**International Journal of Medical and Pharmaceutical Research**

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

# Ki-67 and p53 Expression in Oral Premalignant Lesions: An Immunohistochemical Study Correlating Biomarker Expression with Histopathological Dysplasia

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*Received: 30-04-2026*
*Accepted: 28-06-2026*
*Available online: 04-07-2026*

## ABSTRACT

**Background:** Oral potentially malignant disorders represent a clinically important group of mucosal lesions with a variable risk of progression to oral squamous cell carcinoma. Histopathological grading remains the main method for risk assessment, but it is partly subjective and may not fully reflect the underlying biological activity of the lesion. Ki-67 is a marker of cellular proliferation, while p53 expression reflects cell-cycle dysregulation and possible genomic instability. Their combined assessment may provide additional information in oral premalignant lesions. The Current study is designed to evaluate Ki-67 and p53 immunohistochemical expression in oral premalignant lesions and correlate their expression with histopathological grades of epithelial dysplasia.

**Materials and Methods:** This hospital-based observational study included 80 clinically diagnosed and histopathologically confirmed cases of oral premalignant lesions. Hematoxylin and eosin-stained sections were reviewed and graded as no dysplasia, mild dysplasia, moderate dysplasia, or severe dysplasia. Immunohistochemistry was performed for Ki-67 and p53 using formalin-fixed paraffin-embedded tissue sections. Nuclear staining was assessed semi-quantitatively. Ki-67 labelling index and p53 positivity were compared across dysplasia grades. Statistical analysis was performed using chi-square test, one-way ANOVA, and Pearson correlation. A p value <0.05 was considered statistically significant.

**Results:** Among 80 cases, oral leukoplakia was the most frequent lesion, followed by oral submucous fibrosis, erythroplakia, and oral lichen planus. Histologically, 20 cases showed no dysplasia, 25 showed mild dysplasia, 18 showed moderate dysplasia, and 17 showed severe dysplasia. High Ki-67 expression increased progressively from no dysplasia to severe dysplasia and showed a significant association with dysplasia grade ($\chi^2$=17.12, df=3, p=0.001). Similarly, high p53 expression was significantly more frequent in higher grades of dysplasia ($\chi^2$=15.57, df=3, p=0.001). Mean Ki-67 labelling index increased from 8.3±3.5% in non-dysplastic lesions to 41.6±10.3% in severe dysplasia (F=76.59, p<0.001). Mean p53 expression increased from 5.1±3.0% to 36.8±12.6% across the same groups (F=53.55, p<0.001). Combined high Ki-67 and high p53 expression was most common in severe dysplasia and showed a significant association with increasing dysplasia grade ($\chi^2$=18.48, df=3, p<0.001).

**Conclusion:** Ki-67 and p53 expression increased with the severity of epithelial dysplasia in oral premalignant lesions. Their combined immunohistochemical assessment may support routine histopathological grading and help identify lesions with greater biological activity. However, these markers should be interpreted along with clinical findings, lesion site, habit history, and histological features rather than as independent predictors of malignant transformation.

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***Keywords**: Ki-67, p53, oral premalignant lesions, oral potentially malignant disorders, epithelial dysplasia, immunohistochemistry, oral leukoplakia, oral submucous fibrosis.*

## INTRODUCTION

Oral cancer is a major public health concern, especially in regions where tobacco chewing, smoking, alcohol intake, and areca nut use are common. A considerable proportion of oral squamous cell carcinomas arise from clinically recognizable mucosal lesions that precede invasive malignancy. These lesions are currently described as oral potentially malignant disorders, a term that reflects their increased risk for malignant transformation without implying that every lesion will inevitably progress to cancer [1,2].

Oral leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus, and other clinically suspicious mucosal lesions are included under this broad group. The biological behaviour of these lesions varies widely. Some remain stable for years, while others progress to high-grade dysplasia or invasive squamous cell carcinoma. Therefore, accurate risk assessment is important for deciding follow-up intervals, biopsy site selection, treatment planning, and patient counselling [2,3].

At present, histopathological examination remains the mainstay for evaluating oral premalignant lesions. The presence and grade of epithelial dysplasia are considered important indicators of malignant potential. Dysplasia is assessed using architectural and cytological features such as basal cell hyperplasia, nuclear pleomorphism, altered nuclear-cytoplasmic ratio, abnormal mitotic activity, loss of polarity, drop-shaped rete ridges, and premature keratinization. However, histological grading may vary between observers, particularly in borderline cases. This limitation has encouraged the use of immunohistochemical and molecular markers as adjunctive tools [3,4].

Ki-67 is a nuclear protein expressed during active phases of the cell cycle but absent in resting cells. In normal oral mucosa, Ki-67-positive cells are usually confined to the basal and parabasal layers. In dysplastic epithelium, proliferating cells may extend into the suprabasal and upper epithelial layers. This altered distribution reflects disturbed epithelial maturation and increased proliferative activity [5,6].

p53 is a tumour suppressor protein involved in DNA repair, cell-cycle control, apoptosis, and genomic stability. Under normal conditions, wild-type p53 has a short half-life and is usually not strongly detectable by routine immunohistochemistry. Increased p53 immunostaining may occur due to mutation, protein stabilization, or cellular stress. In oral premalignant lesions, p53 overexpression has been reported more frequently in lesions with higher grades of epithelial dysplasia and in lesions closer to malignant transformation [7,8].

Although Ki-67 and p53 have been individually evaluated in oral epithelial dysplasia and oral squamous cell carcinoma, assessing both markers together may offer a clearer view of the biological changes occurring in premalignant oral mucosa. Ki-67 reflects the proliferative activity of epithelial cells, while p53 indicates possible disturbance in cell-cycle regulation and genomic stability. Therefore, their combined expression may help in identifying lesions with greater dysplastic potential. In this study, Ki-67 and p53 immunohistochemical expression was evaluated in oral premalignant lesions and correlated with the histopathological severity of epithelial dysplasia. The study also aimed to assess the clinicopathological profile of these lesions, grade epithelial dysplasia on hematoxylin and eosin-stained sections, analyze the individual expression patterns of Ki-67 and p53, and determine whether their combined expression showed a meaningful association with increasing grades of dysplasia.

## MATERIALS AND METHODS

### Study Design

This study was designed as a hospital-based observational analytical study. It was conducted in the Department of Pathology in collaboration with the concerned clinical departments, including Oral Medicine, ENT, and Dermatology, depending on the source of patient referral. The study focused on biopsy-proven oral premalignant lesions and evaluated their histopathological features along with immunohistochemical expression of Ki-67 and p53. Since no intervention was given to the patients as part of the study, all observations were based on clinical details, routine biopsy findings, and immunohistochemical assessment of tissue sections.

### Study Duration

The study was carried out over a period of two years. During this period, patients with clinically suspected oral premalignant lesions were screened, and eligible biopsy specimens were included after histopathological confirmation. All cases collected during the study period were processed and evaluated according to a uniform protocol.

**Study Population**
The study population consisted of patients who presented with clinically suspected oral premalignant lesions and underwent biopsy for diagnostic evaluation. The lesions included oral leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus with epithelial changes, and other mucosal lesions considered to have premalignant potential. Only those cases that were confirmed histopathologically as oral premalignant lesions, either with or without epithelial dysplasia, were included in the final analysis. Clinical details such as age, sex, site of lesion, type of lesion, and relevant habit history were recorded from case records and requisition forms.

**Sample Size**
A total of 80 cases were included in the study. These cases fulfilled the predefined inclusion and exclusion criteria and had adequate tissue available for both routine histopathological examination and immunohistochemical staining. The sample size represented the eligible cases collected during the study period.

**Inclusion Criteria**
Patients with clinically suspected oral premalignant lesions were considered eligible for inclusion in the study. Cases were included only when the biopsy showed histopathological features consistent with oral leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus with epithelial changes, or other premalignant oral mucosal lesions. Adequate biopsy tissue had to be available for hematoxylin and eosin staining as well as immunohistochemical evaluation for Ki-67 and p53. Patients who had provided written informed consent for biopsy and use of tissue material for diagnostic and research purposes were included.

**Exclusion Criteria**
Cases with a previous diagnosis of oral squamous cell carcinoma were excluded from the study. Recurrent lesions that had already been treated with chemotherapy, radiotherapy, or other cancer-directed therapy were also excluded, as treatment-related changes could affect histological and immunohistochemical interpretation. Specimens were not included if the tissue was inadequate, poorly preserved, extensively necrotic, or showed marked crush artefact. Biopsies with insufficient epithelial component for grading of dysplasia or immunohistochemical scoring were excluded. Cases with incomplete clinical details were also not considered for final analysis.

**Ethical Considerations**
The study was conducted after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all patients before biopsy. The purpose of tissue evaluation and the use of biopsy material for research analysis were explained to the patients in understandable language. Patient identity was protected throughout the study, and clinical information was used only for academic and research purposes. Confidentiality was maintained during data collection, analysis, and reporting.

**Tissue Processing**
All biopsy specimens were fixed in 10% neutral buffered formalin soon after collection to preserve tissue morphology. After adequate fixation, the tissues were processed routinely through graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections of approximately 3-4 μm thickness were cut using a microtome. Hematoxylin and eosin staining was performed on representative sections for routine histopathological examination. Additional sections were taken on adhesive-coated slides for immunohistochemical staining.

**Histopathological Evaluation**
All hematoxylin and eosin-stained sections were examined under light microscopy. The lesions were first assessed for epithelial thickness, keratinization pattern, inflammatory changes, connective tissue alterations, and other diagnostic features relevant to the clinical lesion. Based on clinical and microscopic findings, the lesions were categorized into their respective diagnostic groups.

Epithelial dysplasia was graded according to the extent and severity of architectural and cytological abnormalities. The cases were classified as no dysplasia, mild dysplasia, moderate dysplasia, or severe dysplasia. Features assessed included basal cell hyperplasia, loss of basal cell polarity, nuclear pleomorphism, nuclear hyperchromasia, increased nuclear-cytoplasmic ratio, abnormal mitotic figures, premature keratinization, drop-shaped rete ridges, and disturbed epithelial maturation. Mild dysplasia was diagnosed when atypical changes were mainly limited to the lower third of the epithelium. Moderate dysplasia was diagnosed when the changes involved the lower and middle thirds. Severe dysplasia was diagnosed when marked cytological atypia and architectural disturbance extended beyond the middle third and approached near full-thickness epithelial involvement.

**Immunohistochemistry**
Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue sections using antibodies against Ki-67 and p53. Sections were first deparaffinized in xylene and rehydrated through descending grades of alcohol. Antigen retrieval was carried out using appropriate buffer solution as per the antibody protocol. Endogenous peroxidase activity was blocked to reduce nonspecific background staining. The sections were then incubated with primary antibodies against Ki-67 and p53 according to manufacturer instructions.

After primary antibody incubation, sections were treated with the appropriate secondary antibody system, followed by chromogen development. Diaminobenzidine was used as the chromogen to produce brown nuclear staining in positive cells. The sections were counterstained with hematoxylin, dehydrated, cleared, and mounted. Appropriate positive and negative controls were used during each staining run to ensure staining reliability (Figure 1).

**Interpretation of Ki-67 Staining**
Ki-67 expression was evaluated by identifying nuclear staining in epithelial cells. Cytoplasmic staining, nonspecific background staining, and staining in inflammatory cells were not considered for scoring. The Ki-67 labelling index was calculated by counting the number of positively stained epithelial nuclei among at least 500 epithelial cells in representative high-power fields. Areas with good epithelial preservation and clear staining were selected for counting.
The expression was categorized as low when fewer than 15% of epithelial nuclei showed positivity and high when 15% or more nuclei were positive. In addition to the percentage of positive cells, the distribution of Ki-67 staining within the epithelium was also recorded. Staining limited to the basal and parabasal layers was considered a lower proliferative pattern, whereas extension of positivity into suprabasal layers was considered suggestive of increased proliferative activity and disturbed epithelial maturation (Figure 1).

**Interpretation of p53 Staining**
p53 expression was assessed by evaluating nuclear staining in epithelial cells. Only distinct nuclear positivity was accepted as true staining. Cytoplasmic staining and weak nonspecific background staining were not included in the assessment. The percentage of p53-positive epithelial nuclei was calculated in representative areas of the lesion.

p53 expression was categorized as low when fewer than 10% of epithelial nuclei showed positivity and high when 10% or more nuclei were positive. The pattern of staining was also noted. Basal-restricted staining, parabasal or suprabasal extension, and diffuse epithelial staining were recorded separately. Increased and more widespread p53 nuclear expression was considered to indicate greater cell-cycle disturbance and possible underlying genetic instability in dysplastic epithelium (Figure 1).

**Statistical Analysis**
The collected data were entered into Microsoft Excel and checked for completeness and accuracy before analysis. Statistical analysis was performed using appropriate statistical software. Categorical variables such as sex, lesion type, dysplasia grade, Ki-67 expression category, and p53 expression category were expressed as numbers and percentages. Continuous variables such as Ki-67 labelling index and p53 expression percentage were expressed as mean±standard deviation.

The chi-square test was used to assess the association between categorical variables, including the relationship between dysplasia grade and marker expression. One-way analysis of variance was used to compare mean Ki-67 and p53 expression across different grades of epithelial dysplasia. Pearson correlation analysis was performed to evaluate the relationship between Ki-67 labelling index and p53 expression. A p value of less than 0.05 was considered statistically significant.

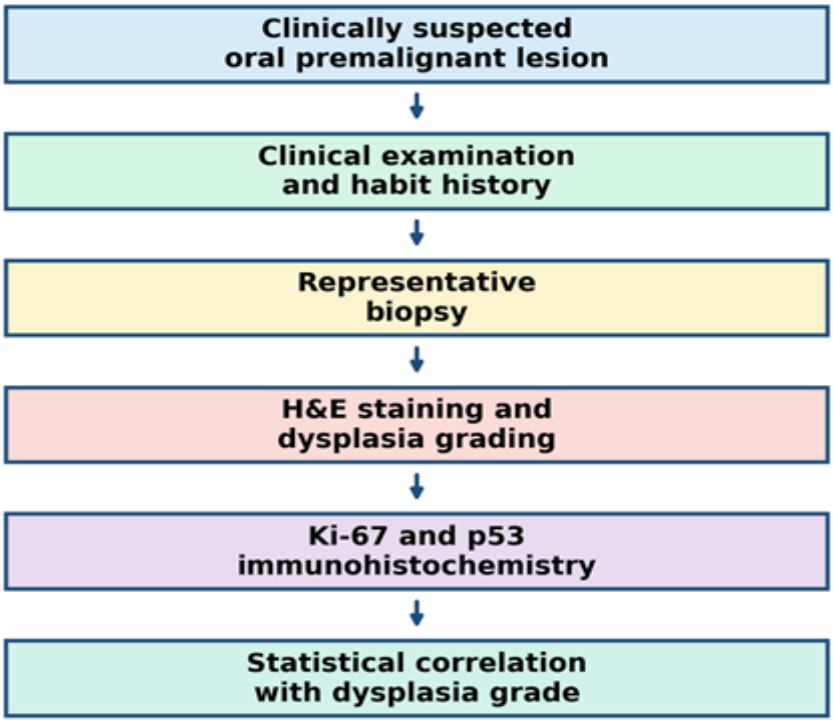


**Figure 1: Study workflow showing clinical assessment, tissue biopsy, histopathological grading, immunohistochemical staining for Ki-67 and p53, and statistical correlation with epithelial dysplasia grade**

## RESULTS

### Clinicopathological Profile

A total of 80 cases of oral premalignant lesions were included. The age of patients ranged from 22 to 74 years, with a mean age of 46.8±12.9 years. Most cases were seen in the fifth and sixth decades. Males were more commonly affected than females. Buccal mucosa was the most frequent site, followed by tongue, labial mucosa, gingivobuccal sulcus, and palate. Tobacco chewing was the most common associated habit.

Oral leukoplakia was the most frequent lesion, accounting for 38 cases (47.5%), followed by oral submucous fibrosis in 22 cases (27.5%), erythroplakia in 10 cases (12.5%), oral lichen planus in 6 cases (7.5%), and other premalignant mucosal lesions in 4 cases (5.0%) (Table 1).

**Table 1: Clinicopathological characteristics of study subjects**

| Variable | Category | Number of cases (n=80) | Percentage (%) |
|---|---|---|---|
| Age group | ≤30 years | 8 | 10.0 |
| | 31-40 years | 18 | 22.5 |
| | 41-50 years | 24 | 30.0 |
| | 51-60 years | 20 | 25.0 |
| | >60 years | 10 | 12.5 |
| Sex | Male | 56 | 70.0 |
| | Female | 24 | 30.0 |
| Site | Buccal mucosa | 36 | 45.0 |
| | Tongue | 16 | 20.0 |
| | Labial mucosa | 10 | 12.5 |
| | Gingivobuccal sulcus | 10 | 12.5 |
| | Palate | 8 | 10.0 |
| Habit history | Tobacco chewing | 42 | 52.5 |
| | Smoking | 12 | 15.0 |
| | Tobacco + alcohol | 14 | 17.5 |
| | Areca nut chewing | 8 | 10.0 |
| | No recorded habit | 4 | 5.0 |

*Data are represented as n and %*

**Distribution of Oral Premalignant Lesions**

Oral leukoplakia was the predominant lesion. Most leukoplakia cases showed mild to moderate dysplasia. Erythroplakia showed a higher proportion of moderate and severe dysplasia compared with other lesions (Table 2; Figure 2).

**Table 2: Distribution of oral premalignant lesions**

| Type of lesion | Number of cases (n=80) | Percentage (%) |
|---|---|---|
| Oral leukoplakia | 38 | 47.5 |
| Oral submucous fibrosis | 22 | 27.5 |
| Erythroplakia | 10 | 12.5 |
| Oral lichen planus with epithelial changes | 6 | 7.5 |
| Other premalignant mucosal lesions | 4 | 5.0 |

*Data are represented as n and %. Lesion classification was based on clinical diagnosis supported by histopathological evaluation*

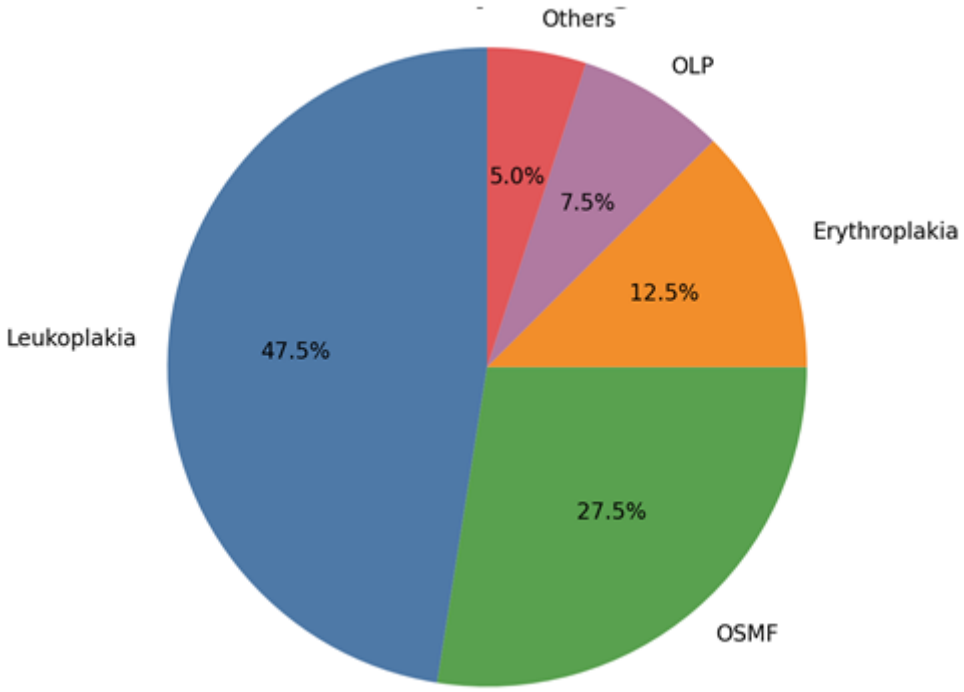


**Figure 2: Distribution of oral premalignant lesions included in the study. Oral leukoplakia was the most common lesion, followed by oral submucous fibrosis (OMSF)**

**Histopathological Grading of Dysplasia**

Out of 80 cases, 20 cases (25.0%) showed no dysplasia, 25 cases (31.3%) showed mild dysplasia, 18 cases (22.5%) showed moderate dysplasia, and 17 cases (21.3%) showed severe dysplasia (Table 3).

**Table 3: Histopathological grades of epithelial dysplasia**

| Histopathological grade | Number of cases (n=80) | Percentage (%) |
|---|---|---|
| No dysplasia | 20 | 25.0 |
| Mild dysplasia | 25 | 31.3 |
| Moderate dysplasia | 18 | 22.5 |
| Severe dysplasia | 17 | 21.3 |

*Data are represented as n and %. Dysplasia grading was performed on hematoxylin and eosin-stained sections*

**Ki-67 Expression and Dysplasia Grade**

Ki-67 expression showed a progressive increase with the severity of epithelial dysplasia. In non-dysplastic lesions, Ki-67-positive nuclei were mostly limited to the basal and parabasal layers. In mild dysplasia, staining extended focally into the lower spinous layer. Moderate and severe dysplasia showed greater suprabasal staining, with severe dysplasia showing diffuse positivity involving multiple epithelial layers.

High Ki-67 expression was observed in 5 of 20 non-dysplastic lesions (25.0%), 13 of 25 mild dysplasia cases (52.0%), 13 of 18 moderate dysplasia cases (72.2%), and 15 of 17 severe dysplasia cases (88.2%). The association between Ki-67 expression and dysplasia grade was statistically significant ($\chi^2$=17.12, df=3, p=0.001) (Table 4; Figure 3).

**Table 4: Association between Ki-67 expression and epithelial dysplasia grade**

| Dysplasia grade | Low Ki-67 expression n (%) | High Ki-67 expression n (%) | Total |
|---|---|---|---|
| No dysplasia | 15 (75.0) | 5 (25.0) | 20 |
| Mild dysplasia | 12 (48.0) | 13 (52.0) | 25 |
| Moderate dysplasia | 5 (27.8) | 13 (72.2) | 18 |
| Severe dysplasia | 2 (11.8) | 15 (88.2) | 17 |
| **Total** | **34 (42.5)** | **46 (57.5)** | **80** |

*Data are represented as n (%). Ki-67 high expression was defined as ≥15% positive epithelial nuclei. Chi-square test: $\chi^2$=17.12, df=3, p=0.001. A p value <0.05 was considered statistically significant*

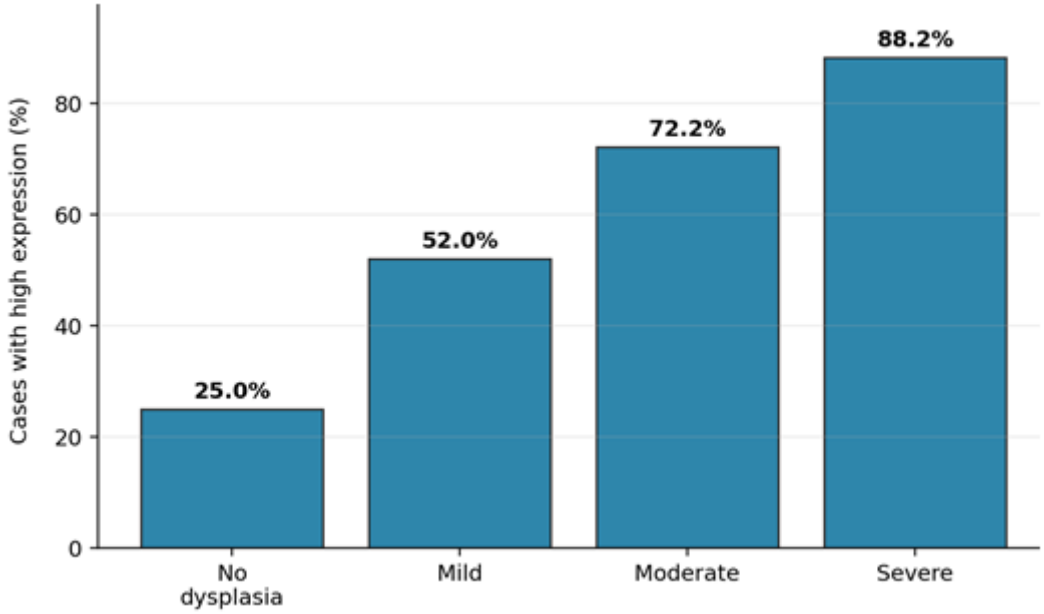


**Figure 3**: **Progressive increase in high Ki-67 expression with increasing grade of epithelial dysplasia**

**p53 Expression and Dysplasia Grade**

p53 expression also increased with dysplasia severity. Low-grade lesions showed either absent staining or basal cell staining, whereas moderate and severe dysplasia showed stronger and more extensive nuclear staining. High p53 expression was seen in 4 of 20 non-dysplastic lesions (20.0%), 11 of 25 mild dysplasia cases (44.0%), 11 of 18 moderate dysplasia cases (61.1%), and 14 of 17 severe dysplasia cases (82.4%). The association between p53 expression and dysplasia grade was statistically significant ($\chi^2$=15.57, df=3, p=0.001) (Table 5; Figure 4).

**Table 5: Association between p53 expression and epithelial dysplasia grade**

| Dysplasia grade | Low p53 expression n (%) | High p53 expression n (%) | Total |
|---|---|---|---|
| No dysplasia | 16 (80.0) | 4 (20.0) | 20 |
| Mild dysplasia | 14 (56.0) | 11 (44.0) | 25 |
| Moderate dysplasia | 7 (38.9) | 11 (61.1) | 18 |
| Severe dysplasia | 3 (17.6) | 14 (82.4) | 17 |
| **Total** | **40 (50.0)** | **40 (50.0)** | **80** |

*Data are represented as n (%). p53 high expression was defined as ≥10% positive epithelial nuclei. Chi-square test: $\chi^2$=15.57, df=3, p=0.001. A p value <0.05 was considered statistically significant.*

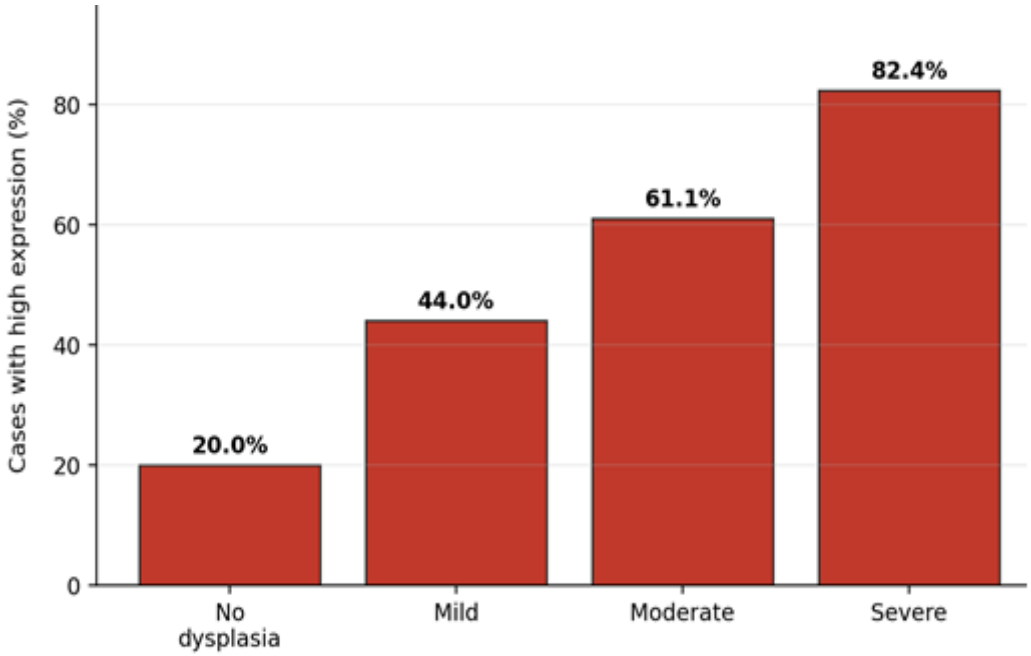


**Figure 4**: **Increased p53 expression in higher grades of oral epithelial dysplasia**

**Mean Ki-67 and p53 Labelling Indices**

The mean Ki-67 labelling index increased progressively across dysplasia grades. The lowest mean value was seen in non-dysplastic lesions and the highest in severe dysplasia. This difference was statistically significant ($F=76.59$, $p<0.001$). Mean p53 expression also increased significantly from non-dysplastic lesions to severe dysplasia ($F=53.55$, $p<0.001$) (Table 6.

**Table 6: Comparison of mean Ki-67 and p53 expression across dysplasia grades**

| Dysplasia grade | Number of cases | Ki-67 labelling index, mean±SD (%) | p53 expression, mean±SD (%) |
|---|---|---|---|
| No dysplasia | 20 | 8.3±3.5 | 5.1±3.0 |
| Mild dysplasia | 25 | 15.9±5.7 | 12.4±6.1 |
| Moderate dysplasia | 18 | 27.8±8.4 | 23.5±9.2 |
| Severe dysplasia | 17 | 41.6±10.3 | 36.8±12.6 |

*Data are represented as mean±SD. Statistical test: one-way ANOVA. Ki-67: $F=76.59$, $p<0.001$. p53: $F=53.55$, $p<0.001$. A p value <0.05 was considered statistically significant*.

**Combined Ki-67 and p53 Expression**

Combined high expression of both Ki-67 and p53 was observed increasingly with higher dysplasia grades. Only 3 of 20 non-dysplastic lesions (15.0%) showed combined high expression, compared with 14 of 17 severe dysplasia cases (82.4%). This association was statistically significant ($\chi^2=18.48$, $df=3$, $p<0.001$) (Table 7; Figure 5-7).

**Table 7: Combined Ki-67 and p53 expression according to dysplasia grade**

| Dysplasia grade | Both markers not high n (%) | Combined high Ki-67 and p53 n (%) | Total |
|---|---|---|---|
| No dysplasia | 17 (85.0) | 3 (15.0) | 20 |
| Mild dysplasia | 16 (64.0) | 9 (36.0) | 25 |
| Moderate dysplasia | 8 (44.4) | 10 (55.6) | 18 |
| Severe dysplasia | 3 (17.6) | 14 (82.4) | 17 |
| **Total** | **44 (55.0)** | **36 (45.0)** | **80** |

*Data are represented as n (%). Combined high expression was defined as Ki-67 ≥15% and p53 ≥10% positive epithelial nuclei. Chi-square test: $\chi^2=18.48$, $df=3$, $p<0.001$*

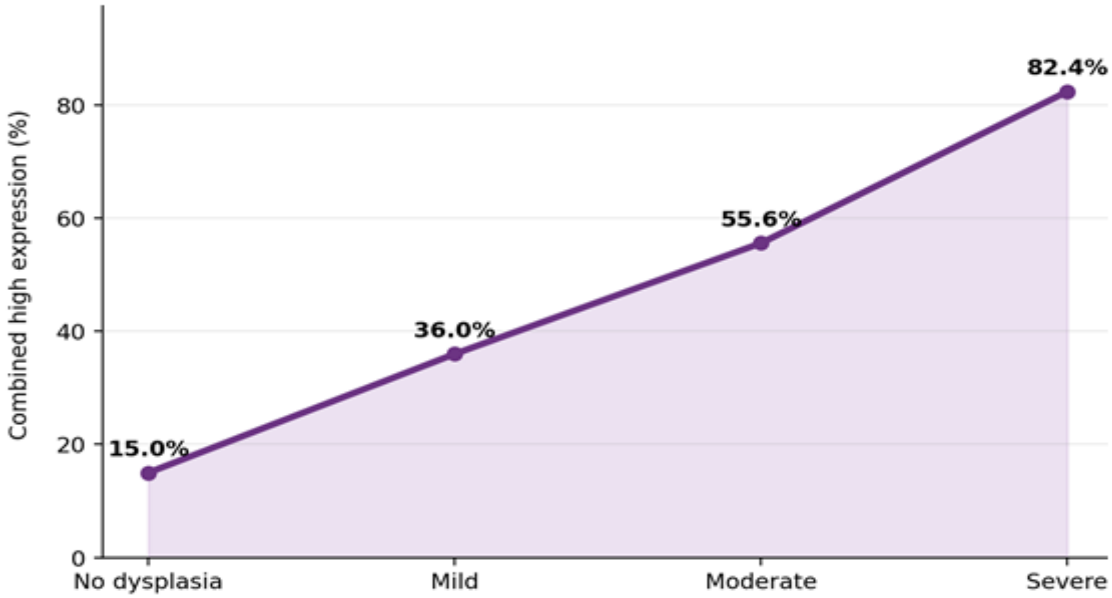


**Figure 5**: **Combined high Ki-67 and p53 expression across grades of epithelial dysplasia**

**Correlation between Ki-67 and p53 Expression**

A positive correlation was observed between Ki-67 labelling index and p53 expression (r=0.68, p<0.001), indicating that lesions with higher proliferative activity also tended to show increased p53 expression (Table 8; Figure 6 & 7).

**Table 8: Correlation between Ki-67 and p53 expression**

| Variables compared | Correlation coefficient (r) | p value |
|---|---|---|
| Ki-67 labelling index and p53 expression | 0.68 | <0.001 |

*Pearson correlation. A positive r value indicates that both markers increased in the same direction*

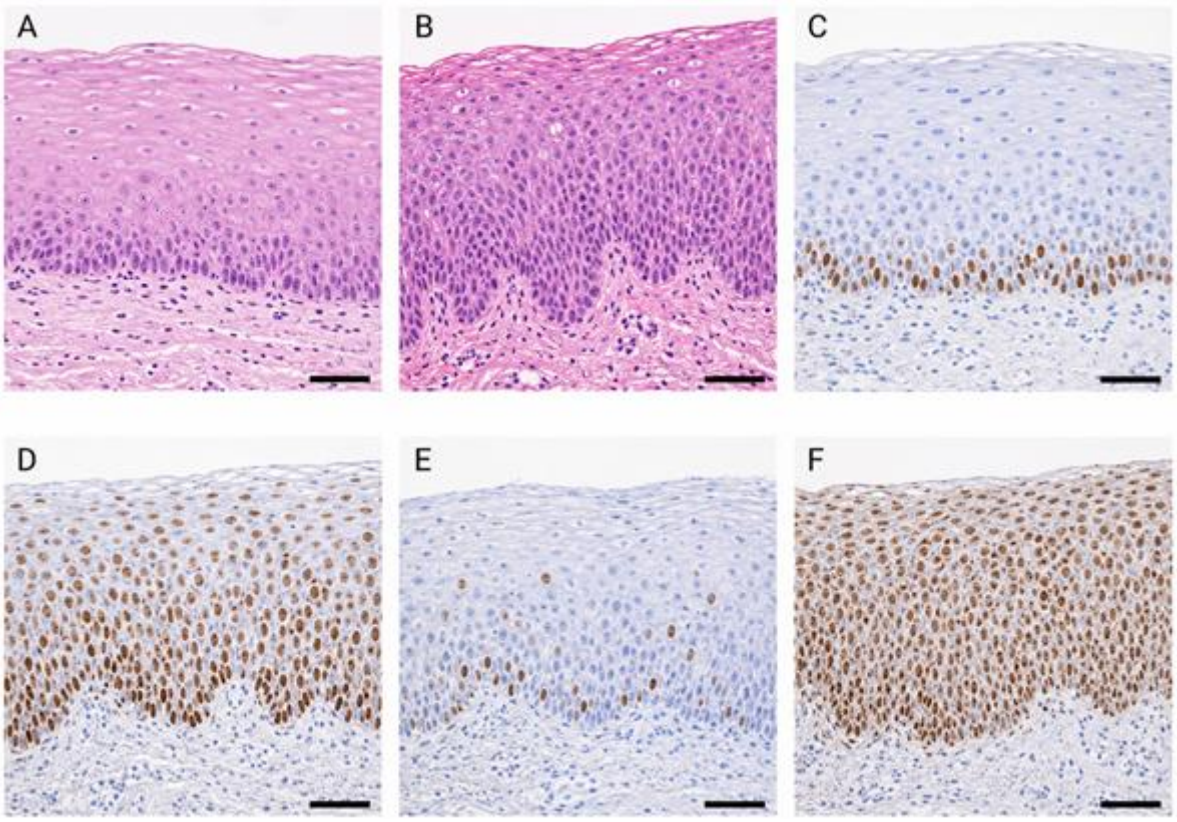


**Figure 6: Representative histopathological and immunohistochemical expression patterns in oral premalignant lesions:** (A) Hematoxylin and eosin-stained section showing mild epithelial dysplasia with relatively preserved epithelial stratification and mild basal/parabasal nuclear atypia. (B) Hematoxylin and eosin-stained section showing severe

epithelial dysplasia with epithelial thickening, nuclear crowding, loss of maturation, and increased cytological atypia. (C) Ki-67 immunostaining in mild dysplasia showing nuclear positivity mainly restricted to the basal and parabasal epithelial layers. (D) Ki-67 immunostaining in severe dysplasia showing increased nuclear positivity with extension into suprabasal epithelial layers, indicating higher proliferative activity. (E) p53 immunostaining in mild dysplasia showing focal nuclear positivity in the lower epithelial compartment. (F) p53 immunostaining in severe dysplasia showing stronger and more extensive nuclear expression across the dysplastic epithelium. Scale bars are shown in each panel

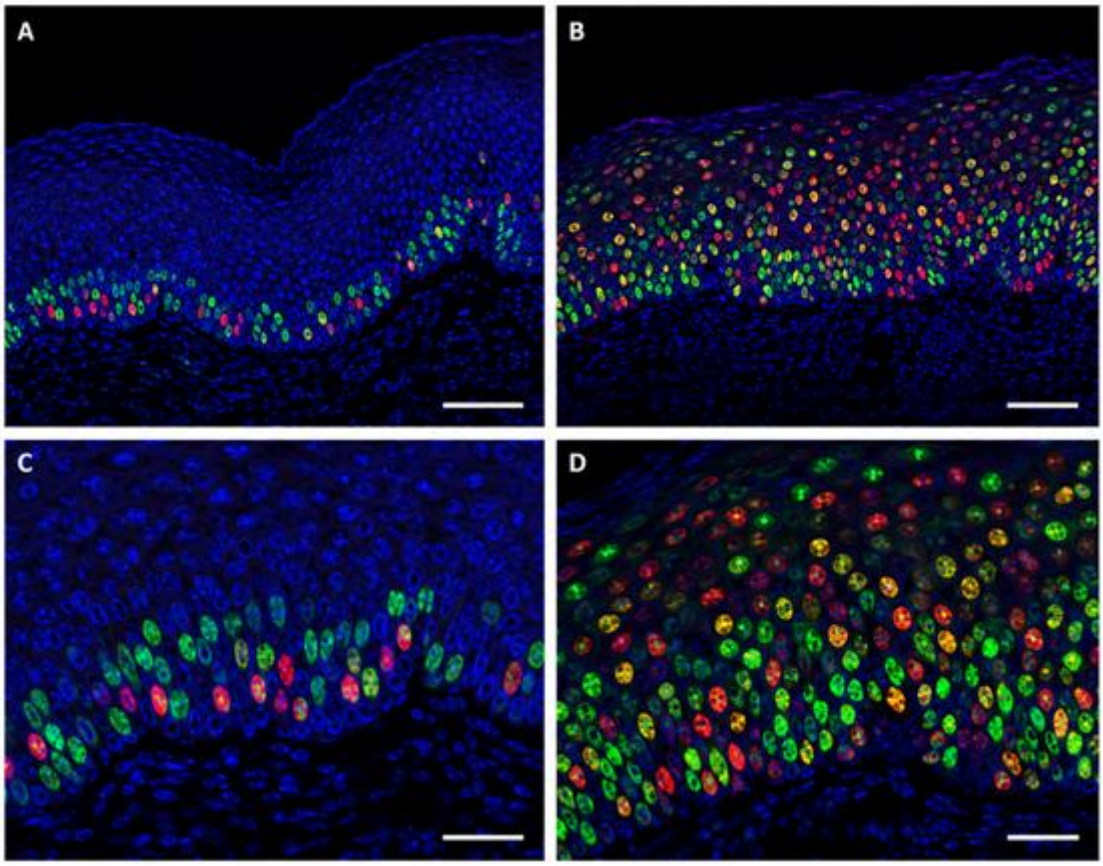


**Figure 7: Representative dual immunofluorescence pattern of Ki-67 and p53 expression in oral epithelial dysplasia:** (A) Mild epithelial dysplasia showing limited basal/parabasal expression of Ki-67 and p53, with DAPI-stained nuclei in blue. (B) Severe epithelial dysplasia showing increased Ki-67 and p53 signals extending into suprabasal epithelial layers. (C) Higher-magnification view of mild dysplasia showing scattered proliferating and p53-positive epithelial cells. (D) Higher-magnification view of severe dysplasia showing numerous positive epithelial nuclei, including areas of overlapping Ki-67 and p53 expression. Blue indicates DAPI nuclear counterstaining, green indicates Ki-67 expression, red indicates p53 expression, and yellow/orange areas indicate overlapping signal. Scale bars are shown in each panel.

## DISCUSSION

The present study evaluated the expression of Ki-67 and p53 in oral premalignant lesions and correlated the findings with histopathological grades of epithelial dysplasia. The results showed a progressive increase in both Ki-67 and p53 expression from non-dysplastic lesions to severe dysplasia. These findings support the concept that oral epithelial dysplasia is associated with increased proliferative activity and altered cell-cycle regulation.

In this study, oral leukoplakia was the most common lesion, followed by oral submucous fibrosis. This pattern is expected in populations with frequent tobacco and areca nut exposure. The buccal mucosa was the most common site, which may be related to prolonged contact of tobacco and areca nut quid with the oral mucosa. Similar clinical patterns have been described in previous studies on oral potentially malignant disorders [1,2].

Histopathological grading remains the standard method for assessing epithelial dysplasia. However, grading is influenced by the quality of biopsy, site of sampling, tissue orientation, inflammatory changes, and observer interpretation. Mild dysplasia may be difficult to distinguish from reactive epithelial atypia, while moderate dysplasia often shows overlapping features. Because of this limitation, adjunctive biomarkers may help in identifying lesions with greater biological activity [3,4].

Ki-67 is widely used as a proliferation marker. In normal oral epithelium, proliferating cells are mainly located in the basal and parabasal compartments. In dysplastic epithelium, the proliferative compartment expands upward into suprabasal layers. In the present study, Ki-67 expression increased significantly with dysplasia grade. High Ki-67 expression was seen in 25.0% of non-dysplastic lesions, 52.0% of mild dysplasia, 72.2% of moderate dysplasia, and 88.2% of severe dysplasia. This upward expansion of Ki-67-positive cells reflects altered epithelial maturation and increased cell turnover. Earlier studies have also reported that suprabasal Ki-67 expression is associated with increasing severity of oral epithelial dysplasia [5,6].

p53 expression also showed a significant association with dysplasia grade in this study. High p53 expression was observed in 20.0% of non-dysplastic lesions and increased to 82.4% in severe dysplasia. This finding suggests that p53 pathway alteration may occur early during oral carcinogenesis and becomes more evident as dysplastic changes progress. p53 immunopositivity may reflect mutation, protein stabilization, DNA damage response, or increased cellular stress. Therefore, p53 staining should not be interpreted as direct proof of TP53 mutation in every case. However, increased p53 expression in dysplastic epithelium remains a useful indicator of altered epithelial biology [7,8].

The combined assessment of Ki-67 and p53 showed a stronger relationship with dysplasia severity than either marker considered alone. Combined high expression was observed in only 15.0% of non-dysplastic lesions but increased to 82.4% in severe dysplasia. This is biologically meaningful because Ki-67 reflects increased proliferation, while p53 reflects cell-cycle disturbance and possible genomic instability. Lesions showing both high proliferative activity and increased p53 expression may represent a subgroup requiring closer clinicopathological attention.

A positive correlation was observed between Ki-67 and p53 expression. This indicates that lesions with increased epithelial proliferation also tended to show greater p53 expression. Such a pattern is consistent with the multistep model of oral carcinogenesis, in which chronic exposure to carcinogens leads to epithelial injury, genetic alterations, clonal expansion, and progressive dysplasia.

The findings of this study are broadly consistent with previous immunohistochemical studies on oral premalignant lesions. Humayun and Prasad reported increasing p53 and Ki-67 expression from normal mucosa to premalignant and malignant lesions [7]. Takkem et al. also found that Ki-67 expression increased with higher histological grades of oral epithelial dysplasia and oral squamous cell carcinoma [6]. Recent studies have further emphasized the value of p53 and Ki-67 as supportive biomarkers in identifying high-risk oral submucous fibrosis and dysplastic lesions [8,9, 13-18].

Despite these observations, Ki-67 and p53 should not replace conventional histopathology. Their expression can be influenced by inflammation, ulceration, fixation quality, antibody clone, antigen retrieval method, scoring criteria, and cut-off values. For this reason, the most practical use of these markers is as adjuncts in selected cases, especially when histological grading is difficult, when clinical suspicion is high despite low-grade histology, or when deciding the intensity of follow-up.
The present study has a few limitations. First, it was a single-centre study with a moderate sample size. Second, follow-up data on malignant transformation were not available. Therefore, the study can demonstrate association with dysplasia grade but cannot establish true predictive value for future cancer development. Third, molecular confirmation of TP53 mutation was not performed. Fourth, the cut-off values for high Ki-67 and p53 expression were based on semi-quantitative immunohistochemical assessment and may vary between laboratories.

Future studies should include larger cohorts, standardized scoring protocols, digital image analysis, interobserver agreement assessment, and long-term follow-up for malignant transformation. Combining Ki-67 and p53 with other molecular markers may further improve risk stratification in oral potentially malignant disorders.

## CONCLUSION

Ki-67 and p53 expression increased significantly with the severity of epithelial dysplasia in oral premalignant lesions. High Ki-67 expression reflected increased proliferative activity, while high p53 expression suggested altered cell-cycle regulation. Combined high expression of both markers was most frequent in severe dysplasia. These findings indicate that Ki-67 and p53 immunohistochemistry may be useful adjuncts to routine histopathological grading. However, they should be interpreted in combination with clinical findings, habit history, lesion site, and conventional microscopic features.

**Acknowledgements**
The authors thank the technical staff of the histopathology and immunohistochemistry laboratory for their assistance in tissue processing and staining.

**Conflicts of Interest**
The authors declare no conflicts of interest.

**Funding**
No external funding was received for this study.

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