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• LIVER CANCER •

Clinical significance of serum vascular cell adhesion molecule-1 levels in patients with hepatocellular carcinoma

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Abstract

AIM: To evaluate the correlation between serum vascular cellular adhesion molecule-1 (VCAM-1) levels and clinicopathological features in patients with hepatocellular carcinoma (HCC).

METHODS: Ninety-six patients who underwent HCC resection were recruited in the study. Preoperative serum levels of soluble VCAM-1 were measured by enzyme-linked immunosorbent assay.

RESULTS: Serum VCAM-1 level in HCC patients was inversely correlated with platelet count (r=-0.431, P<0.001) and serum albumin level (r=-0.279, P<0.001), and positively correlated with serum bilirubin level (r=0.379, P<0.001). Serum VCAM-1 level was not associated with tumor characteristics such as tumor size, venous invasion, presence of microsatellite nodules, tumor grade and tumor stage. Serum VCAM-1 level was significantly higher in HCC patients with cirrhosis compared with those without cirrhosis (median 704 *vs* 546 ng/mL, P<0.001). Furthermore, a significantly better disease-free survival was observed in HCC patients with low VCAM-1 level (P=0.019).

CONCLUSION: Serum VCAM-1 level appears to reflect the severity of underlying chronic liver disease rather than the tumor status in HCC patients, and low preoperative serum VCAM-1 level is predictive of better disease-free survival after surgery.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a tumor characterized by a rich vasculature. The formation of rich vasculature depends on angiogenesis, which is a process that plays an important role in tumor progression, growth, and metastasis. Many angiogenic factors and mediators are involved in the control of angiogenesis.

Vascular cell adhesion molecule-1 (VCAM-1) is one of the adhesion molecules that have been implicated as a mediator of angiogenesis[1]. As one of the cell adhesion molecules in the immunoglobulin superfamily, human VCAM-1 is M_r 100 000-110 000, type 1 transmembrane glycoprotein[2-4]. VCAM-1 is transiently expressed on activated vascular endothelial cells in response to vascular endothelial growth factor (VEGF) and other cytokines, such as tumor necrosis factor α, interleukin 1β, and interferon γ[2,5-7]. Functionally, its expression plays a major role in adhesion of leukocytes to the endothelium in inflammed tissue and in the tumor site[2]. It also plays an important role in providing attachment to the developing endothelium during angiogenesis[8-10]. Furthermore, it may also exert its function as an adhesion molecule to facilitate metastasis[2]. A previous study showed that VCAM-1 expression was associated with the metastasis of melanoma[11].

VCAM-1 is a soluble molecule that can be detected in the circulation. Although the exact mechanism by which VCAM-1 is shredded into the bloodstream is unknown, it may involve both proteolytic processing and alternative splicing[12,13]. Because VCAM-1 can be identified in bloodstream, it is potentially useful as a non-invasive biomarker for the monitoring of disease progression in cancer and other diseases[14-16]. It has been reported that VCAM-1 is over-expressed in various diseases and cancers. Recent studies have demonstrated high serum levels of VCAM-1 in patients with colorectal cancer and gastric cancer[16-17]. One study demonstrated that a high serum VCAM-1 level was significantly associated with advanced disease stage and the presence of distant metastasis in gastric cancer[17]. A high serum VCAM-1 level has been shown to be associated with angiogenesis and poor prognosis in breast cancer[18]. A study has also found that serum VCAM-1 was an independent prognostic marker in patients with Hodgkin's lymphoma[19].

Up-regulated VCAM-1 expression in chronic liver disease has also been reported, suggesting that VCAM-1 may play a role in the pathogenesis of chronic hepatitis or cirrhosis[20-26]. Serum VCAM-1 levels higher than normal have been consistently reported in several studies of chronic liver disease of various etiologies[19-27]. However, the clinical significance of serum VCAM-1 in HCC patients has not yet been reported before. The majority of HCC patients have associated chronic hepatitis or cirrhosis. It is of interest to study serum VCAM-1 in HCC patients because VCAM-1 may be involved in the progression of both the tumor and the underlying chronic liver disease.

The objective of this study was to evaluate the levels of serum soluble VCAM-1 in HCC patients compared with cirrhotic patients without HCC and normal controls, and to analyze the correlation of serum VCAM-1 level with clinicopathological features in HCC patients.

MATERIALS AND METHODS

Patients

Ninety-six patients (71 men and 25 women; median age 55.5 years, range 16-79 years) who underwent resection of HCC in the Department of Surgery of the University of Hong Kong at Queen

Mary Hospital were studied. In the majority of cases (81%, *n*=78), HCC was related to hepatitis B viral infection. None of the 96 patients had received any preoperative treatment. The study was approved by the Ethics Committee of our institution and informed consent was obtained from the patients.

Preoperative serum was collected from the patients. Venous blood samples were drawn into a serum separator tube and centrifuged at 3 000 r/min for 10 min, then stored at -80 ℃ until VCAM-1 levels were determined. Serum samples were also obtained from 19 healthy controls without evidence of any active disease and 23 patients with cirrhosis but no evidence of HCC on ultrasonography and alpha fetoprotein (AFP) surveillance. The majority of the 23 cirrhotic patients (74%, *n*=17) had cirrhosis related to hepatitis B viral infection.

Measurement of serum VCAM-1 level

Serum VCAM-1 levels were quantified using an enzyme-linked immunosorbent assay kit designed to quantitatively measure human soluble VCAM-1 concentration in serum (Human sVCAM-1 Immunoassay; R&D systems, Minneapolis, MN). This assay contains recombinant human VCAM-1 and antibodies against the recombinant factor. The assay can recognize both recombinant and natural human VCAM-1. Briefly, 100 μL of diluted VCAM-1 conjugate (antibody to recombinant VCAM-1 conjugated to horse-radish peroxidase) was added to each well that was pre-coated with monoclonal antibody specific for VCAM-1, after which, 100 μL of serially diluted recombinant VCAM-1 standards and serum samples were added to the wells and incubated at room temperature for 1.5 h. After the wells were washed with wash buffer 6 times to clear any unbound substances, tetramethylbenzidine was added to the wells for color development. The intensity of the developed color was measured by reading absorbance at 450 nm. Each measurement was made in duplicate and the serum VCAM-1 level was determined by extrapolation from a standard curve generated for each set of samples assayed. The sensitivity of the assay was 2 ng/mL, and the coefficients of variation of intraassay and interassay determination were in the range given by the manufacturer (4.3-5.9% and 8.5-10.2%, respectively).

Clinicopathologic and follow-up data

All clinicopathologic and follow-up data were prospectively collected and entered into a computerized database. Detailed preoperative blood tests such as complete blood count, coagulation profile, liver biochemistry, indocyanine green retention at 15 min (ICG_{15}), serum AFP level, and hepatitis viral serology were assessed for all patients. Histopathologic data including Edmonson grade[28] (1-2 or 3-4), any venous invasion, tumor capsule, microsatellite lesion and pTNM stage[29] (I/II or III/IVA) were collected. All patients had a computed tomography (CT) scan 1 mo after the hepatic resection to detect any tumor, which was considered a residual disease. Patients who had a positive margin or residual tumors in the 1-month CT scan were considered having a palliative resection.

Postoperative follow-up included monitoring for tumor recurrence by monthly serum AFP level and chest X-ray detection together with ultrasonography or computed tomography (CT) scan every 3 mo. Recurrence of disease was diagnosed by the detection of any intrahepatic or extrahepatic tumor with a typical enhancement pattern of HCC in contrast CT scan and elevation of serum AFP level on serial measurements. Percutaneous fine-needle aspiration cytology was performed to confirm the diagnosis of recurrence in uncertain cases. Disease-free survival was calculated from the date of hepatic resection to the date when recurrence was diagnosed or, in the absence of detectable recurrence, to the date of death or last follow-up.

Statistical analysis

Continuous data were expressed as median with range in parenthesis. For comparison between groups, Mann-Whitney *U* test was used for analysis of continuous variables, and Chi-square test (or the Fisher's exact test, where appropriate) was used for analysis of discrete variables. Correlation analysis was performed using the Spearman rank correlation test. Cumulative disease-free survival was computed using the Kaplan-Meier method and compared between groups by the log-rank test. Serum VCAM level was entered into a multivariate Cox regression analysis together with 7 other variables that were demonstrated to be of prognostic importance in previous studies from our center and others, namely, serum AFP level, tumor size, presence of venous invasion, presence of microsatellite nodules, presence of underlying cirrhosis and pTNM stage[30,31]. All statistical analyses were performed using the SPSS statistical software (SPSS/PC+, SPSS Inc., Chicago, Illinois). $P<0.05$ was considered statistically significant.

RESULTS

Serum VCAM-1 levels

Figure 1 shows the distribution of serum VCAM levels in normal controls (*n*=19), patients with cirrhosis only (*n*=23) and HCC patients (*n*=96). The median serum VCAM-1 level in healthy individuals, patients with cirrhosis only, and patients with HCC was 631 ng/mL (449-1 103), 780 ng/mL (509-4 120), and 621 ng/mL (179-3 199), respectively. There was no significant difference in serum VCAM-1 levels between HCC patients and normal individuals ($P=0.447$). However, there were significant differences in serum VCAM-1 levels between cirrhotic patients without HCC and normal individuals ($P=0.010$), and between cirrhotic patients with and without HCC ($P<0.001$).

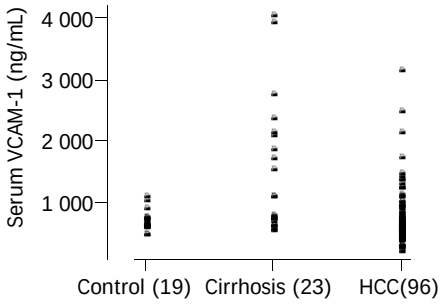

Figure 1 Scatter plot to illustrate the distribution of serum VCAM-1 level (ng / mL) in healthy control subjects (*n*=19), cirrhosis subjects without HCC (*n*=23), and HCC patients (*n*=96).

Correlation between serum VCAM-1 levels and clinicopathological features in HCC patients

Preoperative serum levels of VCAM-1 in the 96 patients with HCC were analyzed to identify any relationship with the clinicopathological parameters. When analyzed in relation to preoperative clinical parameters categorized as binary variables (Table 1), serum VCAM-1 level had no significant relationship with patients' sex, age, hepatitis B surface antigen status, serum AFP level or serum albumin level. However, serum VCAM-1 levels were significantly higher in patients with high ICG_{15} (>10%), which signifies impaired liver function, and in patients with thrombocytopenia, which signifies the presence of significant hypersplenism. When correlated as continuous variables, serum VCAM-1 levels were positively correlated with serum bilirubin level ($r=0.379$, $P<0.001$) and negatively correlated with white cell count ($r=-0.226$, $P=0.027$), platelet count ($r=-0.431$, $P<0.001$) and serum albumin level ($r=-0.279$, $P<0.001$).

Table 1 Preoperative serum VCAM-1 levels categorized by clinical and laboratory variables

Variables	Median serum VCAM-1 (ng/mL)	P^1
Gender		
Male (n=71)	623	0.381
Female (n=25)	594	
Age (yr)		
≤60 (n=56)	615	0.460
>60 (n=40)	639	
HBsAg		
Positive (n=78)	623	0.266
Negative (n=18)	592	
Serum AFP		
≤20 ng/mL (n=29)	608	0.737
>20 ng/mL (n=67)	623	
Serum albumin		
≤40 g/L (n=59)	614	0.228
>40 g/L (n=37)	634	
ICG_{15}		
≤10% (n=45)	531	<0.001
>10% (n=51)	667	
Platelet count		
≤150 $\times 10^9$/L (n=44)	728	<0.001
>150 $\times 10^9$/L (n=52)	547	

HBsAg: Hepatitis B surface antigen; AFP: alpha-fetoprotein; ICG_{15}: indocyanine green retention at 15 min. [1]By Mann-Whitney U test.

Table 2 Preoperative serum VCAM-1 levels categorized by pathological variables

Variables	Median serum VCAM-1 (ng/mL)	P^1
Background liver disease		
Normal (n=7)	462	
Chronic hepatitis (n=39)	548	0.084[2]
Cirrhosis (n=50)	704	<0.001[b]
Cirrhotic liver		
Absent (n=46)	546	<0.001
Present (n=50)	704	
Tumor size		
≤5 cm (n=35)	686	0.158
>5 cm (n=61)	609	
Tumor capsule		
Absent (n=66)	597	0.015
Present (n=30)	736	
Venous invasion		
Absent (n=49)	643	0.248
Present (n=47)	574	
Microsatellite nodules		
Absent (n=50)	643	0.359
Present (n=46)	626	
Edmonson grade		
I/II (n=49)	634	0.629
III/IV (n=47)	642	
pTNM stage		
I/II (n=43)	649	0.134
III/IV (n=53)	592	

pTNM: Pathological tumor-node-metastasis. [1]By Mann-Whitney U test; [2]Compared with normal nontumorous liver; [b]P<0.001 compared with both normal liver and chronic hepatitis.

Table 2 shows the relationship of serum VCAM-1 levels and histopathologic features of the resected HCC. There was no significant relationship with the tumor size (P=0.185), the presence of microsatellite tumor nodules (P=0.359) or venous invasion (P=0.248). Significantly higher serum VCAM-1 levels were observed with the presence of tumor capsule (P=0.015) and the presence of cirrhosis in adjacent non-tumor liver tissues (P<0.001). When categorized according to tumor pTNM staging or Edmonson grading, no significant differences in serum VCAM-1 levels were observed between different tumor stages or grades.

Serum VCAM-1 levels in HCC patients with or without cirrhosis

Fifty out of the 96 HCC patients had cirrhotic liver adjacent to the resected tumor. The median level of serum VCAM-1 in these cases of HCC with cirrhosis was 704 ng/mL (179-3 199), which was significantly higher than that in the HCC cases without cirrhosis (median 546 ng/mL, range 207-1 248, P<0.001), but it was significantly lower than that in the 23 patients with cirrhosis only (P=0.034). All patients with HCC and cirrhosis had Child's A liver function status, whereas among the 23 patients with cirrhosis only, 8 patients were Child's A, 7 patients were Child's B and 8 patients were Child's C. When compared with the healthy individuals, although the median level of serum VCAM-1 in HCC patients with cirrhosis was higher, the difference was not statistically different (P=0.271). In contrast, HCC patients without cirrhosis (n=46) had a significantly lower level of VCAM-1 when compared with healthy individuals (P=0.008). HCC patients with chronic hepatitis (n=39) had a higher serum VCAM-1 level than HCC patients with normal liver, but the difference was not statistically significant (P=0.084).

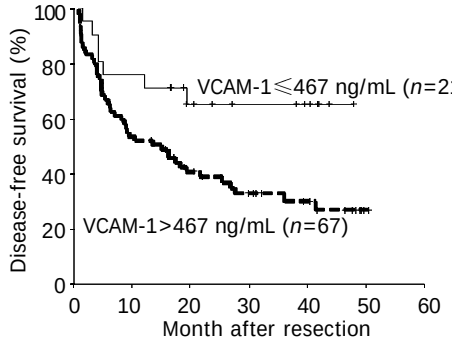

Figure 2 Disease-free survival analysis of HCC patients segregated into low (≤467 ng/mL, n=21) and high (>467 ng/mL, n=67) preoperative serum VCAM-1 levels (P=0.019).

Prognostic value of serum VCAM-1 levels on disease-free survival

Excluding 8 patients with hospital mortality or palliative resection, 88 patients were available for survival analysis to determine the prognostic influence of serum VCAM-1 levels. The median follow-up of the patients was 33 mo (range 15-52 mo). Because of the skewed distribution of serum VCAM-1 levels among the 88 patients, the 25 th percentile of VCAM-1 levels (467 ng/mL) was used as a cut-off point of high and low values of serum VCAM-1 levels in these patients[32]. At the time of analysis, 52 of the 88 HCC patients had postoperative recurrence. The disease-free survival was compared between two groups of patients who were segregated in low (n=21) and high (n=67) serum VCAM-1 levels. Patients with low levels of VCAM-1 had significantly better disease-free survival than those with high levels of VCAM-1 (3-year disease-free survival 66.7% *vs* 32.8%, P=0.019, Figure 2). The overall survival was also better in the group with low serum VCAM-1 levels than in the group with high levels, but the difference was not statistically significant (3-year overall survival 75.0% *vs* 54.2%, P=0.406).

Serum VCAM-1 was entered into a Cox regression analysis of disease-free survival together with serum AFP (> or ≤20 ng/mL), tumor size (> or ≤5 cm), presence of venous invasion, presence of microsatellite lesions, presence of cirrhosis and tumor pTNM stage. Only pTNM stage (risk ratio 1.409, 95% confidence interval 1.080-1.840, *P*=0.012) and serum VCAM-1 (risk ratio 2.329, 95% confidence interval 1.045-5.191, *P*=0.039) were significant prognostic factors for disease-free survival.

DISCUSSION

Evaluation of angiogenic factors has shown important clinical implications in many types of cancer. In particular, the measurement of various circulating angiogenic factors might provide important prognostic values independent of conventional pathological factors in cancer patients[33]. HCC is a highly vascularized malignancy, and angiogenesis has been known to be important for its development and metastasis[34]. Hence, it is of great interest to evaluate the clinical significance of circulating angiogenesis-related markers in HCC patients. Serum VCAM-1 appears to be a promising circulating marker that might have a prognostic value in several types of cancer[16-19]. To our knowledge, this is the first study that evaluated the clinical significance of serum VCAM-1 levels in HCC patients.

Although the median level of VCAM-1 in HCC patients appeared to be similar to that in normal subjects, within the group of HCC patients, those with underlying cirrhosis had a significantly higher VCAM-1 level than those with chronic hepatitis or normal nontumorous liver. Furthermore, high serum VCAM-1 levels in HCC patients correlated positively with serum bilirubin level and inversely with serum albumin level and platelet count. These findings suggested that high serum VCAM-1 levels were related to the severity of underlying chronic liver diseases. In contrast, there was no significant relationship between serum VCAM-1 levels and tumor size, pathological features of invasiveness or tumor stage. This finding in HCC patients was contrary to the findings of higher serum VCAM-1 levels associated with more advanced stage tumors in other cancers[16-19]. Analysis of serum VCAM-1 levels in HCC patients is complicated by the fact that most cases of HCC are associated with chronic liver disease, which could also contribute to the serum VCAM-1 levels. The source of VCAM-1 in the circulation of HCC patients could come from activated endothelial cells in both the tumor and chronic hepatitis or cirrhosis in the nontumorous liver. Our study suggested that serum VCAM-1 level in HCC patients could reflect the severity of underlying chronic liver disease rather than the tumor status. Unlike the case of some other cancers, VCAM-1 may play a less important role as a mediator of angiogenesis or related pathological processes in HCC. One of the main functions of endothelial adhesion molecules in tumor is to facilitate the adhesion of leukocytes into tumor endothelium, which in turn could promote angiogenesis[35]. Yoong *et al*.[36] demonstrated that vascular adhesion protein-1 and intercellular adhesion molecule-1, rather than VCAM-1, supported adhesion of tumor infiltrating lymphocytes to tumor endothelium in HCC. In fact, the serum VCAM-1 levels of the 46 patients with HCC and noncirrhotic liver were significantly lower than those of normal controls, suggesting that VCAM-1 expression may be down-regulated in HCC patients. While most studies in other types of cancer reported the expression of serum VCAM-1 levels was up-regulated, down-regulation of VCAM-1 has also been reported in node positive breast cancer patients[37].

The serum VCAM-1 levels in the 96 HCC patients in this study were significantly lower than those of 23 patients with cirrhosis only. Even when the 50 patients with HCC and background cirrhosis were separately analyzed, their serum VCAM-1 levels were still significantly lower than those of the 23 patients with cirrhosis only. This is probably attributable to the fact that all cirrhotic patients with HCC who underwent hepatic resection had Child's A cirrhosis, whereas the majority of patients in the group with cirrhosis only had Child's B or C cirrhosis. Several previous studies have demonstrated that serum VCAM-1 levels were elevated in cirrhotic patients compared with normal controls, and that serum VCAM-1 level was higher with more severe impairment of liver function or more severe portal hypertension[22,23,26,27]. Two studies demonstrated that serum VCAM-1 levels reflected the degree of fibrosis in hepatitis C related cirrhotic patients[24,25]. Our study suggested a similar relationship between serum VCAM-1 level and severity of cirrhosis in a group of patients with predominantly hepatitis B virus related cirrhosis. VCAM-1 appears to play an important role in liver inflammation and fibrosis, probably by mediating interaction between lymphocytes and endothelium. Further studies to clarify the role of VCAM-1 in chronic liver disease may lead to a better understanding of the pathogenesis of cirrhosis and may provide a novel target of intervention to prevent progression of cirrhosis.

Although serum VCAM-1 level was not related to tumor invasiveness or stage, we observed a significantly better disease-free survival in HCC patients with low level of serum VCAM-1. Our study demonstrated that preoperative circulating level of VCAM-1 had a prognostic value in patients undergoing resection of HCC independent of conventional prognostic factors. The exact mechanism underlying the survival differences between patients with low and high serum VCAM-1 levels is unclear. In view of the relationship between serum VCAM-1 levels and the severity of underlying chronic liver disease or cirrhosis found in this study and other studies[22-27], we speculate that the unfavorable prognostic influence of high serum VCAM-1 level is likely to be related to the underlying cirrhotic or fibrotic condition. The presence of cirrhosis or a higher degree of liver fibrosis has been shown to predispose to multicentric hepatocarcinogenesis and hence postoperative recurrence of HCC[30,31]. An alternative possibility is that HCC with significantly down-regulated VCAM-1 expression may be associated with more favorable prognosis after resection. However, the exact mechanism for the reduction in serum VCAM-1 level in the presence of HCC observed in some patients is far from clear, and such a possibility seems less likely in view of the lack of association between serum VCAM-1 and tumor characteristics. Further studies that correlate serum VCAM-1 level with the expression of VCAM-1 in HCC tumor tissues and adjacent nontumorous liver may help clarify the mechanism underlying the prognostic significance of serum VCAM-1 levels in HCC patients. Irrespective of the underlying mechanism, the predictive value of serum VCAM-1 level might have potential clinical application in selecting patients with a higher risk of postoperative recurrence for some adjuvant therapy to reduce recurrence.

In conclusion, our data demonstrated that serum VCAM-1 levels in HCC patients correlated with the impairment of liver function and the presence of cirrhosis but not tumor size or features of tumor invasiveness. Hence, serum VCAM-1 levels appear to reflect the severity of underlying liver disease rather than the tumor status in HCC patients. A low serum VCAM-1 level predicts better disease-free survival after tumor resection in HCC patients. Further studies are merited to investigate the exact mechanisms involved in these observations and to explore the potential use of serum VCAM-1 level as a novel prognostic marker in HCC patients.

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