

EVALUATION OF P16 EXPRESSION AS A SURROGATE MARKER FOR HIGH-RISK HPV IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background

Oral squamous cell carcinoma (OSCC) is one of the most common global health burden and constitutes the majority of oral cancers in India. Human papillomavirus (HPV), especially high-risk HPV-16 and HPV-18, has emerged as an important etiological factor in a subset of OSCC. p16 immunohistochemistry (IHC) is increasingly being used as a surrogate biomarker for HPV-associated carcinogenesis, alongside detection at molecular level.

Aim

To evaluate p16 expression in OSCC and correlate it with HPV status and clinicopathological parameters. And eventually assess the utility of p16 IHC as a surrogate marker for HPV-associated OSCC.

Materials and Methods

This prospective observational study was conducted in the Department of Pathology over a period of one year. A total of 25 histopathologically confirmed cases of OSCC were included. p16 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections. Cases showing p16 positivity were further evaluated for high-risk HPV DNA using polymerase chain reaction (PCR).

Results

Majority of the patients belonged to 41–50 years age group with male predominance (72%). Tongue was the most common tumour subsite. MDSCC was the most common histopathological grade. p16 positivity was observed in 15 cases (60%). Grade-4 p16 positivity (4 cases 8%) showed the strongest association with high-risk HPV DNA positivity. Most HPV-related cases and Grade-4 p16 positive cases were observed in patients without significant smoking, tobacco chewing or alcohol exposure. p16 positivity showed significant association with degree of differentiation of OSCC, MDSCC being the most common type.

Conclusion

p16 immunohistochemistry is a cost-effective surrogate marker for HPV-associated OSCC. Strong p16 positivity (Grade-3 and Grade-4) demonstrated significant correlation with high-risk HPV infection and may aid in identifying HPV-related oral carcinomas in routine pathology practice. However, combined assessment using p16 immunohistochemistry and molecular techniques such as PCR or ISH provides greater diagnostic accuracy and better therapeutic decision-making in patients with HPV-associated OSCC.

Keywords: Oral squamous cell carcinoma (OSCC), HPV, p16, PCR.

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INTRODUCTION

Oral cancer is one of the major health burden in the world, accounting for a substantial proportion of newly diagnosed malignancies globally^[1]. India contributes significantly to this global healthburden^[2]with almost 90% of them being Oral squamous cell carcinoma (OSCC).^[3,4]

The etiopathogenesis of oral cancer is multifactorial. Traditional risk factors include chronic smoking, tobacco chewing and alcohol consumption.^[5] In the

recent years, infection with high-risk human papillomavirus (HPV), particularly HPV-16, also plays a role in etiopathogenesis.^[6,7]

p16INK4a is a cyclin-dependent kinase inhibitor. It is encoded by the CDKN2A tumor suppressor gene and plays a key role in regulation of cell cycle. Under the normalphysiological conditions, p16 inhibits cyclin-dependent kinase 4 and 6, prevents phosphorylation of the retinoblastoma (Rb) protein and arrests cell cycle progression at the G1-S checkpoint.^[8] In HPV-associated malignancies, the viral E7 oncoprotein

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inactivates Rb, results in uncontrolled cellular proliferation, which in turn leads to overexpression of p16.^[9] Thus p16 can be used a useful surrogate biomarker for high-risk HPV infection.^[10,11]

Immunohistochemistry is an important ancillary diagnostic tool in routine pathology practice owing to its simplicity, reproducibility, and cost-effectiveness.^[4] Nevertheless, the utility of p16 as a standalone biomarker in OSCC remains controversial because p16 positivity can also occur independently

Data Collection:

The detailed history regarding demographic data, risk factors, and relevant clinical data for this study were obtained from the patient's medical record and the histopathology requisition forms.

Histopathological classification and grading:

All the cases were classified based on the WHO criteria and the resected specimens were further reported according to the CAP protocol for tumours

P16 INTERPRETATION	EXPRESSION
Negative	<= 5% cells showing staining or sections showing only nuclear or only cytoplasmic staining.
Positive	>5% cells showing nuclear as well as cytoplasmic staining.
Low/weak positive (Grade-1)	6-10% cells showing golden yellow to light brown staining.
Moderate positive (Grade-2)	>10-30% cells showing dark brown to black staining.
Strong positive (Grade-3)	>30-75% showing dark brown to black staining.
Very strong positive (Grade-4)	>75% tumor cells showing strong, diffuse, darkbrown to black staining.

of HPV infection.^[12]

Molecular techniques such as polymerase chain reaction (PCR) remain the gold standard for detection of HPV DNA in tumor tissues. These are often used along with p16 immunohistochemistry to improve diagnostic accuracy.^[13,14]

Therefore, the present study aims to evaluate the role of p16 immunohistochemical expression in oral squamous cell carcinoma and to determine its association with HPV infection detected by PCR, along with various clinicopathological parameters. And eventually assess the utility of p16 IHC as a surrogate marker for HPV-associated OSCC.

MATERIALS AND METHODS

The current study is a prospective observational study conducted in the Department of Pathology MGM Medical College and Hospital, Kamothe, Navi Mumbai for a period of 1 year from 1st January 2025 to 31st December 2025. All the patients who were newly diagnosed as Oral Squamous Cell Carcinoma (OSCC) on histopathology were included in this study. Patients with past history of surgery, radiotherapy or chemotherapy, recurrent cases and cases other than OSCC were excluded. Thus, a total of 25 cases of OSCC belonging to adults (>18 years of age), of both the genders were enrolled in the study. Approval of the study was taken from the Institutional Ethics Committee before the start of the study. Informed consent was taken from all the participants during the study.

of Oral cavity.

Immunohistochemistry:

The formalin-fixed paraffin-embedded (FFPE) tissue samples of histopathologically diagnosed OSCC cases were further submitted for p16 IHC staining. The 4 microns thick sections were mounted on poly-L-Lysine coated slide and stained by using monoclonal antibody for p16 using standard protocols. A positive control for p16 (cervical cancer) was run on the same slide. The p16 expression was interpreted by counting percentage of cells taking up both nuclear and cytoplasmic stain out of 1000 cells in 10 HPF at 40x magnification (Table 1).

Table 1: Interpretation of p16 IHC expression^[10,15]

HPV- DNA Analysis

The FFPE blocks of cases with p16 positive expression were further subjected to RT-PCR test for detecting the high risk HPV DNA. DNA extraction was carried out from these tissue sections, followed by amplification of HR-HPV DNA, particularly HPV-16 and HPV-18 genotypes, using consensus primers. Appearance of the band in the same range of positive control was considered positive for HR-HPV. The HPV PCR findings were correlated with p16 immunoeexpression and various clinico-pathological parameters to evaluate the utility of p16 as a surrogate marker for HPV-associated OSCC.

Statistical Analysis

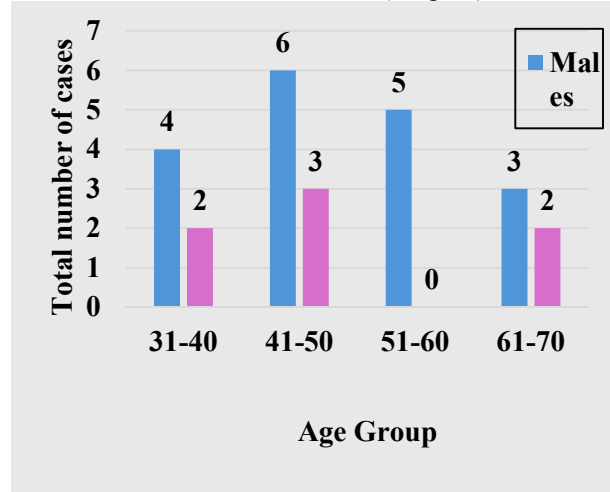
The collected and observed data was analyzed using Microsoft Excel and SPSS software version 23. Chi-

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square test was used for studying the correlation between variables. A p-value of <0.05 was considered to be statistically significant.

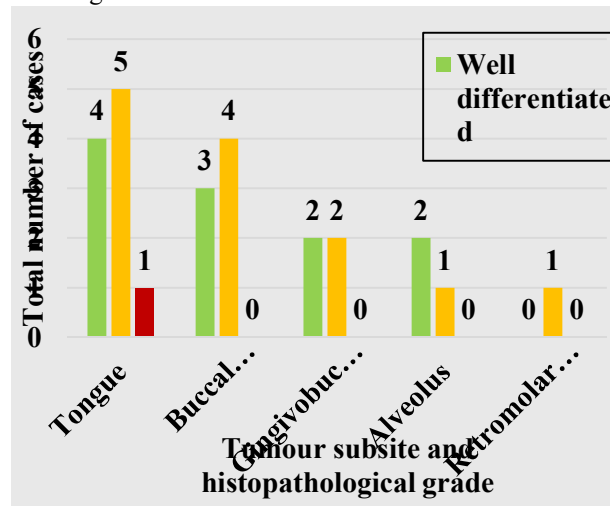
RESULTS

The present study included a total of 25 histopathologically confirmed cases of OSCC. Age ranged from 32 to 66 years, with a mean age of 46.38 years and majority belonged to 41-50 years age group (9 cases, 36%). Male preponderance (72%) was noted with male to female ratio of 2.57:1 (Graph 1).



Graph 1: Age and Gender-wise distribution of cases.

The most common risk factor for OSCC in the study group was smoking (18 cases, 72%), followed by tobacco chewing (12 cases, 48%) and alcohol consumption (8 cases, 32%). 6 cases (24%) did not have any of these three major risk factors indicating that other risk factors also contribute to oral carcinogenesis.



Graph 2: Distribution of cases according to tumour subsite and histopathological grade.

The most commonly involved subsites were tongue (10 cases, 40%), followed by buccal mucosa (7 cases, 28%), gingivobuccal sulcus (4 cases, 16%), alveolus (3 cases, 12%) and retromolar trigone (1 case, 4%). Of the 25 cases, 13 cases (52%) were moderately differentiated SCC, followed by 11 cases (44%) of well-differentiated SCC and 1 case (4%) of poorly differentiated SCC (Graph 2) (Figure 1, 2 and 3).

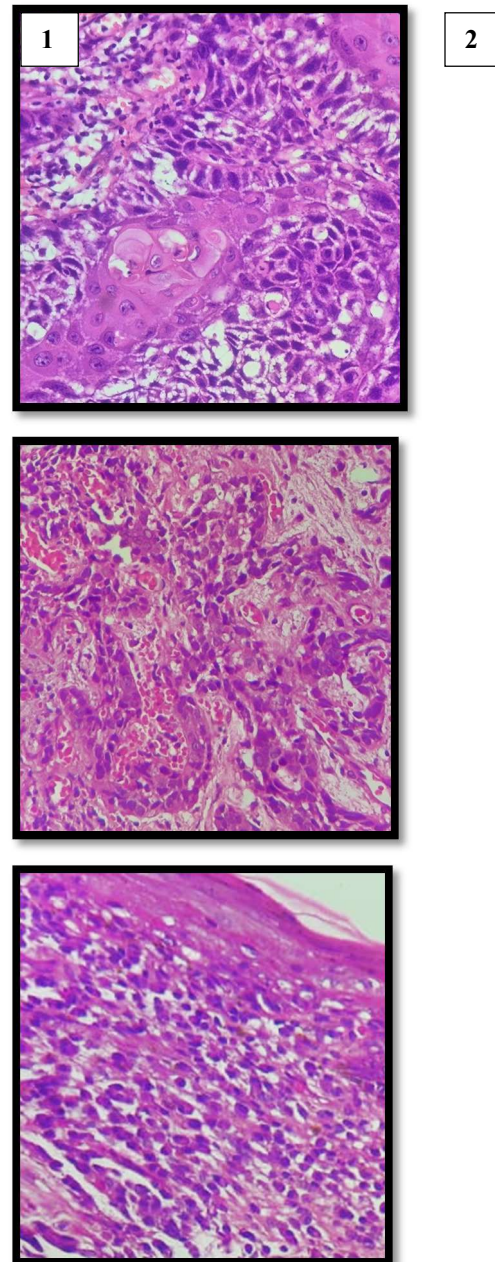
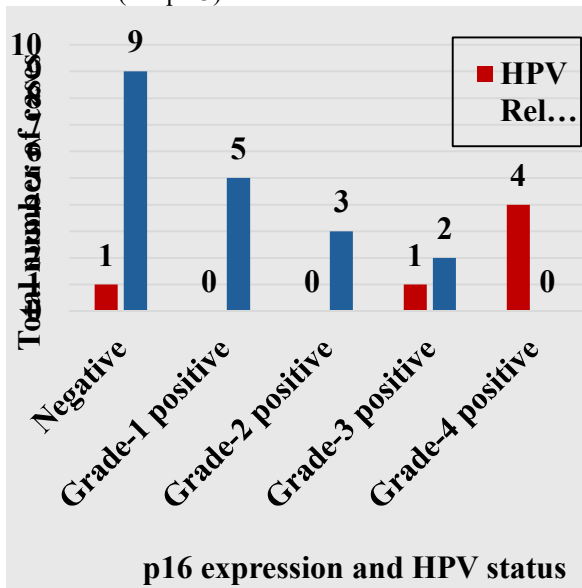


Figure 1: Well-differentiated SCC, Figure 2: Moderately differentiated SCC, Figure 3: Poorly differentiated SCC

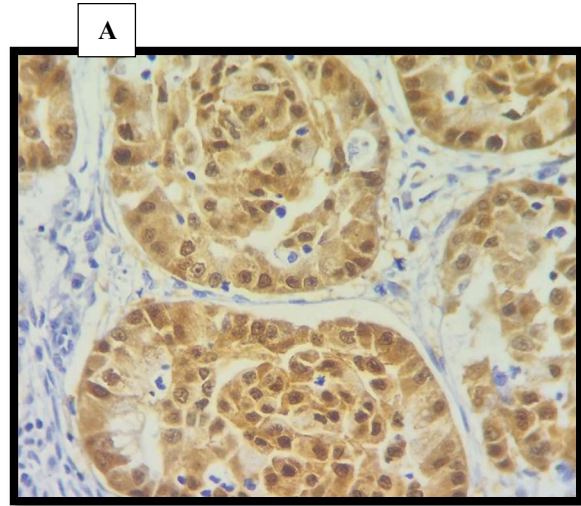
In this study, we noticed that 15 cases (60%) showed p16 positivity. Of these, 5 cases (20%) demonstrated

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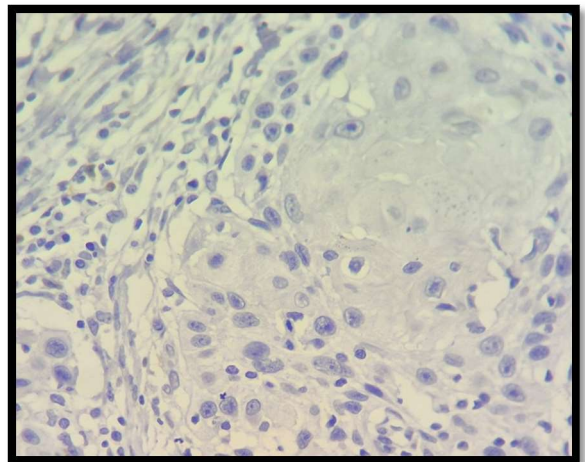
Grade-1 positivity, 3 cases (12%) Grade-2 positivity, 3 cases (12%) Grade-3 p16 positivity, and 4 cases (16%) Grade-4 p16 positivity. The remaining 10 cases (40%) showed p16 negative expression (Figure 4). The HPV DNA analysis was thus carried out in the 15 cases showing p16 positive expression. It was observed that all the grade-4 p16 positive cases had HR-HPV DNA belonging either to HPV-16 or HPV-18 genotypes. Additionally, HR-HPV DNA was also detected in 1 case showing Grade-3 p16 positivity and 1 case showing p16 negativity. All the other cases did not show HR-HPV and were reported as HPV Unrelated (Graph 3).



Graph 3: p16 expression and HPV status of cases.



B



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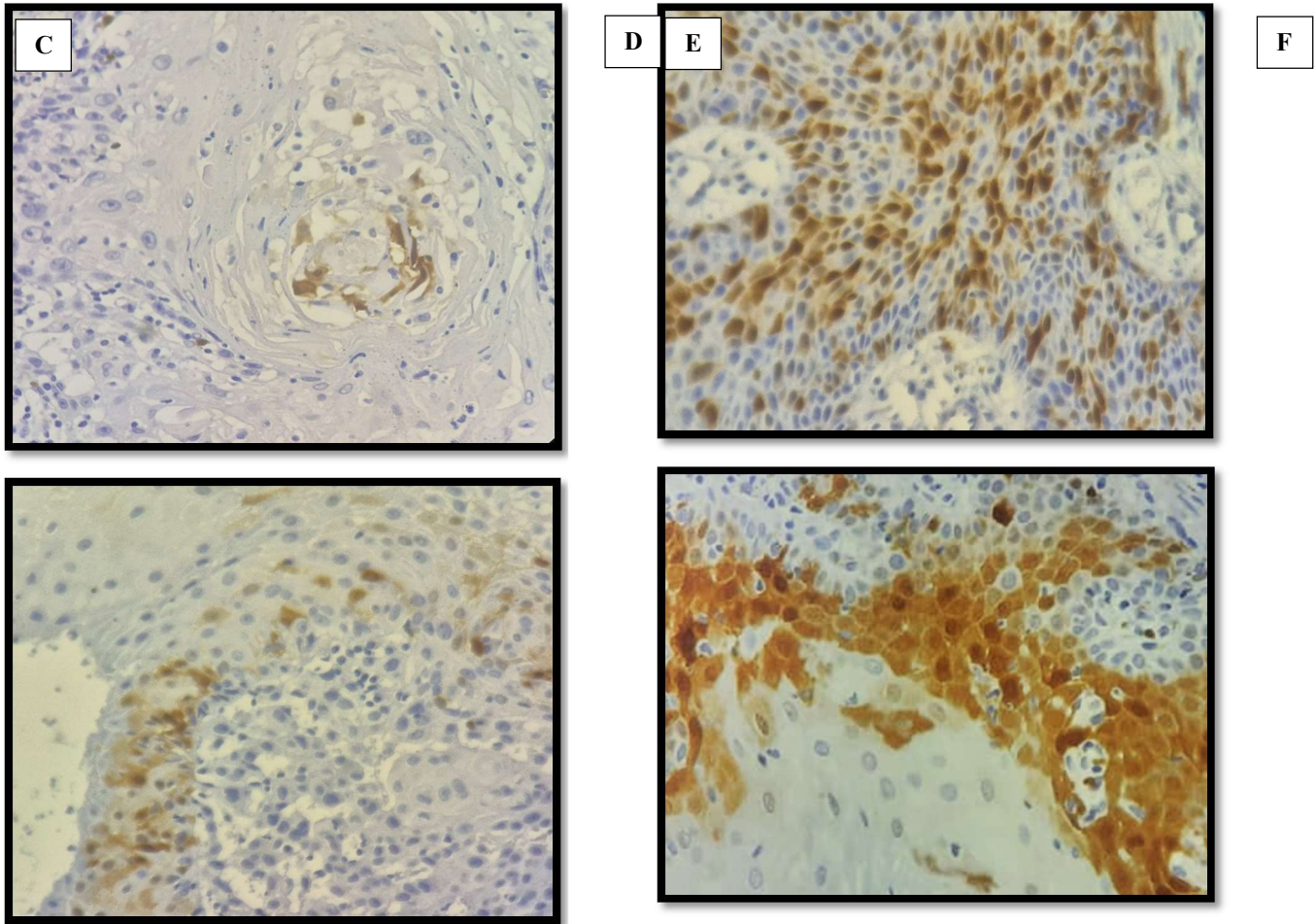


Figure 4: p16 Immunohistochemical Expression (40X): A) Positive Control (Breast Cancer), B) Negative, C) Grade-1 positive (Low/Weak), D) Grade-2 positive (Moderate), E) Grade-3 positive (Strong), F) Grade-4 positive (Very strong – HPV-associated).

On clinicopathological correlation, statistically significant association (p-value 0.002) was observed between p16 expression and HPV status of the individual. The p16 expression showed statistically significant association with histopathological grade (p-value 0.03) with majority of the cases belonging to MDSCC, and individuals with no substance abuse (p-value 0.02). It was found that majority of the p16 positive cases belonged to middle-aged (31-50 years) males. HR-HPV DNA was detected in a total of 5 p16 positive cases, who were not addicted to smoking, tobacco chewing or alcohol consumption, but had complaint of poor oral hygiene or oral thrush suggesting HPV-mediated carcinogenesis. Tongue (6 cases) was the most common subsite showing p16 positive expression, followed by gingivobuccal sulcus (4 cases). (Table 2).

Table 2: Correlation of p16 Immunohistochemical expression with clinico-pathological parameters and HPV status.

Variables	p16 IHC Expression					p-value
	Negative	Positive				
		Grade-1	Grade-2	Grade-3	Grade-4	
Age Group						
31-40	2	0	0	1	3	0.183
41-50	3	3	2	0	1	
51-60	2	1	0	2	0	
61-70	3	1	1	0	0	
Gender						
Male	7	3	2	2	4	0.732
Female	3	2	1	1	0	

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Risk Factors						
Smoking	12	4	1	1	0	0.1 38
Tobacco chewing	8	3	1	0	0	0.0 89
Alcohol consumption	4	3	1	0	0	0.6 18
No substance abuse	1	0	0	1	4	0.0 2
Tumour subsite						
Tongue	4	0	1	2	3	0.1 97
Buccal mucosa	4	2	0	1	0	
Gingivobuccal sulcus	0	2	1	0	1	
Alveolus	2	1	0	0	0	
Retromolar trigone	0	0	1	0	0	
Histopathological Grade						
WDSCC	6	3	2	0	0	0.0 3
MDSCC	3	2	1	3	4	
PDSCC	1	0	0	0	0	
HPV Status						
Positive	1	0	0	1	4	0.0 02
Negative	9	5	3	2	0	

DISCUSSION

Oral squamous cell carcinoma (OSCC) is one of the most prevalent malignancies of the head and neck region, accounting for nearly 90% of all oral cancers. India contributes significantly to this due to widespread tobacco chewing, smoking, alcohol consumption, poor oral hygiene, socioeconomic, psychological and cultural factors. In recent years, the oncogenic role of HR-HPV, particularly HPV-16 and HPV-18, has gained rising evidence in the pathogenesis of a subset of OSCCs. HPV-associated carcinomas are biologically distinct from conventional tobacco-related tumors and show unique molecular pathways and prognostic implications. In the present study, p16 immunohistochemistry was evaluated as a surrogate marker for HPV infection in OSCC and correlated with clinicopathological parameters and HPV DNA status.

The present study included 25 histopathologically confirmed cases of OSCC. Majority of the patients belonged to the age group of 41–50 years with male predominance (72%). This was similar to the demographic findings reported by Ghosh et

al.^[16] who observed higher incidence of OSCC among middle-aged males with tobacco exposure. The higher prevalence of tobacco chewing and smoking habits in men has resulted in male predominance in OSCC. The age distribution observed in the present study also correlates with the cumulative carcinogenic effects of the conventional risk factors.

In the current study, tongue was the most commonly involved subsite, followed by buccal mucosa and gingivobuccal sulcus. Similar observations were made by Naz et al.^[17] and Wang et al.,^[18] who reported tongue as a common site in HPV-associated OSCC. The predilection of HPV-associated carcinomas for tongue and oropharyngeal region may be attributed to reticulated epithelium, deep crypts, and increased susceptibility to microtrauma facilitating viral entry. Buccal mucosa involvement in conventional OSCC reflects the habit of placing tobacco and betel nut in the oral cavity.

Among the 25 cases, 10 cases were p16 negative whereas 15 cases demonstrated varying grades of p16 positivity, of which Grade-4 strong diffuse positivity was noted in 4 cases. Similar observations were made by Ghosh et al.^[16] and Kalsariya et al.,^[19] who emphasized the utility of p16 as a surrogate biomarker for HPV-associated tumors. p16 overexpression results from functional inactivation of retinoblastoma protein (Rb) by the HPV E7 oncoprotein, leading to upregulation of p16 expression. Hence, diffuse and strong p16 staining in >75% tumour cells is considered indicative of transcriptionally active HPV infection.

A statistically significant association between histopathological grade and p16 expression was observed in the present study (p = 0.03). Strong p16 positivity was predominantly noted in moderately differentiated squamous cell carcinoma (MDSCC). Similar findings were reported by Wang et al.^[18] and Kalsariya et al.^[19] Moderately differentiated tumors may represent an intermediate biological state with active HPV-mediated molecular dysregulation while still retaining partial squamous differentiation.

The association between HPV status and p16 expression highly statistically significant (p-value 0.002) in the present study. All Grade-4 p16 positive cases demonstrated HPV positivity on PCR analysis, strongly supporting the role of p16 as a surrogate marker for HPV infection. Naz et al.^[17] observed that nearly 74.8% of p16 positive OSCC cases showed HPV DNA positivity on PCR confirmation. Similar observations were reported by Lewis et al.^[20] and Ang et al.,^[21] who established diffuse p16 positivity as a reliable indicator of transcriptionally active HPV infection.

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Interestingly, HPV-positive cases in the present study lacked traditional risk factors such as smoking, tobacco chewing, and alcohol consumption. Similar findings were reported by Wang et al.,^[18] who demonstrated increased frequency of HPV-associated OSCC among younger individuals and non-tobacco users. This suggests an independent oncogenic role of HPV in oral carcinogenesis.

However, occasional discordance between p16 positivity and HPV DNA status was noted in the current study. Similar observations were reported by Lin et al.,^[22] who concluded that p16 overexpression may occur independent of HPV infection through alternative molecular pathways. Therefore, p16 immunohistochemistry, although highly sensitive, may not always be completely specific for biologically active HPV infection, and molecular confirmation using PCR or ISH remains the gold standard.

The major advantage of p16 IHC lies in its simplicity, reproducibility, cost-effectiveness, and wide availability compared to PCR-based HPV testing. In resource-limited settings, p16 IHC can serve as an effective screening tool and surrogate biomarker for identifying HPV-associated OSCC. However, confirmatory molecular testing remains necessary in selected cases.

CONCLUSION

The present study demonstrated that p16 immunohistochemistry acts as a valuable adjunctive tool in the evaluation of oral squamous cell carcinoma and serves as a promising, surrogate biomarker for HPV-associated OSCC. Statistically significant correlation between p16 positivity and HPV PCR findings supported the role of p16 in detecting HPV-mediated carcinogenesis. Very strong p16 positivity (Grade-4) correlates significantly with high-risk HPV DNA detection and may aid in prognostication and therapeutic stratification.

The findings of the present study are in concordance with several previous studies. However, this study also demonstrated that p16 positivity does not always correlate with HPV DNA positivity, emphasizing that p16 alone cannot be considered a completely reliable marker for HPV infection in oral cavity tumors. Combined assessment of routine histopathology, immunohistochemistry and molecular techniques provides greater diagnostic accuracy.

The present study further supports the concept of HPV-associated OSCC representing a distinct clinicopathological and molecular subgroup. They generally demonstrate better response to radiotherapy and improved overall survival. Hence, early identification of HPV using p16 IHC may therefore contribute to improved diagnostic accuracy,

prognostic assessment and therapeutic decision-making in patients with oral squamous cell carcinoma.

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