Interleukin-8 Serum Levels in Patients with Hepatocellular Carcinoma: Correlations with Clinicopathological Features and Prognosis

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ABSTRACT

Purpose: **In this study, we measured the serum interleukin-8 (IL-8) levels in patients with hepatocellular carcinoma (HCC) to evaluate its correlation with clinicopathological features and prognosis.**

Experimental Design: **ELISA was used to detect the concentrations of IL-8, vascular endothelial growth factor, and macrophage migration inhibitory factor in preoperative sera of 59 patients with resection of HCC and 15 healthy subjects.**

Results: **Preoperative serum IL-8 was found to be significantly elevated in patients with HCC compared with healthy subjects (median, 17.6 *versus* 1.0 pg/ml, $P = 0.046$). The levels of IL-8 correlated significantly with a large tumor size (>5 cm), absence of tumor capsule, presence of venous invasion, and advanced pathological tumor-node-metastasis stage. Serum IL-8 level was a significant prognostic factor in terms of disease-free and overall survival. Patients with a serum IL-8 level of >17.6 pg/ml had a poorer disease-free survival than those with a level of <17.6 pg/ml (median disease-free survival 4.7 *versus* 19.2 months). Multivariate analyses showed that serum IL-8 level was a significant and independent prognostic factor of survival.**

Conclusions: **Significant correlations of serum IL-8 levels with tumor size and tumor stage suggest that IL-8 may be directly or indirectly involved in the progression of HCC. These findings indicate that serum IL-8 may be a useful biological marker of tumor invasiveness and an independent prognostic factor for patients with HCC.**

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INTRODUCTION

HCC[1], one of the most common human cancers worldwide, is the second most common cause of cancer death in China. Although advances in surgical techniques and perioperative management have improved survival, the long-term survival after surgical resection is unsatisfactory because of a high incidence of recurrence (1). The presence of venous invasion was identified as the most important risk factor of recurrence after resection of HCC. This suggests that metastasis is an important mechanism of postoperative recurrence (2). Therefore, the identification of a marker that predicts the risk of extrahepatic or intrahepatic metastasis, and hence prognosis, is highly desirable. Several molecular biological factors have been shown to be associated with the invasiveness of HCC. These, therefore, possess potential prognostic significance. However, routine biomarkers for the prediction of HCC prognosis are not yet available.

Angiogenesis is a prime regulator of tumor growth (3). The induction of tumor angiogenesis is a control point in tumor expansion and in the metastasis of tumor cells to distant sites (4, 5). It has been suggested that angiogenesis is an early event in tumorigenesis (6, 7). Tumor angiogenesis may be regulated by angiogenic factors such as transforming growth factor-β (8), VEGF (9), basic fibroblast growth factor (10), tumor necrosis factor-α (11), MIF (12), and IL-8 (13).

IL-8 is a multifunctional CXC chemokine that affects human neutrophil functions, including chemotaxis, enzyme release, and expression of surface adhesion molecules. IL-8 is produced by a wide variety of cell types, including monocytes, neutrophils, fibroblasts, and endothelial cells (14, 15). IL-8 was identified to be an angiogenesis-regulating molecule that induced angiogenesis (13, 16). The expression of IL-8 has been found in various human cancers (17). Recent studies have demonstrated that IL-8 regulates tumor cell growth and metastasis in melanoma (18), carcinoma of breast (19), stomach (20), pancreas (21), and liver (21, 22). Singh *et al.* (18) have reported that the expression of IL-8 in melanoma cells directly correlated with metastatic potential in nude mice. It has been shown that IL-8 can up-regulate matrix metalloproteinase-2 expression and activity, and increase melanoma cell invasion and migration (23). Thus, multiple mechanisms seem to be involved in IL-8 action, including direct effects on tumor and vascular endothelial cell proliferation, angiogenesis, and migration. Elevated serum level of IL-8 was found to be a prognostic marker in soft tissue sarcoma (24), B-cell chronic lymphocytic leukemia (25),

[1] The abbreviations used are: HCC, hepatocellular carcinoma; IL, interleukin; MIF, macrophage migration inhibitory factor; VEGF, vascular endothelial growth factor; pTNM, pathological tumor-node-metastasis.

primary gastrointestinal non-Hodgkin's lymphoma (26), and malignant melanoma (27). However, no studies have investigated the correlation between the serum IL-8 level and clinicopathological features of HCC. In this study, we measured the preoperative serum IL-8 levels in 59 patients undergoing resection of HCC to evaluate its relationship with tumor stage, invasion, metastasis, and survival.

MATERIALS AND METHODS

Patients and Blood Samples. Fifty-nine consecutive Chinese patients who underwent resection of HCC in the Department of Surgery, The University of Hong Kong, between January 1998 and June 1999 and agreed to participate in the study were recruited. There were 42 males and 17 females. The median age of the patients at diagnosis was 50 years (range from 16 to 73 years). Forty-six patients had solitary tumor, whereas the other 13 patients had multifocal tumor nodules detected grossly by preoperative imaging studies or intraoperative ultrasonography. Thirty-five patients had underlying cirrhosis, and all these patients had Child's A cirrhosis in preoperative assessment. All patients had a potentially curative resection with tumor-free margin macroscopically and microscopically.

Blood samples were collected from the 59 patients with HCC before surgical resection and also from 15 healthy control subjects. Blood samples were also collected from 42 patients with advanced inoperable HCC because of bilobar disease, portal vein invasion, or distant metastasis. The study was approved by the Human Research Ethics Committee, Queen Mary Hospital, The University of Hong Kong. Informed consent was obtained according to the regulations of the committee.

Measurement of the Concentrations of IL-8, VEGF, and MIF in the Sera. MIF, VEGF, and IL-8 in the sera of the 59 patients with resection of HCC were measured by using ELISA kits (R&D Systems, Minneapolis, MN). The ELISA was performed according to the manufacturer's instructions. Serum IL-8 levels were also measured in the 42 patients with inoperable HCC and in the 15 healthy control subjects for comparison.

Statistical Analyses. Continuous variables were expressed as median and interquartile range and compared using the Mann-Whitney *U* test. Correlation between continuous variables was determined using the Pearson correlation coefficient. The cumulative disease-free and overall survival was computed using the Kaplan-Meier method and compared by the log-rank test. The relative prognostic importance of serum IL-8 level and other clinicopathological variables on overall survival was studied by multivariate analyses using a Cox regression model. Statistical significance was taken as $P < 0.05$. All statistical analyses were performed using the SPSS software for windows 9.0 (SPSS, Inc., Chicago, IL).

RESULTS

Serum IL-8 Levels and Clinicopathological Features. The preoperative serum IL-8 levels of 59 patients with resection of HCC ranged from undetectable to 351.6 pg/ml, with a median of 17.6 pg/ml (interquartile range, 0–46.4 pg/ml). Eighteen patients had undetectable serum IL-8 levels. The average level of serum IL-8 in these 59 patients with resectable HCC was significantly higher than that of healthy controls (median, 1.0 pg/ml; interquartile range, 0.2–3.7 pg/ml; $P = 0.046$) but significantly lower than that of the 42 patients with more advanced unresectable HCC (median, 95.8 pg/ml; interquartile range, 49.1–187.3 pg/ml; $P < 0.001$). There was no significant correlation between serum IL-8 concentration and patients' gender ($P = 0.215$), age ($P = 0.574$), hepatitis B surface antigen status ($P = 0.322$), and underlying cirrhosis ($P = 0.772$; Table 1). The serum IL-8 levels were not significantly different between patients with a solitary tumor and those with multiple tumors (P = 0.571). However, high-serum IL-8 levels were significantly associated with large tumors > 5 cm (P = 0.016), absence of tumor capsule ($P = 0.035$), presence of venous invasion, and an advanced disease *stage* ($P = 0.037$; Table 1). The serum IL-8 levels in patients with stage III or IV disease were significantly higher than those of patients with stage I or II disease ($P = 0.037$). However, serum IL-8 levels in patients with stage I or II disease were not significantly different from those of healthy controls. In addition, a significant correlation was noted between serum IL-8 levels and the platelets count ($r = 0.386$, $P = 0.009$).

Table 1 Relationship between serum IL-8 levels and clinicopathological features[a]

Variables	Serum IL-8 (pg/ml)	*P*
Gender		0.215
Male ($n = 42$)	9.7 (2.9–40.7)	
Female ($n = 17$)	21.5 (4.9–46.5)	
Age (yrs)		0.574
≥65 ($n = 48$)	17.6 (0.13–75.0)	
<65 ($n = 11$)	11.7 (0–46.5)	
Etiology of liver disease		0.322
HBV positive ($n = 50$)	9.7 (3.7–45.7)	
HBV negative ($n = 9$)	12.5 (0–27.2)	
Liver cirrhosis		0.772
Present ($n = 35$)	17.6 (2.9–46.5)	
Absent ($n = 24$)	14.1 (0–50.1)	
Tumor number		0.571
Solitary ($n = 46$)	17.0 (0–46.5)	
Multiple ($n = 13$)	17.8 (2.9–82.6)	
Tumor size		0.016[b]
≤5 cm ($n = 22$)	1.3 (0–29.6)	
>5 cm ($n = 37$)	25.3 (4.9–51.3)	
Tumor capsule		0.035[b]
Present ($n = 18$)	4.9 (0–34.9)	
Absent ($n = 41$)	23.2 (6.4–54.9)	
Venous invasion		0.027[b]
Present ($n = 27$)	30.8 (4.1–79.3)	
Absent ($n = 32$)	4.9 (0–34.6)	
Microsatellite nodules		0.558
Present ($n = 31$)	17.6 (4.1–64.2)	
Absent ($n = 28$)	10.3 (0–45.9)	
Edmonson grade		0.806
1–2 ($n = 27$)	18.9 (0.86–46.5)	
3–4 ($n = 32$)	17.6 (0.13–46.5)	
pTNM stage		0.037[b]
I and II ($n = 25$)	1.7 (0–35.3)	
III and IV ($n = 24$)	23.2 (4.1–67.5)	

[a] Serum IL-8 levels are expressed as median (interquartile range).
[b] $P < 0.05$ by Mann-Whitney *U* test.

Prognostic Value of Serum IL-8 on Disease-Free Survival. By the time of data analysis, 40 of the 59 patients with resection of HCC have developed tumor recurrence after a

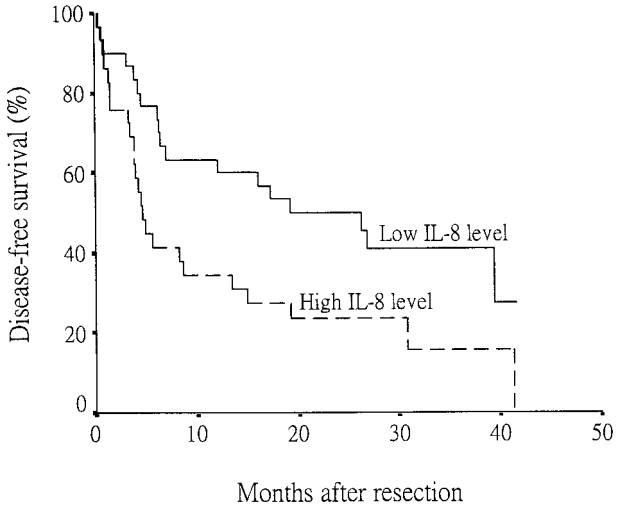

Fig. 1 Cumulative disease-free survival curves of patients with serum IL-8 less than or greater than 17.6 pg/ml ($P = 0.021$).

median follow-up of 42 months. Twenty-five patients developed recurrence within the liver, whereas the other 15 patients developed extrahepatic recurrence. The latter included lung metastasis in 11 patients, i.p. metastasis in 3 patients and bone metastasis in 1 patient. With the median serum IL-8 level (17.6 pg/ml) in the whole group as the cutoff value, patients were divided into high (>17.6 pg/ml) and low serum IL-8 (≤17.6 pg/ml) groups. The median disease-free survival of patients with serum IL-8 levels of ≤17.6 pg/ml was 19.2 months ($n = 30$). This was significantly better than that of patients whose serum IL-8 levels were >17.6 pg/ml (median disease-free survival, 4.7 months, $n = 29$; $P = 0.021$; Fig. 1). The overall cumulative survival of patients with a serum IL-8 level ≤ 17.6 pg/ml was also significantly better than that of those with a serum IL-8 level of >17.6 pg/ml (3-year survival: 71 *versus* 36%, $P = 0.024$; Fig. 2). The overall survival of the subset of 18 patients who had undetectable preoperative serum IL-8 levels was significantly better than that of the other patients with elevated serum IL-8 levels (3-year survival: 80 *versus* 44%, $P = 0.021$). There was no significant difference in the preoperative serum IL-8 levels between patients who developed intrahepatic recurrence and those who developed extrahepatic recurrence (median IL-8 level, 23.2 *versus* 24.5 pg/ml; $P = 0.261$).

Serum IL-8 level was entered into a Cox regression analysis together with other variables that may influence prognosis. These included preoperative serum bilirubin level, serum albumin level, serum α-fetoprotein level, presence of underlying cirrhosis, tumor size, venous invasion, tumor encapsulation, microsatellite nodules, Edmonson grade, and pTNM stage. The multivariate analyses revealed that independent prognostic factors of overall survival included preoperative serum IL-8 level, venous invasion, and pTNM stage (Table 2).

Correlations between Serum IL-8 and other Angiogenic Factors. Serum levels of VEGF and MIF were also evaluated in the HCC patients and healthy control subjects. The levels of VEGF and MIF in the sera of patients with HCC were also significantly increased compared with healthy subjects (Table 3). Furthermore, the level of serum IL-8 correlated positively with the serum level of MIF ($r = 0.327$, $P = 0.028$), suggesting that MIF and IL-8 expression in HCC patients are related. There was also a significant correlation between serum IL-8 and serum VEGF levels ($r = 0.491$, $P = 0.001$).

DISCUSSION

IL-8, originally discovered as a chemotactic factor for leukocytes, has recently been shown to contribute to human cancer progression through its potential functions as mitogenic, angiogenic, and motogenic factor (17). Akiba *et al.* (22) provided evidence that IL-8 produced by HCC is an angiogenic factor of HCC. Therefore, it is of interest to elucidate the role of serum IL-8 as a biological tumor marker in HCC patients.

In the current study, we found that the level of serum IL-8 was markedly elevated in most patients with HCC compared with healthy subjects. In a previous study, IL-8 was found to be overexpressed in the HCC tumor cells compared with the nontumorous livers (22). The high-serum IL-8 levels in HCC patients may be caused by an excessive production in tumor cells and subsequent release into the circulation. We showed that a high-serum IL-8 level was significantly correlated with a more aggressive tumor behavior in patients with resectable HCC. A high-serum IL-8 level was observed more frequently in patients with tumors > 5 cm in diameter and advanced tumor stage (pTNM stages III or IV). Serum IL-8 levels were also found to be significantly correlated with tumor progression, venous in-

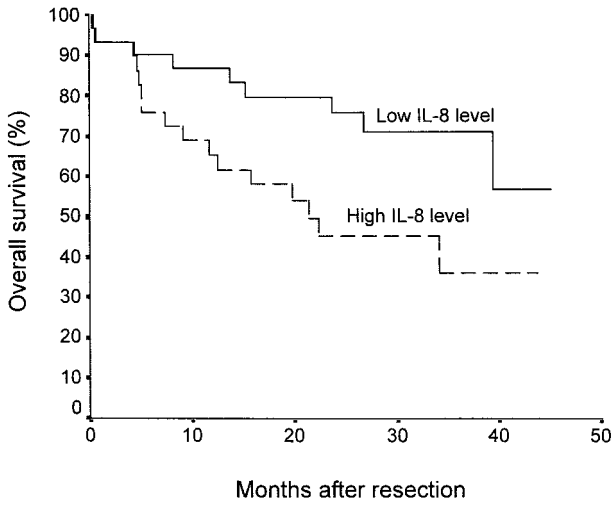

Fig. 2 Overall survival of patients with serum IL-8 less than or greater than 17.6 pg/ml ($P = 0.024$).

Table 2 Multivariate analysis of prognostic factors of survival with Cox's proportional hazards model

Factors	Risk ratio	95% confidence interval	*P*
Serum IL-8 level	3.291	1.142–9.485	0.027
Venous invasion	6.442	1.968–11.186	0.002
pTNM stage	4.093	1.936–8.654	<0.001

Table 3 Serum levels of cytokines in patients with HCC and in healthy controls[a]

	HCC patients ($n = 15$)	Healthy controls ($n = 59$)	P^b
IL-8 (pg/ml)	17.6 (0–46.5)	1.0 (0.2–3.7)	0.046
MIF (ng/ml)	109.6 (64.0–154.0)	2.5 (1.8–3.5)	<0.001
VEGF (pg/ml)	254.9 (150.4–398.4)	140.0 (41.0–230.0)	0.048

[a] Sera was collected from 15 healthy subjects in control group and 59 patients with HCC and assayed for each cytokine by ELISA.
[b] $P < 0.05$ by Mann-Whitney U test.

vasion, and survival. These data suggested that serum IL-8 might be useful in the clinical setting to predict venous invasion and advanced tumor stage. Akiba *et al.* (22) observed a close relationship between the expression of IL-8 in HCC tissues and tumor invasion. This was corroborated by our findings that serum IL-8 was well correlated with venous invasion. We also observed that patients with more advanced unresectable HCC had an even higher serum IL-8 levels than the patients with resectable HCC. This finding additionally supports the role of IL-8 in the progression of HCC. It is much more convenient to measure serum IL-8 levels than to evaluate the expression of IL-8 in tissue specimens as the former approach is noninvasive and reproducible. Furthermore, it does not require tumor tissue specimens. Thus, measurement of preoperative serum IL-8 levels might be more useful and feasible in the clinical setting to predict venous invasion and tumor stage than detection of IL-8 in HCC tumor specimens obtained from surgical resections.

Clinicopathological factors related to the prognosis of HCC have been widely reported. Of these, pTNM stage is a significant prognosticator among the conventional pathological features of HCC (28). In our study, a high-serum IL-8 level was a significant prognostic factor in terms of disease-free and overall survival. Furthermore, multivariate analyses revealed that the serum IL-8 level was an independent predictor of long-term survival in patients with HCC. These results suggest that that IL-8 may play an important role in the progression and tumor dissemination of HCC. A high-serum IL-8 level may reflect active angiogenesis and rapid tumor growth in HCC. Therefore, targeting IL-8 can be a potential approach to control angiogenesis and invasion of HCC. Huang *et al.* (29) reported that targeting of IL-8 by a fully humanized neutralizing antibody can be a potential therapeutic strategy to control angiogenesis, growth, and metastasis of melanoma. More recently, it was reported that the copper chelator trientine has an antiangiogeneic effect against HCC, possibly through inhibition of IL-8 production (30). Inhibition of IL-8 production or activity may be beneficial for HCC patients. Anti-IL-8 therapy using anti-IL-8 antibodies (29), anti-IL-8 receptors (31), and inhibitors of IL-8 production such as trientine (30) may be particularly useful in improving the prognosis of the patient subgroup with high-serum IL-8 levels.

In this study, we found a positive correlation between serum IL-8 level and platelet count. IL-8 receptor has been shown to be present in platelets (32–34), and platelets contain IL-8 (35). Studies on circulating VEGF have suggested that platelet may play a role in the storage of circulating VEGF (36, 37). Platelet may also plays a role in storing and transporting circulating IL-8 in cancer patients. Additional studies are required to clarify the relationship between circulating IL-8 and platelet in cancer patients.

In our study, we also observed a significant increase of serum levels of VEGF and MIF in patients with HCC. VEGF has been shown to be vital for angiogenesis (38). An increased expression of VEGF has also been reported to correlate with tumor progression and intrahepatic metastasis in HCC (39, 40). Furthermore, there is evidence that a high-serum VEGF level is a predictor of intrahepatic metastasis and microscopic venous invasion in HCC (41). Circulating VEGF seems to be reliable surrogate marker of angiogenic activity and tumor progression in cancer patients (42). MIF is a inflammatory cytokine involved in tumor angiogenesis and progression (12). The expression of MIF is increased in various human cancers (43–45), including HCC (46). Our previous study revealed that MIF expression in human esophageal squamous cell carcinoma correlated with tumor differentiation, lymph node metastasis, and survival. Furthermore, the serum MIF levels correlated with the other serum angiogenic factors in patients with esophageal squamous cell carcinoma (unpublished data). On the basis of these data, we tried to analyze the correlations between IL-8 and circulating angiogenic factors VEGF and MIF in HCC patients. In this study, we clearly showed that the serum levels of IL-8 significantly correlated with the levels of MIF and VEGF, suggesting that MIF and VEGF may play a role in regulating serum IL-8 production in patients with HCC. Our ongoing studies are determining the mechanism of MIF regulating IL-8 and VEGF production in tumor cells.

In this study, we only measured the preoperative serum level of IL-8, VEGF, and MIF. Serum levels of some angiogenic factors have been shown to decrease after resection of the tumor in cancer patients (42). Postoperative change in serum IL-8 levels may provide additional predictive value on tumor recurrence and prognosis. The clinical significance of the changes of serum IL-8 levels after resection of HCC warrants additional studies.

In conclusion, our results indicate that there is a high preoperative serum IL-8 level in patients with HCC. A high-serum IL-8 level correlated with large tumor volume and advanced tumor stage. Serum IL-8 may be a useful independent predictor of prognosis in HCC even before resection of the tumor. It may be useful in selecting patients with more aggressive tumors for neoadjuvant or adjuvant therapy. These results suggest that IL-8 is central to the tumor progression of HCC. Therefore, targeting IL-8 can be a potential approach to control angiogenesis and invasion in HCC.

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