**Serum Level of Vascular Endothelial Growth Factor-c in Patients with Head and Neck Squamous Cell Carcinoma**

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**Abstract:** Vascular endothelial growth factor-c (VEGF-C) is a potent lymphangiogenic factor and play a crucial role in the regulation of tumor growth and metastasis. Using an ELISA kit, we assessed the circulating levels of VEGF-C in sera from 25 head and neck squamous cell carcinoma (HNSCC) patients as well as from 14 healthy controls. The serum VEGF-C level in HNSCC patients was significantly higher (142.6 ± 41.1 pg/ml ) compared with the healthy controls (35.5 ± 8.3 pg/ml, p<0.005). There was no apparent correlation in serum VEGF-C concentration with the clinico – pathological features such as stage, tumor size, nodal status, distant metastasis and histological grade. This result suggests that the measurement of serum VEGF-C concentration can be an adjuvant test for establishing the diagnosis of HNSCC beside the other tests.

**Key words:** VEGF-C. Serum. Head and neck squamous cell carcinoma. ELISA. Cytokine.

**INTRODUCTION**

Head and neck squamous cell carcinomas (HNSCC), the sixth most common malignancy throughout the world, originate from the epithelial tissue of the upper aerodigestive tract including the oral cavity, pharynx, and larynx (Mojtahedi, et al., 2010). Despite improvements in diagnosis and conventional therapies, long-term survival rates in HNSCC patients have not increased significantly over the past three decades (Mojtahedi, et al., 2010). These carcinomas are characterized by a marked heterogeneity in their prognosis, and their pathogenesis is incompletely understood (Mojtahedi, et al., 2010).

Neoangiogenesis, the formation of new capillaries from pre-existing blood vessels, is essential for tumor development. Angiogenic cytokines mediated this process that provides tumors not only with nutrients for growth, but also increase the opportunity for tumor cells to enter the circulation and metastasis. The most potent of these cytokines is vascular endothelial growth factor (VEGF) (George, et al., 2001). The VEGFs are dimeric glycoproteins that share a central cystine residue motif and include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor that bind to specific tyrosine kinase receptors (VEGFR) (Mathur, et al., 2005).

VEGF-A and VEGF-B are involved in vasculogenesis while VEGF-C and VEGF-D are lymphogenic. VEGF-A and B bind to VEGFR-1, while VEGF-B,C and D bind to VEGFR-2. VEGF-C and D bind to VEGFR-3, also known as FIT-4 (Mathur, et al., 2005). In addition to VEGFR-3 expression on lymphatic endothelial cells, the VEGFR-3 (FIT-4) has been shown to be expressed in a variety of human malignancies, including lung adenocarcinoma, colorectal adenocarcinoma, head and neck carcinomas, prostate carcinoma,
leukemia and kaposi's sarcoma. These observations suggest that FIT-4's ligand, VEGF-C, may affect cancer development or progression by direct effect on tumor cells (Su JL, et al., 2006). The association of VEGFR-3 and its ligand, VEGF-C, with lymphangio genesis has provided a possible mechanism for primary tumors to metastasis via newly formed lymphatic and has led to studies investigating VEGF-C and lymphatic spread.

Previous studies suggest that the up regulation of VEGF-C strongly correlates with regional lymph node metastasis in thyroid, prostate, stomach, breast, lung, and oral carcinomas (Kyzas PA, et al., 2005). Most of studies focus on the up regulation of VEGF-C mRNA or protein levels at the cellular level, but not in circulation (Su JL, et al., 2006). In similarity to studies on tumor expression, elevated circulating serum VEGF and VEGF-C levels have also been identified to be predictors for poor long-term prognosis, and associated with advanced tumor stages (Yu XM, et al., 2008). Mitsuhashi et al., reported that comparative assays of circulating VEGF-C / VEGF revealed a high specificity for preoperative diagnosis of lymph node metastasis in cervical carcinoma (Mitsuhashi, et al., 2005).

Also circulating VEGF-C levels may provide additional information for distinguishing between the absence and presence of lymph node metastasis in patients with lung cancer (Tamura and Ohta, 2003).

However, no investigations have been conducted regarding serum VEGF-C levels in patients with head and neck squamous cell carcinoma (HNSCC). The current study examined serum VEGF-C levels in patients with HNSCC to elucidate its association with clinicopathologic parameters.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Shiraz University of Medical Sciences. A total of 25 patients with HNSCC (M:12, F:13, mean age: 65.04±14.94) and 14 healthy subjects residing in the same region (M:6, F:8, age: 62.43 ± 5.44) were enrolled in this study. Healthy people didn't have any systemic or inflammatory disease and all of them were non-smoker. All patients were admitted at ENT department of Khalili hospital in Shiraz, Iran and HNSCC was diagnosed histopathologically in all of these patients. Patients with other malignancies were excluded. Control group consists of healthy blood donor with no existence of an inflammatory disease or infection. Serum samples were obtained from clotted blood following centrifugation at 4°C and were then stores at -80°C until analysis.

Concentration of VEGF-C was measured by enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions.

Independent t-test and chi-square were performed to compare results of measurement of serum VEGF-C concentrations in two populations of subjects. Spearman test was used to define the relation between serum VEGF-C and clinical data. Differences were considered significant at P-Value < 0.005.

RESULTS AND DISCUSSION

Table - 1 shows the clinical data of the patients assayed for serum VEGF-C level. There were 25 patients with HNSCC, including 12 male (48%) and 13 female (52%).

At the time of presentation, none of patients were in stage I, II patients (44%) were in stage II, 7 patients (28%) in stage III and 7 patients (28%) were in stage IV. No distant metastasis was detected in the patients.

Thirteen tumors (52%) were well-differentiated, seven (28%) were moderately differentiated and 5(20%) were poorly differentiated. The serum VEGF-C level in HNSCC patients was significantly higher (142± 41.1 pg/ml ; n=25) compared with the healthy control (35.5 + 8.3 pg/ml , n=14 , P <0.005).

There was no significant difference in VEGF-C concentration between males and females, nor was there a correlation in serum VEGF-C concentration with the clinico-pathological features such as stage, tumor size, nodal status, distant metastasis and histological grade.

Table 1: Clinical profiles of 25 HNSCC patients.

| Age (Years) | 65.04±17.03 |
| Sex (male/ female) (%) | 12/13 (48/52) |
| Tumor size (T1, T2, T3, T4) (%) | 1/4/5/5 (4/56/20/20) |
| Regional Lymph node Involvement (N0, N1, N2, N3) (%) | 16/3/6/0 (64/12/24/0) |
| Distant metastases (M0, M1) (%) | 25/0 (100/0) |
| TNM Stage (I/II/III/ IV) (%) | 0/11/7/7 (0/44/28/28) |
| Histological grade (I/II/III) (%) | (13/7/5) (52/28/20) |
Based on data from the patients with HNSCC, Receiver Operating Characteristic (ROC) curve was obtained. The ideal cutoff value of serum VEGF-C concentration was recommended to be 67.4 pg/ml. Using this cutoff value both the sensitivity and specificity were 95%.

VEGF is the most potent of cytokines for tumor angiogenesis (George, et al., 2001). VEGF-C and VEGF-D are known to be involved in lymph angiogenesis (Koyama et al., 2003). VEGF-C may affect cancer development or progression by direct effect on tumor cells (Su JL, et al., 2006).

In human, VEGF-C/ VEGF-D activates VEGFR-2 and VEGFR-3 receptor tyrosine kinases. In various human cancers, a positive correlation was observed between VEGF-C / VEGF-D, VEGFR-3 expression in the primary tumor with lymphatic invasion and lymph node metastasis, implicating their role in the progression of clinical disease (Soumaoro, et al., 2006). Lymph angiogenesis predicted the likelihood of metastasis to distant organs and patient's survival. However, it is not clear whether VEGF-C and tumor lymph-angiogenesis actually promotes tumor metastasis to distant site, or simply serves as an indicator of tumor invasiveness and aggressiveness (Hirakawa, et al., 2007).

In previous studies, it has been found that VEGF-C expression correlated with depth of tumor invasion, tumor stage, venous invasion, lymphatic invasion, lymph node metastasis and histological grade in esophageal cancer (Liu P, et al., 2009). Although previous studies mostly focused on VEGF-C expression, there are a few researching about serum level of VEGF-C especially in HNSCC (Mathur, et al., 2005).

Indeed, some studies have shown that there is no correlation between serum VEGF-C levels and tissue mRNA and protein levels in certain cancers (Mathur, et al., 2005). However, circulating VEGF-C level has been elevated e.g. in lung, liver and cervical cancers (Yu XM, et al., 2008).

We used ELISA kit to measure serum VEGF-C level in patients with HNSCC. To our knowledge, this is the first study using ELISA kit to measure serum VEGF-C concentration, in HNSCC patients. We found that serum VEGF-C level in HNSCC patients was statistically higher than normal control patients (P<0.005). The ideal cutoff value of serum VEGF-C concentration was recommended to be 64.7pg/ml. The sensitivity and specificity of VEGF-C for detecting HNSCC was 95%.

VEGF-C determination has been found to be more accurate marker of lymph node metastasis than chest computed tomography (Krzystek-Korpacka, et al., 2007).

The serum levels of both VEGF-C and VEGF have potential usefulness as biologic marker of SCC of uterine cervix (Mitsuhashi, et al., 2005).

Over expression of VEGF-C in cancer and stromal cells in esophageal SCC and its correlation with some of the clinical and pathological features of the tumors has been confirmed (Krzystek-Korpacka, et al., 2007).

HNSCC metastasis via lymphatic channels to cervical lymph nodes and this regional nodal metastasis is the major cause of locoregional recurrence leading to poor prognosis and a high probability of eventual death from disease (Bock JM, et al., 2008). VEGF-C play a crucial role in the regulation of tumor growth and metastasis (Mitsuhashi, et al., 2005).

However, in the present study there was no apparent correlation in serum VEGF-C concentration with the clinicopathological features, such as stage, tumor size, nodal status and histological grade. In this study all the patients had localized tumor, thus we can't evaluate correlation of VEGF-C level with distant metastasis.

In conclusion, the serum VEGF-C concentration of HNSCC patients was statistically higher than that of controls. This result suggests that the measurement of serum VEGF-C level can be an adjuvant test for establishing the diagnosis of HNSCC beside the other sites.

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Abbreviations:
VEGF-C: Vascular endothelial growth factor-c
HNSCC: Head and neck squamous cell carcinoma
ELISA : Enzyme-linked immunosorbent assay
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