

P16 immunoexpression in oral squamous cell carcinoma: a cross-sectional study from a tertiary care center

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ABSTRACT

Objective: This study delves into the pivotal role of the p16 gene, a tumor suppressor, in OSCC by assessing the frequency of p16 overexpression in OSCC.

Materials and methods: This descriptive cross-sectional study was conducted in the Pathology Department, SZABMU (PIMS), Islamabad, Pakistan, from January 2023 to October 2023. A total of 135 samples were diagnosed with OSCC in 2022. Immunohistochemical staining for p16 was performed on each specimen. The data obtained from the staining were analyzed using appropriate statistical tests to determine any significant associations.

Results: Among 135 samples, p16 tested positive in 20 cases and negative in 115 cases. 65 years was the average age of the patients. A history of addiction was found in 47% of them. The buccal mucosa was the most frequently affected site for the tumor.

Conclusion: In our analysis, we found that oropharyngeal squamous cell carcinoma in our population exhibits low levels of p16 expression, specifically below 50%. These results indicate a limited occurrence of HPV as a contributing factor to oral squamous cell carcinoma in our studied demographic.

Keywords: Immunohistochemistry, Oropharyngeal squamous cell carcinoma, p16

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INTRODUCTION

HNSCC is the sixth most common malignancy in the world and represents a significant cause of cancer-related morbidity and mortality [1]. Although alcohol and tobacco are the most common risk factors, the distribution of the disease varies considerably across regions. In India and Pakistan, widespread long-term consumption of areca nut, often in the form of pan or gutka, contributes substantially to the overall disease burden [2]. Many cases of HNSCC, however, occur without exposure to these carcinogens, indicating involvement of other etiologic pathways.

Western population is also showing an increase in the incidence of OSCC and high-risk HPV, especially HPV-16 is implicated in this case [3]. The p16 gene is responsible for encoding a protein that acts as a tumor suppressor and is involved in the regulation of the cell cycle; its functional inactivation is an important event in carcinogenesis [4].

Overexpression of p16, which can be successfully identified by immunohistochemistry, represents a surrogate marker of oncogenic HPV infection. From the clinical point of view, clinically p16-positive tumors have specific biological behavior and present better treatment response and higher disease-free survival rates compared with p16-negative tumors [5]. Despite the diagnostic and prognostic importance of p16, information on its expression in HNSCC has been very limited in our region. In addition, the true burden of HPV-associated OSCC remains poorly defined among South Asian populations. This study aims to define the prevalence of p16 overexpression in HNSCC in our population and understanding HPV-related carcinogenesis and its potential clinical implications.

MATERIAL AND METHODS

The study was conducted at Histopathology department of SZABMU (PIMS) in 2023, spanning a period of 10 months. It included 135 patients who underwent surgeries for HNSCC. Approval for the was obtained from the institutional ERB and informed written consent was obtained from the patients before surgery. Hematoxylin and eosin-stained slides of these cases were re-evaluated by two surgical pathologists independently, assessing pathological findings such as tumor type and grade, tumor and nodal stage, perineural and lymph vascular invasion.

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Additionally, p16 immunohistochemistry (IHC) was performed on the representative tissue blocks of all cases.

Immunohistochemistry (IHC) for P16 was conducted following the manufacturer's protocol, with both nuclear and cytoplasmic staining of P16 considered. The intensity of staining was categorized as follows: no staining (0), weak (1+), intermediate (2+), and strong (3+). Additionally, the percentage of positively stained cancer cells was calculated as a continuous variable. Intermediate to strong staining in more than 10% of cancer cells was considered positive, while weak to intermediate staining in less than 10% of cancer cells was regarded as focal positive.

Data entry and analysis were performed using version 21 of the Statistical Package for the Social Sciences (SPSS, Chicago, IL). Chi-square analysis was utilized to identify any associations, with a p-value of 0.05 or less deemed statistically significant.

RESULTS

A total of 135 cases of oral squamous cell carcinoma (OSCC) were included in this study. The cohort comprised 74 males (54.8%) and 61 females (45.2%). The most frequent tumor site was the buccal

mucosa (84 cases, 62.2%), followed by the lip (41 cases, 30.4%) and mandible (10 cases, 7.4%) (Table-I).

Histopathological evaluation revealed that 54 tumors (40.0%) were well differentiated, while 81 tumors (60.0%) were moderately differentiated. The depth of invasion (DOI) was < 2 cm in 85 cases (63.0%) and ≥ 2 cm in 50 cases (37.0%) (Table-II).

Immunohistochemical analysis of p16 expression showed block positivity in 20 cases (14.8%), whereas 115 cases (85.2%) were negative. Notably, all well-differentiated tumors were p16-negative, and block positivity was observed exclusively in the moderately differentiated group, with 20 positive and 61 negative cases (Table-III and Table-IV).

Overall, the prevalence of p16 positivity in this cohort was 14.8%, suggesting that high-risk human papillomavirus (HPV) may contribute to a subset of OSCC cases within the local population.

Table-I: Gender and site distribution of oral squamous cell carcinoma (n = 135).

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	74	54.8
	Female	61	45.2
Tumor Site	Buccal mucosa	84	62.2
	Lip	41	30.4
	Mandible	10	7.4

Table-II. Histological grade and depth of invasion of OSCC (n = 135).

Parameter	Category	Frequency (n)	Percentage (%)
Histological Grade	Well differentiated	54	40.0
	Moderately differentiated	81	60.0
Depth of Invasion (DOI)	< 2 cm	85	63.0
	≥ 2 cm	50	37.0

Table-III: p16 Expression in oral squamous cell carcinoma (n = 135).

p16 Expression	Frequency (n)	Percentage (%)
Block positivity	20	14.8
Negative	115	85.2

Table-IV: Correlation between tumor grade and p16 expression (n = 135).

Histological Grade	Block Positivity (n)	Negative (n)	Total (n)
Well differentiated	0	54	54
Moderately differentiated	20	61	81
Total	20	115	135

DISCUSSION

Up to 4% population of the world is affected by OSCC and it remains the sixth most common cancer diagnosis [6]. Statistics show that there are 6.6 new cases of OSCC and 3.1 deaths per 100,000 for men, while for women, there are 2.9 new cases and 1.4

deaths per 100,000. Multiple etiologies are implicated in the development of OSCC such as tobacco chewing, genetic alterations, infection by HPV, and poor nutrition [7]. These etiologies together drive the pathogenesis and progression of the disease [8].

This study examined the expression of p16 in OSCC, given its role as a surrogate marker of infection with HPV. Overall, the expression of p16 was documented in 14.8% of cases. The literature suggests a strong correlation between the overexpression of p16 and infection with HPV. In a recent study by Naz F et al, 17.37% cases of OSCC showed positivity for p16 on immunohistochemistry. When these p16-positive cases were tested for HPV DNA, a total of 74.8% turned out positive [9].

HPV infection prevalence in OSCC varies among different geographical regions. A comprehensive analysis of 148 studies, summing up to 12,163 HNSCC cases, demonstrated that the prevalence of HPV in oral cavity squamous cell carcinoma was 24.2% [10]. Another comprehensive analysis in 3,680 HNSCC cases revealed that HPV DNA was found in 24.9% of oropharyngeal SCC [11]. Further research has also focused on the percentage of tumor cells staining for p16, the presence of confluent staining, and staining intensity as important parameters of evaluation. Lewis *et al.* put forth criteria for determining the infection caused by human papillomavirus: greater than 75% of tumor cells staining for p16, or less than 50% with more than 25% of cells in clusters [12].

HPV infection is a well-known risk factor for head and neck squamous cell carcinoma, especially for the oropharynx. HPV status determination is important because survival outcomes as well as therapeutic responsiveness depend on the HPV-associated tumor biology. Among several methods to infer HPV involvement in cancer cases, the p16 expression on immunohistochemistry is generally used [13].

More recently, Larsen *et al.* in his study showed that >70% p16 staining (nuclear and cytoplasmic) is suggestive of transcriptionally active HPV [14]. Indeed, p16 expression has been shown to be an independent prognostic marker in OSCC; one article stated that p16 overexpression is related to lower T-stage and improved overall survival [15,16].

Several reports have indeed investigated the clinic-pathological implication of p16 expression in HNSCC and its correlation with various prognostic and pathological parameters. A meta-analysis showed that the expression of p16 is associated with favorable clinical and prognostic features, such as a decreased tendency toward nodal metastasis [17]. However, in this series, no correlation was seen between the status of p16 expression and the non-keratinizing phenotype, contradicting some previous studies [18]. A limitation of the study is the lack of molecular analyses for direct correlation of IHC

expression of p16 with the presence of HPV strains in OSCC. Thus, we recommend including molecular testing of HPV in HNSCC, especially with high p16 expression.

CONCLUSION

The prevalence of p16 block positivity detected in 14.8% of cases in the current study suggests that HPV is related to a subset of OSCC in the local population. Additionally, the expression of p16 in this series was correlated with favorable prognostic factors: there were no nodal metastases, lower tumor stage, and increased overall survival. These findings contribute to the position that p16 may serve as an independent prognostic biomarker and stress the potential clinical relevance of p16 determination for the identification of human papillomavirus-driven OSCC, which may have impacts on patient management and treatment decisions.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Maryam Hameed, Armaghana Qamar Khan: Substantial contributions to study design, acquisition of data, manuscript drafting, reviewing it critical for important intellectual content, final approval, accountable for all aspects of publication.

Sana Hanif: Substantial contributions to study design, acquisition of data, final approval, accountable for all aspects of publication

Momina Habib, Ramsha Ali: Substantial contributions to study design, acquisition of data, final approval, accountable for all aspects of publication.

Ahmareen Khalid Sheikh: Substantial contributions to study design, acquisition of data, manuscript drafting, reviewing it critical for important intellectual content, final approval, accountable for all aspects of publication

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