VOLUME-8, ISSUE-6, JUNE-2019 • PRINT ISSN No. 2277 - 8160

Original Research Paper

Pathology

# STUDY OF MALIGNANCY OF ORAL CAVITY AND PHARYNX WITH SPECIAL REFERENCE TO ETIOLOGICAL FACTORS AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION

| | |
|---|---|
| **Dr. Sumit Ray** | Associate Professor, Dept of Pathology, Medical College, Kolkata |
| **Dr. Manoj Saha*** | Consultant Pathologist *Corresponding Author |
| **Dr. Ira Mondal** | Consultant Pathologist |
| **Dr. Debarati Ray** | Assistant Professor, Dept of Oral Pathology, North Bengal Dental College, Siliguri |

**ABSTRACT** Oral cancer holds the eighth position in cancer incidence worldwide, it is the third most common malignancy in South-Central Asia. SCC encompasses at least 90% of all oral malignancies.

The hallmark of SCC is its fast and infiltrative growth, invasion and destruction of adjacent structure, and very high potential for metastatic lympho-haematogenous spread. Tumor development is a multi-stage process, angiogenesis is essential to tumor growth and metastasis.

We evaluated VEGF expression in tumor tissue in patients with oro-pharyngeal SCC in correlation with local and regional disease relapse, and investigated a relationship between the level of VEGF expression with histological type and tumor grading.

Majority of oral and oropharyngeal malignancy were moderately differentiated SCC. VEGF expression was strongly positive in well-differentiated SCC and weakly positive in poorly differentiated SCC.

**KEYWORDS :** Squamous cell carcinoma (SCC), Vascular endothelial growth factor (VEGF), Angiogenesis.

## INTRODUCTION:

Oral squamous cell carcinoma (OSCC) has a remarkable incidence worldwide and a fairly onerous prognosis, encouraging further research on factors that might modify disease outcome. Squamous cell carcinoma (SCC) encompasses at least 90% of all oral malignancies [1,2]. Oral cancer holds the eighth position in cancer incidence worldwide, with epidemiologic variations between different geographic regions (it is the third most common malignancy in South-Central Asia) [3]. In India and some other Asian countries, oral cancer is the most common type of malignancy and may account for more than 50% of all cancer cases. This finding is generally linked to the high prevalence of unique smokeless tobacco habit [4]. Oral squamous cell carcinoma originates from the stratified squamous epithelium of the buccal mucosa, lip, palate, gingiva, tongue, floor of the mouth, tonsils and pharynx. Of the areas in oral cavity, the mortality rate is lowest for lip cancer (0.04 per 1,00,000) and highest for the tongue (0.7 per 1,00,000).[5] Major risk factors for OSCC are smoking, alcohol use, poor oral hygiene, oral human papillomavirus (HPV), AIDS and any other chronic irritation, such as chronic infections, dental caries, etc. The hallmark of squamous cell carcinoma is its fast and infiltrative growth, invasion and destruction of adjacent structure, and very high potential for metastatic lymphatic and haematogenous spread [6]. Although tumor development is a multi-stage process, angiogenesis is essential to tumor growth and metastasis. The most important molecules positively affecting angiogenesis are basic fibroblast growth factor, vascular endothelial growth factor (VEGF), interleukin-8 platelet-derived growth factor, and hepatocyte growth factor. Thrombospondin 1, platelet factor-4, angiostatin, endostatin, and metalloproteinase tissue inhibitors are inhibitors of angiogenesis. The VEGF gene is located on the sixth chromosome. VEGF is a heparin-binding glycoprotein with at least four molecular forms. It enhances vascular permeability and induces endothelial cell growth, proliferation, migration and differentiation.[7] The aim of this study was to assess the level of VEGF expression in tumor tissues in all patients with OSCC , to evaluate the rate of VEGF expression in correlation with local and regional disease relapse, and to investigate a relationship between the level of VEGF expression with histological type and tumor grading.

## MATERIALS & METHODS:

This Instituitional based observational study was conducted in the Dept. of Pathology, Medical College, Kolkata between January 2013 to June 2014 on patients who were histopatho logically diagnosed as oral or pharyngeal carcinoma

Parameters to be studied-

A. Histopathological examination of malignant lesions in oral cavity and pharynx.
B. VEGF expression by the tumour.
C. Evaluation of etiological factors- Age, sex, occupation, blood-grouping, status of oral hygiene, smoking and drinking habits with the following-

Relevant history and clinical examination, Chest X-Ray and CT Scan if indicated, Blood Biochemistry (Tumor Markers if indicated). Cytology, Histopathological examination, Immunohistochemical staining of tissue sections.

A positive VEGF immunostaining is indicated by a reddish-brown precipitate in the cytoplasm. The assessment of VEGF expression was done using a scoring system, which included an intensity parameter correlated with percent of positive tumor cells. The intensity of staining was noted negative staining with 0, weak positive (1+, weak reaction in less than 10% of tumor cells), moderately positive (2+, weak-moderate reaction in 10-50% of tumor cells) and intense positive(3+, strong or moderate intensity in more than 50% of tumour cells).

Immunohistochemistry protocol used in our study:

1. 3-4 micron sections taken on poly-L-lysine coated slide from representative block.
2. Kept in hot air oven overnight.
3. Deparafinization done (Gradual xylene then alcohol then water).
4. Antigen retrieval done by pressure cooking method [2 whistle in citrate buffer (citric acid + trisodium citrate + deionised water )].
5. Cooling by running tap water.
6. Block endogenous peroxidase (By supplied peroxidase block solution) for 8 min.
7. Wash in Tris buffer (pH 7.6) for 5 mins.
8. Protein block (Block background unrelated protein staining by protein block solution) for 8 mins.
9. Primary antibody for 45 mins in moist chamber.

10. Wash in Tris buffer for 5 mins.
12. Post primary block solution for 30 mins (To increase sensitivity).
13. Wash in Tris buffer 5mins.
14. Linking polymer (Secondary antibody) 30 mins.
15. Wash in tris buffer 5 minutes.
16. DAB solution (Freshly prepared) for 3-4 mins.
17. Wash in tap water.
18. Counterstaining by Hematoxylin 2 mins (Progressive method).
19. Wash in tap water.
20. Dehydrate (in gradual alcohol then xylene) and mount in DPX.

## RESULTS:

Maximum number of patients were in the age range of 51-60yrs (40%), followed by 61-70yrs (24%), 71-80yrs (21%) and 41-50yrs (12%). Out of the 50 cases in this study, 29(58%) were males, and 21 (42%) were females. 88% patients were from lower socioeconomic status. 12% patients belonged to middle socio-economic status. Among 50 patients, 28% gave history of smoking only, 14% were both smoking and chewing tobacco. 22% did not have any habits.14% of patients were consuming alcohol with smoking.

60% of the patients presented with the complaint of an oral lesion . Other complaints were oral pain, dysphagia, neck swelling, oral bleeding and speech difficulty. In our study, 80% patients had duration of the symptoms less than 3 months. Most of the specimen (86%) for histopathological examination was incisional biopsy. Cheek was the commonest site involved (24%) followed by tongue (14%) and pharynx (10%).

48 cases (96%) had squamous cell carcinoma, and two had (4%) Verrucous carcinoma . Amongst the 48 cases of squamous cell carcinoma, 22 (44%) had well differentiated, 23 (46%) had moderately differentiated and 3 (6%) had poorly differentiated malignancies.

### VEGF Expression

VEGF expression was found to be present in all specimens. VEGF expression was mainly

confirmed by the presence of brown stained cytoplasm in tumour cells, and was observed in vascular endothelial cells as well.

Out of 50 cases, 20 cases have tumour size less than 1 cm. (13 cases show 2+ staining intensity & 7 cases show 3+ staining intensity). Another 20 cases having tumour size 1-2 cm (14 cases show 3+ staining intensity & 6 cases show 2+ staining intensity). Tumour size less than 2cm associated with good VEGF expression.

| Histopathological diagnosis | VEGF EXPRESSION(staining intensity) | | | Total |
|---|---|---|---|---|
| | 1+ | 2+ | 3+ | |
| Well differentiated SCC | 0 | 1 | 21 | 22 |
| Moderately differentiated SCC | 1 | 21 | 1 | 23 |
| Poorly differentiated SCC | 3 | 0 | 0 | 3 |
| Verrucous carcinoma | 0 | 1 | 1 | 2 |
| Total | 4 | 23 | 23 | 50 |

**Table 1: Correlation of VEGF expression intensity with histopathological factors:**

The correlation between histopathological diagnosis and intensity of VEGF staining is summarized in Table 1. The degree of expression of VEGF was high in well differentiated SCC & moderately differentiated SCC and low in poorly differentiated SCC.

## DISCUSSION:

In our study, majority (58%) of the patients were males. Only 42% were females. Mehrotra Ravi et al[8] from Allahabad, India reported a male: female ratio of 3.27:1. Dias et al[9] from Portugal reported a male: female ratio of 4:1. However gender is not a risk factor per se in oral and oropharyngeal malignancies[9].The difference may be due to the high rate of tobacco and alcohol consumption among males. Moreover tobacco is consumed in both smoking and chewing form in males whereas in our society females do usually not indulge in smoking. This can also be attributed to more males seeking early medical consultation.

Maximum number of patients were in the age range of 51-60(40%), followed by 61-70 (24%) and 71-80 (21%) & 41-50(12%). The youngest age group of patient in our study is 31-40 years old.

Patel MM et al[10] reported 12.9% of oral and oropharyngeal malignancies below 35 years age, 23.8% between 35 and 45, and 63.3% cases over 45 years of age. According to Wahid A et al[11] in Pakistan, the commonest age group affected in oral cavity squamous cell carcinoma was 41-50 years (38%), followed by 51-60 years (34%). Most of the studies found the maximum incidence of oral and oropharyngeal malignancies in people over 50 years of age. Hence, screening programs targeted to men over 50 years, would help in early diagnosis or oral and oropharyngeal malignancy and therefore increase the treatment outcome.

In our study, cheek was the commonest site involved (24%) followed by tongue (14%) and pharynx (10%). Epiglottis(8%) and floor of mouth (8%) were the next common sites involved. Lip(6%), buccal mucosa (6%) & alveolus (6%) Vallecula (2%),tonsil (2%) and retromolar trigone(2%) were the other sites involved. In the study by Patel MM [10], anterior 2/3 of the tongue was the commonest site (23.02%). Next common was posterior 1/3rd of tongue (19.64%), followed by alveolus, lips and cheeks.

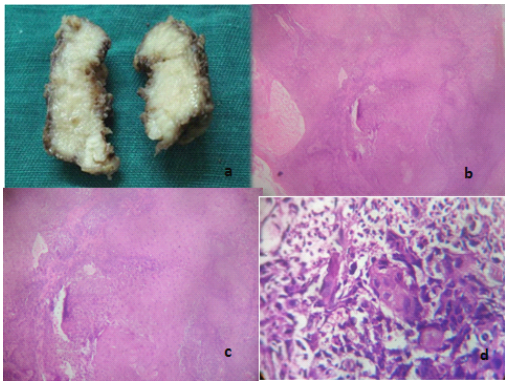


***Fig 1:a- Carcinoma of tonsil - Cut surface, b- Well differentiated SCC showing nests of tumour cells (H&E, X 100), c- Poorly differentiated SCC with keratin pearl (H&E, X 100), d- Well differentiated SCC (H & E, X 400)***

In our study, we have demonstrated that VEGF expression was reduced in tumour size more than 2cm and also in poor differentiated OSCC tumors when compared to moderate and well differentiated forms in accordance with other authors [15,16,17], and in contrast to previously published data [18,19,20,21].

No significant difference of VEGF expression levels in oral squamous cell carcinomas is demonstrated between patients with lymph node metastasis and patients without lymph node metastasis. Accordingly, this would indicate that the expression of VEGF in oral squamous cell carcinoma does not play an important role in regional lymph node metastasis.

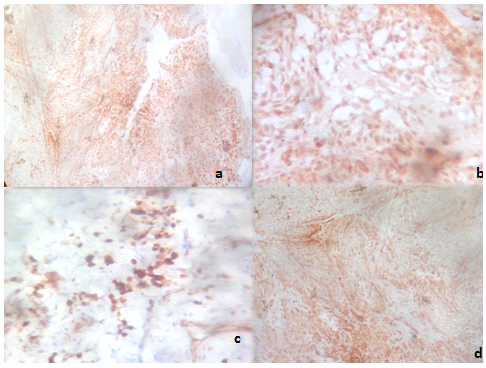


**Fig2:a- *VEGF expression in Well differentiated SCC showing more than 50% cells are positive (strongly positive,3+),(X100),* b- *VEGF expression in Moderately differentiated SCC showing more than 10% cells are positive (moderately positive,2+),( X 400),* c- *VEGF expression in Poorly differentiated SCC showing less than 10% cells are positive (weakly positive,1+),( X 400),* d- *Verrucous carcinoma showing strongly positive 3+ VEGF expression (more than 50%)(X100)***

VEGF plays a key role in tumor angiogenesis and it has been identified in many malignancies, including head and neck squamous cell carcinoma. However, a number of different factors can regulate VEGF expression including hypoxia, cytokines, oncogenes and tumor suppressor genes. Head and neck cancer can be treated effectively with high cure rates if detected at an early stage, but, unfortunately, these patients are usually discovered at a point of advanced disease.

## CONCLUSIONS:

Malignancy of oral cavity and oropharynx is predominantly a disease of males. It usually affects older age group. But younger age group are not completely spared. Tobacco and alcohol consumption are important etiological factor for oral and oropharyngeal malignancy. Presence of oral lesion is the commonest symptom. Cheek was the commonest site involved, followed by tongue. Squamous cell carcinoma was the commonest histological variety. Majority of oral and oropharyngeal malignancy were moderately differentiated squamous cell carcinoma. VEGF expression was strongly positive in well-differentiated SCC and weakly positive in poorly differentiated SCC. VEGF expression was strongly positive in tumour size 2cm or less.

## REFERENCES:

1. Beenken SW, Urist MM. Head and neck tumors. In: Way LW,Doherty GM, editors. Current surgical diagnosis and treatment.11th ed. New York: Lange Medical Books/McGraw-Hill; 2003.p. 282-97.
2. Coleman JJ, Sultan MR. Tumors of the head and neck.In:Schwartz SI, editor. Principles of surgery. 7th edition.New York: McGraw-Hill; 1999. p. 601-65.
3. World Health Organization. The World Oral Health Report2003. Geneva: World Health Organization; 2003. p. 6-7.
4. Regezi JA, Scuibba JJ and Jordan RCK. Oral Pathology-Clinical Pathologic Correlations. 5th Ed. St.Louis: Saunders Elsiever ;2008.
5. Pinborg JJ, Reichart PA, Smith CJ, Van der Waal I. World Health Organization International Histological Classification of Tumours. Histological typing of cancer and precancer of the oral mucosa. Berlin: Springer; 1997.
6. Johnson NW. Etiology and risk factors for oral cancer. Chapter-2. In: Oral cancer. Shah JP,Johnson NW, Batsakis JG, edts. London: Martin Dunitz; 2003
7. Ferrara N: Vascular endothelial growth factor. Eur J Cancer 1996, 32:2413-2422.
8. Mehrotra R, Singh M, Kumar D, Pandey AN, Gupta RK, Sinha US. Age specific incidence rate and pathological spectrum of oral cancer in Allahabad. Indian J Med Sci 2003; 57 (9): 400-4.
9. Dias GS, Almeida AP. A histological and clinical study on oral cancer:Descriptive analyses of 365 cases. Med Oral Patol Oral Cir Bucal. 2007 Nov 1;12(7):E474-8.
10. Patel MM and Pandya AN. Relationship of oral cancer with age, sex, site distribution and habits. Indian J Pathol Microbiol 2004; 47(2): 195-197.
11. Wahid A, Ahmad S, Sajjad M. pattern of carcinoma of oral cavity reporting at dental department of Ayub Medical college. Journal of Ayub Medical College 17(1); Jan- March 2005
12. Durazzo MD, Araujo CEN, Brandao Neto JS, Potenza AS, Costa P et al. Clinical and epidemiological features of oral cancer in a medical school teaching hospital from 1994 to 2002: increasing incidence in women, predominance of advanced local disease, and low incidence of neck metastases. Clinics 2005;60(4):293-8
13. Bhattacharjee A, Chakraborty A, Purkaystha P. Prevalence of head and neck cancers in North East – An institutional study. Indian J Otolaryngol Head Neck Surg 2006; 58(1): 15-19.
14. Khandekar SP, Bagdey PS, Tiwari RR. Oral cancer and some epidemiological factors: a hospital based study. Indian Journal of Community Medicine. Vol 31, No.3, July-September 2006
15. Shintani S, Li C, Ishikawa T, Mihara M et al (2004) Expression of vascular endothelial growth factor A, B, C, and D in oral squamous cell carcinoma. Oral Oncol 40:13–20
16. LI C., SHINTANI S., TERAKADO N., KLOSEK S. K., ISHIKAWA T., NAKASHIRO K., HAMAKAWA H., Microvessel density and expression of vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived endothelial growth factor in oral squamous cell carcinomas, Int J Oral Maxillofac Surg, 2005, 34(5):559–565.
17. Johnstone S, Logan RM (2007) Expression of vascular endothelial growth factor (VEGF) in normal oral mucosa, oral dysplasia and oral squamous cell carcinoma. Int J Oral Maxillofac Surg 36:263–266
18. Chuang HC, Su CY, Huang HY et al (2006) High expression of CD105 as a prognostic predictor of early tongue cancer. Laryngoscope 116:1175–1179
19. Shang ZJ, Li JR (2005) Expression of endothelial nitric oxide synthase and vascular endothelial growth factor in oral squamous cell carcinoma: its correlation with angiogenesis and disease progression. J Oral Pathol Med 34:134–139
20. Smith BD, Smith GL, Carter D, Sasaki CT, Haffty BG (2000) Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. J Clin Oncol 18:2046–2052
21. Kyzas PA, Stefanou D, Agnantis NJ (2004) Immunohistochemical expression of vascular endothelial growth factor correlates with positive surgical margins and recurrence in T1 and T2 squamous cell carcinoma (SCC) of the lower lip. Oral Oncol 40:941–947.