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# A Study to Evaluate the Immunohistochemistry Expression of Ki-67 in Different Histological Grades of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma

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## ABSTRACT

**Background:** Oral squamous cell carcinoma (OSCC) constitutes nearly 90% of oral malignancies and frequently arises through oral epithelial dysplasia (OED), a part of the spectrum of oral potentially malignant disorders. Ki-67, a nuclear proliferation-associated antigen expressed during the active phases of the cell cycle, reflects the proliferative activity of epithelial cells and has been proposed as a marker for grading severity of OED and OSCC.
**Aims and Objectives:** To study the histopathological features of oral neoplastic lesions and to score Ki-67 immunoexpression across different histological grades of OED and OSCC.
**Materials and Methods:** This prospective study was conducted over 1.5 years in the Department of Pathology, Government Medical College, Patiala. 80 cases of OED and OSCC fulfilling the inclusion criteria were studied. Haematoxylin and eosin-stained sections were used for histopathological diagnosis and grading, and immunohistochemistry with Ki-67 monoclonal antibody was performed on all cases. The Ki-67 labelling index (percentage of positive cells per 1000 cells counted at ×400 magnification) and a four-point expression score (0–3) were determined and correlated with histological grade, gender, anatomical site and tobacco use, using the Chi-square test, with $p<0.05$ considered significant.
**Results:** Of 80 cases, 14 (17.5%) were OED (5 mild, 7 moderate, 2 severe) and 66 (82.5%) were OSCC (26 well-differentiated, 34 moderately-differentiated, 6 poorly-differentiated). The mean age was 53.33±13.15 years with male predominance (75%). The mean Ki-67 labelling index increased significantly from mild to severe OED (14.60±5.90 to 25.00±1.41; $p=0.017$) and from well- to poorly-differentiated OSCC (24.92±4.05 to 47.17±10.72; $p<0.001$). The Ki-67 expression score showed a highly significant association with histopathological diagnosis ($\chi^2=19.217$, $p<0.001$), and tobacco use was strongly associated with OSCC ($\chi^2=12.214$, $p<0.001$).
**Conclusion:** Ki-67 expression increases progressively with increasing severity of OED and decreasing differentiation of OSCC. It can therefore serve as a useful adjunct biomarker for

grading oral epithelial lesions, assessing their biological behaviour and identifying lesions at higher risk of malignant transformation.

***Keywords:*** Ki-67; Oral epithelial dysplasia; Oral squamous cell carcinoma; Immunohistochemistry; Labelling index; Proliferation marker.

## INTRODUCTION

Oral cavity cancer is a major global health problem, accounting for nearly 3% of all malignancies and remaining among the most common cancers in India. Oral squamous cell carcinoma (OSCC) constitutes almost 90% of all oral malignancies. Oral cancer commonly develops through a stepwise progression of the oral mucosa from normal epithelium through oral epithelial dysplasia (OED) to invasive carcinoma, driven by the cumulative accumulation of genetic alterations affecting proto-oncogenes, tumour suppressor genes and cell-cycle regulatory genes. [1]

Oral potentially malignant disorders (OPMDs), such as leukoplakia and oral lichen planus, frequently precede oral malignancy, and the histopathological demonstration of OED is the most reliable indicator of their malignant potential. [1]) The World Health Organization (WHO) defines OED as a spectrum of architectural and cytological alterations arising from accumulated genetic abnormalities, and grades it into mild, moderate and severe categories on the basis of the proportion of epithelial thickness exhibiting dysplastic change. [2,3] OSCC, on the other hand, is conventionally graded as well, moderately or poorly differentiated according to Broder's classification, based on the degree of keratinisation and cellular differentiation. [4] Despite advances in management, the five-year survival of OSCC remains around 55%, underscoring the need for objective markers that can supplement morphological grading in predicting the biological behaviour of these lesions.

Cellular proliferation is a key feature of the transition from normal epithelium to dysplasia and carcinoma. In normal oral epithelium, proliferative activity is confined to the basal cell layer, and extension of proliferating cells into the suprabasal layers is regarded as an early indicator of pathological alteration. Ki-67 is a nuclear, non-histone protein expressed during the G1, S, G2 and M phases of the cell cycle but absent in resting (G0) cells, making it a sensitive marker of the growth fraction of a tissue. [5,6] Its expression can be quantified as a labelling index (the percentage of Ki-67-positive nuclei) and several studies have shown that this index increases progressively with increasing grade of OED and with decreasing differentiation of OSCC. [7-11]

The present study was undertaken to evaluate the histopathological features of oral neoplastic lesions and to assess Ki-67 expression across different histological grades of OED and OSCC, and to correlate it with relevant clinicodemographic parameters.

## MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Pathology, Government Medical College, Patiala, over a period of 1.5 years, on cases received from Rajendra Hospital, Patiala. Patients of all age groups presenting with clinically suspected oral lesions were included. Previously biopsy-proven cases of OSCC, patients with a history of chemotherapy or other prior treatment, and cases of recurrent malignancy were excluded. A detailed clinical history, including presenting complaints and history of tobacco use, was recorded for each patient on a structured proforma.

80 cases satisfying the inclusion criteria were enrolled, comprising biopsy and resection specimens of OED, OSCC and normal oral epithelium. Formalin-fixed, paraffin-embedded tissue sections (4 µm) were stained with haematoxylin and eosin for histopathological diagnosis and grading. OED was graded as mild (dysplastic changes

confined to the basal one-third of the epithelium), moderate (involving the basal two-thirds), or severe (involving more than two-thirds of the epithelial thickness), as per WHO criteria. OSCC was graded as well-, moderately- or poorly-differentiated using a modified Broder's classification, based on the percentage of differentiated versus undifferentiated tumour cells.

For immunohistochemistry, sections were deparaffinised and subjected to antigen retrieval followed by blocking of endogenous peroxidase activity with 3% hydrogen peroxide. The sections were incubated with a mouse monoclonal anti-Ki-67 primary antibody followed by secondary antibody and diaminobenzidine (DAB) chromogen were used for visualisation, with Mayer's haematoxylin counterstaining. Ki-67 positivity was identified as brown nuclear staining in proliferating cells. The Ki-67 labelling index (LI) was calculated by counting 1000 cells under ×400 magnification, using the formula: (Ki-67-positive cells) / (total cells counted) × 100. Expression was additionally graded on a four-point scale based on the percentage of positive cells: Score 0 (no expression), Score 1 (1–25%), Score 2 (25–50%) and Score 3 (>50%).

### Statistical Analysis

Data were entered into Microsoft Excel (version 16.0), checked for completeness and consistency, and analysed using appropriate statistical methods. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The mean Ki-67 labelling index and mean Ki-67 expression score were compared across the different histological grades of oral epithelial dysplasia and oral squamous cell carcinoma using one-way analysis of variance (ANOVA). Associations between categorical variables, including histopathological diagnosis, gender, tobacco use, anatomical site and categorical Ki-67 expression score, were assessed using the Pearson chi-square ($\chi^2$) test or Fisher's exact test as applicable. A two-sided p-value <0.05 was considered statistically significant, while p<0.001 was considered highly statistically significant.

## RESULTS

The mean age ± SD of the study participants was 53.33 ± 13.15 years, with the maximum number of cases (56.25%) occurring in the 41–60 year age group. Males predominated, with a male:female ratio of 3:1. The tongue (43.75%) and buccal mucosa (41.25%) were the most commonly involved sites, and growth at these sites was the most frequent clinical presentation. A history of tobacco use was present in 62.50% of cases, and the majority of specimens (93.75%) were biopsies (Table 1).

On histopathological evaluation, 14 cases (17.5%) were diagnosed as OED, comprising 5 mild, 7 moderate and 2 severe dysplasia, while 66 cases (82.5%) were OSCC, comprising 26 well-differentiated, 34 moderately-differentiated and 6 poorly-differentiated tumours (Table 2).

The mean Ki-67 labelling index showed a progressive, statistically significant increase with increasing grade of dysplasia, rising from 14.60 ± 5.90 in mild OED to 22.00 ± 3.21 in moderate OED and 25.00 ± 1.41 in severe OED (p=0.017). A similar and highly significant trend was observed in OSCC, with the labelling index rising from 24.92 ± 4.05 in well-differentiated to 32.76 ± 7.26 in moderately-differentiated and 47.17 ± 10.72 in poorly-differentiated tumours (p<0.001). The mean Ki-67 score (0–3 scale) also increased with grade in both groups, although the increase across OED grades did not reach statistical significance (p=0.258), whereas the increase across OSCC grades was highly significant (p<0.001) (Table 3).

Regarding the categorical Ki-67 expression score, the majority of OED cases (85.7%) showed Score 1, while the majority of OSCC cases (71.3%) showed Score 2, and Score 3 (>50% positive cells) was seen exclusively in OSCC (4.5%); this difference was highly significant ($\chi^2$=19.217, p<0.001). OSCC showed a significant male predominance

(80.3% versus 19.7% females; $\chi^2$=5.657, p=0.017), whereas OED cases were equally distributed between genders. Tobacco use showed a highly significant association with OSCC overall ($\chi^2$=12.214, p<0.001) and specifically among males ($\chi^2$=9.348, p=0.002); no female participant in this study reported tobacco use. The association between anatomical site and histopathological diagnosis was not statistically significant ($\chi^2$=7.186, p=0.066) (Table 4).

**Table 1: Demographic and Clinicopathological Profile of Study Participants (n=80)**

| Parameter | Category | Number | Percentage (%) |
|---|---|---|---|
| Age (years) | 21–40 | 13 | 16.25 |
| | 41–60 | 45 | 56.25 |
| | 61–80 | 21 | 26.25 |
| | 81–100 | 1 | 1.25 |
| Gender | Male | 60 | 75.00 |
| | Female | 20 | 25.00 |
| Anatomical site | Tongue | 35 | 43.75 |
| | Buccal mucosa | 33 | 41.25 |
| | Soft palate | 6 | 7.50 |
| | Hard palate | 6 | 7.50 |
| Tobacco use | Present | 50 | 62.50 |
| | Absent | 30 | 37.50 |
| Specimen type | Biopsy specimens | 75 | 93.75 |
| | Resection specimens* | 5 | 6.25 |

*Mean age ± SD = 53.33 ± 13.15 years. *Resection specimens comprised excision of growth, mandibulectomy, hemimandibulectomy and subtotal glossectomy (1 case each).*

**Table 2: Histopathological Diagnosis and Grading of Study Cases (n=80)**

| Diagnosis | Histological Grade | Number | Percentage (%) |
|---|---|---|---|
| Oral epithelial dysplasia (n=14) | Mild dysplasia | 5 | 6.25 |
| | Moderate dysplasia | 7 | 8.75 |
| | Severe dysplasia | 2 | 2.50 |
| Oral squamous cell carcinoma (n=66) | Well differentiated SCC | 26 | 32.50 |
| | Moderately differentiated SCC | 34 | 42.50 |
| | Poorly differentiated SCC | 6 | 7.50 |
| Total | | 80 | 100.00 |

**Table 3: Association of Histopathological Grading with Ki-67 Labelling Index and Mean Ki-67 Score**

| Diagnosis | Histological Grade | Ki-67 Labelling Index (Mean ± SD) | Ki-67 Score, 0–3 scale (Mean ± SD) |
|---|---|---|---|
| Oral epithelial dysplasia | Mild dysplasia | 14.60 ± 5.90 | 1.00 ± 0.00 |
| | Moderate dysplasia | 22.00 ± 3.21 | 1.14 ± 0.38 |
| | Severe dysplasia | 25.00 ± 1.41 | 1.50 ± 0.71 |
| | **p value** | **0.017 (S)** | **0.258 (NS)** |
| Oral squamous cell carcinoma | Well differentiated SCC | 24.92 ± 4.05 | 1.54 ± 0.51 |
| | Moderately differentiated SCC | 32.76 ± 7.26 | 1.88 ± 0.33 |
| | Poorly differentiated SCC | 47.17 ± 10.72 | 2.50 ± 0.55 |
| | **p value** | **<0.001 (HS)** | **<0.001 (HS)** |

*S = significant; HS = highly significant; NS = not significant.*

**Table 4: Association of Clinicopathological Parameters with Histopathological Diagnosis**

| Parameter | OED (n=14) | OSCC (n=66) | $\chi^2$ value | p value (Significance) |
|---|---|---|---|---|
| Gender – Male | 7 (50.0%) | 53 (80.3%) | 5.657 | 0.017 (S) |
| Gender – Female | 7 (50.0%) | 13 (19.7%) | | |
| Tobacco use – Present | 3 (21.4%) | 47 (71.2%) | 12.214 | <0.001 (HS) |
| Tobacco use – Absent | 11 (78.6%) | 19 (28.8%) | | |
| Site – Tongue | 4 (28.6%) | 31 (47.0%) | 7.186 | 0.066 (NS) |
| Site – Buccal mucosa | 10 (71.4%) | 23 (34.8%) | | |
| Site – Soft / hard palate | 0 (0.0%) | 12 (18.2%) | | |
| Ki-67 Score 0 (negative, no expression) | 0 (0.0%) | 0 (0.0%) | | |
| Ki-67 Score 1 (1–25%) | 12 (85.7%) | 16 (24.2%) | 19.217 | <0.001 (HS) |
| Ki-67 Score 2 (25–50%) | 2 (14.3%) | 47 (71.3%) | | |
| Ki-67 Score 3 (>50%) | 0 (0.0%) | 3 (4.5%) | | |

*S = significant; HS = highly significant; NS = not significant.*

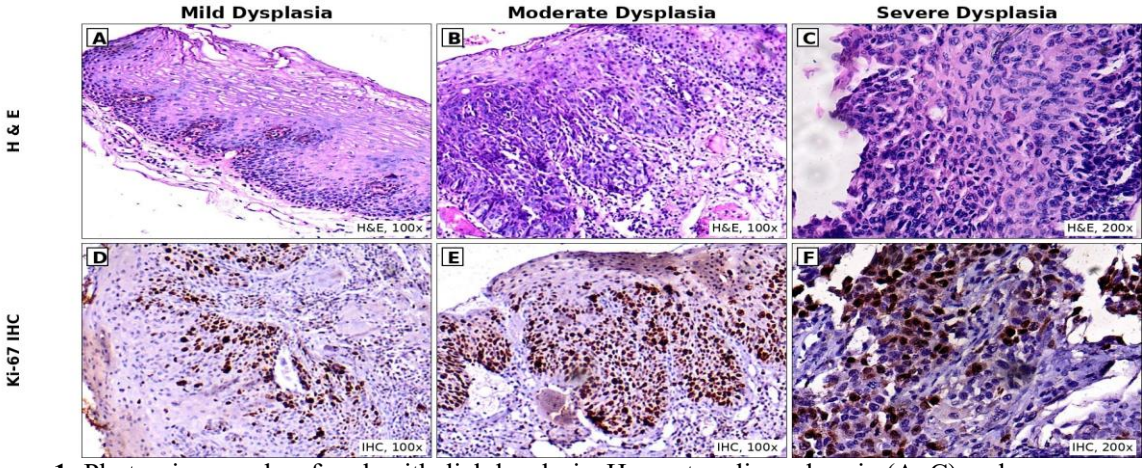


**Figure 1:** Photomicrographs of oral epithelial dysplasia. Haematoxylin and eosin (A–C) and corresponding Ki-67 immunohistochemistry (D–F) in mild (A, D), moderate (B, E) and severe (C, F) dysplasia. Ki-67 positivity is confined to the basal layer in mild dysplasia, extends into suprabasal layers in moderate dysplasia, and becomes diffuse and full-thickness in severe dysplasia.

Representative photomicrographs illustrating H&E morphology and corresponding Ki-67 immunostaining across grades of OED and OSCC are shown in Figures 1 and 2. In mild dysplasia, Ki-67 positivity was confined to the basal cell layer (Figure 1D), extending into suprabasal layers in moderate dysplasia (Figure 1E), and showing diffuse full-thickness positivity in severe dysplasia (Figure 1F). In OSCC, Ki-67 expression was largely restricted to the periphery of tumour nests in well-differentiated carcinoma (Figure 2D), more centrally distributed in moderately-differentiated tumours (Figure 2E), and diffusely and strongly positive throughout poorly-differentiated tumour cells (Figure 2F).

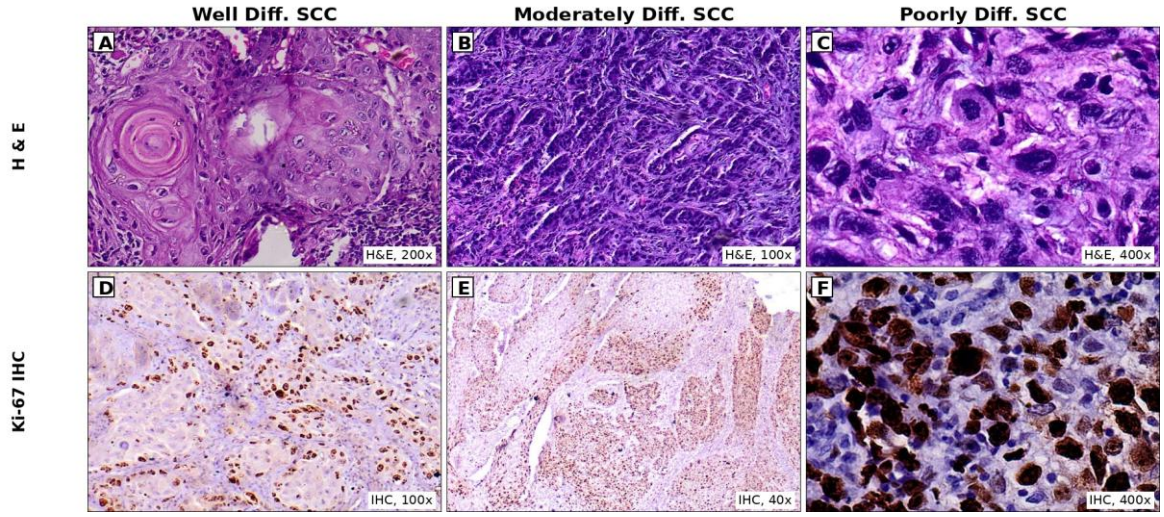


**Figure 2:** Photomicrographs of oral squamous cell carcinoma (OSCC). Haematoxylin and eosin (A–C) and corresponding Ki-67 immunohistochemistry (D–F) in well- (A, D), moderately- (B, E) and poorly-differentiated (C, F) OSCC. Note keratin pearl formation in well-differentiated SCC (A) and the progressive increase in intensity and extent of Ki-67 nuclear positivity from well- to poorly-differentiated tumour**.**

## DISCUSSION

The mean age of patients in the present study (53.33 ± 13.15 years) is comparable to that reported by Meena AK et al [11] (50.13 ± 17.14 years) and Bhardwaj AK et al [13] (45.74 ± 13.72 years), confirming that oral premalignant and malignant lesions predominantly affect individuals in the fifth and sixth decades of life. The male predominance observed in this study (75% males, M:F = 3:1) is also consistent with previous reports by Bhardwaj AK et al [13] (71% males) and Maheshwari V et al [7] (83% males), reflecting the higher prevalence of tobacco-related habits among men in this population.

The tongue and buccal mucosa were the most commonly involved sites in the present study, similar to the findings of Maheshwari V et al [7], who reported the tongue as the predominant site (41.54%). A history of tobacco use was present in 62.5% of cases, comparable with Maheshwari V et al [7] (62%) and Manu K et al [14] (54%). Tobacco use showed a highly significant association with OSCC, particularly among males (p=0.002), a finding in agreement with Thomas P et al [15], who also reported a significant association between tobacco-related habits and gender (p=0.005). Among dysplastic lesions, moderate dysplasia was the most frequent grade, similar to the findings of Bhardwaj AK et al [13], while moderately-differentiated SCC was the predominant subtype of OSCC, in agreement with Doctor P et al [12] and Sharma G et al. [16]

The central finding of this study was the progressive and statistically significant increase in Ki-67 labelling index with increasing severity of OED (from 14.60 ± 5.90 in mild to 25.00 ± 1.41 in severe dysplasia; p=0.017) and with decreasing differentiation of OSCC (from 24.92 ± 4.05 in well-differentiated to 47.17 ± 10.72 in poorly-differentiated tumours; p<0.001). This trend mirrors the findings of Takkem A et al [9], who reported labelling indices rising from 16.80 in mild OED to 60.80 in severe OED, and from 23.3 in well-differentiated to 64.1 in poorly-differentiated OSCC. Similarly, Dash KC et al [10] observed an increase from 15.42 ± 10.13 in mild OED to 58.5 ± 11.00 in severe OED, and from 35.23 ± 14.27 in well-differentiated to 59.13 ± 16.11 in poorly-differentiated OSCC, while Meena AK et al [11] reported an analogous progressive rise across both groups. Although absolute values varied across studies likely reflecting differences in scoring methodology and sample size, the consistent direction of this trend across populations strongly supports the role of Ki-67 as a marker of increasing proliferative

drive accompanying progression from dysplasia to invasive carcinoma.

The categorical Ki-67 expression score also showed a highly significant association with the histopathological diagnosis ($\chi^2$=19.217, p<0.001), with Score 3 (>50% positive cells) observed exclusively in OSCC. This parallels the observations of Bhardwaj AK et al [13], where high Ki-67 expression was likewise restricted to carcinoma cases, while the majority of dysplastic lesions showed low expression.

## CONCLUSION

The present study demonstrates that Ki-67 expression both as a labelling index and as a semi-quantitative score increases progressively with increasing severity of oral epithelial dysplasia and with decreasing differentiation of oral squamous cell carcinoma. A strong association was also observed between tobacco use and malignant transformation. These findings support the use of Ki-67 immunohistochemistry as a reliable and reproducible adjunct biomarker for grading oral epithelial lesions, predicting their biological behaviour, and identifying lesions at increased risk of malignant transformation, thereby aiding early diagnosis and appropriate clinical management.

### *Declaration*

**Ethical Approval:** Approved

**Acknowledgement:** None

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**Conflict of Interest:** The authors declare no conflict of interest.



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