Clinical and Experimental Medicine (2019) 19:235–243
https://doi.org/10.1007/s10238-019-00550-1

ORIGINAL ARTICLE

Microcystin-LR in peripheral circulation worsens the prognosis partly through oxidative stress in patients with hepatocellular carcinoma

Feifei Lei[1] · Xu Lei[1] · Rugui Li[1] · Huabing Tan[1]

Received: 16 January 2019 / Accepted: 5 March 2019 / Published online: 12 March 2019

Abstract

Prognostic significance of serum microcystin in hepatocellular carcinoma has not been well investigated. The aim of the study was to reveal the relationship between serum microcystin-LR and prognosis in these patients. There were 650 early-stage hepatitis B-induced hepatocellular carcinoma patients, who were not affected by hepatitis C, cirrhosis, heavy drinking or excessive aflatoxin exposure. All of them underwent hepatectomy and were followed up for 5 years. Tumor relapse and overall death were recorded. Blood specimens were collected on admission and at the time of relapse. Serum levels of microcystin-LR and fluorescent oxidation products (FlOP_360, FlOP_320 and FlOP_400) were measured separately using enzyme-linked immunosorbent assay and fluorescence spectrometry. Multifactorial COX regression analysis suggested that serum microcystin-LR ≥ 0.97 ng/ml was associated with the increased risk of the tumor relapse (HR: 1.53, 95% CI: 1.35–1.77) and serum microcystin-LR ≥ 1.09 ng/ml was related to the higher risk of the overall death (HR: 1.58, 95% CI: 1.35–1.84) in the follow-up period. Furthermore, there was a linear relationship between serum level of microcystin-LR and serum levels of FlOP_360, FlOP_320 and FlOP_400 ($P = 0.001$, $P = 0.023$, $P = 0.047$). Serum levels of these fluorescent oxidation products were also higher in the patients with tumor relapse ($P < 0.001$, $P < 0.001$, $P = 0.001$) or overall death ($P < 0.001$, $P = 0.001$, $P = 0.002$) compared with the remaining patients. Serum microcystin-LR independently worsens the prognosis partly through promoting oxidative stress in patients with hepatocellular carcinoma.

Keywords Hepatocellular carcinoma · Microcystin · Oxidative stress · Prognosis · Recurrence

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in human digestive system. Compared with the rest of the world, the incidence of HCC is higher in Asia and Africa, especially in China [1, 2]. HCC is a fatal disease. It causes more than six hundred thousand deaths around the world each year, and about half of the deaths occur in China [1, 2]. It is well known that cirrhosis, chronic hepatitis B and C, alcohol abuse and aflatoxin are major risk factors for the tumor [3–5]. Among them, chronic hepatitis virus infection and aflatoxin exposure are more common in China [6, 7]. However, hepatocarcinogenesis is a complex and gradual process, and many carcinogens and risk factors are still unclear.

Microcystins (MCs) are a class of water-borne toxins. At present, more than 90 different toxic variants have been discovered in nature, and MC-LR is the most common and toxic variant [8, 9]. They are produced by some kinds of cyanobacteria, which are distributed all over the world [10]. MCs contaminate drinking water and accumulate in aquatic animals [11, 12]. Human are exposed to these MCs through drinking water, eating aquatic products or direct contact [13, 14]. In recent decades, anthropogenic activities promote water eutrophication and lead to excessive proliferation of MC-related cyanobacteria [15, 16]. Thus, MCs are produced in large quantities and have become a serious threat to public health.

An increasing number of studies have focused on the relationship between MCs and liver diseases. A 10-year epidemiological study from Serbia showed a significant increase in the incidence of primary liver cancer in the regions where people consumed water from cyanobacterial

✉ Huabing Tan
huabing3213@126.com

[1] Department of Infectious Diseases and Lab. of Liver Disease, Renmin Hospital, Hubei University of Medicine, 39 Chaoyang Zhong Road, Maojian District, Shiyan 442000, China

blooming reservoirs [17, 18]. Another study from the USA suggested that the risk of non-alcoholic liver disease increased by 0.3% with each 1% increase in cyanobacterial bloom coverage in the affected county [19]. Li et al. and Chen et al. [20, 21] reported a potential relationship between serum MCs and liver enzymes separately in children and fishermen. Zheng et al. [22] further suggested that serum MC-LR was an independent risk factor for HCC in humans. Furthermore, some in vitro studies reported that MCs induced the formation of reactive oxygen species, caused the disorder of apoptosis regulation and promoted the proliferation in hepatoma carcinoma cells [23, 24]. Therefore, MCs play an important role in hepatocarcinogenesis.

To our knowledge, the prognostic significance of serum MC-LR in HCC patients has not been investigated. So, we conducted this prospective cohort study to reveal the relationship between the prognosis of the patients and the serum level of MC-LR and to preliminarily explore the mechanisms involved.

Materials and methods

Subjects

The study was approved by the Ethics Committee of Shiyan Renmin Hospital and was based on a screening program for early-stage HCC, which had included nearly two thousand HCC patients.

A total of 650 patients with newly diagnosed HCC were continuously included from this screening program. Predefined inclusion criteria were as follows. (1) Outpatients or inpatients in Shiyan Renmin Hospital between January 1, 2000, and December 31, 2012. (2) Patients were permanent residents and always lived in this area. (3) Barcelona Clinic Liver Cancer (BCLC) stage 0 or stage A with only Child–Pugh A liver function. (4) All patients had chronic hepatitis B, but no hepatitis C or cirrhosis. (5) There was no alcohol drinking habit in their lives (less than 3–4 drinks/year). (6) Serum aflatoxin B1-lys adduct levels on admission were less than 1.0 pg/mg, which was the median of this marker in the screening program. (7) Patients did not have any other kinds of tumor. (8) Patients agreed to participate in this study and signed written informed consent forms.

All the patients underwent hepatectomy in this hospital, and the operations were carried out by a single group of liver surgeons. After operation, the patients were followed up for 5 years (60 months). If the tumor relapsed in this period, the patients received transarterial chemoembolization (TACE) or radiofrequency ablation (RFA) according to their conditions.

Data collection

Blood specimens were collected from the patients on admission. If a patient had a tumor relapse in the follow-up, another blood specimen was also collected. All blood specimens were centrifuged at 7000 *g* for 5 min, and the serum specimens were obtained and stored at − 196 °C (liquid nitrogen) for the following determination.

Demographic information, medical history, personal history, family history, serological indicators of viral hepatitis and liver function were obtained from their medical records. Tumor pathological data were obtained from their surgery records.

Definition

Smoking was defined as smoking at least one cigarette per day cumulatively or continuously for 6 months or more in one's life. Obesity was defined as a body mass index ≥ 28 kg/m^2.

Follow-up

The patients were followed up annually by a telephone call or a visit to the house. In the process, the information about clinical manifestation, therapeutic regimen, tumor relapse and survival in the past year was recorded. The follow-ups lasted for 5 years (60 months) unless the patients died. The follow-up of the last patient was ended on December 22, 2017.

Laboratory test

Serum level of MC-LR was measured using a commercial enzyme-linked immunosorbent assay kit (Beacon Analytical Systems, Saco, ME, USA) [22]. Major steps were listed as follows: (1) Microcystin–horseradish peroxidase enzyme conjugate solution (50 ml) was added to each well. (2) The serum specimen (50 ml), negative control (50 ml) or standard solution (50 ml) was separately added into each well. (3) The plate was shaken lightly, incubated in the dark (37 °C, 30 min) and washed five times. (4) The solution was removed from the wells. (5) Substrate solution (100 μL) was added to each well and incubated again in the dark (37 °C, 30 min). (6) Stop solution (mL) was added to each well. (7) Absorbance was measured at 540 nm on a spectrometer. (8) The absorbance of the standard solution was adopted to draw a calibration curve. (9) The levels of MC-LR in the serum specimens were obtained according to their absorbance. The limit of quantification was 0.01 ng/ml.

Fluorescent oxidation product (FlOP) was a global biomarker for oxidative stress. Serum levels of FlOPs were measured according to a proven method described in previous studies [25, 26]. Briefly, the serum specimens were extracted with ethanol ether and the supernatants were measured using a spectrofluorometer. The unit of the biomarker was relative fluorescent intensity per milliliter of serum (FI/ml). The measures were performed at three wavelengths: FlOP_360 (excitation/emission: 360/420 nm), FlOP_320 (320/420 nm) and FlOP_400 (400/475 nm) [26]. FlOP_360 represented the interaction of lipid oxidation productions with amino acids, nucleic acids and carbohydrates. FlOP_320 represented the interaction of lipid oxidative products with nucleic acids and metallic elements. FlOP_400 represented the interaction between amino acids, phospholipids and malondialdehyde [26].

Statistical analysis

Continuous variable was showed as mean and standard deviation, and categorical variable was expressed as frequency and constituent ratio. Difference of two continuous variables was detected using independent sample t test, and difference of two categorical variables was detected using Chi-square test. Kaplan–Meier survival analysis was conducted to compare 5-year relapse or overall death in the groups. Cutoff point of serum MC-LR level was reported using receiver operating characteristic curve (ROC) analysis. Linear relationship between serum MC-LR level and serum FlOP level was measured using a Pearson correlation analysis. If a $P < 0.05$, it was statistically significant. The association of serum MC-LR level with the prognosis of HCC patients was measured using multifactorial COX regression analysis. Hazard ratio (HR) and 95% CI were obtained. If a 95% CI did not include value 1, it was statistically significant. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was adopted to conduct the analysis.

Results

There were 650 patients with confirmed early-stage HCC in the study. All of them were affected by chronic hepatitis B, but no hepatitis C and cirrhosis. They had very low alcohol consumption and aflatoxin exposure.

As shown in Table 1, the average age was 56 ± 12 years old. Most of the patients were male (74.3%). Some of the patients are smokers (38.2%). A total of 88 and 100 patients separately had obesity and a family history of HCC. Many patients had unhealthy diets. About 74.6%, 43.5%, 83.7% and 35.7% of them lacked the intakes of fruits, vegetables, dairy products and eggs. Of the 650 HCC patients, 73 patients had multiple tumors, 185 patients had a main tumor with a diameter of more than 3 cm, and 128 patients had a poor differential tumor. In addition, 355 patients belonged to BCLC stage 0 and 295 patients belonged to BCLC stage A.

In the 5-year follow-up, 396 patients had a tumor relapse and 254 patients did not. In the same period, 311 patients died and 339 patients survived.

Table 1 Characteristics of hepatocellular carcinoma patients on admission

Variables on admission	HCC ($n = 650$)
Age, years, mean ± SD	56 ± 12
Gender *n* (%)	
Male	483 (74.3)
Female	167 (25.7)
Smoking *n* (%)	
Present	248 (38.2)
Absent	402 (61.8)
Obesity *n* (%)	
Present	88 (13.5)
Absent	562 (86.5)
Family history of HCC *n* (%)	
Present	100 (15.4)
Absent	550 (84.6)
Fruits intake *n* (%)	
< 100 g/d	485 (74.6)
≥ 100 g/d	165 (25.4)
Vegetables intake *n* (%)	
< 400 g/d	283 (43.5)
≥ 400 g/d	367 (56.5)
Dairy product intake *n* (%)	
< 100 g/d	544 (83.7)
≥ 100 g/d	106 (16.3)
Egg intake *n* (%)	
< 50 g/d	232 (35.7)
≥ 50 g/d	418 (64.3)
Tumor number *n* (%)	
Single	577 (88.8)
Multiple	73 (11.2)
Tumor size *n* (%)	
< 3 cm	465 (71.5)
≥ 3 cm	185 (28.5)
Tumor differentiation *n* (%)	
Well moderate	522 (80.3)
Poor	128 (19.7)
BCLC stage *n* (%)	
0	355 (54.6)
A	295 (45.4)

HCC Hepatocellular carcinoma, *SD* standard deviation, *BCLC* Barcelona Clinic Liver Cancer

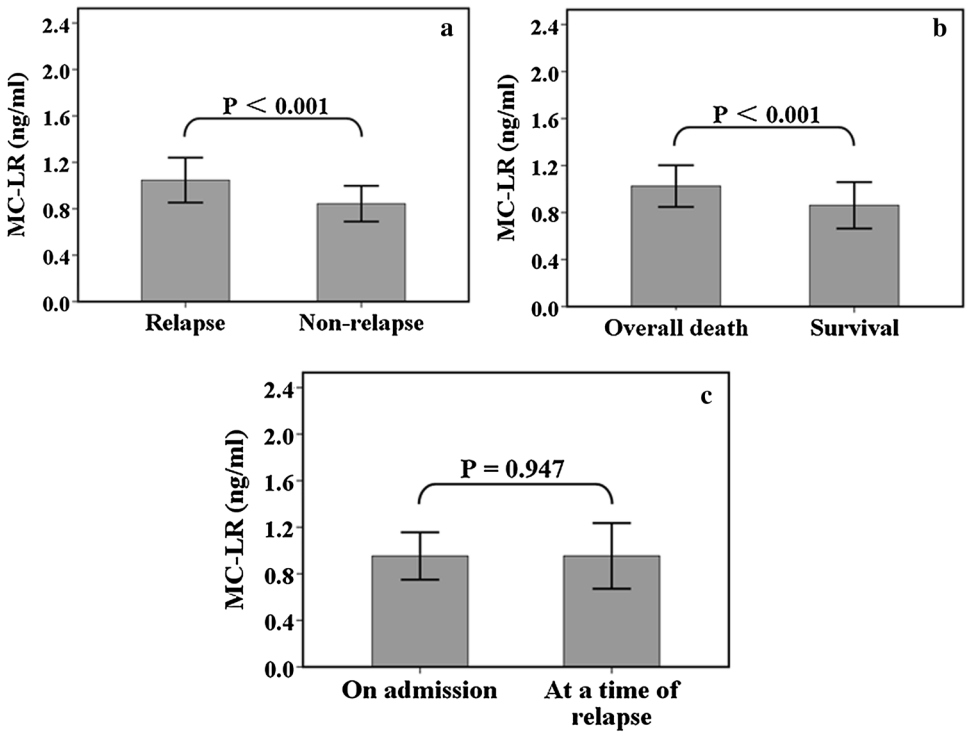

Fig. 1 Serum levels of microcystin-LR in patients with hepatocellular carcinoma. Microcystin-LR = MC-LR. **a** Admission serum level of MC-LR was higher in the relapse patients than in the non-relapse patients ($n = 396$, 1.05 ± 0.19 ng/ml; $n = 254$, 0.84 ± 0.15 ng/ml; $P < 0.001$). **b** Admission serum level of MC-LR was higher in the dead patients than in the surviving patients ($n = 311$, 1.02 ± 0.18 ng/ml; $n = 339$, 0.86 ± 0.20 ng/ml; $P < 0.001$). **c** There was no difference between serum level of MC-LR on admission and serum level of MC-LR at the time of relapse ($n = 396$, 0.95 ± 0.20 ng/ml; $n = 396$, 0.95 ± 0.28 ng/ml; $P = 0.947$)

As shown in Fig. 1a, admission serum level of MC-LR was higher in the relapsed patients than in the non-relapsed patients (1.05 ± 0.19 ng/ml, 0.84 ± 0.15 ng/ml, $P < 0.001$). In Fig. 1b, admission serum level of MC-LR was higher in the dead patients than in the surviving patients (MC-LR: 1.02 ± 0.18 ng/ml, 0.86 ± 0.20 ng/ml, $P < 0.001$). In the study, each relapsed patient provided two blood specimens separately on admission and at the time of relapse. In Fig. 1c, there was no difference between the serum level of MC-LR on admission ($n = 396$) and serum level of MC-LR at the time of relapse ($n = 396$) (0.95 ± 0.20 ng/ml, 0.95 ± 0.28 ng/ml, $P = 0.947$).

As shown in Fig. 2a, a cutoff point of serum MC-LR level for predicting the tumor relapse was 0.97 ng/ml (sensitivity = 61.83%, specificity = 88.04%, area under curve = 0.788, $P < 0.001$). In Fig. 2c, another cutoff point of serum MC-LR level for predicting the overall death was 1.09 ng/ml (sensitivity = 71.44%, specificity = 65.70%, area under curve = 0.732, $P < 0.001$).

The patients were divided into several groups according to the cutoff points of serum MC-LR. In Fig. 2b, the patients with serum MC-LR level ≥ 0.97 ng/ml had a higher risk of the tumor relapse than the patients with serum MC-LR level < 0.97 ng/ml (log rank $P < 0.001$). In Fig. 2d, the patients with serum MC-LR level ≥ 1.09 ng/ml had a higher risk of the overall death than the patients with serum MC-LR level < 1.09 ng/ml (log rank $P < 0.001$).

As shown in Table 2, after adjusting for several confounding factors, serum MC-LR ≥ 0.97 ng/ml was associated with the increased risk of the tumor relapse (HR: 1.53, 95% CI: 1.35–1.77). Then, the patients were divided into four groups according to the quartiles of the serum MC-LR level. With the increase in the serum MC-LR level, the relationship between the serum MC-LR level and the risk of the tumor relapse was strengthened (HR: 1.33, 95% CI: 1.05–1.68; HR: 1.28, 95% CI: 1.06–1.50; HR: 1.30, 95% CI: 1.14–1.48). Serum MC-LR ≥ 1.09 ng/ml was also associated with the higher risk of the overall death (HR: 1.58, 95% CI: 1.35–1.84). With the increase in the serum MC-LR level, the relationship between the serum MC-LR level and the risk of the overall death was also strengthened (HR: 1.46, 95% CI: 1.06–1.99; HR: 1.38, 95% CI: 1.11–1.75; HR: 1.26, 95% CI: 1.07–1.51).

In the study, all the dead patients had experienced a tumor relapse and received a post-relapse therapy. As shown in Table 2, of the 396 relapsed patients, 178 patients had a post-relapse TACE and 218 patients had a post-relapse RFA. Among the 178 patients with TACE, 137 had died at the end of the follow-up. Among the 218 patients with RFA, 174 patients had died when the follow-up ended. In the TACE group, serum MC-LR ≥ 1.09 ng/ml was associated with the increased risk of the overall death in the relapsed patients (HR: 1.20, 95% CI: 1.04–1.38). In the RFA group, serum MC-LR ≥ 1.09 ng/ml was also related to the increased risk of the overall death in the relapsed patients (HR: 1.18, 95% CI: 1.04–1.35).

As shown in Fig. 3a–c, admission serum MC-LR level was linearly related to admission FlOP_360, FlOP_320

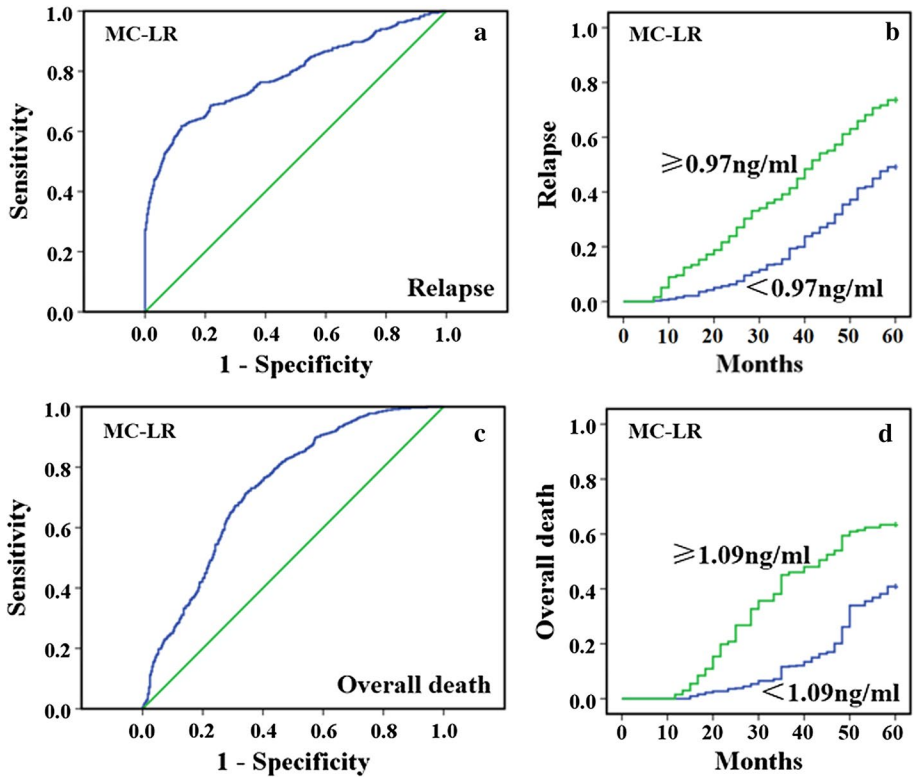

Fig. 2 Results of receiver operating characteristic curve analysis and Kaplan–Meier survival analysis. Microcystin-LR = MC-LR. **a** A cutoff value of serum MC-LR level for predicting the tumor relapse was 0.97 ng/ml with a sensitivity of 61.83% and a specificity of 88.04% (area under curve = 0.788, $P < 0.001$). **b** The patients with serum MC-LR level ≥ 0.97 ng/ml had a higher risk of tumor relapse than the patients with serum MC-LR level < 0.97 ng/ml (log rank $P < 0.001$). **c** A cutoff value of serum MC-LR level for predicting the overall death was 1.09 ng/ml with a sensitivity of 71.44% and a specificity of 65.70% (area under curve = 0.732, $P < 0.001$). **d** The patients with serum MC-LR level ≥ 1.09 ng/ml had a higher risk of overall death than the patients with serum MC-LR level < 1.09 ng/ml (log rank $P < 0.001$)

and FlOP_400 ($r = 0.133$, $P = 0.001$; $r = 0.093$, $P = 0.023$; $r = 0.081$, $P = 0.047$).

In Fig. 4a–c, admission serum levels of FlOP_360, FlOP_320 and FlOP_400 were higher in the relapse patients than in the non-relapse patients (FlOP_360: 225 ± 83 FI/ml, 183 ± 91 FI/ml, $P < 0.001$; FlOP_320: 393 ± 110 FI/ml, 357 ± 119 FI/ml, $P < 0.001$; FlOP_400: 59 ± 24 FI/ml, 53 ± 24 FI/ml, $P = 0.001$). In Fig. 4d–f, admission serum levels of FlOP_360, FlOP_320 and FlOP_400 were also higher in the dead patients than in the surviving patients (FlOP_360: 227 ± 82 FI/ml, 192 ± 91 FI/ml, $P < 0.001$; FlOP_320: 394 ± 111 FI/ml, 364 ± 117 FI/ml, $P = 0.001$; FlOP_400: 60 ± 24 FI/ml, 54 ± 24 FI/ml, $P = 0.002$).

Discussion

A previous study enrolled 214 HCC patients and 214 controls in southwest China and reported that serum MC-LR was an independent risk factor for HCC [22]. However, the prognostic role of serum MC-LR in HCC has not been studied.

It is well known that MC-LR exposure is mainly caused by environmental and dietary pollution. Some people may not be able to realize its harm. Even if the danger is noticed, it is difficult to eliminate the toxin from the environment. So, the exposure should be a chronic process even after the occurrence of HCC. In the study, there was no difference when we compared serum level of MC-LR on admission with that at the time of tumor relapse. This finding may explain the prognostic role of MCLR in HCC.

In the study, this was a homogenous cohort. All the subjects were early-stage hepatitis B-induced HCC patients and were not affected by hepatitis C, cirrhosis, heavy drinking or aflatoxin exposure. The ROC analysis, Kaplan–Meier survival analysis and multifactorial COX regression analysis jointly confirmed the effect of serum MC-LR level on the prognosis of the patients. The results showed that serum level of MC-LR ≥ 0.97 ng/ml and 1.09 ng/ml separately led to an about 50% increased risk of the tumor relapse and overall death in the 5-year follow-up period. Furthermore, the patients were divided into four groups according to the quartiles of serum MC-LR level and found a dose–response relationship between serum MC-LR level and poor prognosis. Therefore, serum MC-LR independently worsened the prognosis of the HCC patients.

Several confounding factors had been taken into account in the study. First, poor dietary habits and obesity were considered to be risk factors for HCC [27–29] and were ignored in the previous study [22]. Our multifactorial analysis had included these factors, and the potential bias had been avoided. Second, post-relapse therapy should be another confounding factor. The patients in the study received two different post-relapse therapies: TACE and FRA. So, a subgroup analysis according to these two therapies was performed and reported that the prognostic significance

Table 2 Relationship between prognosis of patients and serum microcystin-LR

	Relapse/death (*n*)	Total (*n*)	Monofactorial HR (95% CI)	Multifactorial HR (95% CI)[a]
Relapse versus MC-LR	396	650	–	–
MC-LR				
< 0.97 ng/ml	165	336	Reference	Reference
≥ 0.97 ng/ml	231	314	1.52 (1.34–1.76)	1.53 (1.35–1.77)
MC-LR				
< 0.77 ng/ml	68	163	Reference	Reference
0.77–0.96 ng/ml	88	163	1.32 (1.05–1.67)	1.33 (1.05–1.68)
0.96–1.13 ng/ml	107	162	1.27 (1.05–1.49)	1.28 (1.06–1.50)
≥ 1.13 ng/ml	133	162	1.29 (1.13–1.47)	1.30 (1.14–1.48)
Death versus MC-LR	311	650	–	–
MC-LR				
< 1.09 ng/ml	183	448	Reference	Reference
≥ 1.09 ng/ml	128	202	1.57 (1.34–1.83)	1.58 (1.35–1.84)
MC-LR				
< 0.77 ng/ml	46	163	Reference	Reference
0.77–0.96 ng/ml	66	163	1.45 (1.06–1.98)	1.46 (1.06–1.99)
0.96–1.13 ng/ml	89	162	1.37 (1.10–1.75)	1.38 (1.11–1.75)
≥ 1.13 ng/ml	110	162	1.25 (1.06–1.50)	1.26 (1.07–1.51)
Post-relapse TACE	137	178	–	–
MC-LR				
< 1.09 ng/ml	81	112	Reference	Reference
≥ 1.09 ng/ml	56	66	1.19 (1.03–1.38)	1.20 (1.04–1.38)
Post-relapse RFA	174	218	–	–
MC-LR				
< 1.09 ng/ml	102	135	Reference	Reference
≥ 1.09 ng/ml	72	83	1.17 (1.03–1.34)	1.18 (1.04–1.35)

MC Microcystin, *TACE* transarterial chemoembolization, *RFA* radiofrequency ablation, *HR* hazard ratio, *CI* confidence interval

[a]The model was adjusted by age, gender, smoking, dietary status, obesity, family history, tumor number, main tumor size, tumor differentiation and Barcelona Clinic Liver Cancer stage

of serum MC-LR level continuously existed in the TACE-treated patients as well as the FRA-treated patients.

There was clear evidence that oxidative stress was implicated in hepatotoxicity of MC-LR. Several studies suggested that MC-LR in HepG2 cells increased DNA strand breaks, lipid peroxidation and lactate dehydrogenase release, all of which were inhibited by reactive oxygen species scavengers and intracellular antioxidants [30, 31]. In this study, we found that there was a linear relationship between serum MC-LR concentration and oxidative stress level. We also revealed that oxidative stress was more serious in the patients with poor prognosis. Thus, oxidative stress was probably a bridge between the toxin and the poor prognosis of the patients. MC-LR affects the prognosis of the patients partly through promoting oxidative stress in the body.

In the study, FlOP was adopted to reflect the level of oxidative stress in the patients. The reasons why we chose this biomarker were as follows. First, FlOP had been widely adopted in this field, and it can be measured using a simple and reliable method [25, 26]. Second, FlOP represented the interaction of lipid oxidation productions with amino acids, nucleic acids, carbohydrates and metallic elements, which was much more comprehensive than other oxidative stress markers [26]. Third, FlOPs in serum were very stable and can be stored for many years [32].

Besides, there were many other potential mechanisms on the prognostic role of MC-LR. For example, MC-LR promoted the expressions of oncogenes c-fos, c-jun, c-myc, c-met and N-ras, while suppressed tumor-suppressor gene PTEN in HepG2 cells [33]. The toxin also promoted the hepatocyte proliferation, and the activation of Akt/S6K1 and MAPK signaling pathways was proposed to participate in this process [34, 35]. But we failed to explore these mechanisms, which might be a limitation of the study.

In conclusion, the study suggested that serum level of MC-LR might independently worsen the prognosis of the HCC patients partly through promoting the oxidative stress in the body. It is very meaningful to take necessary measures

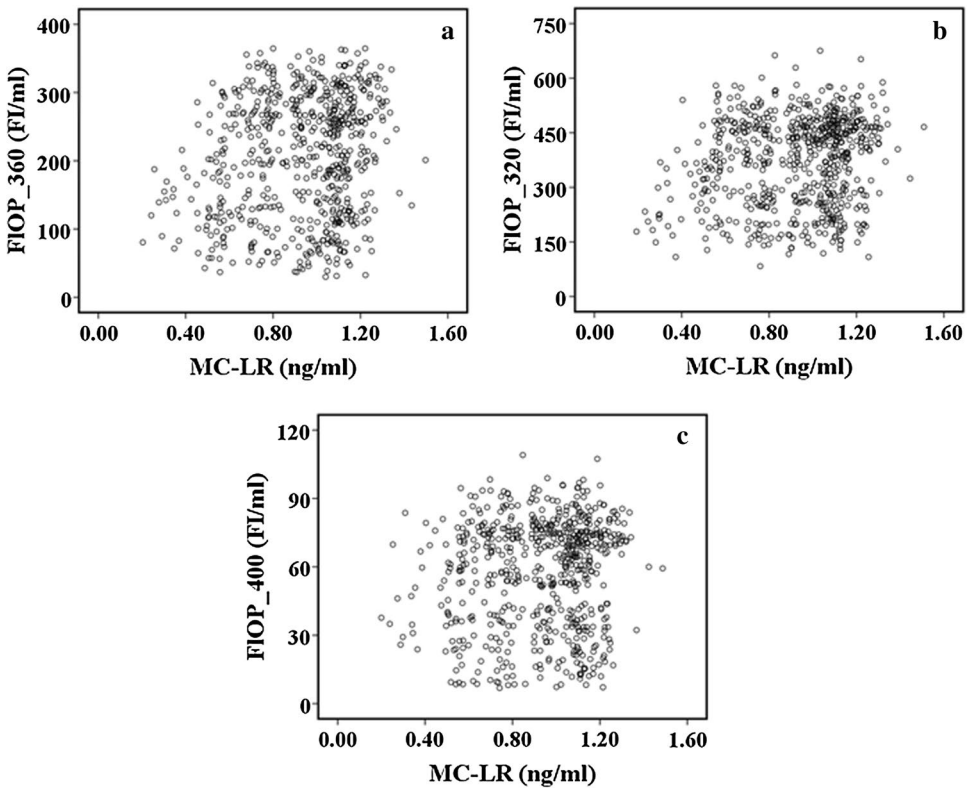

Fig. 3 Linear relationship between serum level of microcystin-LR and serum levels of fluorescent oxidation products. Microcystin-LR = MC-LR; fluorescent oxidation product = FlOP. Serum FlOP levels were measured using a spectrofluorometer at three wavelengths: FlOP_360 (excitation/emission: 360/420 nm), FlOP_320 (320/420 nm) and FlOP_400 (400/475 nm). The unit was relative fluorescent intensity per milliliter of serum (FI/ml). **a**–**c** Serum MC-LR level was linearly related to serum levels of FlOP_360, FlOP_320 and FlOP_400 ($n = 650$, $r = 0.133$, $P = 0.001$; $n = 650$, $r = 0.093$, $P = 0.023$; $n = 650$, $r = 0.081$, $P = 0.047$)

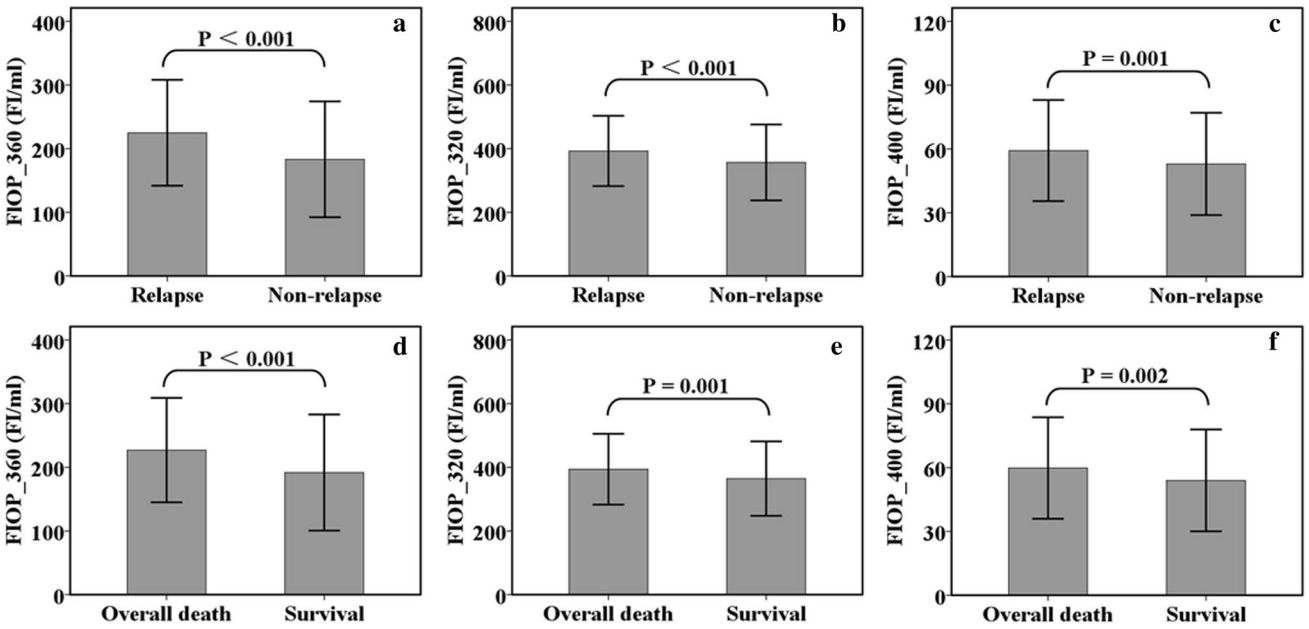

Fig. 4 Serum levels of fluorescent oxidation products in patients with hepatocellular carcinoma. Fluorescent oxidation product = FlOP. **a** Serum level of FlOP_360 was higher in the relapse patients than in the non-relapse patients ($n = 396$, 225 ± 83 FI/ml; $n = 254$, 183 ± 91 FI/ml; $P < 0.001$). **b** Serum level of FlOP_320 was higher in the relapse patients than in the non-relapse patients ($n = 396$, 393 ± 110 FI/ml; $n = 254$, 357 ± 119 FI/ml; $P < 0.001$). **c** Serum level of FlOP_400 was higher in the relapse patients than in the non-relapse patients ($n = 396$, 59 ± 24 FI/ml; $n = 254$, 53 ± 24 FI/ml; $P = 0.001$). **d** Serum level of FlOP_360 was higher in the dead patients than in the surviving patients ($n = 311$, 227 ± 82 FI/ml; $n = 339$, 192 ± 91 FI/ml; $P < 0.001$). **e** Serum level of FlOP_320 was higher in the dead patients than in the surviving patients ($n = 311$, 394 ± 111 FI/ml; $n = 339$, 364 ± 117 FI/ml; $P = 0.001$). **f** Serum level of FlOP_400 was higher in the dead patients than in the surviving patients ($n = 311$, 60 ± 24 FI/ml; $n = 339$, 54 ± 24 FI/ml; $P = 0.002$)

to reduce the exposure of the toxins (such as MC-LR) even after the occurrence of HCC.

Funding The study was supported by the Innovation Team Project of Renmin Hospital Affiliated to Hubei Medical College (201404) and the Free Exploration Fund Project of Hubei Medical College (2017).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. McGlynn KA, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. Clin Liver Dis. 2015;19:223–38.
2. Ozakyol A. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). J Gastrointest Cancer. 2017. https://doi.org/10.1007/s12029-017-9959-0.
3. Heidelbaugh JJ, Bruderly M. Cirrhosis and chronic liver failure: part I. Diagnosis and evaluation. Am Fam Physician. 2006;74:756–62.
4. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007;13:2436–41.
5. Kucukcakan B, Hayrulai-Musliu Z. Challenging role of dietary aflatoxin B1 exposure and hepatitis B infection on risk of hepatocellular carcinoma. Open Access Maced J Med Sci. 2015;3:363–9.
6. Chan SL, Wong VW, Qin S, Chan HL. Infection and cancer: the case of hepatitis B. J Clin Oncol. 2016;34:83–90.
7. Liu Y, Chang CC, Marsh GM, Wu F. Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. Eur J Cancer. 2012;48:2125–36.
8. Ufelmann H, Krüger T, Luckas B, Schrenk D. Human and rat hepatocyte toxicity and protein phosphatase 1 and 2A inhibitory activity of naturally occurring desmethyl-microcystins and nodularins. Toxicology. 2012;293:59–67.
9. Gupta N, Pant SC, Vijayaraghavan R, Rao PV. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology. 2003;188:285–96.
10. Schirrmeister BE, de Vos JM, Antonelli A, Bagheri HC. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. Proc Natl Acad Sci USA. 2013;110:1791–6.
11. Dittmann E, Wiegand C. Cyanobacterial toxins–occurrence, biosynthesis and impact on human affairs. Mol Nutr Food Res. 2006;50:7–17.
12. Smith JL, Haney JF. Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (Lepomis gibbosus). Toxicon. 2006;48:580–9.
13. Cheung MY, Liang S, Lee J. Toxin-producing cyanobacteria in freshwater: a review of the problems, impact on drinking water safety, and efforts for protecting public health. J Microbiol. 2013;51:1–10.
14. Hejkal TW, Larock PA, Winchester JW. Water-to-air fractionation of bacteria. Appl Environ Microbiol. 1980;39:335–8.
15. O'Neil JM, Davis TW, Burford MA, Gobler CJ. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae. 2012;14:313–34.
16. Paerl HW, Huisman J. Climate. Blooms like it hot. Science. 2008;320:57–8.
17. Svirčev Z, Drobac D, Tokodi N, et al. Epidemiology of cancers in Serbia and possible connection with cyanobacterial blooms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2014;32:319–37.
18. Svirčev Z, Drobac D, Tokodi N, et al. Epidemiology of primary liver cancer in Serbia and possible connection with cyanobacterial blooms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2013;31:181–200.
19. Zhang F, Lee J, Liang S, Shum CK. Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. Environ Health. 2015;14:41.
20. Li Y, Chen JA, Zhao Q, et al. A cross-sectional investigation of chronic exposure to microcystin in relationship to childhood liver damage in the Three Gorges Reservoir Region, China. Environ Health Perspect. 2011;119:1483–8.
21. Chen J, Xie P, Li L, Xu J. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. Toxicol Sci. 2009;108:81–9.
22. Zheng C, Zeng H, Lin H, et al. Serum microcystin levels positively linked with risk of hepatocellular carcinoma: a case-control study in southwest China. Hepatology. 2017;66:1519–28.
23. Zegura B, Sedmak B, Filipic M. Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. Toxicon. 2003;41:41–8.
24. Svircev Z, Baltić V, Gantar M, Juković M, Stojanović D, Baltić M. Molecular aspects of microcystin-induced hepatotoxicity and hepatocarcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2010;28:39–59.
25. Wu T, Willett WC, Rifai N, Rimm EB. Plasma fluorescent oxidation products as potential markers of oxidative stress for epidemiologic studies. Am J Epidemiol. 2007;166:552–60.
26. Fortner RT, Tworoger SS, Wu T, Eliassen AH. Plasma florescent oxidation products and breast cancer risk: repeated measures in the Nurses' Health Study. Breast Cancer Res Treat. 2013;141:307–16.
27. Kurozawa Y, Ogimoto I, Shibata A, et al. Dietary habits and risk of death due to hepatocellular carcinoma in a large scale cohort study in Japan Univariate analysis of JACC study data. Kurume Med J. 2004;51:141–9.
28. Pang Q, Qu K, Liu C. Central obesity early in adulthood may affect outcomes of hepatocellular carcinoma. Gastroenterology. 2015;149:1642–3.
29. Funakoshi N, Chaze I, Alary AS, et al. The role of genetic factors in patients with hepatocellular carcinoma and iron overload—a prospective series of 234 patients. Liver Int. 2016;36:746–54.
30. Nong Q, Komatsu M, Izumo K, et al. Involvement of reactive oxygen species in microcystin-LR-induced cytogenotoxicity. Free Radic Res. 2007;41:1326–37.
31. Zegura B, Lah TT, Filipic M. Alteration of intracellular GSH levels and its role in microcystin-LR-induced DNA damage in human hepatoma HepG2 cells. Mutat Res. 2006;611:25–33.
32. Wu T, Rifai N, Roberts LJ 2nd, Willett WC, Rimm EB. Stability of measurements of biomarkers of oxidative stress in blood over 36 hours. Cancer Epidemiol Prev Biomark. 2004;13:1399–402.
33. Li X, Zhang X, Xie W, Zhou C, Li Y, Zhang X. Alterations in transcription and protein expressions of HCC-related genes

in HepG2 cells caused by microcystin-LR. Toxicol In Vitro. 2017;40:115–23.
34. Liu J, Wang B, Huang P, et al. Microcystin-LR promotes cell proliferation in the mice liver by activating Akt and p38/ERK/JNK cascades. Chemosphere. 2016;163:14–21.
35. Liu J, Wang H, Wang B, et al. Microcystin-LR promotes proliferation by activating Akt/S6K1 pathway and disordering apoptosis and cell cycle associated proteins phosphorylation in HL7702 cells. Toxicol Lett. 2016;240:214–25.