Serum sICAM, sVCAM and sE-selectin levels in colorectal cancer patients

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Abstract: Colorectal cancer (CRC) is one of the most common cancers of the gastrointestinal tract and the fourth cause of cancers death in the world. Soluble adhesion molecules (CAMs) are thought to have an important role in host defense against carcinogenesis. They are biomarkers of inflammation and indicators of the immune response to tumors. The study included 40 CRC patients without remote metastases and 24 control subjects. Serum concentrations of sE-selectin, sICAM and sVCAM in patients with CRC were investigated by ELISA method. The level of the sCAMs decreased significantly after radical tumor resection. Preoperative serum concentrations of sICAM and sVCAM in CRC patients were significantly higher compared to the control group, whereas there were no differences regarding serum sE-selectin. Serum levels of sE-selectin, sICAM and sVCAM correlated significantly with each other. There was a significant correlation of serum levels of sICAM-1 and sVCAM-1, but not sE-selectin, with TNM stage and lymph node involvement. No significant relationship was found between serum concentrations of sICAM-1, sVCAM-1 and sE-selectin in CRC patients and patients’ age or gender. Our findings suggest that an improved understanding of the mechanisms of membrane shedding of sICAM, sVCAM and sE-selectin is required to delineate their role in tumor progression.

Key words: sICAM-1, soluble intracellular adhesion molecule-1, sVCAM-1, soluble vascular cell adhesion molecule-1, sE-Selectin, soluble E-Selectin, CRC, colorectal cancer

Introduction

Intracellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion molecules-1 (VCAM-1) are members of the immunoglobulin superfamily. They both participate in adhesion between cells. Soluble forms of ICAM (sICAM) and VCAM (sVCAM) have been previously identified [1]. Elevated serum levels of sICAM, sVCAM and sE-Selectin have recently been described in patients with cancers [2-8]. Several studies suggest that cell adhesion molecules (CAMs) play an important role in cancer progression and metastases [3,9,10]. Guzinska-Ustymowicz and Kemona [11] indicated that evaluation of TGF beta expression of protein in association with histological parameters can be used as a parameter of the aggressiveness of pT1 CRC. Okulczyk et al. indicated no significant correlations between the mutations of codon 12 K- RAS gene and clinical features. Also no correlation was observed with either Dukes or TNM clinical advancement [12].

Metastatic spread of cancer cells is a key event in tumor progression and in determining the prognosis of patients with malignant disease. Malignant cells detached from the primary tumor penetrate into blood or lymph vessels, survive in the circulation, and are then arrested in the capillary endothelium of distant organs, extravasate and grow as a secondary lesion [13]. Therefore, sICAM-1, sVCAM-1 and sE-selectin interactions between endothelial and cancer cells seem to be crucial for the successful development of metastases. These interactions are modulated by a specific cell surface receptor including selectins, integrins and immunoglobulin like family of cell adhesion molecules [1].

It is now increasingly apparent that certain members of these families of CAMs are involved in tumor
Blood analysis. Peripheral venous blood samples in CRC patients have been presented in Table 1. Differentiation grade G2, G3). Demographic data of the CRC patients have previously been recognized. They have been detected in the serum of patients with colorectal cancer, where high circulating levels are generally associated with more advanced and metastatic disease [3]. No study has documented serum levels of E-selectin, ICAM-1 and VCAM-1 in patients with colorectal cancer after radical tumor resection.

The aim of the present study was to assess the effect of tumor advancement and malignancy grade on the levels of soluble ICAM-1, VCAM-1 and E-selectin depending on surgical treatment.

Materials and methods

Patients. The study included 40 colorectal cancer patients (CRC) without remote metastases (20 men and 20 women, aged 42 – 68 years), diagnosed and operated in the Department of General Surgery at the J. Sniadecki's Hospital in Bialystok and in Department of General and Endocrinological Surgery, Medical University in Bialystok, and 24 healthy subjects (control group) (19 men and 5 women, aged 40 – 64 years) (Table 1).

The diagnosis of CRC was based on clinical symptoms and colonoscopy and abdominal ultrasound scanning. The patients underwent surgical procedure of tumor resection with subsequent regional lymphadenectomy. During surgery, samples (primary tumor, lymph node metastasis) were collected and neoplastic lesions were histopathologically verified. All patients had colorectal cancer. None of these patients received chemotherapy or radiation therapy before and after surgical intervention.

The CRC patients qualified for our study did not have any other ailment that could affect the immune response. Those patients who had a recent inflammatory or any acute infection were excluded from the study. Clinical advancement was evaluated based on TNM classification by Hutter and Sobin [14], while tumor type and malignancy grade were determined by histopathological analysis. The CRC patients were divided into two groups: A – 22 patients in stages I and II without metastases (T1N0M0, malignancy differentiation grade G2, G3) and B – 18 patients with lymph node metastases (but not remote metastases) in stage III of clinical advancement (T3N1-2M0, malignancy differentiation grade G2, G3). Demographic data of the CRC patients have been presented in Table 1.

Blood analysis. Peripheral venous blood samples in CRC patients were collected twice: three days prior to surgery and 3 months after surgery, into sterile glass tubes, in the morning after an overnight fast. The samples were allowed to coagulate at room temperature for 30 min and then were centrifuged at 2500 g for 20 min. The serum was separated and stored at -70°C until analysis. Before analysis, samples were thawed slowly and mixed gently.

The soluble forms of CAMs were measured in blood serum using a commercially available immunoenzymatic method. Serum sICAM-1, sVCAM-1 and E-selectin concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Parame-
node. Only serum levels of sICAM and sVCAM, but not sE-selectin, decreased significantly after radical resection of the tumor compared with preoperative levels; sICAM 366.4±114.0 ng/ml vs. 247.3±68.4 ng/ml (p<0.001) and sVCAM 974.3±214.0 vs. 470.1±106.3 ng/ml (p<0.0001) (Table 3).

We compared the concentrations of sICAM-1, sVCAM-1 and sE-selectin in the serum of CRC patients between group A and B. We observed a significantly higher concentration of sICAM (396.2±68.9 ng/ml) and sVCAM (1210.3±199.0 ng/ml) in the serum of the CRC patients with lymph node metastases. There was a significant difference between the two groups (Table 4). No significant difference was observed in sE-selectin concentration in the serum of the CRC patients before surgical intervention (group A: 82.4±23.3 group B: 88.4±29.0) (Table 4). There was also no significant correlation of serum sICAM-1, sVCAM-1 and sE-selectin levels in CRC cases with patients’ age or gender. However, the preoperative levels of all three adhesion molecules were significantly correlated with disease stage and lymph node involvement.

**Discussion**

Adhesion molecules are thought to be essential for maintaining a normal immune defense system in a variety of important biological events including carcinogenesis and metastasis biology. It has been suggested that the process of tumor metastasis includes differential expression of adhesion molecules, with some being over-expressed, others down-regulated and some structurally changed, causing a loss of cell-cell interaction which could facilitate metastasis.

Elevated serum levels of cell adhesion molecules (CAMs) have been described in patients with cancers [17]. In our research, like in the studies of other authors [2], preoperative serum concentrations of sICAM and sVCAM in CRC patients were significantly higher compared to the control group, whereas no differences were found regarding serum sE-selectin levels. Similar results concerning sE-selectin were obtained by other investigators, who concluded that the level of sE-selectin cannot be a predictive marker in colorectal cancer, although it may be useful in the diagnosis of liver metastases [20].

We have confirmed the findings by Alexiou et al. [3] and Kang et al. [21], who found elevated levels of CAMs in CRC patients. In our study, the levels of serum sICAM-1 and sVCAM-1 in healthy controls were similar to those reported by other authors [4,9,20,22,23]. These soluble forms of sICAM, sVCAM and sE-selectin significantly correlated with tumor stage and the development of metastases of colorectal cancer [21,25]. In our study, there was a significant correlation of serum sICAM-1 and sVCAM-1, but not sE-selectin, with the disease stage, which can be explained by low neoangiogenesis in the neoplastic tissue.

As revealed by many authors, these soluble forms of CAMs significantly correlated with TNM stage and the development of metastases of gastric cancer [2,25], colorectal cancer [21], breast cancer [5,7], in urologi-
cal malignancies [6] and in chronic B-lymphocytic leukemia [4]. Numerous investigations in clinical diagnosis have been focused on the evaluation of the adhesion molecule expression to identify the interaction between tumor cells and host immune response. The preoperative levels of sICAM-1, sVCAM-1, elevated in our study, showed relatively high specificity, but had low sensitivity for diagnosis of colorectal cancer. There was a significant correlation of serum sICAM-1 and sVCAM-1 levels, but not sE-selectin, with TNM stage and lymph node involvement. No significant relationship was found between the concentrations and patients’ age or gender. The factors regulating CAMs in colorectal cancer are complex. The association among immunohistochemical CAM expression, tumor vascularity and leukocyte infiltration suggests an important role for these molecules in host immune response and in tumor progression [2].

We observed significantly higher concentrations of sICAM and sVCAM in the serum of the CRC patients with lymph node metastases. Metastatic spread of cancer cells is a key event in tumor progression and in determining the prognosis of patients with malignant disease. CAM expressions have been demonstrated on endothelial cells of small vessels at the invasive margin of tumor cells involved in metastatic spread [9]. In various vascular disorders, activated lymphocytes and macrophages produce cytokines, of which interleukin-1(IL-1), tumor necrosis factor (TNF-α) and interferon-γ are the main inducers of adhesion molecule expression on endothelial cells [26]. We believe that the circulating sCAMs may have a regulatory role, although their biological role has not been completely elucidated. The migration of lymphocytes from intravascular system to tissue is a fundamental event in the immune response to an inflammatory process and it is the result of specific interactions of adhesion molecules on endothelium and their ligands on lymphocytes. Since LFA-1 is the ligand for ICAM-1 and it is involved in the initial steps of adhesion of cytotoxic T lymphocytes to the target tumor cells [27], we suggest that ICAM-1 shedding from tumor cells could block binding of cytotoxic T lymphocytes to cancer. Additional loss of adhesion molecules from activated endothelium could inhibit counter ligands on the tumor cells themselves, preventing their adhesion to endothelium sites and thereby promoting metastases. We have confirmed this assumption, showing a correlation of the level of adhesion molecules with tumor differentiation grade and the process of metastasis. In gastric cancers, serum sICAM-1 and sVCAM-1 levels decreased significantly after radical tumor resection [3]. In our study, serum levels of sICAM and sVCAM, but not sE-selectin, were found to be significantly higher in patients before surgical treatment for CRC compared to those after radical resection. In vitro studies have shown that at least ICAM-1 expressed by tumor cells is shed in the cell culture supernatants [28], which may explain our observations.

These findings seem to suggest that serum concentrations of sCAMs can reflect tumor progression and metastases, and may be clinically useful. We think that an improved understanding of the mechanisms of membrane shedding of sICAM, sVCAM and sE-selectin, their effect on the host immune response, their relationship to disease stage and lymph node involvement is required to delineate their role in tumor progression.

**Acknowledgments:** I would like to thank Krzysztof Kanigowski MD, for collecting samples of patient’s blood at the Department of General Surgery Sniadecki's Hospital of Białystok. This work was supported by the grant 4 09 660F from Medical University of Białystok.

**References**


