# IMMUNOEXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN LARYNGEAL SQUAMOUS CELL CARCINOMA

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## ABSTRACT

Squamous cell carcinoma is the most common neoplasm of the larynx. There were increasing trend of cases of laryngeal squamous cell carcinoma from year to year in Myanmar. Angiogenesis is a necessary part of the process in the progression of cancer from small, localized neoplasms to larger, growing and potentially metastatic tumors. Thus upregulation of angiogenesis is a key step in sustained tumor growth and may also be critical for tumor metastasis. Among the factors causing tumor angiogenesis, vascular endothelial growth factor (VEGF) is a leading candidate. The aim of study was to determine the association between VEGF immunoexpression and different histological grades of laryngeal squamous cell carcinoma. This study was a cross-sectional descriptive study. IHC staining with VEGF monoclonal antibody was done by using the Peroxidase-Antiperoxidase method. Statistical analysis was done with SPSS 16. The association between VEGF immunoexpression and laryngeal squamous cell carcinoma was tested using ANOVA test. Thirty-seven cases ( 95% ) out of total 41 cases of laryngeal squamous cell carcinoma showed positive VEGF immunoexpression and 2 cases ( 5% ) showed negative VEGF immunoexpression. Minimum immunoexpression score was 2 in well differentiated, 3 in moderately differentiated and 4 in poorly differentiated laryngeal squamous cell carcinoma. Maximum immunoexpression score was the same for all grades (score 6). There was no statistically significant association between VEGF immunoexpression and different histological grades. (p = 0.627). The microvascular density in laryngeal squamous cell carcinoma is higher than the normal laryngeal tissue. In patients with VEGF-positive tumours, extensive microvascular formation correlated significantly with VEGF expression. But the VEGF immunoexpression was occurred irrelative to histological grades. Therefore, the findings of this study suggested that VEGF immunoexpression should be tested in all cases of laryngeal squamous cell carcinoma to predict the prognosis and for choice of treatment option.

***Keywords :*** Vascular Endothelial Growth Factor, Laryngeal Squamous Cell Carcinoma

## INTRODUCTION

Head and neck cancer is common in several regions of the world. Laryngeal cancer is one of the most common types of head and neck cancer. Carcinoma of the larynx accounts for 2.2% of all cancers in men and 0.4% in women[1].

According to data from National Cancer Institute of United States, new cases of larynx cancer were 3.1 per 100,000 people per year and the number of deaths was 1.0 per 100,000 people per year based on 2010 to 2014 cases.[2]

According to the data from Otorhino-laryngology- Head and Neck Surgery Specialist Hospital Yangon, the total number of histologically confirmed laryngeal squamous cell carcinoma were 144 cases out of 424 total cancers in 2014 (33.96%), 150 cases out of 433 total cancers in 2015 (34.64%), 200 cases out of

515 total cancers in 2016 (38.83%).[3]Squamous cell carcinoma is the most common cancer of the larynx accounting for ninety percent of laryngeal cancer. Smoking, alcohol abuse, lower socio-economic status, male and age more than 60 years are increase risks of getting laryngeal squamous cell carcinoma .[4]

The prognosis of laryngeal squamous cell carcinoma depends on the size of the lesion, the level of local invasion, cervical lymphatic spread, and presence of distant metastasis. Histologically, squamous cell carcinoma of the larynx is classified as well, moderately, or poorly differentiated depending on the degree of keratinization and cellular atypia.[5]

Carcinoma of the larynx often manifests clinically as persistent hoarseness, dysphagia and dysphonia. There are various treatments depending on clinical staging. Organ preservation techniques (laser surgery, microsurgery and radiation therapy) are being used with greater frequency, particularly with early disease. Combined chemotherapy and radiation therapy with or without salvage laryngectomy may be required for more advanced or recurrent disease.[6]

Although the advances in surgical techniques, radiochemotherapy and immunotherapy greatly improved, the overall survival rate of the patients, postoperative recurrence and metastasis are still the main problems in laryngeal squamous cell carcinoma.[7]

Angiogenesis that is the formation of new capillary blood vessels which originate from a pre-existing vasculature have to begin. VEGF is responsible for vasculogenesis, endothelial cell proliferation and migration. Beginning of the angiogenic process, the activation of some proteolytic enzymes cause stromal decomposition, thereby increasing vascular permeability and causing tumor invasion and metastasis.[8]

Angiogenesis is a necessary part of the process in the progression of cancer from small, localized neoplasms to larger, growing and potentially metastatic tumors. When tumor grows beyond 1 to 2 mm in diameter, it needs an independent blood supply, which is acquired by the expression of growth factors that recruit new vasculature from existing vessels. This process continues even as the tumor matures. Thus upregulation of angiogenesis is a key step in sustained tumor growth and may also be critical for tumor metastasis.[9]

Among the factors causing tumor angiogenesis, vascular endothelial growth factor (VEGF) is a leading candidate. VEGF is a member of a family of 6 structurally related proteins that regulate the growth and differentiation of multiple components of the vascular system, especially blood and lymph vessels. The angiogenic effects of the VEGF family are thought to be primarily mediated through the interaction of VEGF with VEGFR-2. Recently, targeted cancer herapy has spurred attention to angiogenesis inhibitors.[10]

Both VEGFR-1 and VEGFR-2 can promote angiogenesis and VEGFR-3 stimulation leads to lymphangiogenesis. Binding with VEGFs leads to dimerization of VEGFRs and activation of downstream signaling cascades. Activation of the VEGF/VEGFR pathway promotes endothelial cell growth, migration and survival. This pathway also mediates vessel permeability and mobilizes endothelial progenitor cells. There is a general consensus that VEGFR-2 is the dominant receptor in mediating pro-angiogenic functions of VEGF-A and this pathway has been

prioritized for the development of antiangiogenic therapies.[11]

This study was aimed to detect the immunoexpression of VEGF in different histological grades of laryngeal squamous cell carcinoma. VEGF overexpression in laryngeal squamous cell carcinoma would predict tumour angiogenesis, higher grade and worse prognosis. This would support the clinician in considering the usage of anti-angiogenic tumour specific therapy.

## MATERIALS AND METHODS

This study was a cross-sectional descriptive study. This study was carried out within one year from July 2017 to June 2018. Study population was all new cases of laryngeal squamous cell carcinoma admitted to Otorhinolaryngology and Head and Neck Surgery Specialist Hospital, Yangon during study period. All new cases of histologically proven laryngeal squamous cell carcinoma were included in this study.

Haematoxylin and Eosin stained tissue sections were carefully examined using ordinary light microscope. Grading of laryngeal squamous cell carcinoma was done. Results were noted down in pro forma. Then the representative paraffin wax blocks were further processed for IHC staining with VEGF monoclonal antibody by using the Peroxidase-Antiperoxidase method. The immunoreactivity results were interpreted in relation to positive and negative control and recorded in proforma. Scoring for immune-positivity was calculated and noted down in pro forma.

### Interpretation of immuohistochemical result

VEGF immunoexpression was seen as brownish cytoplasmic staining of the cells of laryngeal squamous cell carcinoma. VEGF immune-expression was scored according to the percentage of immunopositive cells and the staining intensity.[12]

### Percentage of cells stained

1000 tumor cells were assessed in ten high power fields (40x) for the presence or absence of staining.

Score 0 = 0% positive tumor cells
Score 1 = 1-25% positive tumor cells
Score 2 = 26-50% positive tumor cells
Score 3 = ≥ 51% positive tumor cells

**Intensity of staining**

Score 0 = negative
Score 1 = weakly positive (light brown)
Score 2 = moderately positive (brown)
Score 3 =strongly positive(dark brown).

The overall immmunoreactivity score was calculated as follows.

Immunoreactivity = percentage score + score (IRS) intensity score

In this study, it will be considered

Positive immunoexpression - IRS is >2
Negative immunoexpression - IRS is 0-2

## RESULTS

Total thirty nine cases were included in this study. In this study, 1 case (2.6%) was seen in 20-39 years age group, 10 cases ( 25.6% ) were found in 40-59 years age group and the remaining 28 cases (71.8%) were >60 years of age. The youngest age of patient was 37 years and the oldest age was 80 years. Majority of the patients were more than 60 years of age. There were 31 male patients (79%) and 8 female patients (21%). Male to female ratio was approximately 4:1.

In this study, laryngeal squamous cell carcinoma was seen mostly in supraglottis (48.7%) and glottis (35.9%). Subglottis involvement was seen in 4 cases (10.3%) and more than one subside was 2 cases (5.1%). There were 14 cases (36%) of well differentiated squamous cell carcinoma, 19 cases (49%) of moderately differentiated squamous cell carcinoma and 6 cases (15%) of poorly differentiated squamous cell carcinoma.

Thirty-seven cases ( 95% ) out of total 41 cases of laryngeal squamous cell carcinoma showed positive VEGF immunoexpression and 2 cases (5%) showed negative VEGF immune-expression. Minimum immunoexpression score was 2 in well differentiated, 3 in moderately differentiated and 4 in poorly differentiated laryngeal squamous cell carcinoma. Maximum immunoexpression score was the same for all grades (score 6). There was no statistically significant association between VEGF immunoexpression and different histological grade. (p = 0.627).

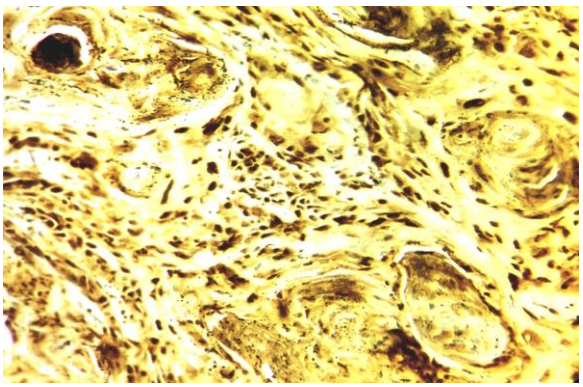

Figure (1). Case no.18: Strongly positive VEGF immunoexpression (Score 6) (IHC stain x 400)

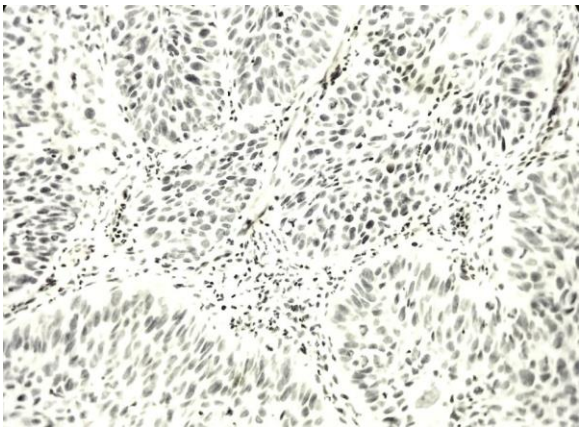

Figure (2). Case no.33: Negative VEGF immunoexpression (Score 1) (IHC stain x 400)

Table (1) Association between VEGF immunoexpression scoring and histological grades of laryngeal squamous cell carcinoma

| Histological grades | N | Mean | Standard Deviation | Minimum score | Maximum score |
|---|---|---|---|---|---|
| well differentiated | 14 | 4.64 | 1.499 | 2 | 6 |
| moderately differentiated | 19 | 4.63 | 1.065 | 3 | 6 |
| poorly differentiated | 6 | 5.17 | .983 | 4 | 6 |
| Total | 39 | 4.72 | 1.213 | 2 | 6 |

## DISCUSSIONS

The age of the patients in present study was comparable to these previous studies of May-Thet-Tun (2017)[19] , Kyaw-Khine-Win (2011)[20] and Vlachtsis et al., (2005)[21].

The findings of these previous studies were consistent with the result in the present study which also showed male preponderance.

Male preponderance is probably due to alcohol consumption and smoking habits. Alcohol

consumption has a stronger relation to laryngeal cancer compared to smoking.[13]

Having of alcohol consumption and smoking habits were not taken from the participants so this association could not be analysed. This factor should be taken for consideration in further studies on laryngeal cancer.

Among the study population in this study, laryngeal squamous cell carcinoma was seen mostly in supraglottis (48.7%) .In the study by May-Thet-Tun in 2017, the most frequent site was supraglottis (70%)[19]. In Kyaw-Khine-Win's study in 2011, the commonest site was supraglottis (58.4%)[20]. Vlachtsis et al. (2005) also stated that there were 46 % in supraglottis.[21] Both previous studies and the present study pointed out that supraglottis was the most common site for laryngeal cancer.

Thirty seven cases (95%) of laryngeal squamous cell carcinoma showed positive VEGF immunoexpression and 2 cases (5%) showed negative VEGF immunoexpression in this study.

Sullu et al in 2010 found, 10 cases (7%) of laryngeal squamous cell carcinoma were VEGF immunoexpression negative and 130 cases (93%) were VEGF immunoexpression positive in their study.[14]

Rueda et al (2010) showed that there were 10 cases (17%) of negative VEGF immunoexpression and 49 cases (83%) were positive VEGF immunoexpression in their study.[15]

Both previous studies showed that most of the laryngeal squamous cell carcinoma were VEGF immunoexpression positive. Therefore, the present study was conclusively consistent with those previous studies. Among positive cases, 12 cases were well differentiated laryngeal squamous cell carcinoma, 19 cases were moderately differentiated laryngeal squamous cell carcinoma and 6 cases were poorly differentiated laryngeal squamous cell carcinoma. Two cases with negative VEGF immunoexpression were well differentiated laryngeal squamous cell carcinoma.

In this study, immunoexpression of VEGF in laryngeal squamous cell carcinoma was tested with ANOVA test. Appropriate statistical analysis was done by using SPSS 16. Minimum immunoexpression score is 2 in well differentiated, 3 in moderately differentiated and 4 in poorly differentiated laryngeal squamous cell carcinoma. Maximum immunoexpression score was the same for all grades (score 6). There was no statistically significant association between VEGF immunoexpression and different histological grade. ($p = 0.627$)

In a study by Riedel et al, in 2000 there were 2 cases of VEGF immunoexpression positive and 1 case of VEGF immune-expression negative in well differentiated squamous cell carcinoma (Grade 1). There were 12 cases of VEGF immunoexpression positive and 10 cases of VEGF immune-expression negative in moderately differentiated squamous cell carcinoma (Grade 2). There were 4 cases of positive VEGF immunoexpression and 4 cases of negative VEGF immunoexpression. There was no correlation between VEGF immune-expression and histological grades of tumours ($p = 0.885$).[16]

In the study of Akdeniz et al,in 2013 the expression of VEGF was positive in 52.63% of well differentiated and 60% of moderately or poorly differentiated tumours. The relationship between the expression of VEGF and differentiation of tumour revealed no significant differences ($p=0.2$).[17]

Most of the previous studies showed findings consistent with this the present study but there were different finding in some studies. In the study by Wang et al in 2016, VEGF expression was closely related to the invasion, depth and differentiation degree ($p < 0.01$).[18] Sullu et al (2010) stated that they used ANOVA test to evaluate the relation between tumour grade and expression score of VEGF and significant correlation was found in their study ($P<0.05$). [14]

Significant association was not found between VEGF immunoexpression and histological grades. Immunoexpression was positive in almost all cases whether histological grade is low or high. It pointed out that immunoexpression of VEGF can be detected in most cases of laryngeal squamous cell carcinoma.

The treatment of LSCC has traditionally been total laryngectomy and radiotherapy. Conservative approaches have been introduced with a view to sparing the larynx (given its crucial role in breathing, speech, and swallowing) and preserving most of its functions without jeopardizing oncological outcomes. Very recent research has focused on targeted molecular therapy, immunotherapy, and latent or residual tumor cell ablation. The latest scientific literature has shown great interest in vascular targeting therapy based on neoplastic antigens.

Bevacizumab, a humanized monoclonal antibody against VEGF, has already been adopted in clinical practice, combined with standard chemotherapy, to treat several human malignancies. VEGF receptor tyrosine kinase inhibitors, such as sunitinib and sorafenib, have also been used in monotherapy for metastatic or advanced-stage diseases.

The second main anti-angiogenic approach is based on evidence that targeting the tumor vasculature with a view to occluding or destroying the blood vessel can significantly reduce the neoplasm's volume. This is due to the reduced oxygen and nutrient flow through the tumor mass and the consequent ischemic necrosis. It has been demonstrated, however, that vascular targeting agents (VTAs) are more effective for early malignancies than for advanced stage disease.

The microvascular density in laryngeal squamous cell carcinoma is higher than the normal laryngeal tissue. In patients with VEGF-positive tumours, extensive micro-vascular formation correlated significantly with VEGF expression. But the VEGF immunoexpression was occurred irrelative to histological grades.

Therefore, the findings of this study suggested that VEGF immunoexpression should be tested in all cases of laryngeal squamous cell carcinoma to predict the prognosis and for choice of treatment option. Since this study was a cross-sectional study with a small sample size, the findings showed no significant association between VEGF immunoexpression and different histological grades. Further studies with larger sample size and longer study period are recommended to find out whether there is significant association between VEGF immunoexpression and histological grades of laryngeal squamous cell carcinoma.

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