

# Vascular Endothelial Growth Factor (VEGF) Expression in Normal, Dysplastic and Neoplastic Squamous Epithelium of Oral Mucosa

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## ABSTRACT

**BACKGROUND:** VEGF (vascular endothelial growth factor) is one of the growth factors, which directly affects vascular endothelial cells and evokes proliferation, migration and chemotaxis of endothelial cells. Studies have provided inconsistent results about the expression of VEGF in the squamous cell carcinoma (SCC) or oral mucosa. The purpose of the present study was to assess the frequency of VEGF expression in oral normal, dysplastic and SCC mucosa

**METHODS:** In this analytical-descriptive study, formalin fixed and paraffin embedded tissue of 20 normal mucosa, 20 dysplastic mucosa, and 20 SCC were studied for the expression of VEGF, using immunohistochemical technique and standard Biotin Streptavidin method. The results were analyzed by Kruskal Wallis and

Mann Whitney tests.

**RESULTS:** The mean expression of VEGF in SCC was 24.65, 16.35 in dysplastic mucosa and 15.5 in normal mucosa. VEGF expression was significantly higher in the SCC group as compared to the dysplastic group ( $p=0.02$ ) or normal group ( $P=0.001$ ). On the other hand, there was no difference in the VEGF expression between the dysplastic mucosa and normal mucosa groups ( $p=0.11$ )

**CONCLUSION:** VEGF expression is significantly higher in the SCC group as compared to dysplastic and normal mucosa. Our results highlight the importance of vascular development in the development of SCC or oral mucosa and suggest possible therapeutic modalities targeted to VEGF and/or vascular development.

Keywords: Immunohistochemical; VEGF; Dysplastic mucosa; SCC; normal mucosa.

## INTRODUCTION

Squamous cell carcinoma (SCC) is by far the most common malignancy of head and neck region with an estimated 5-year survival rate of 40% upon diagnosis. Multifactorial etiology has made this sinister pathology a poor candidate for therapy, with an added burden of poor quality of life despite treatment [1,2]. The characterization of molecular targets involved in initiation, progression and metastasis of SCC can play a vital role in devising the future therapeutics based on these oncotargets [3].

Vascular endothelial growth factor (VEGF) is a protein, which directly affects vascular endothelial cells, stimulates the proliferation of

endothelial cells, and helps in chemotaxis of macrophages and granulocytes. Since malignant cells are rapidly dividing cells and nutrients are required in higher quantities, neovascularization, possibly via the overexpression of VEGF is needed. Previous studies have shown that VEGF is the main factor in neovascularization leading to tumor growth and metastasis [4-7].

Studies have shown that, as compared to normal squamous epithelium, the dysplastic and neoplastic mucosa have high expression of VEGF [8,9]; while other studies have found little or no correlation between VEGF expression and vascularity in oral cancers [10,11,12]. In order to examine the association between VEGF overexpression and malignant SCC, we

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compared the VEGF expression among normal, dysplastic and neoplastic mucosa [11,12].

## METHODS AND MATERIALS

This study was conducted in Department of Oral Pathology, Isfahan University of Medical Sciences dental school. Sixty samples of oral squamous epithelium from sixty individuals were obtained from the archives of the pathology laboratory, 20 in each group (normal, dysplastic and neoplastic). Only samples that had sufficient tissue and little inflammation were considered for this study. The normal mucosae were obtained from the patients who had undergone a dental implant with no mucosal pathology. Our study protocol was approved by Isfahan research and ethical committee.

After standard laboratory protocol for sample handling, the tissues were immunohistochemically stained with the biotin-streptavidin (B-SA) method to detect the specific VEGF antigen [10]. Briefly, the main procedure included serial sectioning (in 3-4  $\mu\text{m}$  sections), deparafinization, rehydration, and antigen retrieval. All specimens were placed in phosphate- buffered saline [PBS], treated with protein block (RS 1102) for 5 minutes to prevent any false staining, and then the specimens were incubated for 30 minutes with primary antibody of VEGF clone VG1 (DAKO, Denmark). Further, the sections were exposed to Novolink polymer (RE7112) or secondary antibody for 30 minutes and washed in PBS. Then they were incubated with DAB (Diamino-benzidin) for 5 minutes for visualization, and after washing, the slides were counter-stained with hematoxylin and finally after drying, the slides were mounted. The sections were observed by two pathologist using 100 and 400 magnifications of light microscope. Cells which were positive for VEGF were scored.

Five non- overlapping fields were selected randomly and based on endothelial stain intensity each specimen was assigned a specific score. Where the epithelial cytoplasm stained stronger than endothelial cell, the field score would be 3 where it was in good harmony with endothelial cell it would be 2 and if it was weaker than endothelium it would receive a score of 1. The score of 0 was recorded if it was not stained. [10] Finally the mean intensity of 5 fields in one specimen recorded. The data were statistically analyzed by Mann Whitney and Kruskalwallis tests (SPSS, ver.10.0).

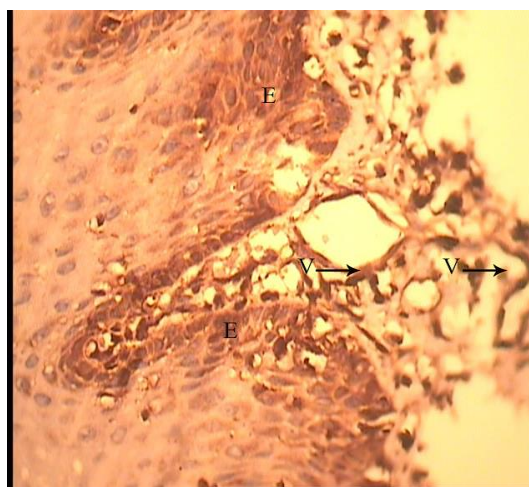
## RESULTS

The study population consisted of 60 paraffin embedded tissues of 20 normal mucosa, 20 dysplastic mucosa and 20 SCC. Immunohistochemical examination found positive reaction for VEGF in all samples of SCC, dysplastic, and normal mucosa. The mean expression of VEGF in SCC was 24.65, 16.35 in dysplastic mucosa, and 15.5 in normal mucosa. VEGF expression was significantly higher in the SCC group as compared to the dysplastic group ( $p=0.02$ ) or normal group ( $P=0.001$ ). On the other hand, there was no difference in the VEGF expression between the dysplastic mucosa and

**Figure 1:** VEGF expression in normal mucosa (x100)



**Figure 2:** VEGF expression in normal mucosa (x400)



## DISCUSSION

Oral SCC is the most common oral cancer. SCC arises from dysplastic epithelial cells and invades the underlying connective tissue in forms of cords and islands [13]. Angiogenesis is a natural essential phenomenon in growth of tumors [4].

The effect of angiogenesis via VEGF on tumor metastasis and prognosis has been well-studied; VEGF directly affects the vascular endothelial cells and promote their proliferation and migration [14].

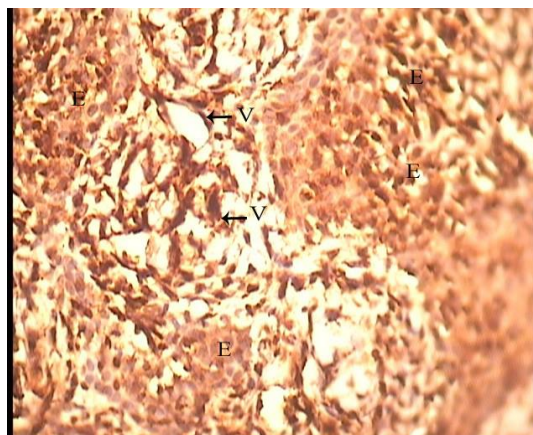
Over expression of VEGF and extensive angiogenesis has been reported in some precancerous oral lesions [6, 14]. About 10-20% of pre-cancerous lesions lead to oral SCC, therefore, recognizing the related molecular mechanism may help to highlight biological progression [15, 16]. In this study normal mucosa, dysplastic mucosa and SCC lesions of oral cavity were evaluated for angiogenic growth factors. To achieve this purpose, VEGF which is one of the important growth factor in angiogenesis, was examined.

The results of this study show that the mean expression of VEGF in SCC is significantly higher than dysplastic or normal mucosa (24.65). Concomitantly, in previous studies, over expression of VEGF in malignant lesions were suggested [9, 12, 17]. Our study validates their findings.

Although the mean VEGF expression in dysplastic mucosa (16.35) was higher than normal mucosa (15.5), the difference was not statically significant ( $P=0.108$ ). A previous study found a significantly higher VEGF expression in dysplastic mucosa as compared to normal mucosa. The authors of that study further noted that VEGF expression may decrease during the tumorigenesis [6]. Thus, their findings are in line with our results that VEGF has a significant role in increased angiogenesis in SCC lesions in comparison with dysplastic and normal mucosa.

In contrast to our and others findings, some studies have found no association between VEGF expression and malignancy. Sauter [18] did not find VEGF overexpression in malignant lesions; while Calile et al [19] stated that VEGF merely has a physiologic role in tumors and no significant role in dysplastic and malignant changes. Moreover, it has been suggested that VEGF overexpression plays significant role in primary transformation of tumors at an earlier stage but later on genetics has greater influence on progression and metastasis [19].

**Figure 3:** VEGF expression in SCC (X400)



## CONCLUSION

In summary, we have shown that SCC has significantly higher expression of VEGF than dysplastic and normal epithelium. This finding confirms the previously reported role of VEGF in neovascularization in malignant cells. Whether VEGF expression is a physiological need of malignant tissues for increased blood flow or it plays some role in malignant transformation needs to be studied.

## REFERENCES

1. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. *N Eng J Med* 1993; 328(3):184-94.
2. Day GL, Blot WL. Second primary tumors in patients with oral cancer. *Cancer J*. 1992; 70(1):14-19.
3. Satoura SH, Yuuji N, Chunnan LI, Mariko M, Kohichi N, Hiroyuki H. HER2/neu expression in oral squamous cell carcinoma *Asian J Oral Maxillofac Surg*. 2004;16:172-176
4. Tammela T, Enholm B, Alitalo K, Paavonen K. The biology of vascular endothelial growth factors. *Cardiovasc Res*. 2005 Feb 15;65(3):550-63
5. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst*. 1990 Jan 3;82(1):4-6
6. Tae K, El-Naggar AK, Yoo E, et al. Expression of vascular endothelial growth factor and microvessel density in head and neck tumorigenesis. *Clin Cancer Res*.2000; 6: 2821–882:4-6.
7. Folkman J. Tumor angiogenesis: Therapeutic complications. *N Eng J Med* 1971(85); 1182-1186.
8. Comon DR, Shelden WF. The significance of hyperemia around tumor implants *Am.J Pathology*. 1946(22):821-26
9. Wang Z, Li R, Zhou B, Liang W, Liang C, Zhang L, Chen Y. Relationships of human laryngeal squamous cell carcinomas with the expression of VEGF-C and VEGFR-3. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*.2009; 26(4):842-6
10. Johnstone S, Logan RM. Expression of vascular endothelial growth factor (VEGF) in normal oral

- mucosa, oral dysplasia and oral squamous cell carcinoma. *Int J Oral Maxillofac Surg.* 2007; 36(3):263-6.
11. El-Gazzar R, Maculskey M, Williams H, Ogden GR. Vascularity and expression of vascular endothelial growth factor in oral squamous cell carcinoma , resection margins, and nodal metastatic. *British J Oral and Maxillofacial Surgery* 2006; 44(3):193-197.
  12. Sun XJ, Ma J, Zhang H, Wang XK, Li JH. The expressions of cyclooxygenase-2 (Cox-2), VEGF in oral squamous cell carcinoma and precancerous lesions and their significances. *Shanghai Kou Qiang Yi Xue.*2005; 14(2):173-6.
  13. Neville B, Damm D, Allen C, Bouquot J. Oral and maxillofacial pathology.2nd ed. Philadelphia:W.B.Saunders; 2009: 449-57,544-47.
  14. Raica M, Cimpeana AM, Ribattib. Angiogenesis in pre-malignant conditions. *European J of Cancer* 2009; 45:1924-1934.
  15. Chaturvedi P, Shan G, Gosseline GJL. Combined modality molecular targeted therapy head/neck squamous cell carcinoma. *Head and neck Oncology.* 2008; 10(1):38-48.
  16. Yamamoto T, Kamata N, Kawano H, Shimizu S, Kuroki T, Toyoshixo K. High incidence of a amplification of the epidermal growth factor receptor gene in human squamous cell carcinoma cell lines. *Cancer Res* 1986; 46(1):414-416.
  17. Pazouki S, Chisholm DM, Adi MM, Carmichael G, Farquharson GR, Ogden GR, et al. The association between tumour progression and vascularity in the oral mucosa. *J Pathol.* 1997; 183(1):39-43.
  18. Sauter ER, Nesbit M, Watson JC, Klein-SzantoAJP, Herlyn M. Vascular endothelial growth factor expression is a marker of tumor invasion and metastasis in squamous cell carcinomas of the head and neck. *Clin. Cancer Res.*1999; 5:775-82.
  19. Calile J, Harada K, Baillie R, Maclusky M. Vascular endothelial growth factor expression in oral tissue: possible relevance to angiogenesis, tumor progression and field cancerisation. *J Oral Pathology Med.* 2001;30 (8):449-57