 2 or more groups of categorical variables as appropriate. Student *t* test and one-way ANOVA test were used to compare parametric variables. Mann-Whitney *U* test and Kruskal-Wallis test were used to compare independent groups for non-parametric variables. Spearman's rank-order correlation is used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and/or ordinal variables.

## Results

### Patient sociodemographic characteristics

The mean age of the HCC group was  $56.21 \pm 4.62$  years and  $54.27 \pm 7.63$  years for the cirrhotic group. There

**Table 1** Aggressiveness index parameters

	1	2	3
Maximum tumour dimension(CM)	< 4.5	4.5–9.6	> 9.6
AFP (ng/ml)	< 100	100–1000	> 1000
PVT	No		Yes
Tumour nodule (number)	$\leq 3$		> 3

was no difference in sex distribution between the two groups. In the HCC group, 31 (42.5%) patients had diabetes and 19 (26.0%) hypertensive in comparison to 27 (31.8%) and 23 (27.1%) in the cirrhotic group, respectively. A statistically significant difference between the two groups was found as regards serum albumin, platelets, aspartate aminotransferase (AST) and AFP with  $P = 0.003$ ,  $0.001$ ,  $0.001$  and  $< 0.001$ , respectively (Table 2).

#### Genotype testing and distribution

On genotype testing, as regards TNF- $\alpha$ , 35 (47.9%) of patients were GG, 22 (30.1%) were GA and 16 (21.9%) were AA in the HCC group. In the cirrhotic group, 27 (31.8%) of patients were GG, 25 (29.4%) were GA and 33 (38.8%) were AA. As regards IL-10, 29 (39.7%) of patients were TT, 21 (28.8%) were CT and 23 (31.5%) were CC in the HCC group. In the cirrhotic group, 16 (18.8%) of patients were TT, 29 (34.1%) were CT and 40 (47.1%) were CC. From these data, it appears that the GG

genotype of TNF- $\alpha$  and TT genotype of IL-10 showed a higher incidence of HCC in comparison to the cirrhotic group with  $P = 0.01$  and  $0.004$  (Table 3 and Fig. 1).

#### Tumour characteristics and aggressiveness concerning genotype polymorphism

As regards IL-10, the portal was found to be thrombosed significantly in the TT haplotype in contrast to the other haplotypes with  $P < 0.001$ . Similarly, ascites was significantly found in the TT haplotype in contrast to the other haplotypes with  $P = 0.006$ . On the other hand, there was no significant difference in the size of the spleen, lymph node metastases or site of the lesions between the haplotypes. On the calculation of the AgI, the TT haplotype was significantly associated with more aggressive tumours in contrast to the other haplotypes with  $P < 0.001$  (Table 4). Table 5 shows the tumour characteristics and aggressiveness of TNF- $\alpha$  genotypes where there was a significant association of portal vein thrombosis, ascites and high AgI with the GG haplotype in contrast to the other haplotypes with  $P = 0.002$ ,  $0.029$

**Table 2** Sociodemographic characteristics of the studied groups

	HCC, $n = 73$	Cirrhotic, $n = 85$	Test of significance
Age/years, mean $\pm$ SD	56.21 $\pm$ 4.62	54.27 $\pm$ 7.63	$t = 1.89$ $P = 0.06$
Sex, $N$ (%)			
Male	43 (58.9%)	39 (45.9%)	$\chi^2 = 2.67$ $P = 0.102$
Female	30 (41.1%)	46 (54.1%)	
DM, $N$ (%)	31 (42.5%)	27 (31.8%)	$\chi^2 = 1.94$ $P = 0.164$
Hypertension, $N$ (%)	19 (26.0%)	23 (27.1%)	$\chi^2 = 0.021$ $P = 0.884$
Albumin (gm/dl), mean $\pm$ SD	3.29 $\pm$ 0.59	3.56 $\pm$ 0.52	$t = 2.98$ $P = 0.003^*$
Bilirubin (mg/dl), median (IQR)	1.2 (0.85–1.7)	1.2 (0.8–1.64)	$z = 0.821$ $P = 0.412$
WBCS, mean $\pm$ SD	4.78 $\pm$ 1.88	4.69 $\pm$ 1.85	$t = 0.33$ $P = 0.739$
HB (gm/dl), mean $\pm$ SD	11.33 $\pm$ 1.66	11.42 $\pm$ 1.53	$t = 0.374$ $P = 0.709$
Platelets $\times 10^3$ , mean $\pm$ SD	101.05 $\pm$ 44.17	123.92 $\pm$ 36.76	$t = 3.55$ $P = 0.001^*$
ALT (U/l), median (IQR)	35.0 (28.5–47.0)	35.0 (29.0–45.0)	$z = 0.452$ $P = 0.65$
AFP (ng/dl), median (IQR)	89.0 (33.0–230.5)	5.2 (2.8–9.7)	$z = 9.98$ $P < 0.001^*$
AST(U/l), median (IQR)	68.0 (54.5–89.0)	40.0(36.0–47.0)	$z = 7.67$ $P < 0.001^*$
INR, mean $\pm$ SD	1.36 $\pm$ 0.20	1.32 $\pm$ 0.19	$t = 1.09$ $P = 0.275$

Parameters described as mean  $\pm$  SD, median (interquartile range) or number (percentage)

$t$  Student  $t$  test,  $Z$  Mann-Whitney  $U$  test,  $\chi^2$  chi-square test

\*Statistically significant

**Table 3** Genotype distribution among the studied groups

	HCC, n = 73 (%)	Cirrhotic, n = 85 (%)	Test of significance	Odds ratio (95% CI)
IL-10				
TT/AA	29 (39.7%)	16 (18.8%)	$\chi^2 = 8.2, P = 0.004^*$	3.15 (1.42–6.99)
CT/CA	21 (28.8%)	29 (34.1%)	$\chi^2 = 0.35, P = 0.55$	1.26 (0.59–2.69)
CC/CC	23 (31.5%)	40 (47.1%)		Reference group
Hardy-Weinberg equation	$P = 0.002^*$	$P = 0.02^*$		
TNF- $\alpha$				
GG	35 (47.9%)	27 (31.8%)	$\chi^2 = 6.24, P = 0.01^*$	2.67 (1.22–5.83)
GA	22 (30.1%)	25 (29.4%)		1.82 (0.79–4.15)
AA	16 (21.9%)	33 (38.8%)	$\chi^2 = 2.01, P = 0.16$	Reference group
Hardy-Weinberg equation	$P = 0.002^*$	$P = 0.001^*$		

$\chi^2$  chi-square test  
\*Statistically significant

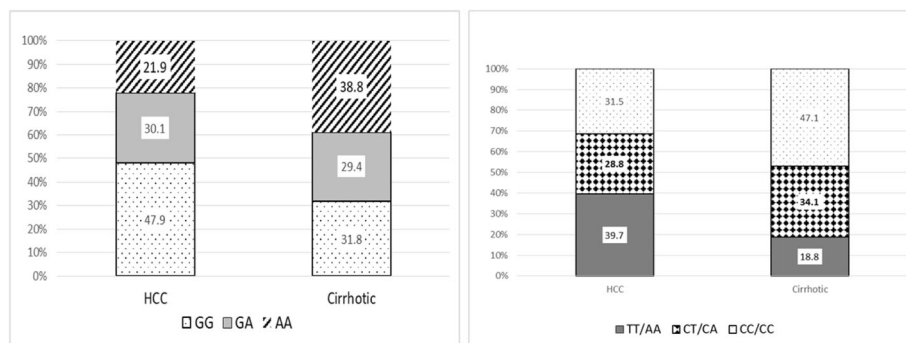
and < 0.001, respectively. Table 6 shows the association of HCC aggressiveness index with sociodemographic and clinical characteristics and genotype of HCC cases where there was no statistically significant difference in the parameters except for AFP and genotype distribution as high AgI (C) was associated with the TT haplotype of IL-10 and GG haplotype of TNF- $\alpha$ .

**Discussion**

HCC represents about 90% or more of primary liver tumours that usually develops in a background of advanced liver disease [8]. Approximately, 10–20% of chronically infected patients with HCV will develop liver cirrhosis, and 1–5% of those patients will develop HCC [9]. The continued cytokines induced hepatocyte damage, and hepatocyte regeneration leads to HCC development. The role of cytokines as IL-1, IL-2, IL-6, IL-10, IL-12 and TNF- $\alpha$  in hepatocarcinogenesis has been reported. The management of patients with HCC represents a challenge as it is often complicated by the heterogenic pattern of the disease, the state of underlying

liver disorders and the need to coordinate a multidisciplinary healthcare team [10].

In our study, the mean age of the HCC group was  $56.21 \pm 4.62$  years and  $54.27 \pm 7.63$  years for the cirrhotic group. There was no difference in sex distribution between the two groups (Table 2). In the HCC group, 31 (42.5%) patients had diabetes and 19 (26.0%) hypertensive in comparison to 27 (31.8%) and 23 (27.1%) in the cirrhotic group, respectively. This runs parallel to many other studies that concluded that advanced age, male gender and DM are well-known risk factors for HCC [11]. Cirrhosis, due to HCV, was found in almost all our patients. This seems logical and in agreement with Seyda et al. who clarified that most of HCC cases develop in a background of cirrhosis [12]. There was no difference in the sociodemographic and clinicolaboratory parameters between the two groups except for serum albumin, platelets, AST and AFP with  $P = 0.003, 0.001, 0.001$  and < 0.001, respectively. This seems logical as platelet count, AST and serum albumin reflect the severity of the liver disease. It also suggests that the two groups are



**Fig. 1** Genotype distribution among the studied groups

**Table 4** Tumour characteristics of HCC cases according to IL-10 polymorphism

	Total, n = 73	Genotype			Test of significance
		TT/AA	CT/CA	CC/CC	
Portal vein					
Thrombosed	10	10 (34.5)	0 (0.0)	0 (0.0)	MC $P < 0.001^*$
Patent	63	19 (65.5)	21 (100.0)	23 (100.0)	
Spleen					
Splenectomy	2	0 (0.0)	2 (9.5)	0 (0.0)	MC $P = 0.154$
Mild	26	7 (24.1)	11 (52.4)	8 (34.8)	
Moderate	39	19 (65.5)	8 (38.1)	12 (52.2)	
Marked	6	2 (6.9)	2 (9.5)	2 (8.7)	
Ascites					
Negative	62	20 (69.0)	19 (90.5)	23 (100.0)	MC $P = 0.006^*$
Positive	11	9 (31.0)	2 (9.5)	0 (0.0)	
Lymph node					
Negative	64	23 (79.3)	21 (100.0)	20 (87.0)	MC $P = 0.089$
Positive	9	6 (20.7)	0 (0.0)	3 (13.0)	
BCLC					
A	11	0 (0.0)	7 (33.3)	4 (17.4)	MC $P < 0.001^*$
B	24	3 (10.3)	7 (33.3)	14 (60.9)	
C	24	13 (44.8)	7 (33.3)	4 (17.4)	
D	14	13 (44.8)	0 (0.0)	1 (4.3)	
Site of lesion					
Right lobe	25	10 (34.5)	6 (28.6)	9 (39.1)	MC $P = 0.929$
Left lobe	12	5 (17.2)	3 (14.3)	4 (17.4)	
Multifocal	36	14 (48.3)	12 (57.1)	10 (43.5)	
AGI					
A	22	2 (6.9)	8 (38.1)	12 (52.2)	MC $P < 0.001^*$
B	23	6 (20.7)	9 (42.9)	8 (34.8)	
C	28	21 (72.4)	4 (19.0)	3 (13.0)	

\* means statistically significant

matched which is extremely important in the inclusion of our patients to avoid the effect of any factor such as age, sex or comorbidities on risk of HCC development, so the increased incidence of HCC is directly related to the effect of gene polymorphism.

In the present study, on genotype testing, as regards TNF- $\alpha$ , 35 (47.9%) of patients were GG, 22 (30.1%) were GA and 16 (21.9%) were AA in the HCC group. In the cirrhotic group, 27 (31.8%) of patients were GG, 25 (29.4%) were GA and 33 (38.8%) were AA. As regards IL-10, 35 (47.9%) of patients were TT, 21 (28.8%) were CT and 23 (31.5%) were CC in the HCC group. In the cirrhotic group, 16 (18.8%) of patients were TT, 29 (34.1%) were CT and 40 (47.1%) were CC. From these data, it appears that the GG genotype of TNF- $\alpha$  and TT

genotype of IL-10 showed a higher incidence of HCC in comparison to the cirrhotic group with  $P = 0.01$  and  $0.004$  (Table 3 and Fig. 1). This goes hand in hand with Aroucha et al. who found that the TT/AT haplotypes of IL-10 and GG haplotype of TNF- $\alpha$  were significantly expressed in patients with HCC [6]. Wei et al. and Cheng et al. agreed [13, 14] also with us but not with Zhou et al. who showed that no association was found between HCC and the TNF- $\alpha$ -238 G/A polymorphism [15]. This contrast may be related to the aetiology of the underlying liver disease, ethnicity or number of included patients.

In 2012, Swiatek found that gene polymorphisms may affect the IL-10 level; he showed that IL-10 -819T was associated with significant low IL-10 expression since it is located in transcript factor binding regions [16]. Aroucha et al. observed an increased frequency of IL-10 -819T genotype in patients with HCC [6]. Moreover, they found a significant association between the TT genotype of IL-10 -819 and multiplicity of lesions and terminal stages of HCC.

As regards TNF- $\alpha$ , the results are conflicting. Talaat et al. and Radwan et al. did not find any significant association between HCC and TNF- $\alpha$  -308 polymorphism in HCV-infected Egyptian patients [17, 18]. On the other hand, Baghel et al. and Karimi et al. demonstrated that patients with TNF- $\alpha$  G allele usually show low TNF- $\alpha$  production in vivo and in vitro [19, 20] despite, Vikram et al. failed to confirm this association [21]. It seems that the balance between IL-10 and TNF- $\alpha$  is crucial for the prevention of development of HCC and that low levels lead to progressive damage to the liver tissue and prevention of wound healing.

Similarly, previous studies found elevated levels of circulating TNF- $\alpha$  in patients with HCC. It is reasonable to speculate that in patients with HCC, the high circulating TNF- $\alpha$  levels found may be attributed to its SNPs. Also, TNF- $\alpha$  may stimulate the release of other inflammatory cytokines and induce the release of other fibrogenic factors, such as interleukin-1, interleukin-6 and tumour growth factor- $\beta$  which can cause or aggravate liver damage [22, 23].

Previously, immunomodulatory cytokines have been described as pre-malignant mediators in different tumour entities in different studies [24]. In HCC, IL-6 promotes multiple stages of tumour development, including initial hepatocyte proliferation, the transformation of hepatocytes into HCC progenitor cells, and progression to HCC nodules and metastases [24].

It seems that the balance between TNF- $\alpha$  and IL-10 is mandatory to the development of HCC, since the shift to Th1 pattern-like cytokines in the liver may lead to more inflammation, necrosis of hepatocytes and subsequent regeneration that leads to mutagenesis and

**Table 5** Tumour characteristics of HCC cases according to TNF- $\alpha$  polymorphism

	Total, n = 73(%)	Genotype			Test of significance
		GG	GA	AA	
Portal vein					
Thrombosed	10	10 (28.6)	0 (0.0)	0 (0.0)	MC $P = 0.002^*$
Patent	63	25 (71.4)	22 (100.0)	16 (100.0)	
Spleen					
Splenectomy	2	0 (0.0)	2 (9.1)	0(0.0)	MC $P = 0.313$
Mild	26	14 (40.0)	8 (36.4)	4(25.0)	
Moderate	39	18 (51.4)	12 (54.5)	9(56.2)	
Marked	6	2 (5.7)	2 (9.1)	2(12.5)	
Ascites					
Negative	62	26 (74.3)	22 (100.0)	14 (87.5)	MC $P = 0.029^*$
Positive	11	9 (25.7)	0 (0.0)	2 (12.5)	
Lymph node					
Negative	64	28 (80.0)	22 (100.0)	14 (87.5)	MC $P = 0.08$
Positive	9	7 (20.0)	0 (0.0)	2 (12.5)	
BCLC					
A	11	0 (0.0)	5 (22.7)	6 (37.5)	MC $P < 0.001^*$
B	24	2 (5.7)	15 (68.2)	7 (43.8)	
C	24	19 (54.3)	2 (9.1)	3 (18.8)	
D	14	14 (40.0)	0 (0.00)	0 (0.0)	
Site of lesion					
Right lobe	25	12 (34.3)	9 (40.9)	4 (25.0)	MC $P = 0.74$
Left lobe	12	7 (20.0)	2 (9.1)	3 (18.8)	
Multifocal	36	16 (45.7)	11 (50.0)	9 (56.2)	
AGI					
A	22	0 (0.0)	12 (54.5)	10 (62.5)	MC $P < 0.001^*$
B	23	9 (25.7)	8 (36.4)	6 (37.5)	
C	28	26 (74.3)	2 (9.1)	0 (0.0)	

\* means statistically significant

activation of protooncogene in the host cells, leading to the development of HCC [25].

A fine tune of the IL-10 and TNF- $\alpha$  balance may exist, and it looks that this balance is controlled by the level of IL-10, where low levels lead to progressive damage to liver tissue and prevention of wound healing. Also, IL-10 can diminish the response to antiviral treatment [26].

Our data showed that the TT haplotype of IL-10 was significantly associated with more aggressive tumours in contrast to the other haplotypes with  $P < 0.001$ . The portal was found to be thrombosed significantly in the TT haplotype in contrast to the other haplotypes with  $P < 0.001$ ; they were also associated with the multiplicity of lesions. Similarly, a significant association of portal vein thrombosis, ascites and high AgI with the GG haplotype in contrast to the other

haplotypes with  $P = 0.002$ , 0.029 and  $< 0.001$ , respectively. These data are available in the absence of a statistically significant difference between AgI and sociodemographic and clinicolaboratory characteristics except for AFP and genotype distribution. This suggests that the aggressive pattern of the tumour noticed in these haplotypes is related to the direct effect of gene polymorphism. Aroucha et al. agree with us as they observed a significant correlation of advanced stages and multiple lesions of HCC with the TT (AA) genotype of IL-10 -819 (-592) [6]. However, studies covering this sector are relatively rare.

Our study may be limited by some factors such as the limited number of cases in the study, the lack of data about overall survival of patients and lastly we included only patients with HCV-related cirrhosis which may affect hepatocarcinogenesis.

**Table 6** Association of HCC aggressiveness index with sociodemographic and clinical characteristics and genotype of HCC cases

	AGI			Test of significance
	A	B	C	
Age/years, mean $\pm$ SD	54.64 $\pm$ 4.44	57.74 $\pm$ 4.98	56.18 $\pm$ 4.15	$F = 2.66$ $P = 0.08$
Sex, $n$ (%)				
Male	13 (59.1)	15 (65.2)	15 (53.6)	$\chi^2 = 0.708$ $P = 0.702$
Female	9 (40.9)	8 (34.8)	13 (46.4)	
Albumin (g/dl), mean $\pm$ SD	3.36 $\pm$ 0.62	3.23 $\pm$ 0.71	3.28 $\pm$ 0.46	$F = 0.280$ $P = 0.757$
Bilirubin (mg/dl), median (IQR)	1.1 (0.90–1.7)	1.68 (0.80–2.4)	1.20 (0.825–1.5)	KW $P = 0.514$
WBCS, mean $\pm$ SD	5.25 $\pm$ 1.89	4.40 $\pm$ 1.21	4.72 $\pm$ 2.28	$F = 1.15$ $P = 0.32$
HB (g/dl), mean $\pm$ SD	11.88 $\pm$ 1.43	10.91 $\pm$ 1.56	11.24 $\pm$ 1.84	$F = 1.15$ $P = 0.32$
Platelet $\times 10^3$ , mean $\pm$ SD	85.09 $\pm$ 29.89	116.09 $\pm$ 56.5	101.25 $\pm$ 38.8	$F = 2.92$ $P = 0.061$
ALT (u/l), median (IQR)	38.0 (27.0–43.0)	36.0 (32.0–55.0)	33.0 (29.25–65.75)	KW $P = 0.482$
AFP (ng/dl), median (IQR)	190 (83.0–536.0) <sup>a</sup>	83.0 (27.0–340.0)	67.0 (32.0–106.25) <sup>a</sup>	KW $P = 0.013^*$
AST(u/l), median (IQR)	56.0 (47.0–88.0)	69.0 (66.0–89.0)	71.0 (53.0–98.0)	KW $P = 0.09$
INR, mean $\pm$ SD	1.36 $\pm$ 0.13	1.41 $\pm$ 0.29	1.31 $\pm$ 0.15	$F = 1.71$ $P = 0.189$
DM, $n$ (%)	8 (36.4)	9 (39.1)	14 (50.0)	$P = 0.58$
Hypertension, $n$ (%)	6 (27.3)	6 (26.1)	7 (25.0)	$P = 0.98$
IL-10 genotypes				
TT/AA	2 (9.1) <sup>a</sup>	6 (26.1) <sup>b</sup>	21 (75.0) <sup>a,b</sup>	MC $P < 0.001^*$
CT/CA	8 (36.4)	9 (39.1)	4 (14.3)	
CC/CC	12 (54.5)	8 (34.8)	3 (10.7)	
TNF- $\alpha$ genotypes				
GG	0 (0.0) <sup>a,b</sup>	9 (39.1) <sup>a,c</sup>	26 (92.9) <sup>b,c</sup>	MC $P < 0.001^*$
GA	12 (54.5)	8 (34.8)	2 (7.1)	
AA	10 (45.5)	6 (26.1)	0 (0.0)	

MC Monte Carlo test, KW Kruskal-Wallis test, F one-way ANOVA test

\* means statistically significant

a significance with group a

b significance with group b

c significance with group c

ab significance with group a, b

bc significance with group b, c

To summarize, specific genotypes of TNF- $\alpha$  and IL-10 may affect the progression of hepatocarcinogenesis in patients with HCV-related liver cirrhosis.

## Conclusion

Our data bring an essential association of IL-10 and TNF polymorphism with the occurrence of HCC in HCV-related liver cirrhosis. The GG haplotype of TNF- $\alpha$  and TT/AT haplotype of IL-10 are associated with the

more aggressive pattern of HCC, so those patients must be treated as early as possible.

## Abbreviations

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; TNF- $\alpha$ : Tumour necrosis factor- $\alpha$ ; MTD: Maximum tumour diameter; PVT: Portal vein thrombosis; AFP: Alpha-fetoprotein; IL: Interleukin; CT: Computed tomography; PCR: Polymerase chain reaction; SNP: Single nucleotide polymorphism; AgI: Aggressiveness index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

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**Authors' contributions**

AA: choosing the idea, patient examination, writing and reviewing. AM: patient examination, writing and reviewing. MM: genotype testing. All authors have read and approved the final manuscript.

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**Availability of data and materials**

Available at the data archive at Specialized Medical Hospital, Faculty of Medicine, Mansoura University, Egypt

**Ethics approval and consent to participate**

Written consents from patients who participated in the study or from their families were obtained and approved by Mansoura Medical Ethics Committee (MMEC) of Faculty of Medicine. Code: R.19.01.411

**Consent for publication**

NA

**Competing interests**

There are no conflicts of interest.

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