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Original Research Article

Immunohistochemical Expression of p16 in Oral Squamous Cell Carcinoma: A Descriptive Study

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Abstract:

Background: Oral squamous cell carcinoma (OSCC) is a major malignancy in India and is strongly associated with tobacco, alcohol and betel-nut consumption. p16^INK4a is a cell-cycle regulatory protein whose overexpression may indicate altered retinoblastoma signalling and possible human papillomavirus involvement. Objectives**:** To evaluate p16 immunohistochemical expression in OSCC and determine its association with histopathological grade.

Materials and Methods: This prospective, hospital-based descriptive study included 184 histopathologically confirmed primary OSCC cases diagnosed in the Department of Pathology, a tertiary care hospital, Mulugu. Tumours were graded histopathologically, and p16 expression was evaluated using immunohistochemistry. Strong, diffuse nuclear and cytoplasmic staining in more than 70% of malignant cells was considered positive. Data were analysed using descriptive statistics and the chi-square test, with $p<0.05$ considered significant.

Results: Most patients were aged 41–60 years (57.61%), and 86.41% were males. The tongue was the most frequently affected site (73.37%). Tobacco smoking (75.4%), alcohol consumption (67.2%) and betel-nut chewing (56.5%) were the major reported risk factors. Well-differentiated SCC was predominant (81.52%). Strong p16 positivity was detected in 28 cases (15.22%), including 25 well-differentiated, one moderately differentiated and two non-keratinising carcinomas. Among conventional differentiated tumours, p16 positivity was significantly more frequent in well-differentiated SCC than in moderately/poorly differentiated SCC (16.67% versus 3.12%; $\chi^2=3.95$, $p=0.047$).

Conclusion: Strong p16 expression was present in a minority of OSCC cases and was significantly associated with well-differentiated tumours. HPV-specific molecular testing is required to confirm HPV-driven carcinogenesis.

Keywords: Oral Squamous Cell Carcinoma; p16^INK4a; Immunohistochemistry; Histopathological Grade; Human Papillomavirus; Oral Cancer.

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Introduction

Oral cancer is an important public health problem, particularly in South and South-East Asia, where exposure to smokeless tobacco, areca nut, betel quid, cigarette smoking and alcohol remains common. Globally, approximately 389,846 new cases of lip and oral cavity cancer were estimated in 2022, with India accounting for a substantial proportion of the disease burden [1]. Oral squamous cell carcinoma (OSCC) is the predominant histological type of oral malignancy. Despite advances in diagnosis, surgery, radiotherapy and systemic treatment, many patients present with locally advanced disease, resulting in considerable morbidity, recurrence and mortality. The heterogeneous clinical behaviour of OSCC has therefore encouraged the investigation of molecular biomarkers that may complement conventional histopathological assessment.

The development and progression of OSCC involve the accumulation of genetic and epigenetic alterations affecting cell-cycle regulation, apoptosis, DNA repair and cellular differentiation. Among the important cell-cycle regulatory proteins is p16^INK4a, a tumour-suppressor protein encoded by the CDKN2A gene located on chromosome 9p21. The p16 protein inhibits cyclin-dependent kinases 4 and 6, prevents phosphorylation of the retinoblastoma protein and restricts progression from the G1 to the S phase of the cell cycle. Loss of this regulatory function through deletion, mutation

or promoter methylation can permit uncontrolled cellular proliferation and contribute to malignant transformation [2].

Conversely, strong p16 overexpression may occur in tumours driven by high-risk human papillomavirus (HPV). The HPV E7 oncoprotein inactivates the retinoblastoma protein, producing compensatory upregulation of p16. Consequently, diffuse nuclear and cytoplasmic p16 immunoreactivity is widely used as a surrogate marker of transcriptionally active HPV infection in oropharyngeal squamous cell carcinoma. Immunohistochemistry is comparatively simple, economical and widely available, making it useful in routine pathology laboratories [3,4]. However, the biological and clinical interpretation of p16 expression in OSCC should be distinguished from that in oropharyngeal carcinoma.

Studies of oral cavity tumours have reported considerable variability in p16 positivity. Patil et al. demonstrated immunohistochemical p16 expression in a proportion of OSCC cases and suggested its potential value in identifying tumours associated with HPV [3]. Duncan et al. found that strong p16 staining correlated significantly with HPV-16 detected by polymerase chain reaction, although weaker staining was frequently unrelated to HPV [4]. In a large Indian cohort of 800 OSCC cases, Naz et al. observed discrepancies between p16 immunopositivity and HPV-DNA positivity, indicating that p16 expression alone may not reliably establish HPV-driven carcinogenesis in every oral tumour [5]. A systematic review by Katirachi et al. similarly demonstrated marked geographical and methodological variation in HPV prevalence among oral cavity squamous cell carcinomas [6].

Apart from its possible relationship with HPV, p16 expression may reflect disturbances in cell-cycle regulation and could be associated with clinicopathological characteristics such as patient age, tumour site, histological grade, tumour size, lymph-node involvement and disease stage. Pandey et al. reported significant relationships between p16 expression and selected clinicopathological variables in patients with head-and-neck squamous cell carcinoma [7]. Nevertheless, the prognostic significance of p16 in OSCC remains uncertain. Maléřová et al. noted that the significance of p16 positivity differs between oral and oropharyngeal carcinomas and that p16 positivity in oral carcinoma does not necessarily indicate the favourable prognosis typically observed in HPV-associated oropharyngeal carcinoma [8]. Current expert guidance consequently does not recommend routine high-risk HPV testing in primary oral cavity carcinoma solely for prognostic purposes, further emphasising the need for site-specific interpretation of p16 findings [9].

Owing to variations in reported p16 expression and its clinicopathological associations, additional studies from different populations and geographical regions are required. Therefore, the present descriptive study aims to evaluate the immunohistochemical expression of p16 in histologically confirmed OSCC and to describe its distribution according to relevant demographic, clinical and histopathological characteristics. The findings may provide baseline information on the pattern of p16 expression in OSCC and support further analytical studies examining its relationship with HPV status, tumour behaviour, treatment response and patient outcomes.

Materials and Method

Study design and setting: This prospective, hospital-based descriptive study was conducted in the Department of Pathology, a tertiary care hospital, Mulugu, Telangana. The study included 184 histopathologically confirmed cases of primary oral squamous cell carcinoma (OSCC).

Study population and sampling: All eligible cases of primary OSCC diagnosed during the study period were included using consecutive sampling. Relevant clinical and demographic information was obtained from histopathology request forms, hospital records and, where available, direct patient interviews.

The variables recorded included age, sex, anatomical site of the tumour and major risk factors, including tobacco smoking, smokeless tobacco or betel-quid use, areca/betel-nut chewing and alcohol consumption. Each risk factor was documented independently.

Inclusion criteria

The study included:

1. Histopathologically confirmed primary squamous cell carcinoma of the oral cavity.
2. Resection or biopsy specimens containing sufficient viable tumour tissue for histopathological and immunohistochemical evaluation.
3. Cases with adequate clinical and demographic information.
4. Newly diagnosed cases received during the specified study period.

Exclusion criteria

The following cases were excluded:

1. Oral lesions diagnosed as malignancies other than squamous cell carcinoma.
2. Recurrent or metastatic squamous cell carcinoma involving the oral cavity.
3. Infective and inflammatory oral lesions.
4. Benign oral lesions, including aphthous ulcers.
5. Specimens showing extensive necrosis or containing insufficient viable tumour cells for

reliable histopathological or immunohistochemical evaluation.
6. Poorly fixed, inadequately preserved or technically unsuitable tissue specimens.
7. Cases for which paraffin blocks or corresponding clinical details were unavailable.

Specimen processing and histopathological examination: All specimens were fixed in 10% neutral-buffered formalin for approximately 24 hours. Following fixation, the tissues were processed using the conventional paraffin-embedding technique. Sections measuring approximately 3–4 µm in thickness were cut from representative paraffin blocks and stained with haematoxylin and eosin (H&E).

The H&E-stained sections were examined under a light microscope to confirm the diagnosis of OSCC. Tumours were histologically graded using the World Health Organization three-tier grading system as:

1. Well-differentiated squamous cell carcinoma.
2. Moderately differentiated squamous cell carcinoma.
3. Poorly differentiated squamous cell carcinoma.

Other histopathological features, including keratinisation, cellular and nuclear pleomorphism, mitotic activity, tumour invasion and lymphovascular or perineural invasion, were recorded wherever assessable. Tumour stage was documented according to the eighth edition of the American Joint Committee on Cancer tumour–node–metastasis classification, wherever adequate clinical and pathological information was available.

Immunohistochemical evaluation of p16: Immunohistochemistry was performed on representative sections from all histopathologically confirmed OSCC cases using a monoclonal antibody against p16^INK4a. Sections of 3–4 µm thickness were placed on poly-L-lysine-coated slides.

A section of cervical squamous cell carcinoma previously demonstrated to show strong and diffuse p16 expression was used as the positive control. A negative reagent control was prepared by replacing the primary antibody with phosphate-buffered saline or the corresponding antibody diluent.

Immunohistochemical staining procedure

The following staining procedure was used:

1. Sections were deparaffinised in xylene.
2. The slides were rehydrated through descending grades of alcohol and washed in distilled water.
3. Tris buffer was transferred to a microwave-compatible container and preheated at 850 W for two minutes.
4. Slides were immersed in the preheated Tris buffer for heat-induced epitope retrieval. Microwave heating was performed at 850 W for five minutes, followed by ten minutes and another five minutes.
5. Slides were removed from the microwave and allowed to cool to room temperature.
6. The sections were washed with wash buffer.
7. Endogenous peroxidase activity was blocked using a peroxidase-blocking solution for five minutes.
8. Slides were washed twice with wash buffer for two minutes each.
9. The entire tissue section was covered with the primary p16 antibody and incubated for one hour.
10. The slides were washed with wash buffer for five minutes.
11. The sections were covered with horseradish peroxidase-conjugated secondary antibody and incubated for 30 minutes.
12. Slides were washed twice with wash buffer for five minutes each.
13. Immunoreactivity was visualised using 3,3′-diaminobenzidine chromogen for approximately five minutes.
14. The slides were washed with wash buffer, followed by tap water.
15. Sections were counterstained with haematoxylin for 30 seconds.
16. Slides were washed in tap water, dehydrated through ascending grades of alcohol and cleared in xylene.
17. The sections were mounted using dibutyl phthalate polystyrene xylene and examined under a light microscope.

The incubation time and chromogen-development period were monitored to prevent excessive background staining.

Assessment of p16 immunohistochemical expression: The stained slides were systematically examined for p16 immunoreactivity. Nuclear and cytoplasmic staining in viable malignant epithelial cells was evaluated. Membranous staining alone was not regarded as specific p16 positivity. Necrotic areas, inflammatory cells, stromal cells and technically damaged areas were excluded from tumour-cell scoring.

Mean labelling index: The mean labelling index for p16 was calculated using the following formula:

$$\text{Mean labelling index (\%)} = \frac{\text{Number of p16} - \text{positive malignant cells}}{\text{Total number of mallignant cell counted}}$$

Final classification of p16 status

For the primary analysis, p16 expression was classified as:

- **p16 positive:** strong and diffuse, continuous nuclear and cytoplasmic staining in more than 70% of viable malignant cells.

- **p16 negative:** absence of staining; weak, focal or patchy staining; cytoplasmic staining without convincing nuclear staining; or strong staining involving 70% or fewer malignant cells.

The percentage-based scores from 0 to 4+ were retained for describing the distribution of p16 expression. However, only cases meeting the above strong and diffuse nuclear and cytoplasmic staining criterion were classified as p16 positive.

Clinicopathological Correlation: The immunohistochemical expression of p16 was evaluated in relation to the following clinicopathological variables:

- Age;
- Sex;
- Anatomical site of the primary tumour;
- Tobacco smoking;
- Smokeless tobacco or betel-quid use;
- Areca/betel-nut chewing;
- Alcohol consumption;
- Histological grade;
- Tumour size and T category;
- Regional lymph-node status;
- Overall clinical or pathological stage; and
- Other histopathological characteristics, wherever available.

Statistical Analysis: The collected data were entered into Microsoft Excel and analysed using SPSS Version 25. Continuous variables, such as age and p16 labelling index, were summarised using mean and standard deviation or median and interquartile range, depending on their distribution. Categorical variables were presented as frequencies and percentages.

The association of p16 expression with demographic, risk-factor and clinicopathological variables was evaluated using the chi-square test or Fisher's exact test, as appropriate. An independent-samples t-test or Mann–Whitney U test could be used for quantitative variables according to data distribution. A two-sided p-value of less than 0.05 was considered statistically significant.

Observation and Results

Table 1: Age- and sex-wise distribution of OSCC cases (N = 184)

Age group (years)	Male, n (%)	Female, n (%)	Total, n (%)
21–40 Years	25 (13.59)	1 (0.54)	26 (14.13)
41–60 Years	92 (50.00)	14 (7.61)	106 (57.61)
≥61 Years	42 (22.83)	10 (5.43)	52 (28.26)
Total	159 (86.41)	25 (13.59)	184 (100.00)

Among the 184 patients with oral squamous cell carcinoma (OSCC), the largest proportion belonged to the 41–60-year age group, comprising 106 cases (57.61%). This was followed by patients aged ≥61 years with 52 cases (28.26%), while 26 cases (14.13%) were aged 21–40 years. A marked male predominance was observed, with 159 males (86.41%) and 25 females (13.59%), giving a male-to-female ratio of approximately 6.4:1. The highest number of both male and female cases was observed in the 41–60-year age group.

Table 2: Distribution of OSCC cases according to anatomical location (N = 184)

Anatomical location	Number of cases	Percentage
Tongue	135	73.37
Buccal mucosa	15	8.15
Lip	14	7.61
Floor of mouth	13	7.07
Palate	5	2.72
Retromolar trigone	1	0.54
Tonsillar fossa	1	0.54
Total	184	100

The tongue was the most frequently involved anatomical site, accounting for 135 of the 184 cases (73.37%). Buccal mucosa was the second most common site with 15 cases (8.15%), followed by the lip with 14 cases (7.61%) and the floor of the mouth with 13 cases (7.07%). Palatal involvement was observed in five cases (2.72%). The retromolar trigone and tonsillar fossa were the least commonly involved sites, with one case (0.54%) each. These findings indicate a strong predominance of tongue involvement among the studied OSCC cases.

Table 3: Distribution of cases according to histopathological type and grade (N = 184)

Histopathological type/grade	Number of cases	Percentage
Well-differentiated SCC	150	81.52
Moderately differentiated SCC	31	16.85
Poorly differentiated SCC	1	0.54
Non-keratinising SCC	2	1.09
Total	184	100

Well-differentiated squamous cell carcinoma was the predominant histopathological grade, observed in 150 cases (81.52%). Moderately differentiated squamous cell carcinoma accounted for 31 cases (16.85%), whereas poorly differentiated squamous cell carcinoma was identified in only one case (0.54%). Two cases (1.09%) were classified as non-keratinising squamous cell carcinoma. Thus, most tumours in the study demonstrated well-differentiated morphology.

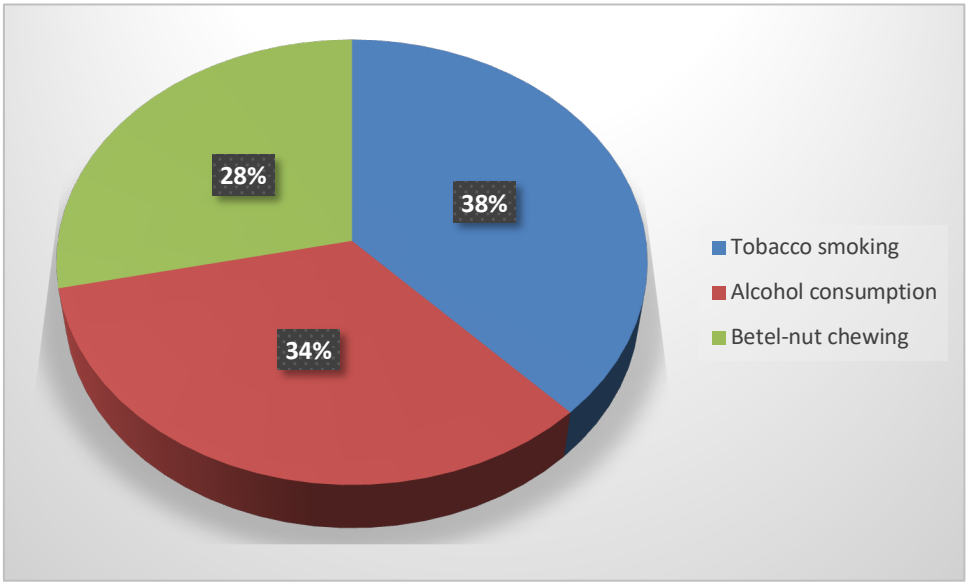


Figure 1: Distribution of reported risk factors among OSCC cases

Tobacco smoking was the most frequently reported risk factor and was present in 75.4% of the patients. Alcohol consumption was reported by 67.2%, while betel-nut chewing was reported by 56.5% of the patients. These findings demonstrate a high prevalence of recognised behavioural risk factors among patients with OSCC. Because the risk factors were recorded independently, individual patients could have more than one exposure; therefore, the percentages are not mutually exclusive and should not be added together.

Table 4: Distribution of cases according to p16 immunohistochemical expression (N = 184)

p16 score	Number of cases	Percentage
0 to 3+(Negative, weak or moderate expression (≤70%))	156	84.78
4+(Strong and diffuse expression (>70%))	28	15.22
Total	184	100

Of the 184 OSCC cases, 156 (84.78%) demonstrated negative, weak or moderate p16 expression involving ≤70% of the malignant cells and were classified as p16 negative. Strong and diffuse nuclear and cytoplasmic p16 expression involving >70% of malignant cells was observed in 28 cases (15.22%), which were classified as p16 positive. Therefore, the overall frequency of strong p16 positivity in the study population was 15.22%.

Table 5: Association between p16 expression and histopathological grade (N = 184)

Histopathological type/grade	Negative/weak/moderate p16, n (% of total)	Strong p16, n (% of total)	Total, n (%)
Well-differentiated SCC	125 (67.93)	25 (13.59)	150 (81.52)
Moderately differentiated SCC	30 (16.30)	1 (0.54)	31 (16.85)
Poorly differentiated SCC	1 (0.54)	0 (0.00)	1 (0.54)
Non-keratinising SCC	0 (0.00)	2 (1.09)	2 (1.09)
Total	156 (84.78)	28 (15.22)	184 (100.00)

Among the 150 well-differentiated SCC cases, 25 showed strong p16 expression, corresponding to 16.67% of well-differentiated tumours. Only one of the 31 moderately differentiated tumours (3.23%) demonstrated strong p16 expression, while the single poorly differentiated tumour was p16 negative. Both non-keratinising SCC cases showed strong p16 positivity. Overall, the 28 strongly p16-positive cases consisted of 25 well-differentiated SCCs, one moderately differentiated SCC and two non-keratinising SCCs. These findings show that strong p16 expression was predominantly observed in well-differentiated tumours, although both non-keratinising tumours were also positive.

Table 6: Association between p16 expression and histopathological grade (N = 184)

Histopathological grade	p16 negative/ weak/ moderate, n (%)	Strong p16 positive, n (%)	χ^2	p-value
Well-differentiated SCC	125 (83.33)	25 (16.67)	3.95	0.047
Moderately/poorly differentiated SCC	31 (96.88)	1 (3.12)		
Total	156 (85.71)	26 (14.29)		

For the statistical analysis, the two non-keratinising SCC cases were excluded, leaving 182 conventional differentiated SCC cases. Among the 150 well-differentiated tumours, 25 (16.67%) demonstrated strong p16 positivity, compared with only one (3.12%) of the 32 moderately or poorly differentiated tumours. Conversely, negative, weak or moderate p16 expression was observed in 125 well-differentiated cases (83.33%) and 31 moderately or poorly differentiated cases (96.88%). The association between p16 expression and conventional histopathological grade was statistically significant ($\chi^2 = 3.95$, $p = 0.047$). Thus, strong p16 expression was significantly more frequent among well-differentiated OSCC cases.

Discussion

Oral squamous cell carcinoma remains an important health problem in India because of the widespread consumption of smoked and smokeless tobacco, areca nut, betel quid and alcohol. The present study evaluated the clinicopathological profile and p16 immunohistochemical expression in 184 histopathologically confirmed OSCC cases. The principal findings were a predominance of middle-aged men, frequent involvement of the tongue, a high prevalence of tobacco and alcohol exposure, predominance of well-differentiated tumours and strong p16 expression in 15.22% of cases.

Age and Sex Distribution: The majority of patients were aged 41–60 years (57.61%), followed by those aged ≥61 years (28.26%). This concentration of cases in middle and older age groups is consistent with the cumulative effect of prolonged exposure to carcinogens. Rai and Ahmed also reported that most Indian OSCC cases occurred between the fifth and seventh decades of life [10]. Sharma et al., in a study from Western Uttar Pradesh, observed the greatest number of cases in the fourth and fifth decades [11]. The presence of 14.13% of cases in patients aged 21–40 years in the present study is nevertheless noteworthy and may reflect an earlier initiation of tobacco, areca-nut or alcohol use, genetic susceptibility or other unidentified factors. Krishnamurthy and Ramshankar similarly reported an increasing occurrence of oral tongue cancer among younger Indian patients, including individuals without conventional tobacco exposure [12].

A marked male predominance was observed, with 159 males (86.41%) and 25 females (13.59%), producing a male-to-female ratio of approximately 6.4:1. Male predominance has been consistently reported in Indian OSCC studies, although its magnitude varies across regions. Sharma et al. reported a ratio of 2.2:1, while Rai and Ahmed found that males constituted 53% of their cases [10,11]. The considerably higher male proportion in the present study may be related to greater exposure to tobacco smoking, alcohol and occupational or lifestyle-related risk factors among men in the study region. It may also reflect differences in healthcare-seeking behaviour and referral patterns.

Anatomical Distribution: The tongue was the most frequently affected anatomical site, accounting for 73.37% of cases. Buccal mucosa, lip and floor of the mouth accounted for 8.15%, 7.61% and 7.07%, respectively. The predominance of tongue carcinoma differs from the findings of Sharma et al., who reported buccal mucosa as the most common

site in Western Uttar Pradesh, representing 63.75% of cases [11]. Rai and Ahmed also identified buccal mucosa as the predominant site in their Indian population and attributed this distribution to the practice of retaining tobacco quid against the cheek [10]. Conversely, oral tongue cancer has been reported as an important and increasing subgroup of OSCC in South Indian populations [12].

These differences demonstrate that the anatomical distribution of OSCC is influenced by regional habits. Placement of smokeless tobacco or betel quid in the gingivobuccal sulcus produces prolonged contact with the buccal mucosa, whereas smoking, alcohol exposure and other biological factors may produce a different distribution. The very high frequency of tongue involvement in the present study should also be interpreted after carefully distinguishing the mobile oral tongue from the base of the tongue. Similarly, the tonsillar fossa is an oropharyngeal rather than an oral cavity site. This distinction is particularly important when interpreting p16 because p16 has a stronger association with transcriptionally active HPV in oropharyngeal carcinoma than in oral cavity carcinoma.

Distribution of Risk Factors: Tobacco smoking was the most frequently reported risk factor, occurring in 75.4% of patients, followed by alcohol consumption in 67.2% and betel-nut chewing in 56.5%. These exposures were recorded independently, indicating that many patients may have had multiple concurrent habits. The findings reinforce the dominant role of preventable behavioural exposures in the development of OSCC in India.

Rai and Ahmed found that tobacco use, either through chewing, smoking or both, was the predominant habit among patients with OSCC [10]. Sharma et al., however, reported that smokeless tobacco consumption was more frequent than smoking among both male and female patients in Western Uttar Pradesh [11]. The predominance of smoking in the present study may therefore represent a regional behavioural pattern. Concurrent tobacco and alcohol use is biologically important because both exposures can act synergistically by causing chronic mucosal injury, facilitating carcinogen penetration and promoting the accumulation of genetic and epigenetic alterations.

Because the present study did not include a cancer-free control group, the reported percentages describe the prevalence of exposures among OSCC patients but do not independently estimate the strength of association between these habits and OSCC. Future case–control studies should quantify the type, frequency and duration of each exposure and calculate adjusted odds ratios.

Histopathological Distribution: Well-differentiated SCC was the predominant histopathological grade, accounting for 81.52% of cases. Moderately differentiated SCC comprised 16.85%, while poorly differentiated and non-keratinising carcinomas were uncommon. The predominance of well-differentiated tumours is consistent with Rai and Ahmed, who reported that most OSCCs in their local Indian population were well differentiated [10]. An Indian systematic review by Nandi et al. also noted that HPV-positive head-and-neck tumours in several Indian studies were frequently well differentiated, although substantial heterogeneity was present across studies [13].

The high proportion of well-differentiated tumours may reflect the histopathological characteristics of the population, biopsy selection or interobserver variation in tumour grading. Histological grade alone does not fully represent tumour aggressiveness, as clinical stage, depth of invasion, pattern of invasion, perineural invasion, lymphovascular invasion and lymph-node status are also important prognostic variables.

Frequency of p16 Expression: Strong and diffuse nuclear and cytoplasmic p16 expression involving more than 70% of malignant cells was observed in 28 cases, giving an overall p16 positivity of 15.22%. This finding is closely comparable to the large Indian study by Naz et al., in which 139 of 800 OSCC cases (17.37%) were p16 immunopositive using a similarly stringent staining threshold [5]. Among their p16-positive cases, 74.8% were positive for HPV-16 or HPV-18 DNA by polymerase chain reaction. Murthy et al. reported p16 overexpression in 20% of 170 Indian patients with broader head-and-neck squamous cell carcinoma, which is also relatively close to the present result [14].

In contrast, Patil et al. reported p16 staining in 26 of 30 OSCC cases (86.67%) [15], while Ralli et al. reported positivity in 78.7% of 75 head-and-neck SCC cases [16]. Pandey et al. observed p16 positivity in 60% of their head-and-neck SCC cases [17]. These substantially higher values may be explained by differences in anatomical sites, antibody clones, staining protocols, sample sizes and definitions of positivity. Some earlier studies counted focal or low-intensity staining as positive, whereas the current study used a stringent criterion of strong and diffuse staining in more than 70% of tumour cells. Moreover, studies combining oral cavity and oropharyngeal tumours generally report higher p16 positivity because HPV-associated cancers occur more frequently in the oropharynx.

The present p16 positivity of 15.22% should not be interpreted as the prevalence of HPV-associated OSCC without confirmatory testing. Although

strong p16 expression is an accepted surrogate for HPV-driven oropharyngeal carcinoma, its specificity is lower in oral cavity carcinoma. p16 may be overexpressed through HPV-independent alterations of the retinoblastoma pathway. Conversely, some HPV-positive oral tumours may fail to meet the conventional p16 threshold. Therefore, HPV DNA polymerase chain reaction or, preferably, detection of transcriptionally active viral E6/E7 mRNA would be required to establish HPV-driven carcinogenesis.

Association between p16 expression and histological grade: Strong p16 expression was observed in 25 of 150 well-differentiated tumours (16.67%), compared with one of 32 moderately or poorly differentiated tumours (3.12%). The association between p16 expression and conventional histological grade was reported as statistically significant ($\chi^2 = 3.95$, $p = 0.047$). Both non-keratinising SCC cases also exhibited strong p16 expression, although they were excluded from the conventional grade comparison.

The predominance of strong p16 expression among well-differentiated tumours agrees with the general pattern identified in the systematic review by Nandi et al., which noted that HPV-positive head-and-neck cancers in Indian studies were often well differentiated [13]. However, Ralli et al. found that p16 expression increased significantly with increasing histological grade ($p = 0.045$), which contrasts with the present findings [16]. Saxena and Prasad also reported heterogeneous p16 staining across oral and oropharyngeal SCC grades [18]. These inconsistent findings suggest that the relationship between p16 and histological differentiation is not uniform and may be influenced by tumour location, HPV status and the method used to define p16 positivity.

The statistical association in the present study should be interpreted cautiously. The p-value of 0.047 is close to the conventional significance threshold, and the moderately/poorly differentiated group contained only one strongly positive case. The presence of small expected cell frequencies may limit the validity of the Pearson chi-square test; therefore, confirmation using Fisher's exact test would strengthen the analysis. Furthermore, a relationship with histological grade alone does not establish p16 as a prognostic marker. Demonstrating prognostic value would require follow-up information on recurrence, disease-free survival and overall survival, preferably with multivariable adjustment for stage and treatment.

Clinical Implications: The present findings demonstrate that a distinct minority of OSCC cases exhibit strong and diffuse p16 expression. The close agreement between the current prevalence and the 17.37% reported by Naz et al. supports the reproducibility of a relatively low p16-positive subgroup in Indian oral cavity carcinoma when strict immunohistochemical criteria are applied [5]. Nevertheless, p16-positive oral tumours should not automatically be labelled HPV driven. Confirmatory molecular testing is particularly important when the tumour arises from a true oral cavity site. For base-of-tongue or tonsillar tumours, reclassification as oropharyngeal carcinoma and site-appropriate HPV testing should be considered.

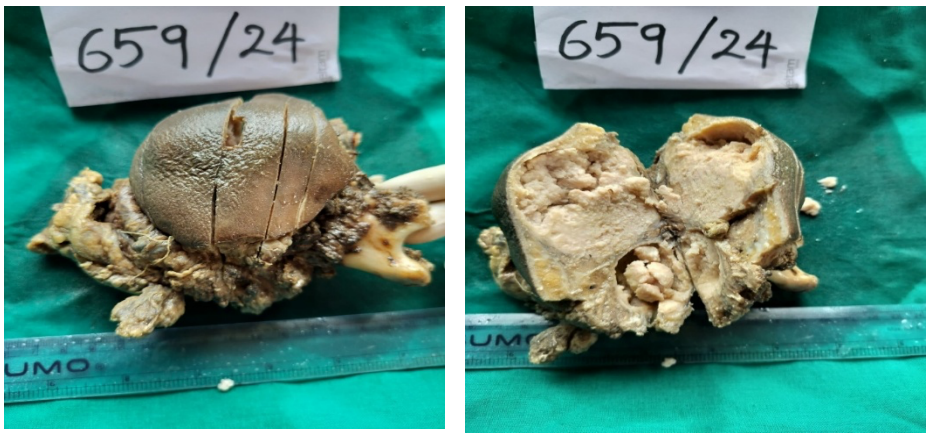
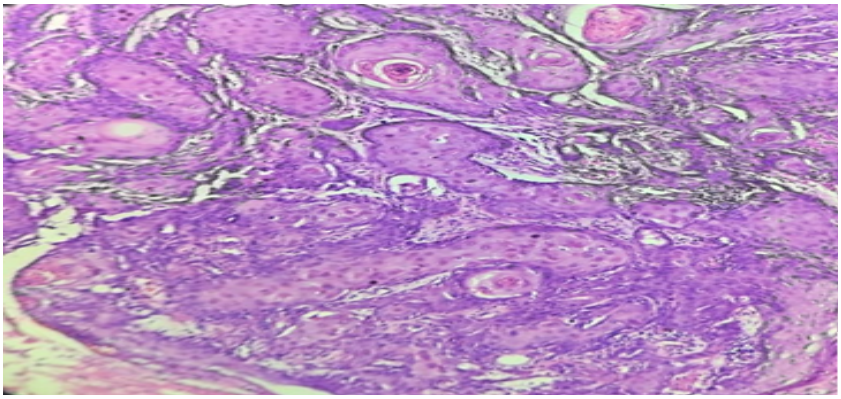
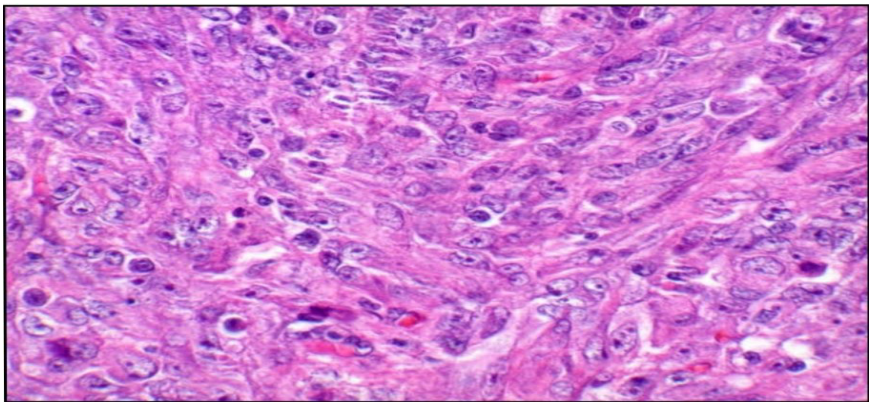


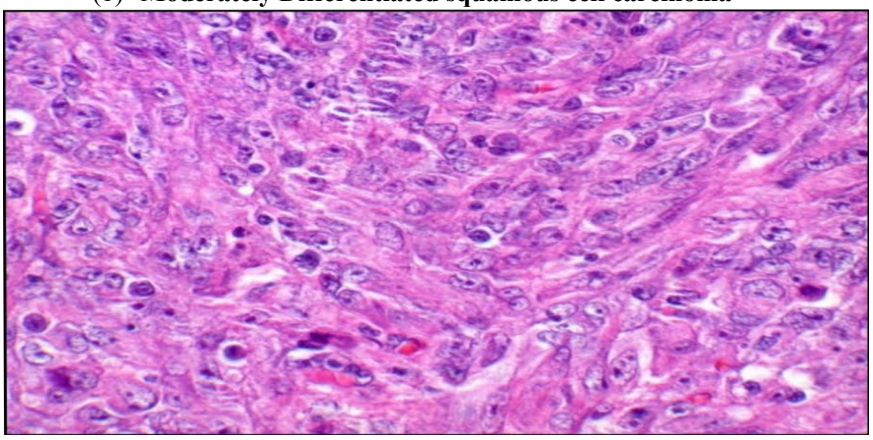
Figure 1: Gross and cut section images OSCC arising from Buccal Mucosa



(a) **Well differentiated squamous cell carcinoma**



(b) **Moderately Differentiated squamous cell carcinoma**



(c) **Poorly differentiated squamous cell carcinoma**

Conclusion

The present study demonstrated that OSCC predominantly affected middle-aged men, with the tongue being the most common site. Tobacco smoking, alcohol consumption and betel-nut chewing were the major associated risk factors, while well-differentiated SCC was the predominant histological grade. Strong and diffuse p16 expression was identified in 15.22% of cases and was significantly associated with well-differentiated tumours ($p = 0.047$). However, p16 positivity alone cannot confirm HPV-driven OSCC; therefore, HPV-specific molecular testing and follow-up studies are recommended to establish its etiological and prognostic significance.

Conflict of Interest: None

Funding: None

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