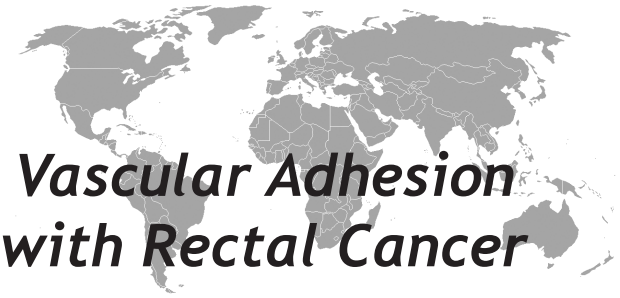

Serum Level of Soluble Vascular Adhesion Molecule 1 in Patients with Rectal Cancer

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ABSTRACT

Aim: *Vascular adhesion molecule 1 (VCAM-1) plays an important role in solid tumor enlargement and/or metastases. This study evaluated the clinical significance of measuring serum levels of soluble VCAM-1 in rectal cancer and aimed to clarify the biologic significance of its local expression.*

Method: *Serum was collected from 90 patients with rectal cancer and 40 healthy volunteers. Cancer tissue was collected from 84 patients. The level of soluble VCAM-1 in serum and cancer tissue was measured enzyme linked immunosorbent assay.*

Result: *The mean soluble VCAM-1 level in patients was significantly higher than that in control subjects. Elevated serum soluble VCAM-1 was significantly associated with clinicopathologic paramaters such as tumor size, lymph node metastasis, distant metastasis, and poor prognosis. The prognosis for stage 2 patients positive for soluble VCAM-1 level was comparable to that for stage 3 patients. In addition, the serum level of soluble VCAM-1 level was not found correlation with the cancer tissue level.*

Conclusion: *The preoperative level of soluble VCAM-1 level reflected disease progression and was a sensitive biomarker for rectal cancer.*
Key words: *Rectal cancer, VCAM-1, preoperative levels*

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Rektum Kanserli Hastalarda Serum VCAM-1 Düzeyleri

Amaç: *Vaskular adhezyon molekül-1 (VCAM-1), solid tümörlerin gelişimi ve/veya metastazında önemli rol oynar. Bu çalışma, rektal kanserde, serum soluble VCAM-1 düzeylerinin klinik önemini vurgulamaktadır ve onun lokal ekspressiyonun biologik önemini açıklamayı amaçlamaktadır.*

Metod: *Rektal kanserli 90 hastanın ve 40 sağlıklı bireyin serum örnekleri, 84 hastanın da doku örnekleri toplandı. Serum ve kanser dokusunda serum VCAM-1 düzeyleri enzim bağlı immunosorbent ölçüm yöntemi ile ölçüldü.*

Bulgular: *Soluble VCAM-1 düzeyleri, hasta grubunda kontrol grubundan anlamlı olarak yüksek bulundu. Artmış serum soluble VCAM-1 düzeyleri, klinikopatolojik parametreler ile ilişkilidir. Örneğin, tümör büyüklüğü, lenf nod metastaz varlığı, uzak metastaz ve zayıf prognoz gibi. Evre 2 ve evre 3 rektal kanserli hastalarda soluble VCAM-1 düzeyleri karşılaştırıldı. Ek olarak, soluble serum VCAM-1 düzeyleri ile kanser dokuları arasında korelasyon bulunamadı.*

Sonuç: *Preoperatif soluble serum VCAM-1 düzeyleri hastalığın progressiyonunu yansıtmaktadır ve rektal kanser için sensitif bir biomarkırdı.*

Anahtar kelimeler: *Rektal kanser, VCAM-1, preoperatif düzeyler*

INTRODUCTION

Metastatic spread of cancer cells is a key event in tumor progression and in determining the prognosis of patients with malignant disease. The process involved in the initiation of metastases from malignant tumors, as in rectal carcinoma, seems to consist of multiple steps. In the metastatic process, tumor cells first detach from the primary tumor, then enter the circulation, adhere to the microvascular endothelium, and finally extravasate and proliferate in the target organ (1, 2). Therefore, adhesive interactions between endothelial and cancer cells seem to be crucial for the successful may play a critical role in infiltrative growth and the metastatic process (3).

Vascular cell adhesion molecule 1 (VCAM-1) is thought to play an important role in the process of metastases. Many studies have suggested that VCAM-1 is a candidate for mediating tumor cell adhesion to vascular endothelial cells and promoting the metastatic process (4-6). Additionally, other studies have suggested a mechanism of tumor immune evasion, by which tumor expression of VCAM-1 may promote T-cell migration away from the tumor, resulting in decreased accumulation of T cells in the tumor microenvironment (7). Therefore, VCAM-1 is considered to play a key role in the process of malignant progression.

This study evaluated the relationship between the circulating level of soluble VCAM-1, clinicopathologic features, established tumor markers, and prognosis in rectal cancer to establish the importance of cell adhesion molecules as prognostic markers.

MATERIALS AND METHODS

90 patients scheduled for rectal cancer surgery were included. Patients were resected with total mesorectal excision, either through abdomino-perineal resection or AR. If deemed necessary, patients undergoing AR received a defunctioning loop ileostomy or a terminal sigmoidostomy (Hartmann' procedure). Venous blood samples were taken in a standardized manner during induction of anaesthesia. The samples were then centrifuged, and the plasma was collected and frozen to -70 ° C until further processed.

The serum concentration of soluble VCAM-1 was measured by enzyme-linked immunosorbent assay (ELISA) (BioSource International, CA). Samples were incubated in duplicate (100 µl) in microtiter plates with a biotinylated anti-sVCAM-1 solution. After incubation at room temperature for two hours and washing, substrate solution was added. Color development was stopped after 30 minutes at room temperature, and the intensity was read at 450 nm within 30 minutes. The results were calculated from a standard curve (recombinant human sVCAM-1; range, 0-75 ng/ml) generated from a four-parameter logistic curve, fitted, and expressed in nanograms per milliliter. The measurement was done in duplicate, and the mean value was used for data presentation. This ELISA was specific for the measurement of soluble VCAM-1 and did not detect membrane-bound VCAM-1. Surgical specimens were stored immediately in liquid nitrogen until use. The samples were thawed, quickly weighed, and placed in 5 ml of phosphate-buffered saline. The tissues were homogenized with a motor-driven Teflon pestle for five minutes on ice in 1 ml of extraction buffer per 100 mg (wet weight) of tis-

***Table 1**. Relationships between serum level of soluble VCAM-1 and clinicopathologic factors in patients.*

Variable	*n*	*serum VCAM-1*	*tissue VCAM-1*	*p*
Gender				
Male	*46*	*701.4 ± 104.6*	*203.5 ± 95.2*	
Female	*44*	*812.4 ± 93.0*	*199.4 ± 67.8*	*<0.001*
Age				
<66 y	*40*	*842.7 ± 110.2*	*213.5 ± 63.6*	
≥66 y	*50*	*813.2 ± 96.2*	*200.1 ± 72.5*	*<0.001*
T classification				
1	*15*	*678.3 ± 70.4*	*147.1 ± 98.5*	
2	*20*	*703.5 ± 78.1*	*159.2 ± 101.3*	
3	*31*	*754.8 ± 80.5*	*160.2 ± 95.3*	
4	*34*	*803.4 ± 91.8*	*189.3 ± 79.8*	*<0.001*
Vessel involvement				
Yes	*75*	*765.9 ± 100.4*	*178.2 ± 85.9*	
No	*15*	*759.3 ± 87.9*	*161.2 ± 68.0*	*>0.05*
Lypmhatic vessel involvement				
Yes	*66*	*801.4 ± 90.2*	*154.7 ± 54.7*	
No	*24*	*785.9 ± 76.8*	*160.1 ± 79.2*	*<0.01*
Lympnh node metastases				
N0	*70*	*789.2 ± 110.3*	*162.1 ± 93.4*	
N1	*20*	*905.6 ± 101.4*	*189.4 ± 89.7*	*<0.001*
Distant metastases				
M0	*68*	*740.2 ± 104.6*	*156.9 ± 78.5*	
M1	*22*	*810.4 ± 98.5*	*181.5 ± 69.5*	*<0.001*
UICC TNM classification				
1	*12*	*713.7 ± 65.9*	*148.2 ± 54.8*	
2	*18*	*734.2 ± 78.2*	*155.9 ± 67.2*	
3	*26*	*789.0 ± 80.6*	*179.2 ± 80.5*	
4	*34*	*810.3 ± 91.2*	*198.3 ± 99.5*	*<0.001*

sue. The tissue extract obtained after centrifugation at 12 000 rpm for 15 minutes at 4° C was placed in 200 µl vial and stored at -80° C. The supernatant was used to measure the concentrations of soluble VCAM-1 and protein in the tumor tissues, using an ELISA kit (BioSource International). The concentrations of soluble VCAM-1 in the tumor tissue were expressed as pictograms per milligram of protein. Protein concentration was measured using a bicinchoninic acid assay (BCATM Protein Assay Kit, IL). The tissue concentrations were expressed in pictograms per milliliter. Informed consent was obtained from each subject. The protocol was approved by the review board of our institute. Results are expressed as mean ± SD. Kruskal-Wallis ANOVA and the Mann-Whitney U test were used to evaluate differences between multiple groups and unpaired observations, respectively. The Spearman rank correlation test was conducted for statistical correlations. P values <0.05 were considered statistically significant.

RESULTS

Serum concentration of soluble VCAM-1 ranged from 20.5 to 2318.9 ng/ml, with a mean level of 789.4 ng/ml. Mean serum soluble VCAM-1 was significantly higher than in healthy volunteers (soluble VCAM-1, 789.4 ± 97.2 vs. 101.3 ± 24.5 ng/ml, p<0.001). The mean soluble VCAM-1 in patients with stage 4 disease was significantly higher than that in patients with stage 1 or stage 2 disease. Table 1 shows the relationship between serum soluble VCAM-1 and clinicopathologic findings. Serum soluble VCAM-1 was associated with disease progression, such as lymph node metastases and distant metastases. In addition, serum soluble VCAM-1 increased significantly in accordance with the progression of UICC classification.

The tissue concentration of soluble VCAM-1 in the tumor ranged from 29.1 to 987.3 ng/mg protein, with a mean of 205.7 ng/mg protein. The tissue concentration of soluble VCAM-1 in the tumor in each classification was 147.1±98.5 ng/mg protein in stage 1, 159.2±101.3

ng/mg protein in stage 2, 160.2±95.3 ng/mg protein in stage 3, and 189.3±79.8 ng/mg protein in stage 4. Significant differences were shown between stage 1 and stage 3, stage 3 and stage 4, stage 1 and stage 4. Serum soluble VCAM-1 was positively correlated with the tissue concentration of soluble VCAM-1 in cancer ($p<0.0001$).

DISCUSSION

VCAM-1 is a 90 kDa glycoprotein that contains six or seven immunoglobulin domains and belongs to the immunoglobulin superfamily of adhesion molecules. VCAM-1 is constitutively expressed on many different types of endothelial and stromal cells and mediates cellular adhesion via integrin α4ß1 (9-11). VCAM-1 exists in a membrane-bound soluble form, and is one of the most important adhesion molecules that play a crucial role in this process (4, 5). Some recent studies have suggested that VCAM-1 is involved in tumor evasion of the immune system by inducing T-cell migration away from the tumor (7, 10), and by mediating adhesion of carcinoma cells to the endothelium during malignant progression of tumors (5, 6). Soluble forms of VCAM-1 have been identified (12), and elevated serum levels of soluble VCAM-1 have recently been described in a number of cancers (9, 13-15). In the present study, rectal cancer patients had significantly higher serum soluble VCAM-1 levels as compared with healthy controls. An elevated serum soluble VCAm-1 level was significantly correlated with factors reflecting disease progression, such as nodal and distant metastases, which are well-known prognostic factors (16).

We also demonstrated that preoperative serum soluble VCAM-1 level was the only prognostic factor for patients with stage 3 and stage 4 rectal cancer. The source of serum soluble VCAM-1 in patients with rectal and colorectal cancer is not fully understood. In vitro, soluble VCAm-1 has been shown to be derived from endothelial, leukocyte, and tumor cell surfaces, perhaps influenced by the intratumoral cytokine environment (17). Pigott et al (18) have reported that soluble VCAM-1 is detected in the supernatants of cytokine-activated endothelial cells in the culture medium of colon cancer cell lines.

Our study demonstrated that serum level of soluble VCAM-1 was significantly positively correlated with the level in cancer tissue. The exact mechanism of this phenomenon is not fully clarified.

In conclusion, we showed that the operative serum concentration of soluble VCAM-1 is a novel prognostic marker for patients with rectal cancer. Furthermore, in patients with stage 3 and stage 4 disease, the serum levels of soluble VCAM-1 were higher.

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