Circulating Soluble Vascular Cell Adhesion Molecule-1 and Vascular Endothelial Growth Factor in Patients with Laryngeal Squamous Cell Cancer

ABSTRACT

Background. Tumor growth and subsequent metastatic spread of cancer cells are multistep processes accompanied by changes in the expressions of many growth factors and vascular adhesion molecules.

Objectives. Investigation whether serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and vascular endothelial growth factor (VEGF) are related to clinicopathological variables of laryngeal squamous cell cancer (LSCC) patients.

Material and Methods. The serum concentrations of sVCAM-1 and VEGF165 were investigated in 41 patients with LSCC and in 27 controls by an enzyme-linked immunosorbent assay (ELISA).

Results. In LSCC cases the mean ± SD serum concentration of sVCAM-1 was 542.1 ± 164.9 ng/ml (range: 388–1284 ng/ml) and of VEGF 519.4 ± 335.9 pg/ml (82–1374 pg/ml). The serum levels of both sVCAM-1 and VEGF were significantly elevated in LSCCs compared with the healthy controls (p < 0.05 and p < 0.001, respectively). In the control group the mean sVCAM-1 concentration was 434.8 ± 76.3 ng/ml (314.7–648.8 ng/ml) and VEGF level 263.6 ± 166.4 pg/ml (70.5–702.9 pg/ml). There were no significant differences between the serum levels of sVCAM-1 and VEGF and clinicopathological variables of the patients with LSCC. In neither the cancer cases nor the control group was a linear correlation between sVCAM-1 and VEGF serum levels found.

Conclusions. The results suggest that the serum levels of sVCAM-1 and VEGF165 are not clinically useful biomarkers for predicting tumor progression or for identifying the metastatic potential of LSCC (Adv Clin Exp Med 2007, 16, 3, 389–394).

Key words: laryngeal cancer, sVCAM-1, VEGF.
The metastatic spread of cancer cells is a key event in tumor progression and, therefore, in determining the prognosis of patients with malignant disease. The development of metastasis includes malignant cells detachment from the primary tumor, penetration of the blood or lymph vessels, cellular arrest in the capillary endothelium, extravasation, and secondary lesion formation. The process is accompanied by changes in the expressions of many cell adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), a 110-kDa glycoprotein which is critical in cell-to-cell interactions. VCAM-1 expression has been demonstrated on the endothelial cells of small vessels at the invasive margin of tumors, suggesting possible interactions between endothelial and tumor cells involved in metastatic spread [1]. It has been shown that endothelial cells expressing VCAM-1 bind melanoma cell lines, which indicates the function of VCAM-1 as an adhesion molecule facilitating metastasis [2].

The phenomenon prerequisite to both tumor growth and metastasis is angiogenesis. The induction of tumor vascularization is mediated in part by the release of angiogenic peptides from tumor cells, macrophages, and the extracellular matrix. Among these angiogenic factors, vascular endothelial growth factor (VEGF) is thought to be one of the most important [6]. VEGF is a dimeric glycoprotein with four spliced variants containing 121, 165, 189, and 206 amino-acid residues, expressing almost identical biological activities as endothelial cell-specific mitogen and vascular permeability factor. Furthermore, VEGF promotes endothelial cell proliferation and stimulates VCAM-1 expression on endothelial cells [3]. The most frequently expressed isoform is VEGF165.

Although there is abundant evidence showing that VEGF and VCAM-1 play central roles in the development and growth of malignant tumors, information regarding the clinical utility of serum VEGF and sVCAM-1 levels in laryngeal squamous cell cancer (LSCC) is limited. Secretion of these biomarkers was detected in a large variety of human solid tumors, including head and neck malignancies [4–7]. Due to the high heterogeneity of head and cancers it seems reasonable to separate particular subgroups of tumors to enhance the reliability of the results. According to the present authors’ knowledge, the number of studies on circulating soluble molecules in LSCC is restricted. On the basis of recent studies it could be suspected that the biological roles of VEGF165 and sVCAM-1 serum levels may also be important in the progression and metastatic process of laryngeal cancer. Thus the aim of this study was to investigate whether serum levels of soluble VEGF165 and sVCAM-1 are related to clinicopathological variables of LSCC patients.

**Material and Methods**

**Subjects**

The concentrations of sVCAM-1 and VEGF165 were investigated in 41 patients (40 male, 1 female) with histologically proven LSCC diagnosed and treated at the Department of Otolaryngology, Silesian Piasts University of Medicine in Wroclaw. The patients’ ages ranged from 40 to 81 years (mean: 58.7 years). Tumor staging was made in accordance with the TNM staging system as follows: pT1 4 cases, pT2 9 cases, pT3 12 cases, and pT4 16 cases. Cervical nodal metastases were observed in 19 (46%) patients. Three cases were in stage I of clinical advancement, 4 in stage II, 17 in stage III, and 22 in stage IV. No patient had received chemo- or radiotherapy or blood transfusion prior to surgery.

The control group consisted of 27 age-matched healthy subjects (25 male and 2 female). The absence of LSCC was assessed by clinical history and endoscopic examination. The presence of a history of rheumatoid arthritis, recent pregnancy, trauma, or surgery (within 1 month) was excluded.

**Blood Samples and Assays**

Peripheral venous blood samples were drawn into sterile glass tubes in the morning. The samples were allowed to coagulate at room temperature for 30 min and then centrifuged at 2000 × g for 10 min. The serum was separated, aliquoted, and stored at −70°C until assay. Before analysis,
the samples were slowly thawed and gently mixed. Serum VEGF_165 and sVCAM-1 concentrations were determined using a solid phase ELISA kit (R&D Systems, Minneapolis, MN, USA). The assays use the quantitative sandwich enzyme immunoassay technique using antibodies raised against recombinant human VEGF and sVCAM-1. For each analysis, 100 µl of sample was used. The measurements were carried out as described by the supplier. To correct for optical imperfections in the plate, the reading was repeated as recommended by the supplier at a different wavelength and these results were subtracted. Each serum sample concentration was calculated automatically from standard curves. All of the analyses and calibrations were carried out in duplicate. VEGF and sVCAM-1 concentrations are reported in pg/ml and ng/ml, respectively.

Statistical analysis using the chi-squared test was performed. Differences were considered statistically significant at $p < 0.05$.

**Results**

Both sVCAM-1 and VEGF were detectable in all cancer patients and control subjects. Among the cancer patients the mean ± SD serum concentration of sVCAM-1 was 542.1 ± 164.9 ng/ml (range: 388–1284 ng/ml) and of VEGF 519.4 ± 335.9 pg/ml (82–1374 pg/ml). The serum levels of the both sVCAM-1 and VEGF were significantly elevated in patients with LSCC compared with the healthy control volunteers ($p < 0.05$ and $p < 0.001$, respectively) (Figs. 1 and 2). In the control group the mean sVCAM-1 concentration was 434.8 ± 76.3 ng/ml (range: 314.7–648.8 ng/ml) and VEGF level 263.6 ± 166.4 pg/ml (70.5–702.9 pg/ml).

There were no significant differences between the serum levels of sVCAM-1 and VEGF and the clinicopathological variables of the patients with LSCC (age, tumor size, nodal status, and clinical stage) (Table 1).

**Table 1.** Characteristics of the cancer patients and associated VEGF and sVCAM-1 levels

<table>
<thead>
<tr>
<th>Variables (Wskazaźni)</th>
<th>n</th>
<th>sVCAM-1 ng/ml (mean – range)</th>
<th>VEGF pg/ml (mean – range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age – years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Wiek pacjent – lata)</td>
<td>≤ 58</td>
<td>560.5 (403–1284)</td>
<td>571.1 (142–1198)</td>
</tr>
<tr>
<td></td>
<td>≥ 58</td>
<td>503.6 (388–680)</td>
<td>418.7 (82–1374)</td>
</tr>
<tr>
<td>Tumor size (Wielkość guza)</td>
<td>pT1 + pT2</td>
<td>472.6 (388–680)</td>
<td>527.8 (219–1198)</td>
</tr>
<tr>
<td></td>
<td>pT3 + pT4</td>
<td>600.3 (429–1284)</td>
<td>511.3 (82–1374)</td>
</tr>
<tr>
<td>Nodal status (Stan węzłów chlonnych)</td>
<td>N0</td>
<td>501.9 (388–680)</td>
<td>470.6 (82–1198)</td>
</tr>
<tr>
<td></td>
<td>N+</td>
<td>560.9 (403–1284)</td>
<td>586.6 (123–1374)</td>
</tr>
<tr>
<td>Clinical stage (Zaawansowanie kliniczne)</td>
<td>I + II</td>
<td>496.7 (388–680)</td>
<td>549.6 (202–1198)</td>
</tr>
<tr>
<td></td>
<td>III + IV</td>
<td>548.8 (403–1284)</td>
<td>517.4 (82–1374)</td>
</tr>
</tbody>
</table>
stage) (Tab. 1). However, elevated serum levels of sVCAM-1 were insignificantly associated with locally advanced tumors (pT3–pT4). The levels of circulating VEGF were slightly higher in the subgroup of patients with lymph node metastases (N+). These changes were not statistically significant ($p = 0.1439$ and $p = 0.2796$, respectively). In both the cancer cases and the control group, no linear correlation between sVCAM-1 and VEGF serum levels was found.

**Discussion**

Laryngeal carcinoma is the second most common malignant neoplasm of the respiratory tract after lung cancer. Despite novel combined adjuvant and neoadjuvant therapeutic approaches, the survival rate is still unsatisfactory. A connection between circulating biological markers and disease prognosis may add interesting insight into the biology of the tumor phenotype and help to identify rational targets for novel therapeutic approaches.

It is now widely accepted that an increase in the tumor cell population must be preceded by an increase in microvessels supplying the neoplasm. It is speculated that the induction of angiogenesis is one of the first events in the transition to a malignant state which further enables the growth of solid tumors and the formation of metastases [2, 8]. In early breast cancers it has been demonstrated that serum sVCAM-1 levels correlated with tumor microvessel density and were an accurate marker for tumor angiogenesis [9].

In the present study, a significant difference in serum sVCAM-1 and VEGF levels between patients with LSCC and healthy controls was demonstrated. The serum concentrations of these markers have also been shown to be elevated in many other malignancies [10]. In some locations, such as in gastric, breast, ovarian, and colorectal cancer, high circulating levels of these markers are generally associated with clinical progression and poor prognosis [5, 6, 11, 12]. In patients with gastric cancer, a positive correlation was observed between the level of soluble VCAM-1 and tumor stage and invasion depth. The concentration of sVCAM-1 in patients with lymph node metastasis was significantly higher than in patients without [12]. In contrast, the levels of sVCAM-1 were higher in healthy controls than in patients with localized prostate cancer; the levels were highest in patients with skeletal metastases [13]. In the same group, circulating levels of VEGF were greatly elevated in patients with regional and distant metastases as compared with those with nonmetastatic prostate cancer or healthy subjects.

It has been stated that preoperative assessment of circulating VEGF may improve early identification of patients which harbor lymph node metastases, thereby providing surgeons with the opportunity of intensive and meticulous lymphadenectomy or early systemic intervention [13]. Similarly, serum VEGF appears to be a useful marker for monitoring the clinical course of ovarian cancers after surgery [11].

In the present study no statistically significant differences in the serum levels of VEGF and sVCAM-1 and clinicopathological parameters were found. These data are consistent with findings reported in the sera of patients with head and neck cancers. In those studies the serum levels before treatment were also significantly higher than in the control groups [4, 7, 14].

In the present study the mean level of VEGF in the sera of LSCC patients was 519.4 pg/ml, which is plainly higher than the findings recorded by Riedel et al. (142 pg/ml) [4]. In that study the serum levels of VEGF grew from 114 pg/ml in oropharyngeal to 181 pg/ml in hypopharyngeal cancer cases. The serum levels of VEGF in the control group of the present study were similar to those obtained by Hyodo et al. [15], who reported a concentration of VEGF in healthy controls as 238 ± 125 pg/ml. In contrast, the mean level of sVCAM-1 in the present study’s patient group was lower than Liu’s et al. data (1155 ng/ml) [7]. In healthy controls the sVCAM-1 concentrations were similar to those previously reported by Alexiou et al. [5] with a mean level of 413 ng/ml.

The results of the present study suggest that serum VEGF and sVCAM-1 might simply not be markers of the extent of disease. The correlation between the serum concentrations of these molecules and clinicopathological parameters has been a subject of controversy in various human malignancies, which is in line with the present data. The sources of soluble VCAM-1 and VEGF are also not fully known. Some studies have reported that soluble VCAM-1 may be related to the inflammation process reflected by white cell count. To eliminate that possibility, only LSCC patients with normal blood tests were included in further investigations. Among head and neck SCC patients, very common factors influencing VEGF serum level are smoking and coronary artery disease [16, 17].

Recently it has been speculated that elevated VEGF serum levels not only originate from increased tumor cell production, but also from alternatives sources, such as platelets during platelet aggregation, activated human neutrophils, T lymphocytes, and blood mononuclear cells [18–20]. Therefore it was postulated that serum samples may not be suitable for the measurement of circulating
VEGF levels because clot formation induces platelet activation and subsequent abundant VEGF release. Circulating VEGF in patients with cancer originates from many sources and reflects the degree of mitogenicity of serum on endothelial cells, which suggests that platelets and leukocytes may scavenge biologically active VEGF [21]. It is also known that not all of the VEGF stored in blood cells is endogenously synthesized, but may originate primarily from plasma [22]. Consequently it is possible that VEGF secreted by neoplastic cells is transmitted to the circulation and accumulates in blood cells. For these reasons, in the present authors’ opinion the measurement of VEGF in the serum of patients with malignancy appears more appropriate than its measurement in plasma.

In conclusion, the results of the present study suggest that the serum levels of the investigated antigens may be helpful in distinguishing invasive cancers. However, circulating sVCAM-1 and VEGF165 are not clinically useful biomarkers for predicting tumor progression or identifying metastatic potential in LSCC. The possible roles of soluble molecules in the prognosis of LSCC deserve further elucidation and evaluation with long-term patient follow-up.

References


Address for correspondence:
Marcin Frączek
Department of Otolaryngology
Silesian Piasts University of Medicine
Chalubińskiego 2
50-368 Wrocław
Poland
Tel.: +48 71 78 42 512
E-mail: raucedo@wp.pl

Conflict of interest: None declared

Received: 8.09.2006
Revised: 30.04.2007
Accepted: 10.05.2007