Considering Vascular Endothelial Growth Factor Elevation in Sera as Marker for Oral Squamous Cell Carcinoma Metastasis

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Abstract

Background: Angiogenesis is the most important contributor for tumor growth and metastasis. Vascular endothelial growth factor (VEGF) is thought to be one of the most contributing angiogenic factors and selective mitogen for vascular endothelial cells. This study was conducted to determine the circulating levels of VEGF in histopathologically proven well defined squamous cell carcinomas of the oral cavity with no metastasis and to compare with serum levels of patients with moderately and poorly differentiate squamous cell carcinomas with metastasis.

Methodology: This study included 20 patients with no metastasis, 20 patients with metastatic squamous cell carcinoma, and 20 healthy individuals as control. The values of healthy volunteers and patients with oral squamous cell carcinoma were compared for VEGF levels only.

Results: Statistical analysis showed that mean VEGF levels in the sera of oral squamous cell carcinoma patients with metastasis were significantly higher than that in patients with no metastasis. Moreover, serum VEGF level was understandably higher in both the groups than in controls.

Conclusion: It may be safe to conclude that VEGF elevation in the serum can be a marker in case of metastatic oral squamous cell carcinomas.

Keywords: Angiogenesis, metastasis, oral squamous cell carcinoma, vascular endothelial growth factor

INTRODUCTION

Multiple studies have proven that vascular endothelial growth factor (VEGF) increases angiogenesis in many tumors, including colorectal carcinoma, breast cancer, liver cancer, lung malignancies, and malignant melanoma. Angiogenesis is the formation of new capillaries by budding of endothelial cells residing in the surrounding preexisting vessels. VEGF induces the permeability of fluids and proteins 50,000 times more than histamine. VEGF potently increases microvascular permeability, stimulates endothelial cell proliferation, and induces proteolytic enzymes and migration of endothelial cell and monocytes, which are essential for angiogenesis. Various factors secreted by tumor cells can regulate angiogenesis of solid tumors. Angiogenic factor VEGF, also called as vascular permeability factor, is the most potent one for induction of the angiogenesis in tumor growth. Tumors cannot exceed 1–2 mm volume without developing neovascularization. It is known that metastasis of malignant tumors depends on neovascularization too. An in vitro study showed that oral squamous cell carcinoma cell line could promote angiogenesis via expression of VEGF and upregulation of their receptor KDK/flk-1 expression in endothelial cells. One study proved that increased levels of VEGF in oral squamous cell carcinoma were associated with microvessel density in tumor. Not much explained on the serum levels of VEGF in literature, with patients having metastatic oral squamous cell carcinoma. The purpose of this study is to evaluate the possibility of VEGF as a marker for metastasis of oral squamous cell carcinoma into deeper planes, lymph nodes, or distant places.

MATERIALS AND METHODS

This study consists of three groups. The first group consists of 20 patients, irrespective of the age and sex, devoid of any other systemic illness. Patients confirmed well-differentiated squamous cell carcinoma by histopathological diagnosis and failed to show any signs of metastasis clinically, cytologically (fine-needle aspiration cytology lymph nodes), and radiographically (positron emission tomography-computed tomography of head and neck) are included (T1 N0 M0 only).
Those who had positive metastatic nodes after performance of prophylactic supraomohoid neck dissection or any other signs of metastasis during the definitive therapy are excluded from this group. The second group consisted of 20 patients with histopathologically proven moderately or poorly differentiated squamous cell carcinoma. Patients in this group varied in TNM staging but definitively proved nodal metastasis or metastasis before definitive treatment. The third group consisted of 20 healthy adults who volunteered for this as controls. Care was taken to rule out possible substance dependence in healthy individuals, and such individuals were not included in the third group.

**Collection of samples and determination of serum levels of vascular endothelial growth factor**

Five milliliters blood from all subjects was drawn from the peripheral vein into a tube containing EDTA. The patients asked to fast for 12 h before obtaining the sample and it was obtained presurgically. This blood samples were centrifuged at 3000 rpm for 10 min immediately to obtain serum sample and then stored for analysis. Samples with hemolysis were discarded. The concentration of VEGF was determined using commercially available enzyme-linked immunosorbent assay kit (R&D system Minneapolis, USA), as instructed by the manufacturer.

**RESULTS**

The first group of 20 patients included 10 males and 10 females. Group 2 consisted of nine males and 11 females. Patients with squamous cell carcinoma metastasis consisted of 12 male and eight female patients [Table 1]. Mean age of the healthy controls was 43.6 years. In patients with oral squamous cell carcinoma without metastasis, the mean age was 52.8 years, whereas in a group of patients with carcinoma metastasis, the mean age was 56.3 years [Table 2].

When comparisons made within the group on VEGF levels based on gender, values were statistically insignificant.

**DISCUSSION**

VEGF, also known as vascular permeability factor or vasculotropin, belongs to platelet-derived growth factor family. It was described by Ferrara and Gispharowiz in 1989. Several members of VEGF family have been described including VEGF-A, PIGF, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. VEGF-A is a dimeric glycoprotein which has at least nine subtypes due to the alternative splicing of the single gene; VEGF 121, VEGF 145, VEGF 148, VEGF 165b, VEGF 183, VEGF 189, and VEGF 206. VEGF has a role in physiological (menstrual cycle, pregnancy, and wound healing) and pathological conditions (arthritis, diabetes, psoriasis, etc.). Memory and learning have also contribution from VEGF. Exaggerated levels of VEGF-A have been detected in the tissues and biological samples from the subjects of asthma where these levels correlate directly with disease. In case of malignant tumor cells, four VEGF isoforms, including 121,165, 189, and 206 amino acid residues, have been well described. VEGF can act as a vascular endothelial cell motogen and mitogen and as a

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**Table 1: Gender frequency and percentage**

| Groups | Males (%) | Females |
|--------|-----------|---------|
| Group 1 | 10 (50)   | 10 (50) |
| Group 2 | 9 (45)    | 11 (55) |
| Group 3 | 12 (60)   | 8 (40)  |

**Table 2: Comparison of age among the three groups**

|                          | n   | Mean   | SD    | SE   |
|--------------------------|-----|--------|-------|------|
| Healthy controls         | 20  | 43.600 | 8.94074| 1.99921|
| Without metastasis       | 20  | 52.850 | 11.05620| 2.47224|
| With metastasis          | 20  | 56.350 | 9.05698| 2.02520|
| Total                    | 60  | 50.9333| 10.99748| 1.41977|

|                          | 95% CI for mean | Minimum | Maximum |
|--------------------------|-----------------|---------|---------|
| Healthy controls         | 39.4156, 47.7844| 31.00   | 61.00   |
| Without metastasis       | 47.6755, 58.0245| 35.00   | 70.00   |
| With metastasis          | 52.1112, 60.5888| 43.00   | 73.00   |

F=9.162, P=0.001 HS. HS: Highly significant, CI: Confidence interval, SE: Standard error, SD: Standard deviation
mediator of increasing vascular permeability by binding to specific receptors.[13]

Recent advances suggest major role of VEGF in the metastasis and tumor growth making us design this study.

Studies have shown that VEGF was overexpressed by a variety of human cancer tissues. VEGF protein levels were the important prognostic factor in the study conducted by Smith et al. in cohort of patients with oral and oropharyngeal squamous cell carcinomas.[14] Shemirani and Crowe also confirmed that head and neck squamous cell carcinoma lines could produce biologically active angiogenic factors, including VEGF and fibroblast growth factor by immunoprecipitation.[15]

One study notes that serum VEGF concentration in the patients with lung cancer could reach as high as 664 pg/ml.[16] In contrast, it is interesting to know that serum VEGF did not increase in patients with angiosarcoma and was not useful in diagnosing lung cancer.[17]

Kazakydasan et al. in a March 2017 article concluded that high expression of VEGF-C in the primary tumor may be a good determinant for detection of occult tumor cells in the lymph nodes of oral squamous cell carcinoma cases.[19] Another research paper also noted strong podoplanin and VEGF-C expression by malignant cells is associated with perineural invasion in patients with oral squamous cell carcinoma.[19] Kim et al. stated that in VEGF quantitative RT-PCR analysis, progressive cancer showed more VEGF expression than carcinoma in situ.[20] A study performed on oral squamous cell carcinoma patients in Taiwan to assess expression of VEGF and to evaluate the possible influence on progression concluding VEGF as biomarker for prediction of progression.[21] These findings are similar to our study which showed definitive excess presentation of VEGF in oral squamous cell carcinoma. The mechanism by which VEGF participate in carcinogenesis is well known. It has been reported that VEGF is a selective mitogen for vascular endothelial cells and may directly simulate the development of the new blood vessels. At the same time, elevation of VEGF will result in extravasation of plasma proteins (fibrinogen) into the extravascular space through increasing microvascular permeability. Then, extravasated fibrinogen clots and other proteins, such as fibronectin, may be incorporated into fibrin clot which is an essential matrix for endothelial cell growth. In this study, VEGF levels in the sera of squamous cell carcinoma patients were significantly higher than in controls. This finding can be correlated with findings of the other authors as described. With regard to the relationship between VEGF and squamous cell progression, it is found that serum VEGF concentration correlated with regional lymph node metastasis. This is similar to the study showing patients with VEGF-positive gastric carcinoma having poor prognosis than those with VEGF-negative gastric carcinoma patients by Maeda et al.[22] Higher levels of serum VEGF were closely associated with lymph node metastasis in this study which correlates with pathophysiology of carcinogenesis and participation of VEGF.

**Conclusion**

The present study suggests that serum VEGF concentration was obviously increased in the patients with oral squamous cell carcinoma metastasis when compared with squamous cell carcinoma without metastasis and controls. This suggests that screening for the serum VEGF levels in cases of oral squamous cell carcinoma may be helpful for the clinicians to have a clue on possibility of metastasis. Elevated levels of VEGF may help in planning a better definitive treatment effecting prognosis.
Better understanding of possible metastasis may help the specialist in drawing a definitive treatment plan even in cases of nil clinical evidence.

Further, detailed study of the subject with wider sample size, which may help in drawing the range for serum VEGF levels suggestive of metastasis, was suggested. It is also possible to have elevated levels of VEGF in other diseases or physiological condition like pregnancy, which may mask the carcinoma spread. Hence, this might be another major limitation for considering VEGF alone as a marker for metastasis. In our study, care was taken to identify the patients across all the groups devoid of any other systemic conditions. At present, it might be safer to conclude that VEGF levels may be considered as one of the markers for diagnosis of oral squamous cell carcinoma metastasis.

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Conflicts of interest
There are no conflicts of interest.

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