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# Association Between Human Papillomavirus (HPV) Oncoproteins E6 and E7 and VEGF Expression in Oral and Oropharyngeal Squamous Cell Carcinoma

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**ABSTRACT**

**Background:** Oral and oropharyngeal squamous cell carcinoma (OSCC) represents a significant global health challenge. While human papillomavirus (HPV) infection and angiogenesis are known contributors to carcinogenesis, the relationship between HPV oncoproteins and vascular endothelial growth factor (VEGF) expression remains poorly understood.

**Objective:** This study investigated the immunohistochemical expression of VEGF and its association with HPV E6 and E7 oncoproteins in oral and oropharyngeal squamous cell carcinoma, along with their correlation to clinicopathological parameters.

**Methods:** We conducted a cross-sectional study of 80 newly diagnosed OSCC cases. Immunohistochemical analysis was performed to detect VEGF expression and HPV E6/E7 oncoproteins. Expression patterns were correlated with clinicopathological features including tumor differentiation, stage, and lymph node involvement.

**Results:** VEGF expression was detected in 85% of cases, with significantly higher expression in carcinoma compared to adjacent dysplastic tissue (p=0.004). HPV16/18 E6 positivity was observed in 37.5% of cases, while E7 (HPV16) and E7 (HPV18) were positive in 26.25% and 20% of cases respectively. VEGF expression significantly correlated with tumor differentiation (p=0.012) but showed no significant association with HPV oncoprotein expression. No significant correlations were found between VEGF expression and other clinicopathological parameters including age, gender, tumor size, and lymph node status.

**Conclusion:** Although VEGF expression and HPV infection are crucial elements in oral and oropharyngeal tumor progression, their mechanisms appear to be independent. The significant increase in VEGF expression during malignant

transformation highlights its role in promoting vasculogenesis within the tumor microenvironment, suggesting VEGF as a potential therapeutic target, particularly in early-stage disease. These findings contribute to the understanding of angiogenic mechanisms in OSCC and may support the development of targeted therapies.
**Keywords:** Oral squamous cell carcinoma, Human papillomavirus, VEGF, Angiogenesis, E6/E7 oncoproteins, Immunohistochemistry

## INTRODUCTION

Oral and oropharyngeal squamous cell carcinoma (OSCC) represents a significant global health burden, with an estimated worldwide incidence of 2.1% in oral cavity and 1% in pharynx [1]. Despite advances in treatment modalities, the prognosis remains poor, largely due to late diagnosis and aggressive tumor behavior [2]. Recent evidence has established human papillomavirus (HPV) infection as a major etiologic factor in oropharyngeal cancer, particularly in younger patients without traditional risk factors like tobacco and alcohol use [3, 4].

The oncogenic potential of high-risk HPV types (primarily HPV-16 and HPV-18) is largely attributed to their E6 and E7 oncoproteins. These viral proteins interfere with critical cell cycle regulators - E6 promotes p53 degradation while E7 inactivates the retinoblastoma protein (pRb), leading to unrestricted cell proliferation [5, 6]. Additionally, emerging evidence suggests that these oncoproteins may play a crucial role in tumor angiogenesis by modulating the expression of vascular endothelial growth factor (VEGF) [7].

VEGF is a key regulator of angiogenesis and plays a central role in tumor growth and metastasis. It promotes endothelial cell survival, migration, and proliferation, leading to the formation of new blood vessels essential for sustaining tumor progression [8]. Previous studies have demonstrated increased VEGF expression in OSCC, correlating with poor prognosis and reduced survival rates [9]. However, the relationship between HPV oncoproteins and VEGF expression in oral and oropharyngeal cancers remains poorly understood.

Recent molecular studies suggest that HPV E6 and E7 oncoproteins may enhance VEGF expression through multiple mechanisms. E6 leads to p53 inactivation, resulting in HIF-1α induction, which subsequently upregulates VEGF expression. Similarly, E7 can directly induce HIF-1α and stimulate VEGF expression through pRb phosphorylation [10, 11]. Understanding these interactions could have significant implications for developing targeted therapies, particularly anti-angiogenic treatments for HPV-positive oral cancers.

The present study aims to investigate the immunohistochemical expression of VEGF and its association with HPV E6 and E7 oncoproteins in oral and oropharyngeal squamous cell carcinoma. This research could provide valuable insights into the role of HPV in tumor angiogenesis and potentially guide the development of more effective therapeutic strategies for HPV-positive oral cancers.

## MATERIALS AND METHODS

### Study Design and Population

A hospital-based descriptive observational cross-sectional study was conducted from November 2014 to March 2016 at Lady Hardinge Medical College and Associated Hospitals, New Delhi, in collaboration with the Institute of Cytology and Preventive Oncology (ICMR), Noida. The study included 80 newly diagnosed, histologically proven cases of oral or oropharyngeal squamous cell carcinoma. Patients already undergoing therapy or with malignancies other than squamous cell carcinoma were excluded [12].

### Tissue Processing and Histopathology

Biopsy specimens received in 10% neutral buffered formalin were processed following standard protocols. The minimum fixation time was 4 hours. Paraffin-embedded tissue blocks were sectioned at 3-4 μm thickness and mounted on poly-L-lysine coated slides. Hematoxylin and eosin (H&E) staining was performed for histopathological examination and tumor grading [13].

### Immunohistochemistry

Immunohistochemical analysis was performed using a two-step process involving unconjugated primary antibody

binding followed by detection using a secondary antibody conjugated to horseradish peroxidase-labeled polymer chain. Antigen retrieval was carried out using a decloaking chamber (Biocare Medical) with citrate buffer (pH 6.0) [14].

The following primary antibodies were used:

- VEGF: Concentrated rabbit monoclonal antibody IgG Clone EP1176Y (Biocare Medical, dilution 1:100-1:200)
- HPV16/18 E6 Antibody (C1P5): Mouse monoclonal IgG1 (Santa Cruz Biotech)
- HPV16 E7 Antibody (ED17): Mouse monoclonal IgG1 (Santa Cruz Biotech)
- HPV18 E7 Antibody (N-19): Goat polyclonal IgG (Santa Cruz Biotech)

Visualization was achieved using the Dako REAL™ EnVision™ Detection System with DAB chromogen. Appropriate positive controls were included for each marker [15].

**Scoring Systems For VEGF**

An immunoreactivity score (IRS) ranging from 1-12 was calculated by multiplying staining intensity (0-3) with percentage positivity (1-4). The scores were categorized as: low (1-4), medium (6-8), and high (9-12) [16].

For HPV oncoproteins (E6 and E7), immunoreactivity scores (0-7) were calculated by adding percentage positivity scores (0-4) and staining intensity scores (0-3). For statistical analysis, scores were grouped as low (0-3) and high (4-7) expression [17].

**Clinicopathological Parameters**

Clinical staging was performed according to the TNM classification system. Tumors were histologically graded as well, moderately, or poorly differentiated squamous cell carcinoma. Additional parameters recorded included patient age, gender, addiction habits, tumor site, and lymph node involvement [18].

**Statistical Analysis**

Data analysis was performed using SPSS version 16.0. Pearson's chi-square test was used for categorical variables, and Pearson's correlation was used for measurement scale variables. A p-value of 0.05 was considered statistically significant [19].

**RESULTS**

**Study Population Demographics**

Among the 80 patients studied, ages ranged from 32 to 80 years (mean age: 54 years), with the majority (51.25%) between 41-60 years. Males predominated with a male-to-female ratio of 3.4:1 (77.50% males, 22.50% females). Tobacco and/or alcohol addiction was present in 65% of cases.

**Table 1:** Demographic and Clinical Characteristics (n=80)

| Characteristic | Number | Percentage (%) |
|---|---|---|
| **Age Distribution** | | |
| 21-40 years | 17 | 21.25 |
| 41-60 years | 41 | 51.25 |
| >60 years | 22 | 27.50 |
| **Gender** | | |
| Male | 62 | 77.50 |
| Female | 18 | 22.50 |
| **Addiction Status** | | |
| Present | 52 | 65.00 |
| Absent | 28 | 35.00 |

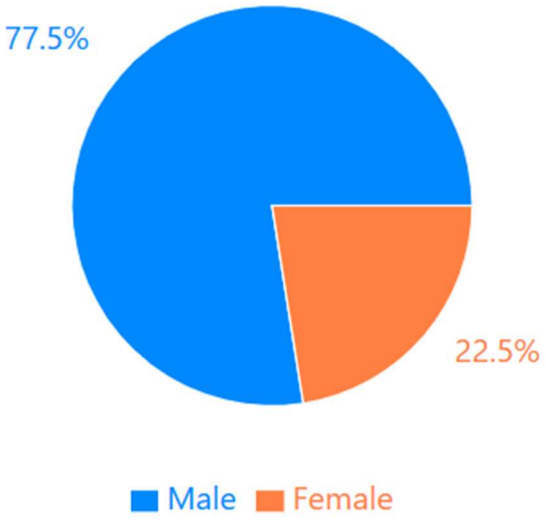


**Fig 1:** Pie chart showing gender distribution.

**Tumor Characteristics**

The tongue was the most common tumor site (28.75%), followed by cheek (26.2%). Most tumors (47.50%) measured ≤2 cm, with 46.2% classified as T1 stage. Lymph node involvement was observed in 41.25% of cases, predominantly affecting cervical nodes (66.66%).

**Table 2:** Tumor Site Distribution and Staging (n=80)

| Parameter | Number | Percentage (%) |
|---|---|---|
| **Tumor Site** | | |
| Tongue | 23 | 28.75 |
| Cheek | 21 | 26.20 |
| Tonsil | 10 | 12.50 |
| Alveolus | 8 | 10.00 |
| Other sites | 18 | 22.55 |
| **T Stage** | | |
| T1 | 37 | 46.20 |
| T2 | 32 | 40.00 |
| T3 | 10 | 12.50 |
| T4 | 1 | 1.20 |

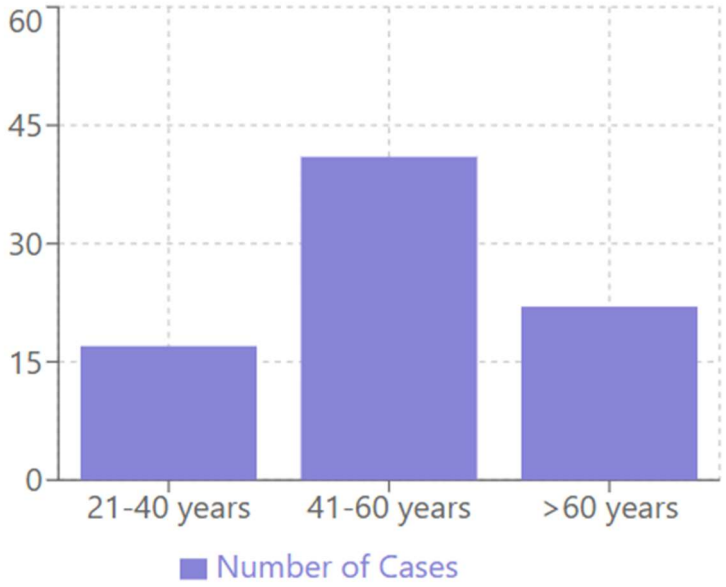


**Fig 2:** Age Distribution of OSCC Cases (n=80)

**Histopathological Findings**

Moderately differentiated squamous cell carcinoma (MDSCC) was most common (51.25%), followed by well-differentiated (WDSCC, 47.50%). All cases showed severe dysplasia in adjacent mucosa.

VEGF Expression Analysis VEGF positivity was observed in 68 cases (85%), with varying expression levels:

- High expression (9-12): 24 cases (30%)
- Medium expression (6-8): 19 cases (23.75%)
- Low expression (1-4): 25 cases (31.25%)
- Negative: 12 cases (15%)

Table 3: VEGF Expression in Tumor and Adjacent Dysplasia

| VEGF Score | Tumor | Dysplasia | p-value |
|---|---|---|---|
| 0-4 | 37 (46.25%) | 55 (68.75%) | 0.004 |
| 6-12 | 43 (53.75%) | 25 (31.25%) | |

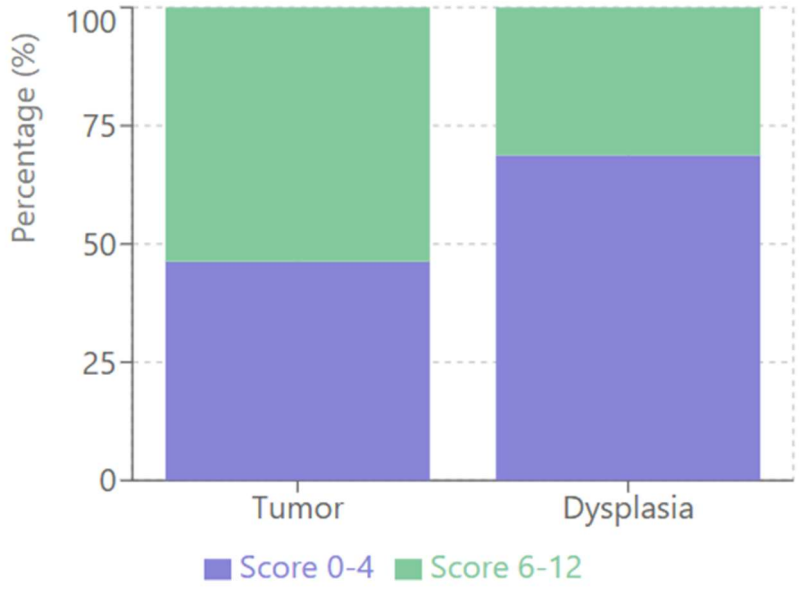


**Fig 3** : VEGF Expression in Tumor vs Dysplasia

**HPV Oncoprotein Expression**

E6 (HPV16/18) positivity was detected in 30 cases (37.5%), while E7 (HPV16) and E7 (HPV18) were positive in 21 (26.25%) and 16 (20%) cases respectively.

**Table 4:** Correlation of VEGF Expression with HPV Oncoprotein Status

| Oncoprotein | VEGF Score 0-4 | VEGF Score 6-12 | p-value |
|---|---|---|---|
| **E6 (HPV16/18)** | | | |
| Score 0-3 | 29 (50.00%) | 29 (50.00%) | 0.275 |
| Score 4-7 | 8 (36.36%) | 14 (63.63%) | |
| **E7 (HPV16)** | | | |
| Score 0-3 | 32 (48.48%) | 34 (51.51%) | 0.384 |
| Score 4-7 | 5 (35.71%) | 9 (64.28%) | |
| **E7 (HPV18)** | | | |
| Score 0-3 | 33 (45.83%) | 39 (54.16%) | 0.823 |
| Score 4-7 | 4 (50.00%) | 4 (50.00%) | |

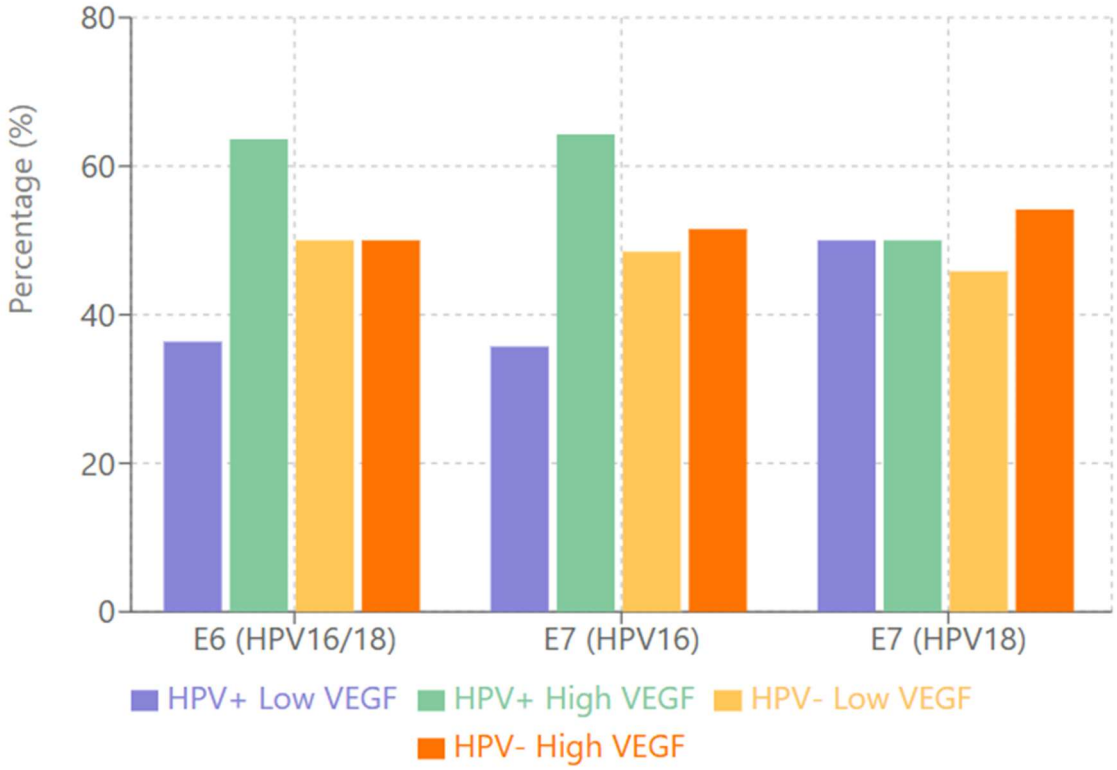


**Fig 4:** VEGF Expression Patterns in HPV-positive vs Negative Cases

**Clinicopathological Correlations**

A significant correlation was found between VEGF expression and tumor differentiation (p=0.012), with higher expression in WDSCC compared to MDSCC/PDSCC. No significant associations were observed between VEGF expression and age (p=0.658), gender (p=0.368), tumor size (p=0.093), lymph node status (p=0.303), or clinical stage (p=0.226).

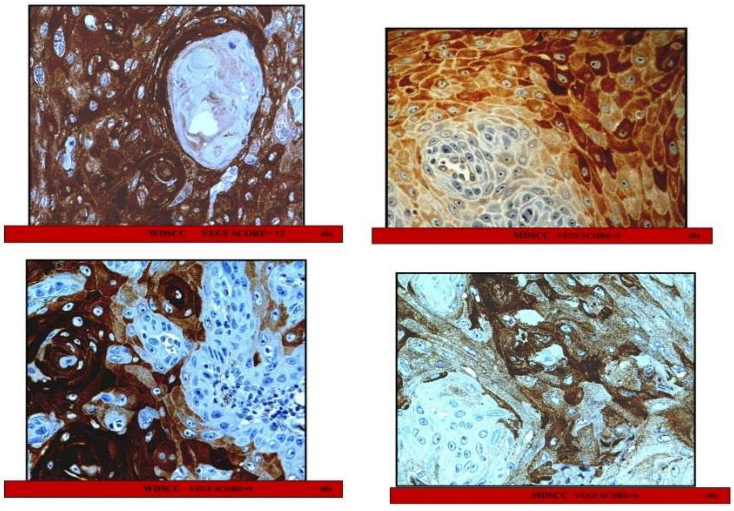

**Fig 5:** Immunohistochemical staining result of histopathological findings VEGF Score

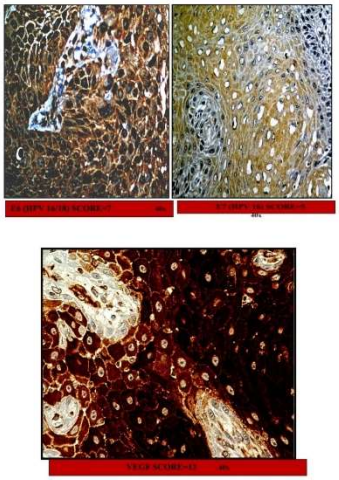

**Fig 6:** Histopathological findings of HPV E6/E7 Oncoprotein Expression

**DISCUSSION**

The present study investigated the complex relationship between HPV oncoproteins and VEGF expression in oral and oropharyngeal squamous cell carcinoma, providing insights into potential angiogenic mechanisms in these malignancies.

**VEGF Expression Patterns** : Our finding of VEGF positivity in 85% of OSCC cases aligns closely with previous studies. Sappayatosok et al. [20] reported 89.39% VEGF positivity, while Henriques et al. [21] found 91.4% positive cases in their analysis. The high prevalence of VEGF expression underscores its significance in oral cancer progression. Notably, we observed a significant increase in VEGF expression in carcinoma compared to adjacent dysplastic tissue (p=0.004), supporting findings by Shilpi Arora et al. [22] who demonstrated VEGF as a significant predictor for transition from precancerous to malignant phenotype.

**Tumor Differentiation and VEGF:** A significant correlation emerged between VEGF expression and tumor differentiation (p=0.012), with higher expression in well-differentiated tumors. This finding parallels observations by Marinescu et al. [23], who reported higher immunostaining percentages in well-differentiated carcinomas compared to moderately and poorly differentiated variants. However, this relationship remains controversial, as studies by Yanase et al. [24] found no correlation between VEGF expression and histological differentiation.

**HPV Status and VEGF Expression**: Our study revealed HPV16/18 E6 positivity in 37.5% of cases, with E7 (HPV16) and E7 (HPV18) detected in 26.25% and 20% of cases respectively. These frequencies align with global prevalence rates reported in meta-analyses [25]. Interestingly, we found no significant correlation between HPV oncoprotein expression and VEGF levels, consistent with findings by Troy et al. [26] who also reported no association between HPV status and VEGF expression (p=0.82).

This lack of correlation contrasts with some molecular studies suggesting that HPV E6 and E7 oncoproteins enhance VEGF expression through HIF-1α pathways [27]. The discrepancy might reflect the complexity of angiogenic regulation in vivo, where multiple pathways potentially compensate for or modify HPV-related effects.

**Clinicopathological Parameters**: Our analysis found no significant associations between VEGF expression and various clinical parameters including age, gender, tumor size, and lymph node status. While some studies like Cheng et al. [28] reported correlations between VEGF expression and lymph node metastasis, others align with our findings. Naruse et al. [29] similarly found no significant relationship between VEGF expression and clinicopathological features.

**Therapeutic Implications**: The high prevalence of VEGF expression in our cohort, independent of HPV status, suggests that anti-angiogenic therapies might benefit both HPV-positive and negative cases. However, the complex relationship between HPV oncoproteins and angiogenesis warrants further investigation, particularly regarding potential differences in treatment response between HPV-positive and negative tumors [30].

**Study Limitations and Future Directions**: While our study provides valuable insights, several limitations should be acknowledged. The cross-sectional design prevents assessment of temporal relationships between HPV infection and VEGF expression. Future longitudinal studies incorporating larger sample sizes and molecular analyses of HPV-mediated angiogenic pathways could better elucidate these associations.

## CONCLUSION

This comprehensive analysis of 80 oral and oropharyngeal squamous cell carcinoma cases reveals important insights into the relationship between VEGF expression and HPV status. The high prevalence of VEGF expression (85% of cases) and its significant increase during progression from dysplasia to carcinoma emphasizes its crucial role in oral cancer development. The study demonstrates a significant correlation between VEGF expression and tumor differentiation, with well-differentiated tumors showing higher expression levels.

Notably, while HPV infection was present in a substantial proportion of cases, with E6 and E7 oncoproteins detected in 37.5% and 26.25% of cases respectively, no significant correlation emerged between HPV status and VEGF expression. This finding suggests that HPV-mediated carcinogenesis may involve angiogenic pathways independent of or parallel to VEGF regulation.

The absence of significant associations between VEGF expression and clinical parameters such as tumor size, lymph node involvement, and disease stage indicates that VEGF's role in tumor progression may be more complex than previously understood. However, the marked increase in VEGF expression during malignant transformation suggests its potential utility as a therapeutic target, particularly in early-stage disease.

These findings have important therapeutic implications, suggesting that anti-angiogenic strategies might be valuable in treating oral and oropharyngeal cancers regardless of HPV status. Furthermore, the study highlights the need for individualized treatment approaches that consider both HPV status and angiogenic markers.

Future research should focus on elucidating the molecular mechanisms underlying the interaction between HPV oncoproteins and angiogenic pathways, potentially leading to more effective targeted therapies for both HPV-positive and HPV-negative oral cancers. Additionally, longitudinal studies examining VEGF expression patterns during disease progression could provide valuable insights for developing stage-specific therapeutic strategies.

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