Prognostic value of interleukin-10 and tumor necrosis factor-α polymorphisms in patients with hepatocellular carcinoma treated with transarterial chemoembolization

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Background
Transarterial chemoembolization (TACE), a locoregional therapy, is widely recommended as first-line treatment for intermediate-stage hepatocellular carcinoma (HCC). Several prognostic indices have been used to predict overall survival in HCC patients undergoing the procedure.
Patients and methods
A total of 73 patients with HCC, candidate for TACE attending to HCC clinic, Specialized Medical Hospital, Mansoura University, were subjected to full history taking, physical examination, laboratory profile and testing for interleukin (IL)-10 and tumor necrosis factor (TNF)-α polymorphisms. Aggressiveness index is calculated for all patients and followed-up for 4 weeks after TACE to asses response. According to IL-10 and TNF-α polymorphisms results, patients were divided into groups and compared.
Results
The aggressiveness index is significantly higher in the TT/AT haplotype of IL-10 and GG haplotype of TNF-α in comparison with the other haplotypes. The TT/AT haplotype of IL-10 and GG haplotype of TNF-α are significantly associated with less favorable outcome after TACE, wherein 64.3 and 56.25% of patients showed residual active tumor tissue, respectively.
Conclusion
The TT/AT haplotype of IL-10 and GG haplotype of TNF-α are associated with more aggressive pattern of HCC and less favorable outcome after TACE; hence, these patients must be treated as early as possible.

Keywords:
hepatocellular carcinoma, interleukin, tumor necrosis factor

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and worldwide it is the third leading cause of cancer-related deaths. The incidence of HCC has been rising in developed countries due to an increase in the incidence of hepatitis C virus (HCV) and nonalcoholic steatohepatitis (NASH)-related cirrhosis [1].

Barcelona Clinic Liver Cancer (BCLC) is a staging system that is currently advised as the best method for staging HCC and choosing treatment modality. The system incorporates different parameters such as the diameter of the lesion, presence of extrahepatic spread or vascular invasion, performance status and degree of severity of the underlying liver disease according to the Child–Pugh–Turcot score [2].

Transarterial chemoembolization (TACE), a locoregional therapy, is widely recommended as first-line treatment for intermediate-stage HCC (BCLC stage B) [3]. To maximize response and survival, correct patient selection for treatment within BCLC stage B is crucial. This is not a simple process, as, sometimes, choices on the ground may not go with evidence-based recommendations [4].

Identification of prognostic indices to predict overall survival in HCC patients undergoing TACE is very important due to the variability in response and complexity of TACE. Staging systems such as BCLC and different inflammation scores such as neutrophil to lymphocyte ratio and the Glasgow prognostic score are currently used for this purpose [5].

An 'HCC aggressiveness' scoring system was recently described, which incorporates four tumor-related

parameters such as maximum tumor diameter, number of lesions, presence of portal vein thrombosis (PVT) and serum α-fetoprotein (AFP) levels. The score was shown to predict survival in patients with HCC [6].

Aroucha *et al*. [7] concluded that polymorphisms in tumor necrosis factor (TNF)-α and interleukin (IL)-10 were associated with increased risk of HCC development in HCV chronically infected patients. The GG genotype of TNF-α 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 correlated significantly 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 a bad prognosis in these patients [7].

Aim

In this study, we tried to assess the validity of TNF-α and IL-10 polymorphisms as predictors of response in patients with HCC treated by TACE.

Patients and methods

Patients

This was a prospective, descriptive, cross-sectional study that was conducted on 73 patients attending the HCC Clinic, Specialized Medical Hospital, Mansoura University, from June 2017 to September 2018 for follow-up of HCC. All patients were not candidates for surgical resection and prepared for TACE. Baseline tumor characteristics including maximum tumor diameter, number of focal lesions and presence of PVT were collected from imaging reports performed at the Specialized Medical Hospital.

Laboratory assessment

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

Interleukin-10 and tumor necrosis factor-α polymorphisms' determination

Peripheral blood was used to extract genomic DNA using the Wizard Genomic Blood DNA Isolation Kit (Promega, Madison, Wisconsin, USA). We stored samples at −80°C until single nucleotide polymorphism (SNPs') genotyping by real-time PCR was carried out. In the IL-10 gene, we tested one substitution at position −819 C>T (rs1800871). In TNF-α, the substitution at position −308 G>A (rs1800629) was tested. We used TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA), according to the instructions of the manufacturer.

Transarterial chemoembolization

Cannulation and angiography of the celiac trunk or the superior mesenteric artery via the right femoral artery was carried out to perform hepatography using a 5 French (F) sheath and exactly localize the site and arterial supply of the tumor. The catheter was used for both embolization and drug injection. Selective injection was performed unless there were anatomical difficulties that interfered with selective catheterization. The emulsion, which is formed of adriamycin and lipiodol, was injected into the feeding vessels to the tumor. We utilized gelatin sponge particles, 1–2 mm in diameter, to embolize the feeding vessels to the tumor, until we observed a significant decrease in blood flow.

The response to TACE was assessed one month after the procedure by an expert radiologist who depended on two main parameters, percentage of lipidol uptake by the tumor and need for a second session or not.

According to Ventura *et al*. [8], aggressiveness index (AgI) score was divided into three categories: (a) score −<4; (b) 4<score ≤7; and (c) score ≥8 (Table 1).

AgI was calculated for all patients before TACE. According to the result of genotype frequencies' distribution of IL-10 −819 (rs1800871) and TNF-α −308 (rs1800629), patients were divided into groups and compared.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percentage. Quantitative data were described using median (minimum and maximum) for nonparametric data, and mean and SD for parametric data after testing normality using the Shapiro–Wilk test. Significance of the obtained results was judged at the 5% level, and all tests were two tailed.

Table 1 Aggressiveness index parameters

	1	2	3
Maximum tumor dimension (cm)	<4.5	4.5–9.6	>9.6
AFP (ng/ml)	<100	100–1000	>1000
PVT	No		Yes
Tumor nodule (number)	≤3		>3

AFP, α-fetoprotein; PVT, portal vein thrombosis.

Table 2 Basline clinical, laboratory and tumor characteristics of the studied group

Age [mean±SD (minimum–maximum)] (years)	58.73±5.07 (55.0–62.0)
Sex [*n* (%)]	
Male	48 (65.8)
Female	25 (34.2)
DM [*n* (%)]	28 (38.4)
Hypertension [*n* (%)]	19 (26.0)
Liver disease [*n* (%)]	67 (91.8)
Albumin [mean±SD (minimum–maximum)]	3.29±0.59 (2.0–4.60)
WBCs [mean±SD (minimum–maximum)]	4.78±1.89 (2.0–10.0)
HB [mean±SD (minimum–maximum)]	11.33±1.66 (7.80–16.0)
INR [mean±SD (minimum–maximum)]	1.36±0.20 (1.0–1.90)
Bilirubin [median (minimum–maximum)]	1.2 (0.6–24.0)
Platelet	100.0 (22.0–249.0)
AFP	89.0 (3.9–2000.0)
ALT	35.0 (20.0–182.0)
AST	68.0 (32.0–203.0)
HFL (*n*=73) [*n* (%)]	
Left lobe	12 (16.4)
Multifocal	37 (50.7)
Right lobe	24 (32.9)
Portal vein [*n* (%)]	
Patent, dilated	63 (86.3)
PVT	10 (13.7)
Spleen [*n* (%)]	
Absent	2 (2.7)
Mild	24 (32.9)
Moderate	38 (52.1)
Marked	9 (12.3)
Ascites [*n* (%)]	
Absent	63 (86.3)
Present	10 (13.7)
Lymph nodes [*n* (%)]	
Absent	65 (89.0)
Present	8 (11.0)
AgI [*n* (%)]	
a	22 (30.1)
b	22 (30.1)
c	29 (39.8)

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; HB, hemoglobin; HFL, Hepatic focal lesion; INR, international normalized ratio; PVT, portal vein thrombosis; WBC, white blood cell.

χ^2-test was used for categorical variables, to compare between different groups, as appropriate. One-way analysis of variance was used for parametric quantitative variables, to compare between more than two studied groups with post-hoc least significant difference test.

Kruskal–Wallis test for nonparametric quantitative variables, to compare between more than two studied groups, was used, and the Mann–Whitney test was used for nonparametric quantitative variables, to compare between two studied groups.

Table 3 Distribution of patients according to gene polymorphism

	n=73 [*n* (%)]
IL-10 genotype	
CC/CA	25 (34.2)
CT/CA	20 (27.4)
TT/AT	28 (38.4)
TNF-α genotype	
AA	18 (24.7)
GA	23 (31.5)
GG	32 (43.8)

IL-10, interleukin-10; TNF-α, tumor necrosis factor-α.

Ethics

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.

Results

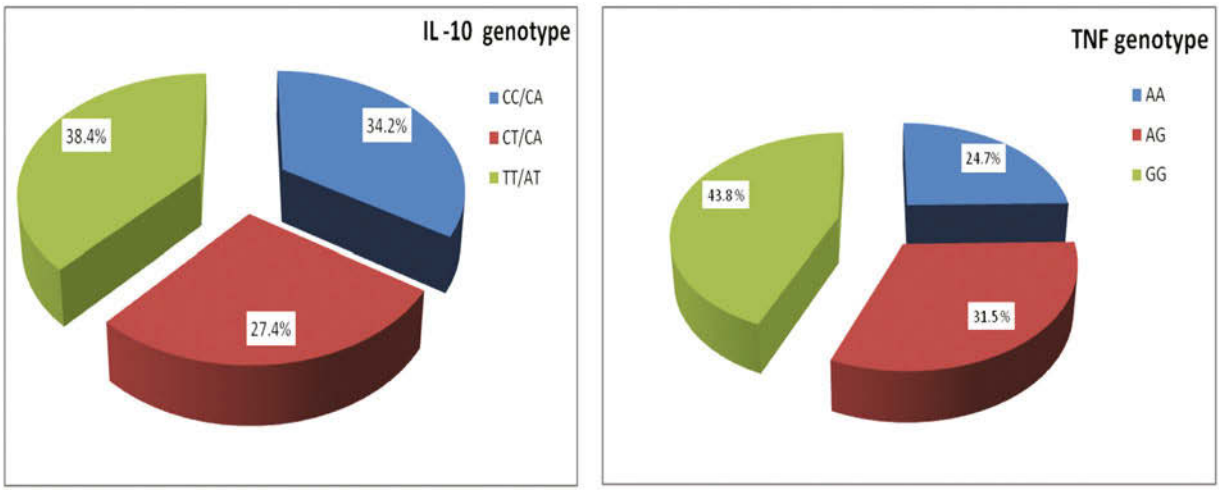
The study involved 73 patients with HCC, 48 (65.8%) male individuals and 25 (34.2%) female individuals, with a mean age of 58.73±5.07 years. The basline clinical, laboratory, and tumor characteristics of the studied patients are shown in Table 2. The AgI of all patients was calculated and included in Table 2. Table 3 and Fig. 1 show the distribution of the studied patients according to IL-10 and TNF-α gene testing.

After genotype testing, the laboratory characteristics and AgI of different genotypes of IL-10 and TNF-α were compared and shown in Tables 4 and 5, respectively. In Tables 6 and 7, we summarized the response to TACE parameters one month after the procedure of IL-10 and TNF-α genotypes, respectively.

Discussion

HCC represents about 90% or more of primary liver cancers that usually develops in a background of advanced liver disease [9]. Approximately, 10–20% of chronically infected patients with HCV will develop liver cirrhosis, and, of these patients, 1–5% will develop HCC [10]. Continued cytokine-induced hepatocyte damage followed by hepatocyte regeneration leads to HCC development. The role of cytokines such as IL-1, IL-2, IL-6, IL-10, IL-12, and TNF-α in hepatocarcinogenesis has been reported. The management of patients with HCC represents a challenge. It is often complicated by the heterogenic pattern of the disease, the presence of underlying advanced liver disorders, and the need to coordinate a multidisciplinary healthcare team [11].

Figure 1



Distribution of patients according to gene polymorphism.

Table 4 Laboratory characteristics of interleukin-10 genotypes

	IL-10			Test of significance
	CC/CC	CT/CA	TT/AT	
Albumin (mean±SD)	3.37±0.52	3.39±0.48	3.15±0.71	F=1.31 P=0.28
WBCs (mean±SD)	4.64±1.7	4.73±2.4	4.94±1.7	F=0.18 P=0.84
HB (mean±SD)	11.47±1.87	10.96±1.39	11.46±1.7	F=0.67 P=0.52
INR (mean±SD)	1.30±0.16[a]	1.31±0.23[b]	1.44±0.19[a,b]	F=4.07 P=0.02*
Bilirubin [median (minimum–maximum)]	1.2 (0.79–2.90)	0.90 (0.60–2.0)[a]	1.5 (0.7–24.0)[a]	KW P=0.01*
Platelets [median (minimum–maximum)]	103.0 (61.0–170.0)	104.5 (31.0–157.0)	80.5 (22.0–249.0)	KW P=0.46
AFP [median (minimum–maximum)]	44.0 (3.90–133.0)[a]	76.0 (15.3–221.0)[b]	290.5 (28.0–2000.0)[a,b]	KW P<0.001*
ALT [median (minimum–maximum)]	33.0 (20.0–109.0)	32.5 (24.0–58.0)	42.0 (22.0–182.0)	KW P=0.23
AST [median (minimum–maximum)]	82.0 (43.0–156.0)	66.0 (44.0–76.0)	67.5 (32.0–203.0)	KW P=0.18
AgI				
a	12	8	2	χ^2=21.08 P<0.001*
b	8	8	6	
c	5	4	20	

Similar superscripted letters denote significant difference between groups. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; *F*, one-way analysis of variance test; HB, hemoglobin; INR, international normalized ratio; KW, Kruskal–Wallis test. *P<0.05, statistically significant.

Table 5 Laboratory characteristics of tumor necrosis factor-α genotypes

	AA	AG	GG	
Albumin (mean±SD)	3.17±0.4	3.39±0.54	3.29±0.71	F=0.69 P=0.51
WBCs (mean±SD)	4.51±1.93	5.19±2.22	4.63±1.6	F=0.84 P=0.44
HB (mean±SD)	11.39±2.13	10.73±1.40	11.73±1.44	F=2.54 P=0.08
INR (mean±SD)	1.39±0.23	1.29±0.17	1.38±0.20	F=1.71 P=0.19
Bilirubin [median (minimum–maximum)]	1.2 (0.80–2.90)	1.1 (0.60–2.50)	1.22 (0.7–24.0)	KW P=0.49
Platelet [median (minimum–maximum)]	103.0 (43.0–157.0)	110.0 (31.0–249.0)	88.5 (22.0–187.0)	KW P=0.18
AFP [median (minimum–maximum)]	48.0 (3.9–111.0)[a]	83.0 (15.6–340.0)[b]	230.5 (28.0–2000.0)[a,b]	KW P<0.001*
ALT [median (minimum–maximum)]	28.0 (20.0–109.0)[a]	33.0 (29.0–182.0)[a]	42.0 (22.0–83.0)	KW P=0.09
AST [median (minimum–maximum)]	65.0 (43.0–156.0)	66.0 (44.0–203.0)	68.5 (32.0–122.0)	KW P=0.63
AgI				
a	12	8	2	χ^2=21.08 P<0.001*
b	8	8	6	
c	5	4	20	

Similar superscripted letters denote significant difference between groups. AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; *F*, one-way analysis of variance test; HB, hemoglobin; INR, international normalized ratio; KW, Kruskal–Wallis test. *P<0.05, statistically significant.

Table 6 Parameters of response to transarterial chemoembolization among interleukin-10 genotypes

	IL-10			Test of significance
	CC/CC (*n*=25)	CT/CA (*n*=20)	TT/AT (*n*=28)	
lipidol uptake [median (minimum–maximum)]	90.0 (20.0–100.0)	87.5 (40.0–100.0)	60.0 (20.0–95.0)	KW *P*=0.19
Second session	7 (28.0)	8 (40.0)	18 (64.3)	χ^2=7.3 *P*=0.02*

IL-10, interleukin-10; KW, Kruskal–Wallis test. *$P<0.05$, statistically significant.

Table 7 Parameters of response to transarterial chemoembolization among tumor necrosis factor-α genotypes

	AA (*n*=18)	AG (*n*=23)	GG (*n*=32)	Test of significance
Lipidol uptake [median (minimum–maximum)]	90.0 (20.0–100.0)[a]	90.0 (40.0–100.0)[b]	60.0 (20.0–95.0)[a,b]	KW *P*=0.018*
Second session	7 (38.8)	8 (34.7)	18 (56.25)	χ^2=7.32 *P*=0.02*

Similar superscripted letters denote significant difference between groups. KW, Kruskal–Wallis test. *$P<0.05$, statistically significant.

Treatment options for HCC include surgical resection, locoregional therapy including TACE, targeted therapy and liver transplantation. According to the American and European Association for the Study of Liver Diseases, TACE is currently indicated for treatment of patients with intermediate-stage HCC and as a bridge therapy for liver transplantation candidates while on the waiting list [12]. TACE, if applied correctly, can improve patient survival without affecting the functional reserve of the liver [13]. Therefore, good patient selection is crucial to maximize the response.

In our study, the included patients had a mean age of 58.73±5.07 years with a male to female ratio of 1.92 : 1. Diabetes mellitus was found in 28 (38.4%) cases (Table 2). This runs parallel to many other studies that concluded that advanced age, male sex, and diabetes mellitus are well-known risk factors for HCC [14]. Cirrhosis, regardless of its cause, was found in almost all our patients (Table 2). This seems logical and in agreement with Seyda Seydel *et al*. [15] who clarified that most of the HCC cases develop in a background of cirrhosis.

In the present study, on testing IL-10 gene polymorphism at site-819 C>T (rs1800871), we found that 34.2% of patients carry the haplotype CC/CA, 27.4% carry the haplotype CT/CA and 38.4% carry the haplotype TT/AT. As regards, TNF-α genotyping, we found that 24.7% carry the haplotype AA, 31.5% carry the haplotype GA and 43.8% carry the haplotype GG (Table 3 and Fig. 1). Aroucha *et al*. [7] also found that haplotype TT/AT of IL-10 and GG haplotype of TNF-α were significantly expressed in patients with HCC.

In this study, we compared IL-10 genotypes as regards laboratory and tumor characteristics assembled in the AgI. A statistically significant difference was found between the TT/AT haplotype and the CC/CA and CT/CA haplotypes as regards international normalized ratio, serum bilirubin, AFP and AgI with *P* values 0.02, 0.01, less than 0.001, and less than 0.001, respectively (Table 4). From these parameters that represent both the synthetic and excretory functions of the liver and more aggressive tumor characteristics such as number and diameter of nodules, the presence of PVT and level of AFP, we can clarify that the TT/AT haplotype of IL-10 is associated with more aggressive forms of HCC. In contrast, when we compared the TNF-α genotypes, we found a statistically significant difference between the GG haplotype and the GA and AA haplotypes in the text of AFP and AgI, with *P* value less than 0.001 for both (Table 5).

Swiatek [16] in 2012 found that the IL-10 level may be affected by gene polymorphisms; he showed that IL-10 −819T was associated with significant low IL-10 expression, as it is located in transcript factor binding regions. Aroucha *et al*. [7] observed that the frequency of IL-10 −819T genotype is increased in patients with HCC. Moreover, they found a significant association between the TT genotype of IL-10 −819 and multifocal lesions and terminal stages of HCC.

As regards TNF-α, the results are conflicting, wherein Talaat *et al*. [17] and Radwan *et al*. [18] did not find any significant correlation between HCC and TNF-α −308 polymorphism in HCV-infected Egyptian patients. In contrast, Baghel *et al*. [19] and Karimi *et al*. [20] demonstrated that patients with TNF-α G allele usually show low TNF-α production *in vivo* and *in vitro*. Vikram *et al*. [21] failed to confirm this association. It seems that the balance between IL-10 and TNF-α is crucial for prevention of development of HCC and that low levels lead to progressive damage to liver tissue and prevents wound healing. In our study, we found that the TT/AT haplotype of IL-10 and GG haplotype of TNF-α were associated with a more aggressive pattern of HCC, as the AgI is

significantly high in the TT/AT and GG haplotypes (Tables 4 and 5).

When we followed-up our patients one month after doing TACE by postcontrast computed tomography, we depended mainly on two main parameters: the first was the percentage of lipidol uptake by the lesion and the second was whether the lesion needed a second session or not, which was indicative of the presence of residual active tumor tissue. On comparing IL-10 genotypes, we found that the TT/AT haplotype has a less favorable outcome, wherein the median percentage of lipidol uptake by the lesion was 60, and 64.3% of patients needed a second session in contrast to the other haplotypes (Table 6). Furthermore, the GG haplotype of TNF-α has a poor prognosis, wherein the median percentage of lipidol uptake by the lesion was 60, and 56.25% of patients needed a second session in contrast to the other haplotypes (Table 7).From these results, it seems like the TT/AT haplotype of IL-10 and the GG haplotype of TNF-α are poor prognostic factors for HCC patients treated with TACE, but whether this is due to the direct effect of these cytokines or due to the fact that these haplotypes are associated with more aggressive forms of the tumor is still controversial. Parallel to our study, Loosen *et al.* [22] showed that serum levels of IL-6 and IL-8 before TACE not only predict patients' local tumor response after the procedure but are also indicative of the overall survival of patients. Cytokines could potentially reflect distinct inflammatory mechanisms during tumor progression and might, therefore, be used as prognostic biomarkers in cancer patients [23].

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

It seems that the balance between IL-10 and TNF-α is mandatory to the development of HCC, as 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 activation of proto-oncogene in the host cells, leading to HCC [25].

There may be a fine tuning of the IL-10 and TNF-α balance, and it seems to be controlled by the level of IL-10, wherein low levels lead to progressive damage to liver tissue and prevention of wound healing. Moreover, IL-10 can diminish the response to antiviral treatment [26].

Our study may be limited by some factors such as the limited number of cases in the study, whether we took the most suitable decision to the patients, lack of data about overall survival of patients and, lastly, we included patients with underlying liver cirrhosis regardless of its cause, which may affect oncogenesis.

To summarize, certain genotypes of TNF-α and IL-10 may affect the balance between them, which may lead to progression of hepatocarcinogenesis and refractory response to TACE.

Conclusion

The TT/AT haplotype of IL-10 and GG haplotype of TNF-α are associated with a more aggressive pattern of HCC and less favorable outcome after TACE; hence, these patients must be treated as early as possible.

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Conflicts of interest

There are no conflicts of interest.

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