



Prognostic value of soluble factors of angiogenesis and adhesion processes in head and neck squamous cell carcinomas

Head and neck squamous cell carcinomas

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Abstract

Aim: In this study, we aimed to define the prognostic value of two factors related to angiogenesis and adhesion processes of head and neck squamous cell carcinomas. The prominent angiogenesis molecule is vascular endothelial growth factor (VEGF). The vascular cell adhesion molecule (VCAM) first attracted attention more than two decades ago as endothelial adhesion receptor with key function for leukocyte recruitment in term of cellular immune response. **Material and Method:** 35 patients with head and neck squamous cell carcinomas were enrolled in this study. The control group consisted of 20 people who had no sign of regional or systemic diseases. 33 patients were male and 2 patients were female. Mean age was 59.7 years (28-76). **Results:** We showed that VEGF levels in the patient group were significantly higher than in the control group ($p=0.001$). However, when comparing the VEGF levels of different stages, there was no statistical significant difference between the stages. **Discussion:** Serum VEGF levels can provide sufficient information for the early diagnosis of the disease but prognosis may not be evaluated according to the results of our study. VCAM levels were not specific and sensitive to use as a tumor marker but VCAM may be a valuable factor to determine the prognosis and tumor aggression in cancer patients.

Keywords

Vascular Cell Adhesion Molecule; Vascular Endothelial Growth Factor; Prognosis

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Introduction

Head and neck cancers include malignant tumors involving various anatomical sites, such as the oral cavity, oropharynx, hypopharynx, and larynx. Predominantly, these tumors are squamous cell carcinomas originating from the epithelium that lines the upper aerodigestive tract [1]. Squamous cell carcinomas of the head and neck (HNSCC) have the sixth highest incidence rate globally [1].

Tumor cells produce many biochemically detectable molecules. Many molecules have been studied for the use of early detection of squamous carcinomas of the head and neck. There is still a need for highly specific as well as sensitive tumor markers for early detection of head and neck squamous carcinomas. Angiogenesis and adhesion processes are two important mechanisms of tumor progression and metastasis in tumor biology. Angiogenesis is consequential for the growth of both primary and metastatic tumors demanding blood supply to grow over the size of 1 or 2 mm (1-5). The prominent angiogenesis molecule is vascular endothelial growth factor (VEGF) [5-14].

Adhesive relationship between extracellular matrix and the cells in cancer biology is important. Adhesion molecules such as vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and N-CAM like adhesion molecule have been described [15-21]. All of these molecules are thought to play a role in tumor invasiveness and tumor aggression but the mechanism of this function is still not clear [15-21]. Adhesion molecules are believed to take part in the process of metastasis but here again the exact mechanism is not clear. The possible roles of soluble adhesion molecules in the prognosis of head and neck carcinoma deserve further elucidation and evaluation. However, some studies show that the level of circulating adhesion molecules such as VCAM is higher in cancer patients than in the normal population [15-21].

As new treatment options against angiogenesis and adhesion processes have been introduced, the determination of angiogenesis and adhesion profiles in carcinomas has become more important.

In this study, we aimed to define the prognostic value of serum VEGF and VCAM.

Material and Method

35 patients with head and neck squamous cell carcinomas were enrolled in this study. The control group consisted of 20 people who had no sign of regional or systemic diseases. 33 patients were male and 2 patients were female. Mean age was 59.7 years (28-76). In 29 patients, the American Joint Committee on Cancer (AJCC) staging manual was used to determine the stage of the diseases. In all patients, the pathological diagnosis was squamous cell carcinoma. Blood samples were obtained intravenously, placed in an EDTA tube, and transferred to Ankara University Medical School Immunology Laboratory. The study protocol was approved by the local ethics committee and conducted in accordance with the ethical principles of the Declaration of Helsinki.

A quantitative enzyme-linked immunosorbent assay (ELISA) was performed twice on each sample to measure the concentration of soluble serum VEGF and soluble serum VCAM-1. A commercially available ELISA kit was used (Cytelisa Human

VEGF Immunoassay, College Park, MD, USA). Serum samples from all patients were incubated for 30 minutes at room temperature and centrifuged for 10 minutes at 5000 rpm. The CYT ELISA kit measures the free cytokine in serum using the sandwich enzyme immunoassay technique. The kit includes VEGF 165 variant. Serums were duplicated on microtiter plates coated with a monoclonal antibody specific for VEGF. Next, any unbound substances were washed away and an enzyme-linked polyclonal antibody specific for VEGF was introduced. This was allowed to incubate for two hours at room temperature, and the plates were washed to remove unbound antibody. A substrate solution was added and color development was stopped after 25 minutes at room temperature. A microplate reader was then used to determine colorimetric densities at 490 nm for each sample. Results were calculated from a standard curve generated by a form parametric logistic curve fit and expressed in pg/mL of serum. VCAM levels were measured with Biosource kit (Biosource, Inc., Camarillo, CA, USA) used according to the manufacturer's instructions.

Results

Statistical analysis of the study includes 55 persons, of whom 35 were enrolled in the patient group. Mean age was 59.7 years. Stages were T1N0 (7 patients), T2N0 (21 patients), T3N0 (4 patients), T3N1 (2 patients), and T4N2 (1 patient).

The mean serum concentration of VEGF for the healthy control group was 19 pg/ml (13-168 pg/ml) and interquartile range was 8. The mean serum VEGF level for the patient group was 45 pg/ml (21-472 pg/mL) and IQR was 69. In the patient group, VEGF levels were not uniformly distributed ($p < 0.0001$ Kolmogorov-Smirnov test and $p = 0.010$ Shapiro-Wilkinson test). Also in the control group, VEGF levels were not uniformly distributed ($p < 0.0001$ Kolmogorov-Smirnov test and $p = 0.010$ Shapiro-Wilkinson test). The mean serum concentration of VCAM for the healthy control group was 415.90 ng/mL (260-550 ng/mL) and standard deviation was 101.92. The mean serum VCAM level for the patient group was 450.5 ng/mL (260-1125 ng/mL) and IQR was 145.50. In the patient group, VCAM levels were not uniformly distributed ($p = 0.002$ Kolmogorov-Smirnov test and $p = 0.010$ Shapiro-Wilkinson test). In the control group, VCAM levels were uniformly distributed ($p = 0.200$ Kolmogorov-Smirnov test and $p = 0.436$ Shapiro-Wilkinson test).

Mean VEGF levels compared with Mann-Whitney U test between the patient and the control group were significantly higher in the patient group ($p = 0.001$).

Mean VCAM levels compared with Mann-Whitney U test between the control and the patient group showed no significant difference between the groups ($p = 0.602$).

In the patient group, for VEGF levels compared on the basis of the T stage of the disease, there was no difference between the groups ($p = 0.392$) (Table 1).

In the patient group, for VEGF levels compared on the basis of the N stage of the disease, there was no difference between the groups ($p = 0.402$) (Table 2).

In the patient group, for VCAM levels compared on the basis of the stage of the disease, there was a significant difference between the groups ($p < 0.0001$) (Table 3).

In the patient group, for VCAM levels compared on the basis of the N stage of the disease, there was a significant difference between the groups ($p < 0.0001$) (Table 4).

Table 1. Comparison of VEGF levels and T Stage

T Stage	Value	Mean VEGF level(pg/ml)	p(Jonckheere Tersptra test)
T1	7	41.3	0.392
T2	21	65.0	
T3	6	39.0	
T4	1	37.0	

Table 2. Comparison of VEGF levels and N Stage

N Stage	Value	Mean VEGF level(pg/ml)	p(Jonckheere Tersptra test)
N0	31	40.8	0.402
N1	3	39.0	
N2	1	37.0	

Table 3. Comparison of VCAM levels and T Stage

T Stage	Value	MeanVCAM level(pg/ml)	p(Jonckheere Tersptra test)
T1	7	352	<0.0001
T2	21	434	
T3	6	505	
T4	1	1125	

Table 4. Comparison of VCAM levels and N Stage

T Stage	Value	MeanVCAM level(pg/ml)	p(Jonckheere Tersptra test)
N0	31	443.0	<0.0001
N1	3	522.5	
N2	1	1125.0	

Discussion

Head and neck cancers include a heterogeneous group of malignant tumors involving various anatomical sites, such as the oral cavity, oropharynx, hypopharynx, and larynx. Predominantly, these tumors are squamous cell carcinomas originating from the epithelium that lines the upper aerodigestive tract. Squamous cell carcinomas of the head and neck have the sixth highest incidence rate globally. There is considerable geographical heterogeneity in incidence rates that can be attributed to the differences in the distribution of associated risk factors. Despite advances in the treatment, including surgery, radiotherapy, and chemotherapy, the 5-year survival is still <50%. Thus HNSCC is associated with considerable disease-related mortality and treatment-related morbidity [18]. Loco-regional recurrences are seen in 30-40% of patients, distant metastasis in 20-30% of cases, while still others present with secondary primary tumors [18]. Tumor site, size, thickness, involvement of adjacent structures, presence of extracapsular spread, and perivascular and perineural invasion are some of the factors that have prognostic significance. However, the most important prognostic indicator affecting the overall and disease-free survival is the presence of regional cervical lymph node metastases. This factor alone reduces the survival rates by 50%.

Early diagnosis and evaluation of prognosis at the time of detection of head and neck squamous cell carcinomas (HNSCC) are important. However, there is still a lack of dependable his-

tological or biochemical parameters. Some studies have concluded that the definition of sensitive and specific parameters for prognosis and for treatment protocols needs to be more accurate and specific [13]. Recent prognostic parameters have become important for the use of different treatment modalities [13]. However, there is still no consensus on these molecules for clinical purposes [14].

In the literature, many molecules have been proposed as prognostic factors [13-15]. It has been shown that elevated expression of VEGF has a negative effect on the prognosis of tongue squamous cell carcinomas [14]. Bowden et al. also determined that elevated expression of VEGF mRNA increases the tumor aggression and risk of metastasis in skin squamous cell carcinomas [13]. In the literature, there are studies on the levels of VEGF in the circulation of different cancer types. Teknos et al. studied serum VEGF levels in high stage laryngeal carcinoma patients and showed that serum VEGF levels have prognostic value since high levels of VEGF correlate with low survival rates [4]. After discovering the relation of angiogenesis to tumor growth and metastasis, studies focused on treatment. Torabian et al. showed significantly higher expression of VEGF (as assessed by immunostaining) in squamous cell carcinoma of the oral cavity (OSCC), which is clinically one of the most common sites of the origin of head and neck cancers, as compared to dysplastic or normal mucosa. Other studies have also assessed the role of VEGF family members, receptors and other angiogenic factors in OSCC in relation to tumor stage, severity, presence of metastasis, and survival [18].

Use of chemotherapy decreases tumor microvessel density and decreases VEGF levels in the circulation [4]. It is believed that VEGF levels can be a predictive factor. However, studies conducted to show the relationship between the VEGF levels and tumor aggression and metastatic capability in premalign lesions and dysplasias have concluded that VEGF levels may not predict the tumor aggression or premalign lesions [1,2,3]. Ninck et al. suggested that therapies targeting only one factor would fail because there are many other factors affecting the angiogenesis [5].

In our study, we showed that VEGF levels in the patient group were significantly higher than in the control group ($p = 0.001$). However, when comparing the VEGF levels of different stages, there was no statistically significant difference between the stages. Based on this finding, determination of serum VEGF levels can provide sufficient information for the early diagnosis of the disease but cannot be used to predict prognosis. With this finding, we concluded that VEGF may not be used as a prognostic factor, but further studies are needed to confirm the results. Recently conducted studies revealed the importance of adhesion process in tumor biology [17]. The existence of adhesion molecules in circulation has been shown in head and neck carcinomas [15-21]. One of the most important adhesion molecules is the vascular cell adhesion molecule. VCAM-1 is suggested as a new and heretofore underestimated target in cancer treatment and in clinical diagnosis of malignancies.

One of the earliest studies on adhesion molecules in circulation stated that transformation of the tumor from benign form to malign form, tumor cell invasion, extracellular matrix degradation, and tumor cell adhesion processes should be functional

[17]. Another study on adhesion molecules postulated that adhesion molecules in circulation facilitates the metastatic process of the neoplasms [16]. Kuzu et al. showed that VCAM is found only in tumor tissues and not in normal tissues [15]. Banks reported elevated VCAM-1 levels in serum of 110 cancer patients for the first time [16].

There is a connection between VEGF and VCAM [20]. Studies reported on an upregulation of VCAM-1 after VEGF stimulation of endothelial cells [20]. Most of the studies concluded that augmented levels of VCAM-1 are associated with advanced oncogenesis, as for instance in stage 4 breast cancer patients [19,21]. Furthermore, the development of lymphedema after breast cancer surgery also correlated with VCAM expression and VCAM was markedly increased after a dose-dense regimen of chemotherapy [21]. Similar findings could be obtained in colorectal cancer patients, where VCAM was revealed as a biomarker for overall survival and post-operative recurrence [20]. Beside the utilization of VCAM-1 as a marker for cancer progression, blockade of endothelial VCAM-1 adhesive functions appears promising to interfere with cancer diseases [19]. However, direct blocking approaches of VCAM-1 by small molecules or antibodies have not been described yet. Hence, several drugs with miscellaneous indications exhibit anti-adhesive effects on T cells due to a downregulation of VCAM-1 and other adhesion molecules on endothelial cells [19].

Our study showed that the difference of VCAM levels between the patient and the control groups were statistically insignificant ($p=0.602$). With this result, we believe that the VCAM molecule is not sufficiently sensitive and specific to be a tumor marker, but to make distinction with other positive studies, future studies are needed.

We also found out that VCAM levels between disease stages were statistically significant ($p<0.0001$). VCAM levels were also significant between the stages on the basis of lymph node metastasis.

Outcome of the study showed that VCAM levels were not specific and sensitive to use as a tumor marker but it may be a valuable factor to determine the prognosis and tumor aggression in cancer patients.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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References

- Sauter EM, Nesbit M, Watson JC, Klein-Szanto A, Litwin S, Herlyn M. Vascular Endothelial Growth Factor is a marker of invasion and metastasis in squamous cell carcinomas of the head and neck. *Clin Cancer Res.* 1999;5(4):775-82.
- Tae K, El-Naggar AK, Yoo E, Feng L, Lee JJ, Hong WK. Expression of vascular endothelial growth factor and microvessel density in head and neck tumorigenesis. *Clin Cancer Res.* 2000;6(7):2821-8.
- Chen Z, Malhotra PS, Thomas RG, Ondrey FG, Duffey DC, Smith CW, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res.* 1999;5(6):1369-79.
- Teknos NT, Cox C, Yoo S, Chepeda BD, Wolf GT, Bradford CR, et al. Elevated serum vascular endothelial growth factor and decreased survival in advanced laryngeal carcinoma. *Head Neck.* 2002;24(11):1004-11.
- Ninck S, Reisser C, Dyckhoff G, Helmke B, Bauer H, Herold-Mende C. Expression profiles of angiogenic growth factors in squamous cell carcinomas of the head and neck. *Int J Cancer.* 2003;106(1):34-44.
- Senger DR, Van de Water L, Brown LF, Nagy JA, Yeo KT, Yeo TK, et al. Vascular permeability factor in tumor biology. *Cancer Metastasis Rev.* 1993;12(3-4):303-24.
- Eisma RJ, Spiro DJ, Kreutzer DL. Role of angiogenic factors: coexpression of interleukin-8 and vascular endothelial growth factor in patients with head and neck cell carcinoma. *Laryngoscope.* 1999;109(5):687-93.
- Petruzelli GJ, Benefield J, Taitz MD, Fowler S, Kalkanis J, Scobercea S, et al. Heparin binding growth factors derived from head and neck squamous cell carcinomas induce endothelial cell proliferation. *Head Neck.* 1997;19(7):576-82.
- Neufeld G, Cohen T, Gitay-Goren H, Poltorak Z, Tessler S, Sharon R, et al. Similarities and differences between the vascular endothelial growth factor splice variants. *Cancer Metastasis Rev.* 1996;15(2):153-8.
- Kato H, Yoshikawa M, Miyazaki T, Nakajima M, Fukai Y, Masuda N, et al. Vascular permeability factor and its receptors in esophageal squamous cell carcinomas. *Anticancer Res.* 2002;22(6C):3977-84.
- Shih CH, Ozawa S, Ando N, Ueda M, Kitajima M. Expression of vascular growth factor expression predicts outcome and lymph node metastasis in squamous cell carcinomas of the oesophagus. *Clin Cancer Res.* 2000;6(3):1161-68.
- Zhao ZH, Tian Y, Yang JP, Zhou J, Chen KS. RhoC, vascular endothelial growth factor and microvascular density in esophageal squamous cell carcinoma. *World J Gastroenterol.* 2015;21(3):905-12.
- Bowden J, Brennan PA, Umar T, Cronin A. Expression of vascular endothelial growth factor in basal cell carcinoma and cutaneous squamous cell carcinoma of head and neck. *J Cutan Pathol.* 2002;29(10):585-9.
- Mineta H, Miura K, Ogino T, Takebayashi S, Misawa K, Ueda Y. Vascular endothelial growth factor expression correlates with p53 and Ki-67 expression in tongue squamous cell carcinoma. *Anticancer Res.* 2002;22(2B):1039-44.
- Kuzu I, Bicknell R, Fletcher CDM, Gatter K. Expression of adhesion molecules on the endothelium of normal tissue vessels and vascular tumours. *Lab Invest.* 1993;69(3):322-8.
- Banks RE, Gearing AJH, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule-1, E-selectin and vascular cell adhesion molecule in human malignancies. *Br J Cancer.* 1993;68(1):122-4.
- Liu CM, Sheen TS, Shun CT. Circulating intercellular adhesion molecule -1, E-selectin and vascular cell adhesion molecule 1 molecule in head and cancer. *Br J Cancer.* 1999;79(2):360-2.
- Torabinia N, Razavi SM, Tahririan D. Vascular endothelial growth factor expression in normal, dysplastic and neoplastic squamous epithelium of oral mucosa. *J Pioneer Med Sci.* 2014;4(3):115-8.
- Eggeman H, Stöblen F, Thill M, Korlach S, Schmid P, Lüftner D, et al. Influence of a dose-dense adjuvant chemotherapy on sVCAM-1/sICAM-1 serum levels in breast cancer patients with 1-3 positive lymph nodes. *Anticancer Res.* 2011;31(8):2617-22.
- Dymicka-Piekarska V, Guzinska-Ustymowicz K, Kuklinski A, Kemona H. Prognostic significance of adhesion molecules (sICAM-1, sVCAM-1) and VEGF in colorectal cancer patients. *Thromb Res.* 2012;129(4):47-50.
- Miaskowski C, Dodd M, Paul SM, West C, Hamolsky D, Abrams G, et al. Lymphatic and angiogenic candidate genes predict the development of secondary lymphedema following breast cancer surgery. *PLoS One.* 2013;8(4):e6016.

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