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ORIGINAL ARTICLE

Retrospective Study

High level of intercellular adhesion molecule-1 affects prognosis of patients with hepatocellular carcinoma

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Abstract

AIM: To determine the cut-off value of intercellular adhesion molecule-1 (ICAM-1) and assess the correlation of ICAM-1 with clinicopathological features and the prognosis of hepatocellular carcinoma (HCC) patients who underwent surgical resection.

METHODS: We prospectively collected clinicopathological data from 236 HCC patients who had undergone successful hepatectomy. Receiver operating characteristic curve analysis was performed to determine the optimal cut-off value of ICAM-1. Enzyme-linked immunosorbent assay was used to measure the concentration of ICAM-1 in 236 serum samples isolated from HCC patients and the stratified analysis was used to compare the serum level of ICAM-1 in different HCC subgroups. Immunohistochemistry was performed to test the expression level of the ICAM-1 protein in 76 cases of HCC tissues and their adjacent normal liver tissues (ANLT). The survival probability of HCC patients was estimated using Kaplan-Meier plots and differences between the groups were obtained using the log-rank test. Furthermore, independent indicators

of the prognosis were acquired using a stepwise Cox proportional hazard model to analyze a series of predictors that were associated with disease-free survival (DFS) and overall survival (OS) in HCC patients.

RESULTS: Our findings suggested that ICAM-1 promotes HCC metastasis and high serum ICAM-1 is significantly associated with alpha-fetoprotein (AFP) (P = 0.022), clinical tumor-node-metastasis stage (P < 0.001), portal vein tumor thrombus (P = 0.005), distant metastasis (P = 0.016) and recurrence (P = 0.034). We further detected the ICAM-1 protein in HCC specimens and found that 56 of 76 (73.7%) HCC tissues had ICAM-1 positive staining while only 23 of 76 (30.3%) ANLT were positively stained (P < 0.0001). Survival analysis indicated that HCC patients with increased ICAM-1 concentrations had significantly shorter DFS and OS after resection. A multivariate analysis showed that ICAM-1 > 684 ng/mL was an independent factor for DFS (HR = 1.643; 95%CI: 1.125-2.401; P = 0.010) and OS (HR = 1.692; 95%CI: 1.152-2.486; P = 0.007).

CONCLUSION: ICAM-1 may be a promising serological biomarker for HCC diagnosis and an independent predictor of DFS and OS after surgical resection and may provide a useful reference for the prediction of intra- and extrahepatic metastasis.

Key words: Hepatocellular carcinoma; Intercellular adhesion molecule-1; Prognosis; Biomarker; Metastasis

Core tip: Our previous research and other studies found that intercellular adhesion molecule-1 (ICAM-1) is overexpressed in hepatocellular carcinoma (HCC) and might be a biomarker for HCC diagnosis. However, the correlation between ICAM-1 and clinicopathological features and its prognostic significance for HCC have not been explored. In this paper, we validate that ICAM-1 promotes HCC metastasis and that high serum ICAM-1 is significantly associated with alpha-fetoprotein, clinical tumor-node-metastasis stage, portal vein tumor thrombus, distant metastasis, recurrence, disease-free survival and overall survival. ICAM-1 may be a promising serological biomarker for HCC diagnosis and prognosis and may provide a useful reference for the prediction of intra- and extrahepatic metastasis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor worldwide[1]. Recurrence and intra- and extrahepatic metastases contribute predominantly to the high mortality rate of HCC patients after curative resection[2,3]. Despite some well-known improvements in HCC, including epidemiology, etiology, fundamental biology, chemotherapy, radio-frequency, transarterial therapy and surgical resection, there is still a majority of patients with HCC who have an inferior prognosis[4]. Therefore, it is urgent for us to search for novel biomarkers that are useful to evaluate the survival of HCC patients.

Mounting evidence has demonstrated that the pathogenesis of HCC is partially based on systemic inflammation, as factors based on systemic inflammation can predict the outcome of various malignancies including HCC[5,6]. A number of studies have shown that systemic inflammation can be measured by widely available inflammatory cytokines, such as intercellular adhesion molecule-1 (ICAM-1)[7,8], C-reactive protein[9] and neutrophil-to-lymphocyte ratio (NLR)[10]. Our previous research and other studies found that ICAM-1 is overexpressed in malignant diseases, such as HCC[7,8], bladder cancer[11], gastric cancer, lung cancer[12] and renal cancer[13]. The expression of ICAM-1 has been reported to mediate the interaction of cells with one another and with their microenvironment, which plays an important role in cell differentiation and movement. ICAM-1 has also been shown to be positively correlated with tumor generation, metastasis and recurrence in HCC[14]. Elevated circulating ICAM-1 concentration may proportionally relate to a poor prognosis in patients with HCC. However, the cut-off value of ICAM-1 has not been confirmed at present, which is a major clinical problem. Therefore, it is of great importance to ascertain the cut-off value of ICAM-1, which can help clinicians to make the most use of ICAM-1 to evaluate the prognosis of patients with HCC. The aim of our study is to determine the cut-off value of ICAM-1 and to assess the correlation of ICAM-1 with the clinicopathological features and prognosis of HCC patients who have undergone surgical resection.

MATERIALS AND METHODS

Patients and specimens

A total of 236 HCC patients who underwent surgical resection from December 1997 to July 2006 at the Affiliated Hospital of Guilin Medical University in China were analyzed retrospectively. Clinical and pathological evaluations of all patients were assessed using the Primary Liver Cancer Clinical Diagnosis and Staging Criteria[15] and patients were diagnosed by clinical symptoms, laboratory tests, ultrasonography (US),

Table 1 Clinical and biochemical data of examined patients

Parameter	mean ± SD
Age (yr)	49.38 ± 12.01
Gender, female/male, *n*	29/207
Alcohol abuse, yes/no, *n*	133/103
Cirrhosis, yes/no, *n*	211/25
HBsAg, negative/positive, *n*	34/202
ICAM-1	984.32 ± 370.48
AFP (ng/mL)	1809.97 ± 5533.23
WBC (10^9/L)	6.35 ± 2.37
Platelets (10^9/L)	175.65 ± 78.02
Albumin (g/L)	40.67 ± 4.61
TBIL(μmol/L)	18.52 ± 24.98
ALT (U/L)	50.27 ± 47.91
AST (U/L)	63.78 ± 86.73
NLR	2.83 ± 2.01
γ-GT (U/L)	117.55 ± 108.79

HBsAg: Hepatitis B surface antigen; ICAM-1: Intercellular adhesion molecule-1; AFP: Alpha-fetoprotein; WBC: White blood cell; TBIL: Total bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NLR: Neutrophil-to-lymphocyte ratio; γ-GT: γ-glutamyl transpeptidase.

computed tomography (CT) scans and magnetic resonance imaging (MRI). The clinical and biochemical data of the examined patients are listed in Table 1 and Table 2, including age, gender, family history, alcohol abuse, cirrhosis, hepatitis B surface antigen (HBsAg), ICAM-1, alpha-fetoprotein (AFP), median size, tumor number, clinical tumor-node-metastasis (TNM) stage, portal vein tumor thrombus (PVTT), distant metastasis, recurrence, white blood cell (WBC), platelets, albumin, total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), NLR and γ-glutamyl transpeptidase (γ-GT). All of the surgical samples that used for immunohistochemistry assay were fixed with formalin and then embedded with paraffin. In each case, blood samples were collected and the serum was separated and stored at -80 ℃ until use. All 236 HCC patients met the inclusion criteria and provided complete clinical background information for our study.

Follow-up

Patients were regularly followed-up from the date of operation; we inspected their serum AFP and performed US every 2 mo and chest radiography every 6 mo during the first two postoperative years and every 3-6 mo thereafter. Patients with abnormal AFP or suspected US examination underwent further examinations and recurrence was confirmed by contrast ultrasonography, magnetic resonance imaging and computerized tomography. The mean postoperative follow-up period was 39.6 mo (median, 25.0 mo; range, 2.0 to 120.0 mo). Disease-free survival (DFS) was measured from the date of surgery to the date of recurrence, metastasis, death or last follow-up. Overall survival (OS) was measured from the date of surgery to the date of death or last follow-up. The study was approved by the Ethical Committee of Affiliated Hospital of Guilin Medical University, which was in accordance with the Helsinki Declaration of 1975. Written informed consent was provided by all examined patients or their guardians.

Selection cutoff value of ICAM-1 and clinicopathological features

The cutoff score for ICAM-1 in the serum of patients with HCC was analyzed using MedCalc analysis. We defined the optimal cutoff value for ICAM-1, which was closest to the key point with not only maximum sensitivity but also specificity. In addition, we adopted a dichotomy to categorize the rest of the clinicopathological features and then analyzed the correlation between circulating ICAM-1 concentrations and the prognostic significance of patients with HCC.

Enzyme-linked immunosorbent assays

All serum samples in the study were assembled into anticoagulant-containing tubes during their preoperative inspection, after centrifuging, we separated the supernatants and stored them at -80 ℃ until use. The serum level of ICAM-1 was measured using commercially available ICAM-1 ELISA kits (Biosource Europe, Fleurus, Belgium) by two independent researchers according to the manufacturer's instructions. The testing details of ICAM-1 were described previously[8]; briefly, standard and experimental samples were added to 96-well flexible microtiter plates and pre-coated with anti-human ICAM-1 monoclonal antibodies for 2 h at 37 ℃. Subsequently, the wells were washed four times with washing buffer and further incubated with biotinylated antibody for 1 h at 37 ℃. After washing away unbound biotinylated antibody, streptavidin-HRP solution was added for 1 h at 37 ℃. Lastly, color developing agents A and B were performed and the reaction was blocked with stop buffer. The OD value of all wells was read at 450 nm. The concentration of ICAM-1 of each well was calculated by standard curves.

Immunohistochemistry assay

The 76 pairs of tissue specimens were deparaffinized with xylene, rehydrated using graded alcohols and pressure cooked for 3 min in citrate buffer (pH = 6.0) for antigen retrieval. Phosphate buffered saline (PBS) was used to wash the slides, followed by treatment with 3% hydrogen peroxide for 20 min to quench endogenous peroxidase activity. Then, the samples were preincubated with 10% goat serum at room temperature for 30 min to prevent nonspecific staining. Additionally, the cancerous foci and non-tumorous samples were incubated with rabbit polyclonal anti ICAM-1 antibody (catalog 25464, BioVision Company, 1:200 dilution) overnight in a humidified container at 4 ℃; the next day, they were washed with PBS and the tissue slides were treated with a biotinylated horseradish-peroxidase detection system according to the manufacturer's instructions and stained with

Table 2 Correlation between the clinicopathological variables and serum intercellular adhesion molecule-1 level in hepatocellular carcinoma *n* (%)

Clinical character	Variable	Number of patients	ICAM-1 level		χ^2	*P* value
			Low[1]	High[2]		
Age (yr)	≤ 55	161	37 (23.0)	124 (77.0)	0.265	0.607
	> 55	75	15 (20.0)	60 (80.0)		
Gender	Male	207	44 (21.3)	163 (78.7)	0.593	0.441
	Female	29	8 (27.6)	21 (72.4)		
Family history	No	203	42 (20.7)	161 (79.3)	1.527	0.217
	Yes	33	10 (30.3)	23 (69.7)		
HBsAg	Negative	34	7 (20.6)	27 (79.4)	0.048	0.826
	Positive	202	45 (22.3)	157 (77.7)		
AFP (ng/mL)	≤ 100	99	29 (29.3)	70 (70.7)	5.231	0.022
	> 100	137	23 (16.8)	114 (83.2)		
Median size (cm)	≤ 5	32	9 (28.1)	23 (71.9)	0.800	0.371
	> 5	204	43 (21.1)	161 (78.9)		
Cirrhosis	No	25	2 (8.0)	23 (92.0)	3.206	0.073
	Yes	211	50 (23.7)	161 (76.3)		
Tumor number	Single	149	37 (24.8)	112 (75.2)	1.842	0.175
	Multiple	87	15 (17.2)	72 (82.8)		
NLR	≤ 2.31	130	31 (23.8)	99 (76.2)	0.553	0.457
	> 2.31	106	21 (19.8)	85 (80.2)		
TNM stage	I-Ⅱ	108	37 (34.3)	71 (65.7)	17.324	< 0.001
	Ⅲ-Ⅳ	128	15 (11.7)	113 (88.3)		
PVTT	No	184	48 (26.1)	136 (73.9)	7.985	0.005
	Yes	52	4 (7.7)	48 (92.3)		
Distant metastasis	No	217	52 (24.0)	165 (76.0)	5.840	0.016
	Yes	19	0 (0)	19 (100)		
Recurrence	No	139	24 (17.3)	115 (82.7)	4.475	0.034
	Yes	97	28 (28.9)	69 (71.1)		

[1]Low, ICAM-1 ≤ 684 ng/mL; [2]High, ICAM-1 > 684 ng/mL. HBsAg: Hepatitis B surface antigen; ICAM-1: Intercellular adhesion molecule-1; AFP: Alpha-fetoprotein; NLR: Neutrophil-to-lymphocyte ratio; TNM: Tumor-node-metastasis; PVTT: Portal vein tumor thrombus.

3,3-diaminobenzidine tetrahydrochloride. Lastly, the sections were counterstained with Mayer's hematoxylin, dehydrated and mounted. We replaced the primary antibody with normal goat serum to obtain a negative control. The semi-quantitative immunohistochemistry results were evaluated by two independent pathologists who were blinded to the patients' clinical and biochemical information and the stained tissue sections were evaluated using a 4 point scale as follows: the percentage of positive cells, grades 0-3 (0: no positive cells; 1: < 25% positive cells; 2: 25-50% positive cells; 3: > 50% positive cells).

Statistical analysis

SPSS 13.0 (SPSS Inc., Chicago, IL) and MedCalc statistical software version 11.3.0.0 (MedCalc Software, Broekstraat 52 Mariakerke, Belgium) were used to analyze the statistical data. The χ^2 test was used for the correlation analysis. Quantitative variables were analyzed by the independent *t* test. Statistical significance was considered when *P* value < 0.05. Survival curves for the HCC patients were determined by the Kaplan-Meier method and the differences between groups were estimated by the log-rank test. Independent prognostic indicators for DFS and OS were assessed in the univariate and multivariate analyses using Cox's proportional hazard model.

RESULTS

Receiver operating characteristic curves

Receiver operating characteristic (ROC) curves were plotted to determine the optimal cut-off value of ICAM-1. As a result, a cutoff value of 684 ng/mL was found to have a relatively high specificity. The area under the ROC curves (AUC) was 0.636 with a 95%CI of 0.571 to 0.698, a sensitivity of 60.2% and a specificity of 77.3% (Figure 1A).

Serum level of ICAM-1 in different groups of HCC patients

We compared the serum level of ICAM-1 in two groups of HCC patients with a series of different clinical features, including PVTT, TNM stage, distant metastasis, recurrence and AFP. We found that the level of ICAM-1 in serum was significantly associated with the presence of PVTT (*P* = 0.0005), TNM stage (*P* = 0.001) and distant metastasis (*P* = 0.0174) (Figure 1B). Therefore, we supposed that the overexpression of ICAM-1 may be associated with cellular invasion and venous permeation and may have contributed to tumor metastasis in HCC. It is therefore possible to use the serum ICAM-1 level to evaluate the curative effectiveness of patients with HCC.

The data from the two different groups are shown separately. The median ICAM-1 concentration in

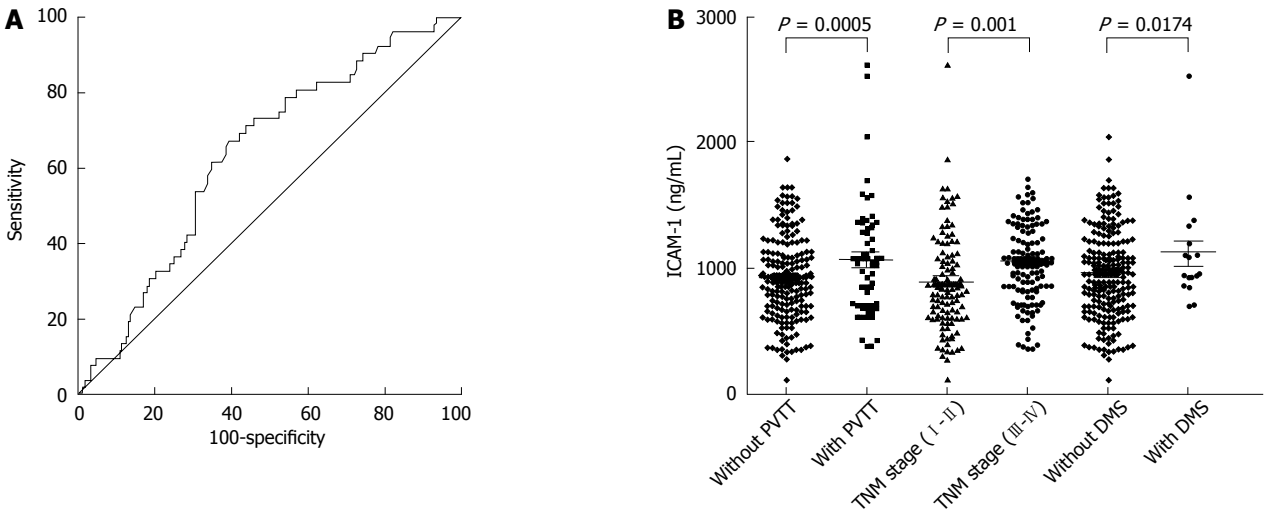


Figure 1 Receiver operating characteristic curve and serum level of intercellular adhesion molecule-1 in different hepatocellular carcinoma subgroups. A: Receiver operating characteristic curves to assess hepatocellular carcinoma (HCC) patients with prognosis by the appropriate cutoff values of preoperative intercellular adhesion molecule-1 (ICAM-1); B: Two hundred and thirty-six cases of HCC patients were stratified according to portal vein tumor thrombus (PVTT), tumor-node-metastasis (TNM) stage and distant metastasis (DMS), comparing the serum level of ICAM-1 in each subgroup. The percentage of high ICAM-1 patients with PVTT, TNM stage Ⅲ-Ⅳ and with DMS are much higher than those without PVTT, TNM stage Ⅰ-Ⅱ and without DMS, respectively ($P < 0.05$).

patients with PVTT was significantly higher than that in patients without PVTT, and that in the TNM stage Ⅲ-Ⅳ group was significantly higher than that in the TNM stage Ⅰ-Ⅱ group. Furthermore, the median ICAM-1 concentration in patients with distant metastasis was significantly higher than that in patients without distant metastasis (Figure 1B).

ICAM-1 overexpression is closely correlated with intrahepatic metastasis

To further confirm that the concentration of ICAM-1 can be used as a tumor marker in HCC, we next detected ICAM-1 expression in HCC tissues by using immunohistochemical assays and found that 56 of 76 (73.7%) HCC tissues had ICAM-1 positive staining, while only 23 of 76 (30.3%) peritumoral tissues were positively stained ($P < 0.0001$; Figure 2A-C), suggesting that ICAM-1 may maintain the extracellular microenvironment for tumor cell growth. In addition, we showed positive staining in 20 of 21 (95.2%) HCC specimens with PVTT, when compared with 30 of 50 (60%) in those without PVTT ($P = 0.008$; Figure 2D), suggesting that the overexpression of ICAM-1 may be a key factor that promotes tumor cell proliferation, invasion and metastasis in the tumor microenvironment.

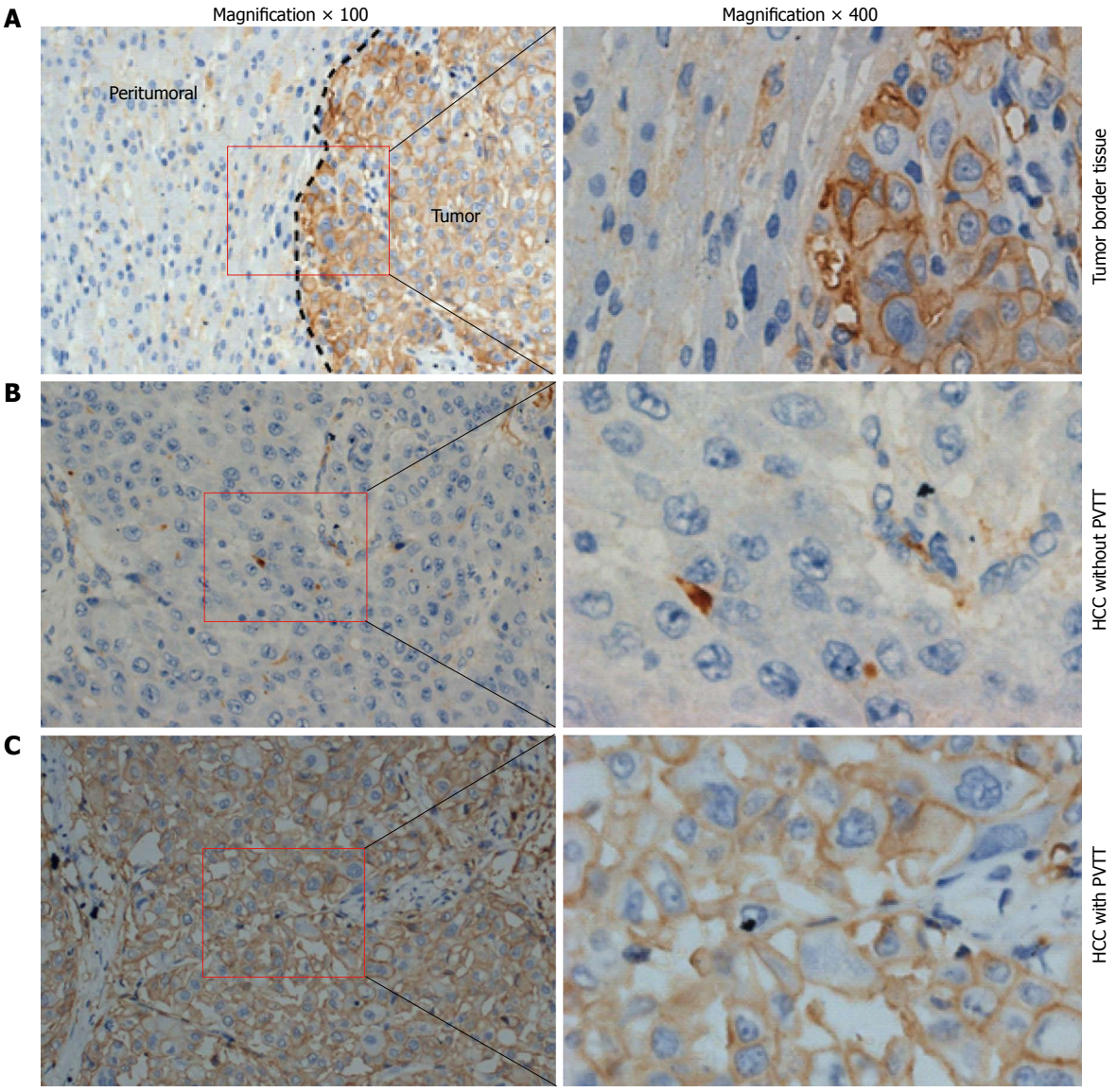
Relationship between high concentrations of ICAM-1 and the clinicopathological data

On the basis of the above findings, we next considered whether serum ICAM-1 correlates well with clinicopathological characteristics. Two hundred and thirty-six patients with HCC were divided into two groups according to their preoperative serum level of ICAM-1. Patients were classified into either the high ICAM-1 group (> 684 ng/mL, $n = 184$) or the low ICAM-1 group (≤ 684 ng/mL, $n = 52$). We found that the serum level of ICAM-1 was significantly associated with AFP ($\chi^2 = 5.231$; $P = 0.022$), clinical TNM stage ($\chi^2 = 17.324$; $P < 0.001$), PVTT ($\chi^2 = 7.985$; $P = 0.005$), distant metastasis ($\chi^2 = 5.840$; $P = 0.016$) and recurrence ($\chi^2 = 4.475$; $P = 0.034$). However, the serum level of ICAM-1 was not significantly associated with age, gender, tumor family history, HBsAg, median size, liver cirrhosis, the number of tumors or NLR (all $P > 0.05$), as shown in Table 2.

Interestingly, from the results of serological examination, we found that the increase in ICAM-1 (> 684 ng/mL) and AFP (> 100 ng/mL) in sera from HCC patients did not happen concurrently. Both increased in 114 cases (48.3%). Beyond that, the rise in ICAM-1 was not noticeable in 23 cases (9.75%) in which AFP alone increased; however, ICAM-1, but not AFP, increased in 70 cases (29.66%) (Figure 2E), demonstrating that it is valuable to link serum ICAM-1 and AFP for the final confirmatory diagnosis for HCC, which effectively guides the therapeutic schedule. When combining serum AFP and ICAM-1, the HCC diagnosis rate reached more than 87% in our study.

Association of ICAM-1 concentration, clinicopathological index and postoperative DFS or OS

To further investigate the correlations between ICAM-1 concentration and survival of patients with HCC, Kaplan Meier analyses were performed. As shown in Figure 3, the median DFS time in patients with ICAM-1 > 684 ng/mL was 36.96 mo (95%CI: 30.51-43.40), which was remarkably shorter than that of ICAM-1 ≤ 684 ng/mL (58.18 mo, 95%CI: 44.77-71.58; $P = 0.003$; Figure 3A). Furthermore, the mean OS in patients with ICAM-1 > 684 ng/mL was 42.75 (36.48-49.01) mo, significantly shorter than that of the ICAM-1 ≤



D

Clinical character	Variable	No. of patients	ICAM-1$^+$ *n* (%)	ICAM-1$^-$ *n* (%)	*P* value
PVTT	Yes	21	20 (95.2)	1 (4.8)	0.008
	No	55	36 (65.4)	19 (34.5)	

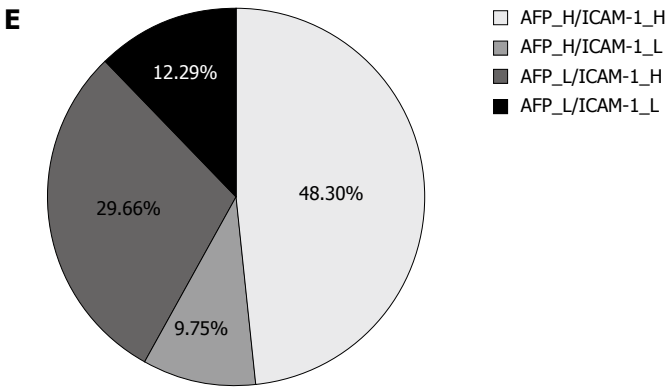


Figure 2 Level of intercellular adhesion molecule-1 in hepatocellular carcinoma specimens. A-C: Intercellular adhesion molecule-1 (ICAM-1) expression in hepatocellular carcinoma (HCC) was confirmed by immunohistochemical staining (*n* = 76). Representative pictures are shown, the nuclei were counterstained with hematoxylin, original magnification: left (× 100) and right side (× 400). A: Tumor border tissue of representative intratumoral high density staining (right) and peritumoral low density staining (left) of ICAM-1 are shown. The dark dotted line represents the interface of the tumor and adjacent liver tissues; B and C: Representative HCC tissues without portal vein tumor thrombus (PVTT) low density staining (B) and with PVTT high density staining (C) of ICAM-1 are shown, respectively; D: Statistical analysis was performed using the chi-square test to compare the relative levels of ICAM-1 negative (ICAM-1$^-$) or positive (ICAM-1$^+$) between HCC with PVTT and HCC without PVTT (P = 0.008); E: Combined measurement of serum ICAM-1 and alpha-fetoprotein (AFP). The distribution of the ICAM-1 and AFP levels in the HCC specimens. The numbers indicate the percentages of ICAM-1 and/or AFP higher (H) or lower (L) HCC specimens.

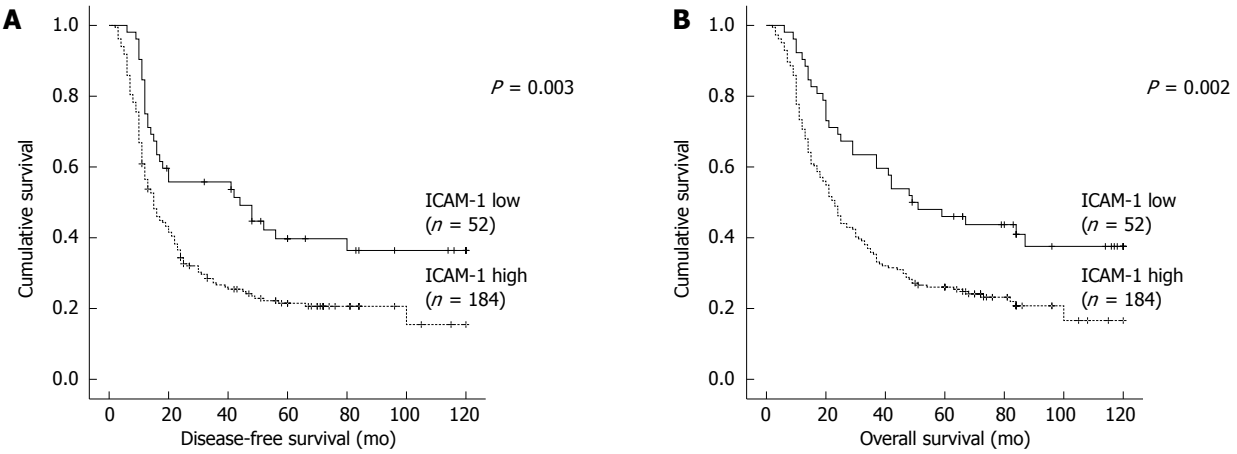


Figure 3 Relationship between intercellular adhesion molecule-1 level and disease-free survival or overall survival. Patients with high intercellular adhesion molecule-1 (ICAM-1) level had a shorter disease-free survival (DFS) (A) and overall survival (OS) (B). The solid line represents the patients with low ICAM-1 level, whereas the dashed line represents the patients with high ICAM-1 level.

684 ng/mL group (64.57 mo, 95%CI: 52.14-77.01; *P* = 0.002; Figure 3B). Univariate analysis revealed an obvious association between clinical parameters and both DFS and OS (Table 3). In addition to the high ICAM-1 (> 684 ng/mL) group, the size of tumor > 5 cm, multiple tumors, NLR > 2.31, Ⅲ-Ⅳ of TNM stage and PVTT were associated with a shorter DFS and OS (Table 3).

High ICAM-1 concentration as an independent predictor of DFS or OS

To identify the association between clinicopathological factors and DFS/OS in HCC patients with surgical resection, multivariate analyses were conducted by using the Cox proportional hazards mode. Six factors (high ICAM-1 level, multiple tumor number, Ⅲ-Ⅳ of TNM stage, PVTT, size of tumor > 5 cm and high NLR) were analyzed with the stepwise multivariate Cox proportional hazard model for both DFS and OS. The results showed that high ICAM-1 level (HR = 1.643; 95%CI: 1.125-2.401; *P* = 0.010), PVTT (HR = 1.397; 95%CI: 1.016-1.920; *P* = 0.040) and high NLR (HR = 1.578; 95%CI: 1.156-2.153; *P* = 0.004) were independent predictors for DFS (Table 4). High ICAM-1 level (HR = 1.692; 95%CI: 1.152-2.486; *P* = 0.007), Ⅲ-Ⅳ of TNM stage (HR = 1.468; 95%CI: 1.074-2.015; *P* = 0.016), PVTT (HR = 1.514; 95%CI: 1.031-2.225; *P* = 0.035) and high NLR (HR = 1.485; 95%CI: 1.086-2.030; *P* = 0.013) were independent predictors for OS (Table 4).

DISCUSSION

During the last few decades, the postoperative survival rate of HCC patients has improved. However, due to the difficulty in early diagnosis and the presence of tumor invasiveness, metastasis and recurrence of HCC, the prognosis of HCC is still not satisfactory, which is demonstrated by the low DFS rate in HCC patients within 5 years after surgical resection. Although the serum AFP level has been widely used for the diagnosis of HCC, the sensitivity and specificity of AFP for HCC were demonstrated to be limited. To improve the prognosis in HCC patients, seeking more effective biomarkers in diagnosis of HCC at very early stages and monitoring tumor recurrence are very important.

It has been shown that a series of hematological and biochemical examinations, including AFP[16], albumin[17], TBIL, ALT[18], AST[19], NLR[10] and γ-GT, were proposed as effective aids to the early diagnosis and prognosis of HCC. However, the diagnostic specificity and sensitivity were still not accurate. Our previous research and other studies found that ICAM-1 is overexpressed in HCC. Other research has shown that serum ICAM-1 is produced and secreted by tumor cells[14], ICAM-1 has the capability to induce the adhesion between cancer cells and the vascular endothelium, and the expression of ICAM-1 is a dominating step that may be involved in the metastatic process of HCC[20,21], suggesting that ICAM-1 may be able to serve as a new marker to predict the progression and prognosis of patients with HCC.

Our previous research showed that the mRNA and protein levels of ICAM-1 in patients with HCC were significantly higher than those in patients with chronic hepatitis B virus and healthy subjects[8] and that the malignant degree of the tumor was related to the ICAM-1 level, indicating that ICAM-1 contributed to the progression of HCC. In the present study, using the ROC curve, we identified 684 ng/mL as the optimal cutoff value of ICAM-1; the patients were divided into the low ICAM-1 group (ICAM-1 ⩽ 684 ng/mL) and the high ICAM-1 group (ICAM-1 > 684 ng/mL). From the results of the correlation analysis, we also found that high levels of ICAM-1 were significantly associated with AFP, clinical TNM stage, PVTT, distant metastasis and recurrence in patients with HCC. In the present study, our data show that the combination of serum ICAM-1

Table 3 Association between intercellular adhesion molecule-1 level, clinical parameters and disease-free survival/overall survival

Clinical character	Category	Number of patients	Disease-free survival (mo)			Overall survival (mo)		
			mean	95%CI	*P* value	mean	95%CI	*P* value
ICAM-1 (ng/mL)	≤ 684	52	58.18	44.77-71.58	0.003	64.57	52.14-77.01	0.002
	> 684	184	36.96	30.51-43.40		42.75	36.48-49.01	
Age (yr)	≤ 55	161	40.37	33.32-47.43	0.325	45.89	39.05-52.74	0.262
	> 55	75	45.38	34.62-56.14		51.57	41.37-61.77	
Gender	Female	29	57.84	38.90-76.78	0.066	62.96	43.72-82.19	0.076
	Male	207	39.73	33.60-45.86		44.66	38.58-50.74	
Family history	No	203	39.83	33.62-46.05	0.077	45.69	39.68-51.69	0.092
	Yes	33	53.83	37.30-70.36		59.63	43.90-75.36	
HBsAg	Negative	34	47.54	30.65-64.43	0.501	52.62	36.64-68.60	0.462
	Positive	202	40.74	34.47-47.01		46.72	40.65-52.79	
AFP (ng/mL)	≤ 100	99	46.01	36.52-55.49	0.286	52.18	43.07-61.28	0.214
	> 100	137	39.00	31.53-46.47		44.75	37.51-51.99	
Tumor size (cm)	≤ 5	32	70.51	53.82-87.19	< 0.001	77.42	62.56-92.28	< 0.001
	> 5	204	37.57	31.49-43.66		43.36	37.44-49.28	
Cirrhosis	No	25	34.60	17.57-51.64	0.190	39.16	22.83-55.49	0.237
	Yes	211	42.81	36.53-49.09		48.83	42.77-54.89	
Tumor number	Single	149	50.25	42.28-58.21	< 0.001	55.27	47.66-62.87	< 0.001
	Multiple	87	27.72	20.32-35.12		34.92	27.37-42.48	
NLR	≤ 2.31	130	53.58	45.00-62.17	< 0.001	59.00	50.88-67.12	< 0.001
	> 2.31	106	27.50	20.58-34.43		34.23	27.17-41.29	
TNM stage	Ⅰ-Ⅱ	108	59.65	50.13-69.18	< 0.001	66.86	58.17-75.54	< 0.001
	Ⅲ-Ⅳ	128	27.17	20.87-33.46		31.88	25.56-38.21	
PVTT	No	184	47.62	40.59-54.64	< 0.001	54.25	47.61-60.90	< 0.001
	Yes	52	22.06	13.88-30.24		24.73	16.55-32.91	
Distant metastasis	No	217	42.85	36.62-49.08	0.313	48.89	42.90-54.88	0.166
	Yes	19	26.79	14.76-38.82		31.21	18.29-44.13	
Recurrence	No					44.59	36.73-52.46	0.051
	Yes					51.58	43.77-59.39	

ICAM-1: Intercellular adhesion molecule-1; HBsAg: Hepatitis B surface antigen; AFP: Alpha-fetoprotein; NLR: Neutrophil-to-lymphocyte ratio; TNM: Tumor-node-metastasis; PVTT: Portal vein tumor thrombus.

Table 4 Cox multivariate proportional hazard model of independent predictors on disease-free and overall survival

Variable	Disease-free survival		Overall survival	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
ICAM-1 (ng/mL) (> 684 *vs* ≤ 684)	1.643 (1.125-2.401)	0.010	1.692 (1.152-2.486)	0.007
Tumor number (multiple *vs* single)	1.324 (0.902-1.944)	0.152	1.273 (0.924-1.755)	0.140
TNM stage (Ⅲ-Ⅳ *vs* Ⅰ-Ⅱ)	1.390 (0.931-2.077)	0.108	1.468 (1.074-2.015)	0.016
PVTT (yes *vs* no)	1.397 (1.016-1.920)	0.040	1.514 (1.031-2.225)	0.035
Tumor size (cm) (> 5 *vs* ≤ 5)	0.666 (0.389-1.149)	0.144	1.551 (0.899-2.656)	0.115
NLR (> 2.31 *vs* ≤ 2.31)	1.578 (1.156-2.153)	0.004	1.485 (1.086-2.030)	0.013

ICAM-1: Intercellular adhesion molecule-1; TNM: Tumor-node-metastasis; PVTT: Portal vein tumor thrombus; NLR: Neutrophil-to-lymphocyte ratio.

and AFP significantly increased the positive diagnostic ratio for HCC.

PVTT, the main form of intrahepatic metastasis of HCC, affects the prognosis of HCC patients. It was reported that a total of 34% to 50% of patients with advanced HCC may suffer from PVTT and may be predisposed to metastasis and recurrence[22,23]. In this study, we also found that the ICAM-1 level was significantly higher in the HCC specimens with PVTT than in those without PVTT (Figure 2B-D). Although no substantive progress of finding effective indicators for PVTT has been made, the ICAM-1 protein level had the ability to forecast the incidence rate of PVTT, which merits a further large sample prospective study and which indicates the diagnostic value of ICAM-1 for further clinical practice. In addition, we compared serum ICAM-1 in two subgroups of HCC patients with different clinical features, including PVTT, TNM stage and distant metastasis, and found that the level of serum ICAM-1 was significantly associated with the presence of PVTT, the TNM stage and distant metastasis, which provided evidence that ICAM-1 overexpression is associated with cellular invasion, venous permeation and perhaps even metastasis in HCC.

From the results of the univariate analysis, we observed that ICAM-1 > 684 ng/mL, size of tumor > 5 cm, multiple tumor number, NLR > 2.31, Ⅲ-

Ⅳ of TNM stage together with PVTT were associated with a shorter DFS and OS. These observations are consistent with previous studies that multiple tumors are a useful prognostic factor for the recurrence of HCC[24]. Additionally, our previous studies found that elevated NLR (> 2.31) effectively reflected systemic inflammation, tumor invasion and even metastasis of HCC[10].

We also found that except Ⅲ-Ⅳ of TNM stage was independent predictor for OS, and high ICAM-1 level, PVTT and high NLR were independent predictors for both DFS and OS. An elevated NLR may suppress antitumor immunity with the help of peritumoral macrophages[25,26] and myeloid-derived suppressor cells[27,28]. In addition, an elevated NLR may promote the production of vascular endothelial growth factor[29] and matrix metalloproteinases[30] and thus promote tumor angiogenesis and conductive tumor growth and metastasis.

In short, the value of 684 ng/mL as a cut-off point for ICAM-1 in this retrospective study has a substantial sensitivity and specificity, which is of great importance for the diagnosis of HCC. The serum ICAM-1 test is not only a cost-effective examination but also an optimal choice for HCC screening and it helps clinicians to make clinical decisions. We believe that ICAM-1 is a valuable marker for the early diagnosis of HCC, recurrence monitoring and the prediction of intrahepatic (PVTT) and extrahepatic metastasis for HCC. However, our results need to be further validated in a large cohort and prospective studies are warranted to verify the effectiveness of our results.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor that is associated with recurrence and intra- and extrahepatic metastases. Elevated circulating intercellular adhesion molecule-1 (ICAM-1) concentration may proportionally relate to a poor prognosis in patients with HCC. However, the cut-off value of ICAM-1 and its clinical significance has not been further investigated.

Research frontiers

ICAM-1 mediated the interaction of cells with one another and with their microenvironment and is involved in cell differentiation and movement. Its overexpression is greatly associated with tumor initiation, metastasis and recurrence in many human cancers. The cut-off value of ICAM-1 has also not been confirmed and the correlations of ICAM-1 with clinicopathological features and prognosis of HCC patients are still unavailable. All these questions are hot topics in the present research fields.

Innovations and breakthroughs

This is the first study to demonstrate that 684 ng/mL may serve as a cut-off point of ICAM-1 for the diagnosis of HCC patients. We believe that serum ICAM-1 may provide a useful reference for the prediction of intra- and extrahepatic metastasis.

Applications

Serum ICAM-1 can be used to predict the diagnosis and prognosis of patients with HCC, which may serve as a therapeutic target for the purpose of improving clinical treatment.

Terminology

ICAM-1 is an important member of the immunoglobulin superfamily; it mediates the interaction of cells with one another and with their microenvironment and is involved in tumor initiation, metastasis and recurrence in many human cancers.

Peer-review

In this study, the authors confirmed that 684 ng/mL may serve as a cut-off point for ICAM-1 in improving the diagnosis and prognosis of patients with HCC. These findings are informative and suggest that ICAM-1 could be a potential biomarker for therapy for HCC. The results deserve publication in the journal.

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