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Original Article

Serum interleukin-8 as an indicator of tumor recurrence in hepatocellular carcinoma after transarterial chemoembolization

Maofeng Zhong[1,2*], Xiaofeng Zhai[2*], Wanfu Lin[2*], Qun Liu[2], Man Yao[2], Yuyu Guo[2], Binbin Cheng[2], Changquan Ling[2]

[1]Department of Gastroenterology, Shanghai Municipal Hospital of Traditional Chinese Medicine Affiliated to Shanghai University of TCM, Shanghai 201900, P. R. China; [2]Department of Traditional Chinese Medicine, Changhai Hospital, Naval Medical University, Shanghai 200433, P. R. China. [*]Equal contributors.

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Abstract: To investigate the relationship between inflammatory cytokines and the recurrence of hepatocellular carcinoma (HCC) patients post transarterial chemoembolization (TACE), sixty patients with HCC who received TACE for the first time from February 2014 to October 2016 were enrolled. Peripheral blood was collected before and 3 days after TACE to examine C-reactive protein (CRP) and inflammatory cytokines such as IL-1β and IL-8. Clinicopathological features including liver function, the number and size of tumors, and the Barcelona Clinic Liver Cancer (BCLC) staging were all taken into consideration for further analysis. A COX proportional hazards model was applied to perform a multivariate analysis of the prognostic factors which may predict the relapse free survival (RFS) of HCC. Finally, a Kaplan-Meier curve analysis was used to analyze the relationship between the cumulative recurrence rate and inflammatory cytokines. The results indicated that the levels of IL-2R, IL-6, IL-8, TNF-α, and CRP on the third day after TACE were significantly higher than the levels before TACE, while IL-1, IL-10 decreased significantly ($P < 0.05$). A COX analysis showed that the IL-8 increase ratio (ΔIL-8/pre-IL-8) was an independent risk factor for recurrence after TACE ($P = 0.02$, HR = 1.21). The relationship between the cumulative recurrence rate and the IL-8 increase ratio was plotted with a Kaplan-Meier curve, and the log rank analysis verified the role of increased IL-8 as a predictive factor ($\chi^2 = 4.67$, $P = 0.031$). In conclusion, TACE causes significant changes in CRP and inflammatory cytokines in patients with HCC, and IL-8 may be used as a potential predictor of tumor recurrence post TACE.

Keywords: Hepatocellular carcinoma, inflammation, serum interleukin-8, transarterial chemoembolization, recurrence

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, ranking as the sixth most common cancer and the second most common cause of cancer-related deaths worldwide. As reported, approximately 850,000 new cases of HCC are diagnosed each year globally [1-3]. Currently, surgical resection and liver transplantation are still the optimal therapeutic methods for early-stage HCC. However, only 15% of patients are eligible for these therapies since most patients are diagnosed at the advance stages [4, 5]. For these inoperable HCC patients, transarterial chemoembolization (TACE) is widely used as a palliative therapy [6]. TACE, which directly delivers chemotherapeutic drugs to the tumor and blocks tumor-feeding arteries with iodipin, induces hypoxia and necrosis in tumors. However, it is almost impossible for TACE to reach a complete embolization and necrosis of the hepatoma cells in clinical practice. The hypoxia microenvironment caused by TACE can clearly induce the upregulation of hypoxia-inducible factor-1 (HIF-1). TACE also leads to acute hepatic injury and to an inflammatory response in the local tissue. In addition, there is also a fast increase in the inflammatory related cytokines in the peripheral blood of HCC patients after TACE, such as interleukin-6 (IL-6), IL-8, and the tumor necrosis factor-alpha (TNFα). More and more studies in recent years

indicate that the tumor inflammatory microenvironment is related to the prognosis of HCC patients [7]. However, whether these increased inflammation-related cytokines are available to predict the risk of tumor progression and metastasis for advanced hepatocellular carcinoma after TACE has not been investigated. Therefore, in the present study, we recruited HCC patients treated with TACE for the first time and analyzed the correlation of the elevation of inflammatory related cytokines after TACE and the prognoses of these HCC patients.

Materials and methods

Patients

Patients who underwent TACE as an initial therapy in the Department of Traditional Chinese Medicine, Changhai Hospital from February 2014 through October 2016 were recruited. The diagnosis of HCC was made according to the diagnostic criteria of the American Association for the Study of Liver Diseases [8].

Inclusion criteria: (1) Patients with a newly diagnosed, unresectable HCC tumor without evidence of main portal vein thrombosis or extrahepatic metastasis; (2) Stage A or B according to the Barcelona Clinic Liver Cancer (BCLC) staging system; (3) Patients received TACE as an initial therapy without any other anticancer therapies before the operation.

Exclusion criteria: (1) Serious complications; (2) Liver function Child-Pugh classification C-Class; (3) The presence of other malignant tumors simultaneously; (4) Lost to follow-up or without complete information.

The information of the enrolled patients, including their demographic data, blood routines, liver function, prothrombin time (PT), alpha fetal protein (AFP) and B ultrasound, CT or MRI of the liver was collected. Since we retrospectively evaluated the patients, and their records or information was anonymized and deidentified prior to our analysis, an approval by the Ethic Committee was not necessary.

Treatment procedure and cytokine assays

A uniform treatment protocol of TACE was performed for each included patient at the Radiology Department of Changhai Hospital. Briefly, Seldinger's method was performed with local anesthesia and chemotherapeutic agents suspended in lipiodol were injected into the hepatic segmental artery where the target tumor was located through the femoral artery [9]. None of the patients received glucocorticoids during the therapy. Before the TACE operation and on the 3rd day after TACE, blood samples were collected. The levels of interleukin (IL)-1, IL-2 receptor (IL-2R), IL-6, IL-8, L-10 and TNF-α were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. AFP and C-reactive protein (CRP) were determined by radioimmunoassay and transmission immunoturbidimetry, respectively.

Follow-up

The clinical and laboratory data of the enrolled patients were collected during the follow-up period according to our established guidelines. Liver ultrasonography, CT or MRI, liver and kidney functions, blood routine and AFP were all performed at 6 weeks from the procedure to assess the response to TACE. After that, a follow up of the enrolled patients was performed every 3 months. Patients' recurrences were evaluated according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) [10]. The last follow-up date was February 28, 2017.

Statistical analysis

SPSS version 22 statistical software (SPSS Inc., Chicago, IL, USA) was applied for the statistical analysis. Continuous variables were expressed as the means and ranges and analyzed though the Kruskal Wallis rank test, and if the variables were less than 10 then Fisher's exact test was used alternatively. For the categorical variables, a chi-square test and Fisher's exact test were used. A multivariate analysis with the Cox regression model was used to evaluate the relapse free survival (RFS) of the enrolled patients. The cumulative recurrence rate, which was defined as the date of TACE to the date of recurrence or last follow-up, was analyzed using the Kaplan-Meier method, and a log-rank test was used to identify the differences. A *P* value of less than 0.05 was considered statistically significant.

Table 1. Baseline characteristics

Characteristics	Value
Included patients' number	58
Age (year; mean ± SD)	57.0 ± 9.6
Sex	
Female	15 (25.9%)
Male	43 (74.1%)
Tumor size (cm; mean ± SD)	6.2 ± 4.3
Tumor number (%)	
Single	26 (44.8%)
Multiple	32 (55.2%)
BCLC stage (%)	
BCLC-A	19 (32.8%)
BCLC-B	39 (67.2%)
Recurrence (days; mean ± SD)	154.2 ± 137.1

SD: standard deviation; BCLC: Barcelona Clinic Liver Cancer staging system.

Results

Baseline characteristics

According to the aforementioned criteria, a total of 60 HCC patients who underwent TACE as their initial treatment in Department of Traditional Chinese Medicine, Changhai Hospital from February 2014 to October 2016 were retrospectively enrolled in this study. Among them, two patients were excluded due to loss of follow-up, and 58 patients were analyzed finally. As shown in **Table 1**, these patients included 43 males and 15 females and ranged from 37 to 81 years old with an average of 57 ± 9.6 years. Among them, 26 patients were diagnosed with single tumor nodules, and the remaining patients were found to have multiple tumor nodules. The mean diameter of the tumors was 6.2 ± 4.3 cm. After 436 days' follow-up till February 2017, 43 cases of postoperative recurrence were found, in patients with an average age of 57 years. Therefore, the overall recurrence rate was 74.14% and the median time to relapse was 154.2 ± 137.1 days.

Cytokine levels after TACE of HCC patients

At the third day after TACE, the concentrations of IL-2R, IL-6, IL-8, TNF-α and CRP after TACE were 921.7 ± 653.1 U/mL, 70.7 ± 79.0 pg/mL, 168.2 ± 475.7 pg/mL, 17.3 ± 11.3 pg/mL and 101.3 ± 75.4 mg/L, respectively (**Table 2**). The changes of the cytokine profiles were statistically significant when compared with the levels before TACE. Among which, the mean changes of IL-2R, IL-8 and CRP were 348.84 U/mL, 111.14 pg/mL and 92.28 mg/L, respectively, and that means IL-2R, IL-8 and CRP increased significantly after TACE. On the other hand, IL-1β and IL-10 decreased significantly after TACE.

Multivariate analysis of prognostic factors of RFS after TACE

Considering other factors such as the number of tumor nodules, tumor size, BCLC stage, AFP, etc. may also influence patients' RFS after TACE, the patients without recurrence up to the last day of follow-up were categorized into the control group, and the others were categorized into the recurrence group. And then a multivariate analysis with a COX proportional hazard model was performed. As shown in **Table 3**, among the inflammatory cytokines tested, the increase of IL-8 was an independent risk factor for recurrence after TACE ($P = 0.02$, HR = 1.21), although the BCLC stage may also determine recurrence after TACE.

IL-8 predicts the cumulative recurrence rate after TACE

Since IL-8 was indicated as an independent risk factor for recurrence after TACE, we further analyzed whether IL-8 may predict recurrence after TACE in the HCC patients. Taking IL-8 as a variable, we defined patients with ΔIL-8 < 0 as control group and put the others in the observation group and calculated the cumulative recurrence rate after TACE using a Kaplan-Meier curve. The results of the log rank analysis verified that an increase in IL-8 could be considered a predictive factor ($\chi^2 = 4.67$, $P = 0.03$) of recurrence post TACE in HCC patients (**Figure 1**).

Discussion

Most HCC develops as the result of chronic inflammatory liver diseases, such as viral hepatitis, liver cirrhosis, and nonalcoholic steatohepatitis. Numerous studies have shown that inflammation is a major risk factor for the progression of HCC [11]. The microenvironment of HCC is infiltrated with a variety of inflammatory cytokines that interact with hepatoma cells thereby regulating the signaling pathways relat-

Table 2. Cytokine levels change after TACE of the included patients

Inflammatory cytokines	Before TACE	The third day after TACE	difference in means	95% confidence interval	*P* value
IL-1β (pg/mL)	14.8 ± 1.2	13.6 ± 3.2	-1.15	[0.25, 2.05]	0.01*
IL-2R (U/mL)	572.9 ± 278.2	921.7 ± 653.1	348.84	[-534.02, -163.65]	0.00*
IL-6 (pg/mL)	8.4 ± 7.7	70.7 ± 79.0	62.28	[-82.93, -41.63]	0.00*
IL-8 (pg/mL)	57.1 ± 123.0	168.2 ± 475.7	111.14	[-239.09, 16.82]	0.01*
IL-10 (pg/mL)	13.9 ± 2.7	12.5 ± 3.9	-1.40	[0.16, 2.65]	0.03*
TFN-α (pg/mL)	12.8 ± 13.1	17.3 ± 11.3	4.48	[-9.03, 0.07]	0.00*
CRP (mg/L)	9.1 ± 13.6	101.3 ± 75.4	92.28	[-72.32, -112.23]	0.00*

The data are presented as the mean ± standard deviation; *represents $P < 0.05$ when compared with the cytokine levels before TACE.

Table 3. Multivariate analysis of prognostic factors of RFS after TACE

Factors	Recurrence group	Control group	RFS	
			P value	HR
Included patients' number	43 (74.1%)	15 (25.9%)		
Male	31 (72.1%)	12 (27.9%)	0.84	1.11
Age (year; mean ± SD)	57.0 ± 9.9	57.0 ± 9.1	0.93	1.00
Tumor size (cm; mean ± SD)	6.7 ± 4.0	4.9 ± 4.9	0.16	0.92
Tumor number (n, %)			0.08	0.27
Single	18 (41.9%)	8 (53.3%)		
Multiple	25 (58.1%)	7 (46.7%)		
BCLC stage (n, %)			0.00*	17.42
BCLC-A	10 (23.3%)	9 (60.0%)		
BCLC-B	33 (76.7%)	6 (40.0%)		
HBsAg (n, %)			0.76	1.24
Negative	8 (19.51%)	3 (20%)		
Positive	33 (80.49%)	12 (80%)		
PT (S)	13.84 ± 2.09	14.18 ± 1.00	0.84	1.13
AFP (ng/mL)	520.83 ± 708.20	349.16 ± 653.64	0.10	1.00
WBC ($\times 10^9$)	5.31 ± 1.77	4.59 ± 1.12	0.22	1.26
TB (μmol/L)	15.76 ± 8.47	15.94 ± 6.39	0.05	0.92
AST (U/L)	47.35 ± 29.93	31.80 ± 13.05	0.84	1.00
ΔIL-1/pre-IL-1	-1.43	-0.35	0.08	12.33
ΔIL-2/pre-IL-2	381.3	245.8	0.09	1.26
ΔIL-6/pre-IL-6	65.2	53.58	0.33	0.97
ΔIL-8/pre-IL-8	234.5	69.96	0.02*	1.21
ΔIL-10/pre-IL-10	-1.82	-0.18	0.19	2.16
ΔTFN-a/pre-TFN-a	6.18	-0.34	0.24	0.60

There were two persons in the Recurrence group who did not screen the HBsAg. *Means $P < 0.05$ when Recurrence group *vs.* Control group. RFS: relapse free survival; HR: hazard ratio; SD: standard deviation; BCLC: Barcelona Clinic Liver Cancer staging system; PT: prothrombin time; AFP: alpha fetal protein; WBC: white blood cell; TB: total bilirubin; AST: aspartate aminotransferase; ΔIL: mean the difference between the interleukin after and before TACE; Pre-IL: interleukin before TACE.

ed to cancer proliferation and metastasis [12]. As one of the effective therapeutic methods for HCC, TACE works by cutting off the tumor's nutrition supply. However, TACE causes a local hypoxic-ischemic state and induces HIF-α accumulation, which stimulates the expression of inflammatory cytokines and influences the progression and prognosis of HCC [13, 14].

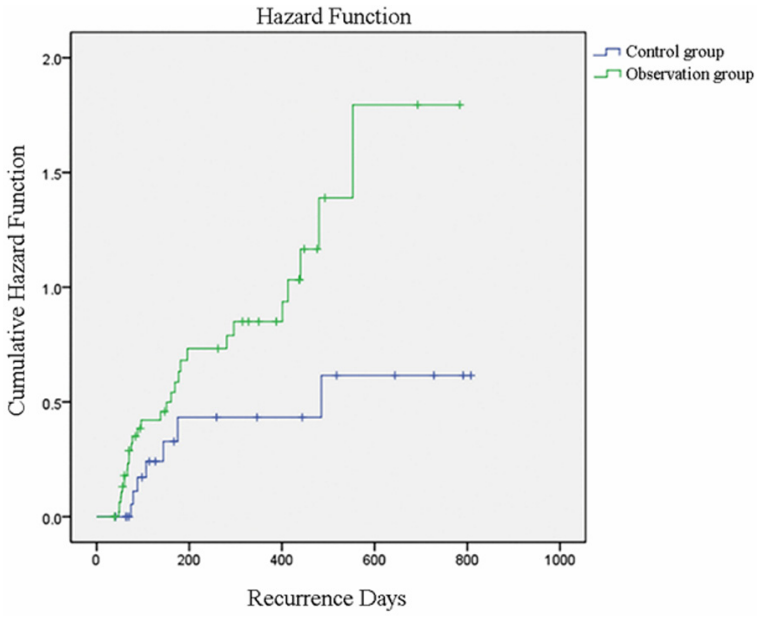

Figure 1. A Kaplan-Meier curve of the cumulative recurrence rates of the different groups.

The elevation of proinflammatory cytokines in the serum of HCC patients after TACE has been widely reported. Kim et al. [15] evaluated the changes of several cytokines in HCC patients after TACE, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-22, and TNF-α and found that IL-4, IL-5, IL-6, and IL-10 are increased after TACE, but only IL-6 level positively correlates with both tumor size and the Child-Pugh score. Chao et al. [16] also found that serum IL-6 levels were higher after TACE, which may correlate with post-TACE fever. Ikei [17] showed that serum IL-6 and plasma IL-8 increased after TACE and reached a peak on day 3. In the present study, we retrospectively evaluated the serum levels of IL-1β, IL-2R, IL-6, IL-8, IL-10, TNF-α and CRP pre-operation and on the 3rd day after TACE. In accordance with previous studies, our results showed that IL-1β and IL-10 decreased, but IL-2R, IL-6, IL-8, TNF-α and CRP increased significantly after TACE.

Although Kim et al. [18] showed that a high pre-TACE serum IL-8 level is a useful prognostic marker for TACE refractoriness and liver transplantation (LT)-free survival in TACE-treated patients with HBV-associated HCC, the relationship between TACE-induced cytokine changes and the prognosis of HCC patients after TACE has not been investigated completely. In this study, a multivariate analysis showed that the increase of IL-8 is an independent risk for recurrence after TACE. IL-8, also called CXCL8, is a pro-inflammatory CXC-chemokine which was originally identified as a neutrophil chemoattractant, and its effects are mediated by two heterotrimeric G protein-coupled receptors, CXCR1 and CXCR2 [19]. IL-8 is a key factor in the survival and angiogenesis of endothelial cells. In human umbilical vein endothelial cells (HUVEC), the inhibition of IL-8 inhibits angiogenesis [20, 21]. HIF-1α can induce the expression of IL-8 and VEGF [22, 23], but IL-8 can also promote the VEGF expression independent HIF-1α [24]. A combined knockdown of the expressions of HIF-1α and IL-8 inhibits tube formation and invasion and induces the apoptosis of endothelial cells induced by the conditioned medium of the hepatoma cells [25, 26]. Therefore, the high level of IL-8 after TACE may promote the angiogenesis of HCC and thereby lead to tumor recurrence and metastasis.

IL-6 is another one of the most extensively studied cytokines before and after TACE. IL-6 could induce strong immunosuppression in the HCC microenvironment by recruiting immunosuppressive cells [27]. A recent study by Jang et al. [28] found that the levels of IL-4, IL-6 and CRP are higher in patients with HCC than they are in healthy subjects, and higher CRP and IL-6 levels correlated well with shorter survival in HCC patients, indicating that inflammatory activation of the IL-6/CRP network may be a potential therapeutic target and biomarker for HCC. In addition, they also showed that tumor response positively relates to CRP decline, but tumor progression positively relates to CRP rise during the treatment period. However, our study did not show a positive relationship between IL-6/CRP and the prognosis of HCC patients after TACE. The difference may be due to the diversity in the main outcome measures and the sample size. In the current study, we mainly focused on the recurrence of HCC post TACE and its relationship with the change of inflam-

matory cytokines. Overall survival was not investigated in this study.

Like the previous studies, we found the changes in the other inflammatory cytokines, such as IL-2 and IL-4, among HCC patients after TACE. However, we cannot determine their predictive role after TACE. We also found that these inflammatory factors may rather play a static and unidirectional role, but a complex role according to their specific environment. For example, IL-2 induces immunosuppression by inducing the expression of the transcription factor FOXP3 to promote $CD4^+$ T-regulatory cell differentiation [29]. However, in our study, IL-2 increased after TACE. Such results indicate that different inflammatory cytokines may play different roles in a specific microenvironment, which may conflict with the traditional understanding. Therefore, it helps to understand the complexity of the tumor more deeply when viewing different inflammatory factors as a whole in the specific microenvironment, and it is also more conducive to making better multi-target interventions.

To sum up, our study showed that several inflammatory cytokines in patients with HCC are increased post TACE, among which the level of IL-8 may be used as a potential predictor of recurrence after TACE. There are also several limitations to the present study. Firstly, the sample size was relatively small, and larger sample sizes in multiple centers are still needed. Next, more cytokines such as IL-12, IL-17 are required to make a more comprehensive understanding of the inflammatory cytokines in HCC after TACE. Furthermore, intervention such as traditional Chinese medicine may be applied, since it is made up of different compounds that may function in multi-targeted and dynamic ways [30-32]. In the future, we will further investigate the underlying mechanisms that IL-8 influences in the progression of HCC, and whether suppressing the secretion of IL-8 post TACE may benefit the prognosis of HCC.

Conclusion

In conclusion, our present study found that TACE causes significant changes of CRP and inflammatory cytokines in patients with HCC, and IL-8 may be used as a potential predictor of tumor recurrence post TACE. Further investigation with larger sample size and more comprehensive cytokines are warranted to make the conclusion more convincing, and the underlying mechanisms of why IL-8 influences the progression of HCC need to be studied further.

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Disclosure of conflict of interest

None.

Address correspondence to: Binbin Cheng and Changquan Ling, Department of Traditional Chinese Medicine, Changhai Hospital, Naval Medical University, 168 Changhai Road, Shanghai 200433, China. Tel: +86-21-31161966; E-mail: cbb8202@126.com (BBC); Tel: +86-21-81871559; Fax: +86-21-81871559; E-mail: changquanling@smmu.edu.cn (CQL)

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