Biomedical and Biopharmaceutical Research
Abbreviation: Biomed. Biopharm. Res.
Volume: 22: Issue: 02 |Year: 2025
Page Number: 663-667

Molecular Pathological Epidemiology of Oral Cancer in Rural India

Rahul Gupta[1], Abha Gupta[2]
[1]Department of Surgical Oncolgy, Mahatma Gandhi Medical College, Jaipur
[2]Department of Pathology, Geetanjali Institute of Medical Sciences, Jaipur

Corresponding Author

Rahul Gupta
Department of Surgical Oncolgy, Mahatma Gandhi Medical College, Jaipur

Article Received:27-06-2025

Article Accepted:28-07-2025

Abstract

Background: Oral squamous cell carcinoma (OSCC) remains a leading malignancy in India, with a significant burden in rural populations. While tobacco and areca nut use are well-established risk factors, the molecular characteristics of OSCC in rural patients remain underexplored. This study integrates traditional epidemiology with molecular pathology to identify key risk patterns and biological behaviour in rural Indian OSCC cases.

Methods: A cross-sectional observational study was conducted on 100 histologically confirmed OSCC patients from rural northern India. Epidemiological data were collected via structured interviews. Tissue samples underwent immunohistochemical (IHC) analysis for p53, Ki-67, and Cyclin D1 expression. HPV status was assessed using p16 IHC. Associations between risk factors, molecular markers, and clinicopathological parameters were analysed.

Results: The majority (83%) had a history of tobacco or areca nut use. HPV positivity (p16+) was found in 20% of cases, predominantly among younger, non-smoking patients. p53 overexpression was noted in 57% of cases and correlated with high-grade tumours and nodal metastasis ($p < 0.05$). Cyclin D1 and Ki-67 were significantly associated with tumor stage and depth of invasion.

Conclusion: OSCC in rural India demonstrates high p53 mutation prevalence and moderate HPV association. Integration of molecular markers with epidemiologic data could enable risk stratification and tailored treatment in resource-limited settings.

Keywords: Oral squamous cell carcinoma, rural India, molecular pathology, p53, HPV, epidemiology.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) remains a major public health burden globally, with India bearing a disproportionate share of the incidence and mortality. According to GLOBOCAN 2020, India accounted for approximately one-third of the global oral cancer burden, with particularly high rates in rural regions (1). This epidemiological trend is closely linked to the widespread consumption of smokeless tobacco, betel quid, and areca nut products, which are deeply embedded in the socio-cultural practices of rural communities (2).

In these settings, barriers such as delayed diagnosis, low health literacy, inadequate access to specialized care, and reliance on traditional or unqualified practitioners contribute to a higher proportion of patients presenting with advanced-stage disease (3). While numerous epidemiological studies have reported on OSCC patterns in urban populations, data from rural regions—where socioeconomic and environmental conditions differ markedly—remain limited.

Molecular pathological epidemiology (MPE) is an emerging interdisciplinary field that combines molecular biology with population-based epidemiology to understand cancer heterogeneity and identify biologically distinct subgroups (4). In OSCC, several molecular markers, including **p53**, **Ki-67**, **Cyclin D1**, and **p16 (a surrogate for HPV infection)**, have been associated with tumor aggressiveness, prognosis, and therapeutic response (5,6).

Of particular interest is the role of high-risk human papillomavirus (HPV), which has been established as a key driver of oropharyngeal squamous cell carcinoma but is now increasingly reported in a subset of oral cavity cancers, especially among younger patients without significant tobacco or alcohol exposure (7). The use of p16 immunohistochemistry as a proxy for transcriptionally active HPV makes its evaluation feasible in resource-limited environments where PCR may not be readily available.

The **p53** tumor suppressor gene is among the most frequently mutated in OSCC, particularly in cases linked to carcinogen exposure, and its overexpression often correlates with poor differentiation, nodal involvement, and worse survival (8). Similarly, **Cyclin D1** dysregulation contributes to uncontrolled cell cycle progression, while **Ki-67** serves as a proliferation marker, both of which have prognostic implications in OSCC (9).

Despite the recognized value of these markers, their integration into routine cancer profiling in rural Indian populations remains underutilized. Bridging this gap could enable more precise risk stratification and guide personalized management approaches. Therefore, this study aims to explore the molecular pathological epidemiology of OSCC in rural India by examining the expression of key molecular markers and correlating them with demographic, clinical, and behavioural risk factors. Understanding these relationships is critical for improving outcomes in a population that continues to face disproportionate cancer burden and limited access to advanced care.

STUDY OBJECTIVES

The primary objective of this study is to investigate the association between molecular markers—specifically p53, Ki-67, p16, and Cyclin D1—and the clinicopathological characteristics of oral squamous cell carcinoma (OSCC) in patients from rural India. The secondary objectives include determining the prevalence of these key molecular alterations in OSCC tissues within this rural cohort, and assessing their correlation with established risk factors such as tobacco use, alcohol consumption, and areca nut chewing. Additionally, the study aims to evaluate the influence of molecular alterations on tumor aggressiveness, including parameters like depth of invasion (DOI), perineural invasion (PNI), lymphovascular invasion (LVI), and lymph node metastasis. An exploration of diagnostic delay patterns and their potential impact on the molecular and pathological profiles of OSCC will also be undertaken. Finally, the study seeks to develop a risk-based stratification model that integrates molecular and clinicopathological predictors for the early identification of biologically aggressive disease.

MATERIALS AND METHODS

Study Design

This was a prospective, cross-sectional observational study conducted over a 12-month period (January 2024 to December 2024) at a tertiary care cancer centre located in North India. The institution caters to a predominantly rural population from neighbouring districts and serves as a regional referral centre for head and neck cancers. Ethical clearance was obtained from the Institutional Ethics Committee (IEC/2025/019-HNC), and the study adhered to the principles outlined in the Declaration of Helsinki.

Study Population

Consecutive patients presenting to the surgical oncology outpatient department with newly diagnosed oral squamous cell carcinoma (OSCC) were screened for eligibility. Inclusion criteria comprised histologically confirmed squamous cell carcinoma of the oral cavity, age 18 years or older, permanent rural residence as per the definition of the Census of India, no prior treatment for cancer including surgery, chemotherapy, or radiotherapy, availability of adequate biopsy or surgical tissue for molecular analysis, and provision of written informed consent for participation and tissue use. Patients were excluded if they presented with recurrent or metastatic disease, had a non-squamous histology, were immunocompromised (e.g., HIV-positive), or had incomplete clinical or pathological records.

Data Collection and Variables

A structured proforma was utilized to systematically collect relevant data from each participant. Sociodemographic information included age, gender, occupation, education level, and socioeconomic status, assessed using the Modified BG Prasad Scale. Behavioral risk factors were recorded, including the type and duration of tobacco use (both smoking and smokeless forms), alcohol consumption, areca nut chewing habits, and oral hygiene practices. Clinical details captured included the tumor subsite, duration of symptoms, TNM staging as per the AJCC 8th edition, and diagnostic delay, which was defined as the time interval between the onset of first symptom and the pathological diagnosis. Pathological parameters documented comprised tumor grade, presence of perineural invasion (PNI), lymphovascular invasion (LVI), depth of invasion (DOI), and the worst pattern of invasion (WPOI).

Histopathological and Immunohistochemical Analysis

Formalin-fixed paraffin-embedded (FFPE) tumor tissues were retrieved from the pathology department. Representative 4 µm-thick sections were cut for haematoxylin and eosin (H&E) staining and immunohistochemical (IHC) evaluation.

The following markers were studied:

- **p53** (Clone DO-7): tumor suppressor gene mutation marker
- **Ki-67** (Clone MIB-1): cellular proliferation index
- **Cyclin D1** (Clone SP4): cell cycle regulator
- **p16INK4a**: surrogate marker for transcriptionally active high-risk HPV infection

IHC was performed using a standardized automated immunostaining platform. Antigen retrieval and staining protocols were followed as per manufacturer instructions. Positive and negative controls were included for each run.

Scoring Criteria

- **p53**: Nuclear staining in ≥10% of tumor cells was considered positive.
- **Ki-67**: Proliferation index calculated by counting positive nuclei in 500 tumor cells; >20% considered high.
- **Cyclin D1**: Moderate-to-strong nuclear staining in ≥10% of cells considered overexpression.
- **p16**: Strong and diffuse nuclear and cytoplasmic staining in ≥70% of tumor cells considered HPV-positive.

Slides were independently reviewed by two experienced pathologists blinded to clinical data. Discordant cases were resolved by consensus.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY). Continuous variables were summarized as mean ± standard deviation or median (IQR) and compared using Student's t-test or Mann–Whitney U test as appropriate. Categorical variables were expressed as frequencies and percentages; comparisons were done using Chi-square or Fisher's exact test.

Univariate analysis was performed to identify associations between molecular markers and clinicopathological features. Variables with p-value <0.05 in univariate analysis were included in a multivariate logistic regression model to determine independent predictors of advanced stage and nodal metastasis. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were reported.

Receiver Operating Characteristic (ROC) curve analysis was used to determine optimal cut-off points for depth of invasion (DOI) in predicting nodal involvement. A simple risk stratification model was created by assigning 1 point to each significant molecular or clinicopathological predictor.

A p-value <0.05 was considered statistically significant.

RESULTS

1. Patient Demographics and Risk Factors

A total of 100 patients were included in the study, with a mean age of 53.4 ± 9.1 years (range: 28–73). The majority were male (75%), and 83% of patients reported tobacco use, primarily in smokeless form. Alcohol consumption was noted in 58%, while areca nut use was reported by 67% of patients.

2. Tumor Characteristics

In the study cohort, the tongue was the most frequently involved subsite, accounting for 31% of cases, followed closely by the buccal mucosa (30%), floor of the mouth (21%), and gingiva (18%). Histologically, the majority of tumors were well-differentiated (64%), while 26% were moderately differentiated and 10% were poorly differentiated. The mean depth of invasion (DOI) was 5.86 mm ± 1.93, with a DOI greater than 5 mm observed in 58% of cases. Perineural invasion (PNI) was identified in 37% of tumours, whereas lymphovascular invasion (LVI) was present in 26%.

3. Nodal Metastasis

Clinically N0 patients were evaluated for pathological lymph node metastasis, and positive nodal involvement was identified in 37% of cases. Univariate analysis revealed that a depth of invasion (DOI) greater than 5 mm, presence of perineural invasion (PNI), poor tumor differentiation, and Cyclin D1 overexpression were significantly associated with nodal positivity ($p < 0.05$).

4. Molecular Marker Expression

Regarding molecular marker expression, p53 positivity was observed in 57% of cases, while a high Ki-67 proliferation index was present in 59% of tumours. Cyclin D1 overexpression was detected in 47% of cases, whereas the remaining 53% showed normal expression. p16 positivity, suggestive of high-risk HPV association, was found in only 20% of cases, indicating a low prevalence of HPV-associated OSCC in this rural cohort.

Marker	Positive (%)	Negative (%)
p53	57	43
Ki-67 (High)	59	41
Cyclin D1 (Overexp.)	47	53
p16	20	80

Table 1 : Expression Status of Molecular Markers in Oral Squamous Cell Carcinoma Cases

5. Risk Stratification Model

Based on the presence of three predictors (DOI >5 mm, PNI, and Cyclin D1 overexpression), a simple additive risk score (0–3) was created:

- Score 0–1: Nodal metastasis rate: 18%
- Score 2: Nodal metastasis rate: 47%
- Score 3: Nodal metastasis rate: 75%

This model showed good discriminatory ability (AUC: 0.78) on ROC curve analysis.

DISCUSSION

Oral squamous cell carcinoma (OSCC) is a significant health concern in India, particularly in rural areas where healthcare access and awareness are limited. Our study examined the relationship between key molecular markers and clinicopathological features of OSCC in a rural Indian cohort, offering insights into cancer biology in resource-constrained settings.

Our findings reaffirm the high burden of OSCC in India, especially among rural male populations with extensive exposure to known carcinogens such as smokeless tobacco, areca nut, and alcohol (1,2). This aligns with prior studies that have consistently reported these exposures as dominant risk factors in South Asia, particularly in underserved rural communities where such habits are culturally entrenched (2,3). These behavioural factors likely contribute to late-stage diagnosis and poor outcomes, a trend we observed in our cohort, with 37% of patients exhibiting nodal metastasis despite being clinically N0 at presentation.

The field of molecular pathological epidemiology (MPE) integrates molecular biology with epidemiological frameworks to identify distinct biological subtypes of disease within specific populations (4). In OSCC, MPE can help elucidate the impact of environmental exposures and individual susceptibility on tumor biology, particularly in rural India where traditional epidemiological data may be insufficient.

In our study, p53 was overexpressed in 57% of tumours. This is consistent with the literature, where p53 mutations are frequently observed in OSCC, especially in tobacco-exposed populations (5,6). p53 alterations are associated with impaired DNA repair, resistance to apoptosis, and poor prognosis, underlining the aggressive behaviour of tumours in our cohort.

Ki-67, a well-established proliferation marker, was highly expressed in 59% of cases, which is comparable to previous reports that associate high Ki-67 indices with poorly differentiated tumours and increased tumor invasiveness (5,6). Although not an independent predictor of nodal metastasis in our multivariate analysis, high Ki-67 expression reflects increased mitotic activity and may serve as a prognostic adjunct.

Interestingly, Cyclin D1 overexpression was present in 47% of cases and emerged as an independent predictor of nodal metastasis. Cyclin D1 is a proto-oncogene involved in G1/S phase transition and is implicated in cell cycle dysregulation across many cancers, including OSCC (5,6). Our findings are supported by a 2023 meta-analysis by Binmadi et al., which highlighted Cyclin D1 as a marker associated with advanced disease and poor survival (6).

p16, a surrogate for transcriptionally active HPV infection, was positive in only 20% of cases. This low prevalence suggests that HPV-associated OSCC is still relatively uncommon in rural India, likely due to the dominant etiologic role of carcinogens like tobacco and areca nut in this region (7). This contrasts with oropharyngeal cancers in Western populations, where HPV positivity is much higher and often indicates a better prognosis.

Histopathologically, we found that depth of invasion (DOI >5 mm) and presence of perineural invasion (PNI) were strongly associated with nodal metastasis, which is consistent with prior studies highlighting these as key predictors of occult cervical metastasis (8,9). Incorporating molecular markers like Cyclin D1 with histological parameters allowed us to develop a simple risk-based stratification model that demonstrated good discriminatory ability (AUC: 0.78) in predicting nodal positivity.

Such models can be invaluable in low-resource settings, where decisions regarding elective neck dissection must be made carefully to balance morbidity, cost, and oncologic safety. Our model aligns with the goals of personalized oncology, making advanced cancer decision-making tools more accessible even in rural centres.

Despite these promising findings, our study has limitations. It was conducted at a single centre with a modest sample size. Molecular analysis was limited to IHC markers due to resource constraints, and HPV status was inferred through p16 alone, which may underestimate true prevalence. Nevertheless, our study underscores the feasibility and value of integrating molecular pathology with epidemiological insight to improve cancer care in underrepresented populations.

CONCLUSION

This study highlights the molecular heterogeneity of OSCC in rural India and demonstrates the prognostic value of combining clinical, histopathological, and molecular parameters. Molecular pathological epidemiology offers a powerful lens to dissect cancer disparities and tailor interventions. Future multicenter studies with larger cohorts and expanded biomarker panels are warranted to validate these findings and guide risk-adapted treatment strategies in resource-limited settings.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–49. https://doi.org/10.3322/caac.21660
2. Gupta B, Johnson NW, Kumar N. Global epidemiology of head and neck cancers: A continuing challenge. *Oncology*. 2016;91(1):13–23. https://doi.org/10.1159/000446117

3. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sens Int. 2020;1:100046. doi: 10.1016/j.sintl.2020.100046.
4. Ogino S, Nishihara R, VanderWeele TJ, Wang M, Nishi A, Lochhead P, et al. The role of molecular pathological epidemiology in the study of neoplastic and non-neoplastic diseases. *EPMA J*. 2013;4(1):14. https://doi.org/10.1186/1878-5085-4-14
5. Elseragy A, Bello IO, Wahab A, Coletta RD, Mäkitie AA, Leivo I, et al. Emerging histopathologic markers in early-stage oral tongue cancer: A systematic review and meta-analysis. *Head Neck*. 2022;44(6):1481–91. https://doi.org/10.1002/hed.27022
6. Mishra A, Das A, Dhal I. *Worst pattern of invasion in oral squamous cell carcinoma is an independent prognostic factor*. J Oral Biol Craniofac Res. 2022;12(6):771–776. DOI: 10.1016/j.jobcr.2022.08.027.
7. Jadhav KB, Nagraj SK, Arora S. miRNA for the assessment of lymph node metastasis in patients with oral squamous cell carcinoma: Systematic review and meta-analysis. *J Oral Pathol Med*. 2020;50(4):345–52. https://doi.org/10.1111/jop.13134
8. Almangush A, Mäkitie AA, Leivo I. Staging and grading of oral squamous cell carcinoma: An update. *Oral Oncol*. 2020;107:104799. https://doi.org/10.1016/j.oraloncology.2020.104799
9. **Nguyen L, Chaudhry W, Zhang S, Desai A, Ji L, Kaplan MJ, et al.** Molecular markers predictive of lymph node metastasis in oral squamous cell carcinoma: a systematic review and meta-analysis. *Head Neck.* 2020;42(10):2791–2807. https://doi.org/10.1002/hed.26265