Quest Journals
Journal of Medical and Dental Science Research
Volume 12~ Issue 8 (August 2025) pp: 20-30
ISSN(Online): 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Comparative Immunohistochemical Evaluation of Ki-67 and p53 in Oral Premalignant and Malignant Lesions

[1.] Sailendra Nath Biswas, *Professor, Department of Oral & Maxillofacial Pathology and Oral Microbiology, Burdwan Dental College & Hospital, Burdwan, West Bengal, India*
[2.] Sayan Chattopadhyay, *Associate Professor, Department of Oral Medicine & Radiology, Dr. R. Ahmed Dental College & Hospital, Kolkata, West Bengal, India*
[3.] Rachita Arora, *Associate Professor, Department of Pediatric & Preventive Dentistry, Dr. R. Ahmed Dental College & Hospital, Kolkata, West Bengal, India*
[4.] Sumit Dutta, *Professor, Department of Oral Medicine & Radiology, Kalka Dental College, Partapur Bypass Road, Meerut, Uttar Pradesh, India*
Corresponding author: *Sailendra Nath Biswas*

Abstract
Background:
Oral premalignant lesions may exhibit progressive cellular and molecular alterations before the development of oral squamous cell carcinoma (OSCC). Ki-67 reflects cellular proliferative activity, whereas p53 is associated with cell-cycle regulation and genomic integrity. Evaluation of these markers may provide additional insight into the biological changes accompanying oral carcinogenesis.
Aim:
To evaluate and compare the immunohistochemical expression of Ki-67 and p53 in oral premalignant and malignant lesions and to determine their association with the histological grade of OSCC
Materials and Methods:
This study included 150 patients with histopathologically diagnosed oral lesions, comprising leukoplakia (n=45), oral submucous fibrosis (OSMF) (n=30), oral lichen planus/lichenoid lesions (n=20), erythroplakia (n=10), and OSCC (n=45). Immunohistochemical staining was performed to assess Ki-67 and p53 expression. The percentage of positive cells was recorded and compared among the diagnostic groups. OSCC cases were further categorized according to histological differentiation. Statistical analysis included one-way analysis of variance (ANOVA), independent-samples t-test, post-hoc comparisons, and correlation analysis, with $p < 0.05$ considered statistically significant.
Results:
Ki-67 expression varied significantly among the lesion groups ($p < 0.001$), with mean values ranging from 6.9 ± 3.1% in OSMF to 38.5 ± 11.6% in OSCC. Similarly, p53 expression increased from 11.8 ± 5.9% in OSMF to 55.8 ± 15.2% in OSCC ($p < 0.001$). Comparison between premalignant and malignant lesions demonstrated significantly greater Ki-67 and p53 expression in OSCC ($p < 0.001$ for both). A significant positive correlation was observed between Ki-67 and p53 expression ($r = 0.68$, $p < 0.001$). Among OSCC cases, both markers increased progressively with poorer histological differentiation, with the highest expression observed in poorly differentiated tumors.
Conclusion:
Ki-67 and p53 expression showed significant differences across oral premalignant and malignant lesions and increased with advancing histological grade of OSCC. Their positive correlation suggests that enhanced cellular proliferation and altered p53-associated regulatory mechanisms may occur concurrently during malignant progression. Combined assessment of these markers may therefore provide useful adjunctive information for biological characterization of oral lesions. However, further prospective studies with larger cohorts and molecular validation are required to establish their independent diagnostic and prognostic value.
Keywords: Biomarkers, Cell proliferation, Ki-67, Oral lichen planus, Oral premalignant lesions, Oral squamous cell carcinoma, Oral submucous fibrosis, p53, Tumor grade

***R**eceived 09 Aug., 2025; **R**evised 22 Aug., 2025; **A**ccepted 25 Aug., 2025 © The author(s) 2025.*
P**ublished with open access at **www.questjournals.org

I. Introduction

Oral potentially malignant disorders (OPMDs) represent a heterogeneous group of oral mucosal conditions that are associated with an increased risk of progression to oral squamous cell carcinoma (OSCC). Their clinical and microscopic features can vary considerably, and the likelihood of malignant transformation is influenced by both the type of lesion and the presence and severity of epithelial abnormalities. Histopathological assessment of epithelial dysplasia remains an important component of risk evaluation; however, morphological grading alone may not completely reflect the underlying biological alterations within a lesion [1].

The development of oral cancer is a multistep process characterized by progressive genetic and molecular changes that disturb normal mechanisms of cellular proliferation, differentiation, DNA repair, and apoptosis. During this process, alterations may occur before the development of overt malignant morphology, creating a need for biomarkers that can provide additional information regarding the biological behavior of premalignant lesions. Immunohistochemistry (IHC) has consequently become an important adjunct to conventional histopathological examination because it permits visualization of specific molecular and cellular alterations within tissue sections [2].

Among the biomarkers investigated in oral carcinogenesis, Ki-67 is widely used as an indicator of cellular proliferative activity. Ki-67 is a nuclear protein expressed during the active phases of the cell cycle but is largely absent in resting cells. In normal oral epithelium, proliferating cells are predominantly confined to the basal and parabasal compartments. Increased Ki-67 expression and expansion of positive cells toward the upper epithelial layers have been associated with progressive epithelial abnormalities and may provide an indication of disturbed proliferative control [3].

The expression of Ki-67 has been investigated extensively in oral epithelial dysplasia and OSCC, with studies suggesting that differences in the intensity and distribution of staining may assist in distinguishing normal mucosa, dysplastic epithelium, and malignant tissue. Nevertheless, variations in tissue processing, antibody selection, scoring methods, and interpretation criteria have contributed to differences between individual studies. Recent systematic evidence indicates that Ki-67 can be useful in the assessment of oral potentially malignant and malignant lesions, although its ability to predict aggressive clinical behavior or recurrence remains less consistent [4].

p53 represents another important biomarker involved in the molecular pathway of oral carcinogenesis. The TP53 gene encodes a tumor suppressor protein that contributes to genomic stability by regulating cell-cycle arrest, DNA repair, senescence, and apoptosis following cellular stress or DNA damage. Genetic alterations involving TP53 may result in abnormal accumulation of p53 protein within cells, which can be detected immunohistochemically. Consequently, aberrant p53 expression has been investigated as a marker of molecular alterations occurring during the transition from potentially malignant epithelium to carcinoma [5].

Evidence from longitudinal investigations has indicated that p53 overexpression may have prognostic relevance in OPMDs. A systematic review and meta-analysis demonstrated an association between p53 overexpression and an increased risk of malignant transformation, supporting its potential value in identifying lesions with greater biological risk. However, the strength of this association may vary according to lesion subtype and immunohistochemical methodology, emphasizing the importance of standardized assessment [6].

The biological information provided by Ki-67 and p53 is complementary. Ki-67 primarily reflects the proliferative status of epithelial cells, whereas p53 provides information related to abnormalities in cell-cycle regulation and genomic integrity. Evaluation of these markers in combination may therefore provide a broader representation of the molecular changes accompanying oral carcinogenesis. Both markers have also been among the most frequently investigated immunohistochemical biomarkers in studies addressing field cancerization and malignant progression within the oral cavity [7].

Despite substantial research, the diagnostic significance of Ki-67 and p53 remains influenced by differences in lesion classification, histological grade, staining protocols, antibody clones, cutoff values, and scoring systems. Furthermore, OPMDs demonstrate considerable biological heterogeneity, and not every lesion showing dysplastic or biomarker alterations will progress to invasive carcinoma. These factors highlight the

importance of comparative studies that evaluate biomarker expression alongside established histopathological characteristics rather than relying on a single marker in isolation [8].

In this context, comparative evaluation of Ki-67 and p53 expression may help clarify the relationship between proliferative activity, cell-cycle dysregulation, and malignant transformation in oral lesions. A recent synthesis of the literature has further emphasized the continuing role of these biomarkers in the histopathological evaluation of OPMDs and OSCC, while also identifying limitations related to methodological heterogeneity and interpretation [9]. Therefore, the present study was undertaken to comparatively evaluate the immunohistochemical expression of Ki-67 and p53 in oral premalignant and malignant lesions and to assess their potential relationship with the histopathological progression of oral epithelial disease.

II. Materials & Methods

Study Design and Study Population

This cross-sectional observational study was conducted at a single tertiary care centre to evaluate the immunohistochemical expression of Ki-67 and p53 in oral premalignant and malignant lesions from January to March 2025. A total of 150 patients with clinically suspected and histopathologically confirmed oral lesions were included in the study. The study population comprised 45 cases of leukoplakia, 30 cases of OSMF, 20 cases of oral lichen planus/lichenoid lesions, 10 cases of erythroplakia, and 45 cases of OSCC.

Inclusion Criteria

- Patients with histopathologically confirmed oral premalignant or malignant lesions
- Patients diagnosed with leukoplakia, oral submucous fibrosis (OSMF), oral lichen planus/lichenoid lesions, erythroplakia, or OSCC
- Patients with adequate tissue specimens suitable for histopathological and immunohistochemical evaluation
- Patients with complete demographic and relevant clinical information available for analysis

Exclusion Criteria

- Cases with inadequate or insufficient tissue specimens for immunohistochemical evaluation
- Specimens showing poor preservation or significant tissue degeneration that could interfere with interpretation
- Cases with incomplete demographic or clinical records
- Patients with lesions that could not be definitively classified histopathologically
- Specimens in which immunohistochemical staining was technically inadequate or uninterpretable

Clinical and Histopathological Evaluation

Relevant demographic and clinical information, including age and sex, was recorded for each participant. Representative tissue specimens obtained during diagnostic biopsy or surgical procedures were processed routinely for histopathological examination. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy to establish the final histopathological diagnosis.

The OSCC cases were further classified according to their degree of histological differentiation into well-differentiated, moderately differentiated, and poorly differentiated categories.

Immunohistochemical Evaluation

For immunohistochemical analysis, representative paraffin-embedded tissue sections were prepared and subjected to staining for Ki-67 and p53. Sections were processed using standard immunohistochemical procedures, including deparaffinization, rehydration, antigen retrieval, blocking of endogenous activity, incubation with the respective primary antibodies, and application of an appropriate detection system. Appropriate positive and negative controls were included to ensure the reliability of the staining procedure. Ki-67 immunoreactivity was identified as brown nuclear staining, while p53 positivity was assessed based on the presence of distinct nuclear immunoreactivity in lesional epithelial or tumor cells.

Assessment of Ki-67 and p53 Expression

Immunostained sections were examined microscopically, and the proportion of positive cells was recorded as a percentage of the total cells evaluated. The Ki-67 labeling index was calculated as the percentage of cells showing nuclear Ki-67 positivity. Similarly, p53 expression was expressed as the percentage of cells demonstrating positive nuclear staining.

The staining results were evaluated across the different diagnostic groups to determine differences in proliferative activity and p53 expression. In OSCC, marker expression was additionally compared according to histological grade.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables such as age, Ki-67 labeling index, and p53 expression were expressed as mean ± standard deviation (SD), while categorical variables were summarized as frequencies and percentages. The distribution of continuous variables was assessed for normality before applying parametric tests. For comparison of Ki-67 and p53 expression across the five lesion groups (leukoplakia, OSMF, OLP/lichenoid lesions, erythroplakia, and OSCC), one-way analysis of variance (ANOVA) was used Comparison of Ki-67 and p53 expression between premalignant and malignant lesions was performed using the independent-samples Student's t-test**.** The relationship between Ki-67 and p53 expression was assessed using Pearson's correlation coefficient (r). Among OSCC cases, differences in Ki-67 and p53 expression according to histological grade (well, moderately, and poorly differentiated) were evaluated using one-way ANOVA, followed by post-hoc multiple comparison testing. All statistical tests were two-tailed**,** and a p-value <0.05 was considered statistically significant, while p<0.001 was considered highly statistically significant. The results were presented in tables and graphical form wherever appropriate.

III. Results

Table 1 & Figure 1 summarize the demographic profile of the 150 study participants. The mean age of the participants was 49.2 ± 12.4 years. The 41–50-year age group constituted the largest proportion, with 43 participants (28.7%), followed by those aged 51–60 years, comprising 37 participants (24.7%). Participants aged >60 years accounted for 28 (18.7%), while 25 (16.7%) were in the 31–40-year age group and 17 (11.3%) were aged 18–30 years. A male predominance was observed, with 96 males (64.0%) and 54 females (36.0%). Overall, the study population was predominantly middle-aged, with males representing approximately two-thirds of the participants.

Table 1: Distribution of patients according to demographic characteristics

	Parameter	**n (%) / Mean ± SD**
	Age (years)	49.2 ± 12.4
Age Group	18–30 years	17 (11.3%)
	31–40 years	25 (16.7%)
	41–50 years	43 (28.7%)
	51–60 years	37 (24.7%)
	>60 years	28 (18.7%)
Gender	Male	96 (64.0%)
	Female	54 (36.0%)

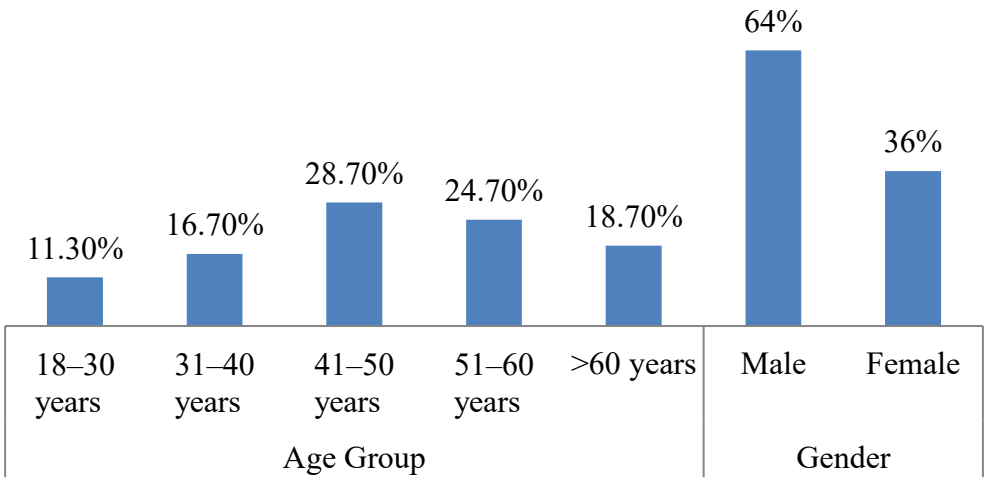

Figure 1: Age-wise and sex-wise distribution of the study participants

Table 2 & Figure 2 illustrate the distribution of histopathological diagnoses among the 150 study participants. Leukoplakia and OSCC were the most commonly observed diagnostic categories, with 45 cases (30.0%) each. OSMF was diagnosed in 30 patients (20.0%), whereas oral lichen planus/lichenoid lesions

(OLP/LL) accounted for 20 cases (13.3%). Erythroplakia was the least frequently identified lesion, with 10 cases (6.7%). Overall, the study encompassed a diverse range of histopathological conditions, extending from potentially malignant oral lesions to established invasive malignancy. Leukoplakia and OSCC together constituted 60.0% of the study population.

Table 2: Histopathological distribution of study lesions

Diagnosis	**n**	**%**
Leukoplakia	45	30.0
OSMF	30	20.0
OLP/lichenoid lesion	20	13.3
Erythroplakia	10	6.7
OSCC	45	30.0
Total	150	100.0

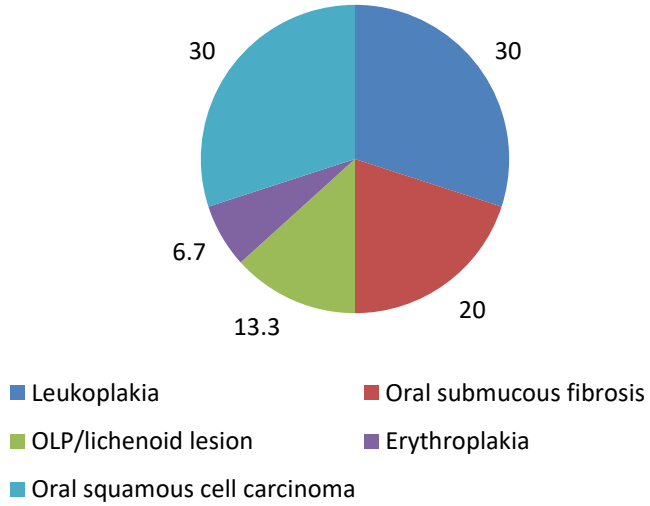

Figure 2: Distribution of study participants according to histopathological diagnosis

Table 3 & Figure 3 demonstrate substantial differences in Ki-67 immunohistochemical expression among the evaluated lesion groups. The lowest mean Ki-67 labeling index was recorded in OSMF (6.9 ± 3.1%), followed by oral lichen planus/lichenoid lesions (7.8 ± 3.4%) and leukoplakia (8.6 ± 3.7%). Erythroplakia exhibited a comparatively higher mean labeling index of 14.7 ± 5.2%, whereas OSCC showed the highest value at 38.5 ± 11.6%.

The standard error ranged from 0.55 to 1.73, with minimum and maximum Ki-67 values varying across the diagnostic groups. Overall, Ki-67 expression demonstrated a progressive increase from potentially malignant lesions to OSCC, suggesting greater cellular proliferative activity with malignant progression. The differences in Ki-67 expression among the lesion groups were statistically highly significant (one-way ANOVA, $p < 0.001$).

Table 3: Comparative Ki-67 labeling index across the different lesion groups

Lesion group	**n**	**Mean ± SD (%)**	**SE**	**Minimum (%)**	**Maximum (%)**
Leukoplakia	45	8.6 ± 3.7	0.55	1.2	16.0
OSMF	30	6.9 ± 3.1	0.57	0.7	13.1
OLP/Lichenoid	20	7.8 ± 3.4	0.76	1.0	14.6
Erythroplakia	10	14.7 ± 5.2	1.64	4.3	25.1
OSCC	45	38.5 ± 11.6	1.73	15.3	61.7

(ANOVA, $p < 0.001$)

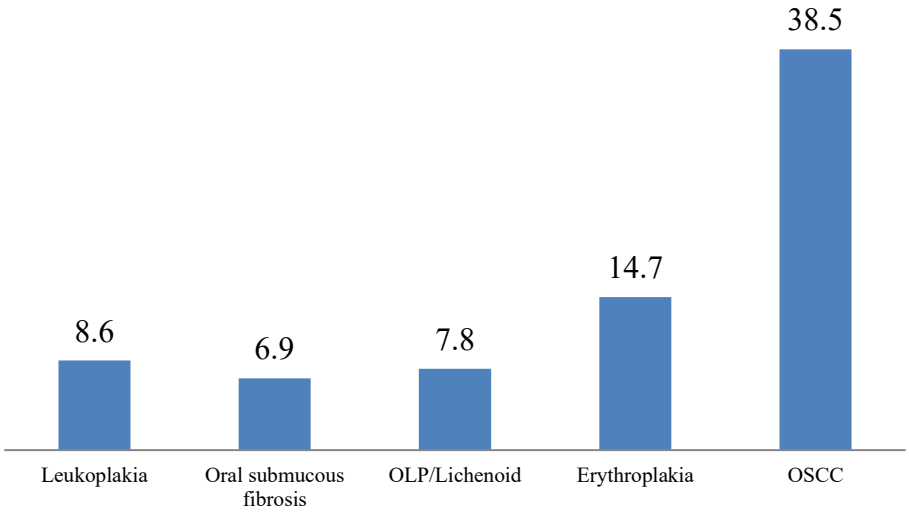

Table 4 & Figure 4 illustrate the variation in p53 immunohistochemical expression among the different lesion groups. The lowest mean p53 expression was observed in OSMF (11.8 ± 5.9%), followed by oral lichen planus/lichenoid lesions (13.6 ± 6.3%) and leukoplakia (15.2 ± 7.1%). Erythroplakia showed a higher mean p53 expression of 28.4 ± 9.7%, whereas the highest expression was recorded in OSCC (55.8 ± 15.2%). The standard error ranged from 1.06 to 3.07, while the minimum and maximum expression values varied across the diagnostic groups. Overall, p53 expression showed a progressive increase from potentially malignant lesions to malignant disease, indicating greater accumulation of p53-positive cells with malignant progression. The differences in p53 expression among the lesion groups were statistically highly significant (one-way ANOVA, $p < 0.001$).

Table 4: Comparative p53 expression across the different lesion groups

Lesion group	n	Mean ± SD (%)	SE	Minimum (%)	Maximum (%)
Leukoplakia	45	15.2 ± 7.1	1.06	3.1	29.0
OSMF	30	11.8 ± 5.9	1.08	2.4	23.5
OLP/Lichenoid	20	13.6 ± 6.3	1.41	2.7	26.1
Erythroplakia	10	28.4 ± 9.7	3.07	11.2	44.8
OSCC	45	55.8 ± 15.2	2.27	24.6	84.7

(ANOVA, $p < 0.001$)

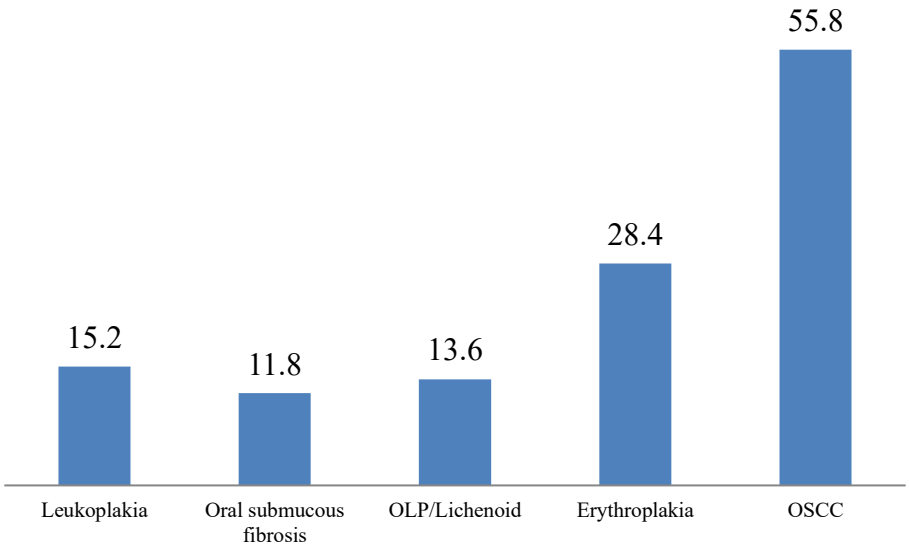

Figure 4: Comparison of mean p53 immunohistochemical expression among different oral lesion groups

Table 5 & Figure 5 compare the immunohistochemical expression of Ki-67 and p53 between premalignant and malignant lesions. Both markers demonstrated substantially higher expression in the malignant group. The mean Ki-67 labeling index increased from 8.5 ± 4.0% in premalignant lesions to 38.5 ± 11.6% in malignant lesions, with a statistically highly significant difference ($t = 16.4$, $p < 0.001$).
Similarly, mean p53 expression increased from 14.3 ± 7.0% among premalignant lesions to 55.8 ± 15.2% in malignant lesions. This difference was also statistically highly significant ($t = 17.1$, $p < 0.001$). Overall, both Ki-67 and p53 exhibited significantly greater expression in malignant lesions, supporting their potential value in assessing increased cellular proliferation and malignant progression.

Table 5: Comparison of immunohistochemical markers

Marker	Premalignant Mean ± SD	Malignant Mean ± SD	Test	p-value
Ki-67 (%)	8.5 ± 4.0	38.5 ± 11.6	t = 16.4	<0.001
p53 (%)	14.3 ± 7.0	55.8 ± 15.2	t = 17.1	<0.001

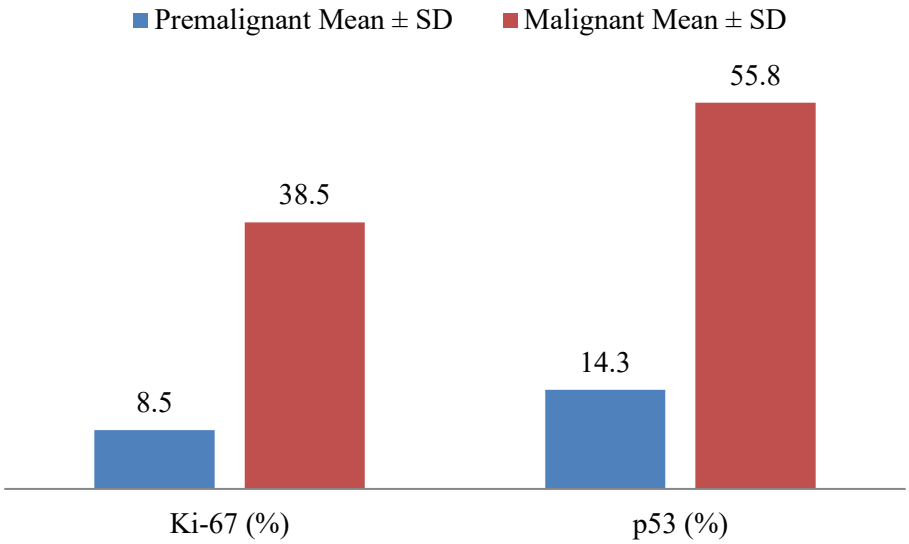

Figure 5: Comparative mean expression of Ki-67 and p53 in premalignant and malignant lesions

Table 6 demonstrates a significant positive correlation between Ki-67 and p53 immunohistochemical expression across the study population. A correlation coefficient of $r = 0.68$ indicated a moderately strong positive association, showing that higher p53 expression tended to occur alongside increased Ki-67 labeling. This relationship was statistically highly significant ($p < 0.001$).

These findings indicate that increased p53 expression was associated with greater cellular proliferative activity, suggesting that Ki-67 and p53 may provide complementary information when evaluating cellular alterations across oral premalignant and malignant lesions.

Table 6: Correlation between Ki-67 and p53

Variables	R	p-value	Interpretation
Ki-67 vs p53	0.68	<0.001	Significant positive correlation

Table 7 & Figure 7 demonstrates a progressive increase in both Ki-67 and p53 expression with worsening histological differentiation of OSCC. Well-differentiated OSCC showed the lowest mean Ki-67 labeling index (29.4 ± 8.2%) and p53 expression (43.7 ± 11.5%). These values increased to 39.2 ± 9.6% and 56.8 ± 13.4%, respectively, in moderately differentiated tumors. The highest expression was observed in poorly differentiated OSCC, with mean Ki-67 and p53 values of 52.7 ± 10.8% and 69.3 ± 12.7%, respectively.

Table 7: Ki-67 and p53 expression according to OSCC grade

OSCC grade	n	Ki-67 (%) Mean ± SD	p53 (%) Mean ± SD
Well differentiated	18	29.4 ± 8.2	43.7 ± 11.5
Moderately differentiated	17	39.2 ± 9.6	56.8 ± 13.4
Poorly differentiated	10	52.7 ± 10.8	69.3 ± 12.7

Table 8 presents the post-hoc comparison between the histological grades. Ki-67 expression differed significantly across all three grades. Expression was higher in moderately differentiated tumors than in well-differentiated tumors (mean difference = −9.8, p = 0.003). Similarly, poorly differentiated tumors showed significantly higher Ki-67 expression than both well-differentiated (mean difference = −23.3, p < 0.001) and moderately differentiated tumors (mean difference = −13.5, p = 0.004).

A similar grade-related pattern was observed for p53 expression. Significant differences were found between well- and moderately differentiated tumors (mean difference = −13.1, p = 0.004), well- and poorly differentiated tumors (mean difference = −25.6, p < 0.001), and moderately and poorly differentiated tumors (mean difference = −12.5, p = 0.025).

Overall, both markers exhibited a significant increase with advancing histological grade, with the highest Ki-67 and p53 expression observed in poorly differentiated OSCC. This pattern suggests that greater proliferative activity and altered p53 expression may be associated with poorer tumor differentiation and potentially more aggressive disease.

Table 8: Post Hoc Comparison

	Ki-67expression		p53 expression	
Comparison	**Mean Difference**	**p-value**	**Mean Difference**	**p-value**
Well differentiated vs. Moderately differentiated	−9.8	**0.003**	−13.1	**0.004**
Well differentiated vs. Poorly differentiated	−23.3	**<0.001**	−25.6	**<0.001**
Moderately differentiated vs. Poorly differentiated	−13.5	**0.004**	−12.5	**0.025**

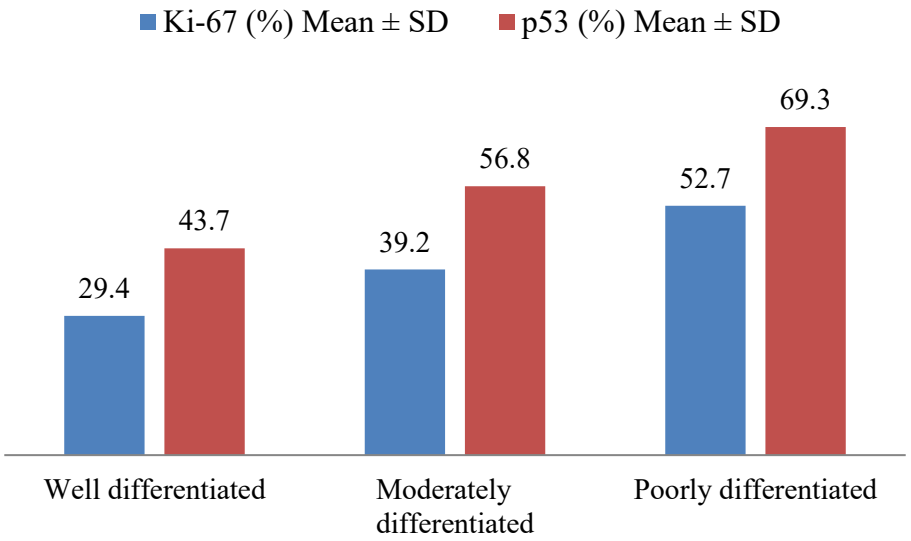

Figure 6: Comparison of mean Ki-67 and p53 expression according to histological grade of OSCC

IV. Discussion

The present study comparatively evaluated the immunohistochemical expression of Ki-67 and p53 in oral premalignant and malignant lesions to assess their potential role in identifying progressive epithelial alterations and malignant transformation. These biomarkers represent two complementary aspects of tumor biology. While p53 is involved in genomic surveillance, DNA-damage response, cell-cycle regulation, and apoptosis, Ki-67 is primarily associated with cellular proliferation. Their combined assessment may therefore provide a broader understanding of the biological behavior of oral epithelial lesions.

Wood et al. (1994) [10] demonstrated accumulation of the p53 tumor-suppressor gene product in oral leukoplakia, indicating that alterations involving the p53 pathway may occur during the premalignant stage of oral carcinogenesis. Similarly, Langdon and Partridge (1992) [11] reported p53 expression in oral cancer and suggested an association between abnormalities of the p53 tumor-suppressor pathway and malignant

transformation. These findings support the concept that disruption of p53-mediated cell-cycle control may allow genetically altered epithelial cells to escape apoptosis and continue proliferating, thereby contributing to carcinogenesis.

The detection of p53 immunoreactivity in premalignant lesions is particularly important because it may indicate alterations in cellular regulatory mechanisms before the development of frank malignancy. However, immunohistochemical p53 positivity should not be considered synonymous with a TP53 gene mutation, as protein accumulation can result from different molecular mechanisms. Therefore, p53 expression is best interpreted in conjunction with histopathological findings and other biomarkers rather than being considered an isolated diagnostic parameter.

In this context, Varun et al. (2014) [12] evaluated p53 and p63 expression immunohistochemically in OSCC, oral leukoplakia, and OSMF. Their observations demonstrated differential expression of these markers among oral lesions and highlighted the usefulness of immunohistochemistry in investigating molecular alterations associated with progression from potentially malignant disorders to carcinoma. These findings further support the relevance of p53 as a marker of altered epithelial biology in oral potentially malignant and malignant lesions.

Ki-67 provides complementary information because its expression reflects the proliferative activity of cells. Increased Ki-67 labeling indicates expansion of the proliferative cell population and may accompany progressive epithelial atypia and malignant transformation. Thus, assessment of Ki-67 expression can provide an indication of the biological activity of a lesion and may help differentiate lesions exhibiting relatively limited epithelial alteration from those with more pronounced proliferative behavior.

The clinical significance of Ki-67 in OSCC has also been investigated. Gadbail et al. (2021) [13] reported that the Ki-67 labeling index was associated with clinical outcome and survival in patients with OSCC, emphasizing the potential relationship between proliferative activity and tumor behavior. Their findings provide biological support for incorporating Ki-67 assessment into the evaluation of oral malignancies. However, the prognostic utility of Ki-67 remains controversial. Gonzalez-Moles et al. (2010) [14] concluded that Ki-67 should not necessarily be regarded as an independent prognostic indicator in OSCC. Variations in tumor heterogeneity, staining methodology, evaluation criteria, cutoff values, and clinical characteristics may contribute to differences among studies.

The combined evaluation of Ki-67 and p53 may consequently provide greater biological insight than assessment of either marker independently. p53 expression primarily reflects abnormalities associated with cell-cycle regulation and tumor-suppressor function, whereas Ki-67 reflects the proliferative status of the epithelial or tumor cell population. Increased expression of both markers may therefore indicate a lesion in which regulatory mechanisms are compromised while cellular proliferation is simultaneously enhanced. This complementary relationship is particularly relevant when comparing premalignant lesions with established OSCC.

Further support for the biological relevance of these markers was provided by Verma et al. (2014) [15], who evaluated Ki-67 and p53 expression at the invasive tumor front of OSCC. Their study emphasized the importance of these biomarkers in relation to tumor progression and invasive behavior. The invasive front represents a biologically active region of the tumor characterized by interactions between malignant cells and the surrounding tissue. Consequently, biomarker expression in this region may provide information that is not completely reflected by evaluation of the tumor bulk alone.

From a diagnostic and pathological perspective, the present comparative assessment is relevant because histopathological examination remains the principal approach for diagnosing oral epithelial dysplasia and OSCC. Nevertheless, interpretation of dysplastic changes may be affected by morphological heterogeneity and interobserver variation. Immunohistochemical markers can provide additional biological information and may complement conventional microscopic evaluation. In this regard, Ki-67 offers an estimate of proliferative activity, whereas p53 provides information regarding abnormalities in tumor-suppressor-associated pathways.

Despite their potential value, neither Ki-67 nor p53 should currently be considered a replacement for conventional histopathological diagnosis. Immunohistochemical findings may be influenced by antibody selection, staining protocols, tissue preparation, scoring methods, cutoff values, and the specific region selected for evaluation. In addition, p53 staining patterns may have different biological interpretations, while Ki-67

expression may demonstrate considerable intratumoral variation. These methodological factors may explain, at least in part, the inconsistent prognostic findings reported in the literature.

Overall, the findings from previous studies suggest that Ki-67 and p53 have complementary roles in characterizing oral premalignant and malignant lesions and demonstrate the biological and potential prognostic relevance of Ki-67 and its relationship with p53. Taken together, these findings indicate that combined assessment of proliferative activity and cell-cycle dysregulation may improve the biological characterization of oral lesions. However, larger prospective investigations using standardized immunohistochemical scoring systems and molecular validation are required to establish the independent diagnostic and prognostic value of Ki-67 and p53 in oral premalignant and malignant lesions.

V. Limitations

The present study had several limitations. The relatively limited sample size may restrict the generalizability of the findings to larger and more diverse populations. The cross-sectional design permitted assessment of Ki-67 and p53 expression at a single time point and therefore did not allow evaluation of changes in marker expression over time or establish a causal relationship with malignant transformation. Furthermore, the study was conducted in a single setting, which may have introduced selection bias and limited the applicability of the findings to other populations and clinical environments. Immunohistochemical results may also be influenced by tissue processing, antibody characteristics, staining procedures, and scoring methods, potentially affecting comparisons with other studies. Ki-67 and p53 expression were assessed immunohistochemically without molecular confirmation; particularly, p53 positivity does not necessarily indicate an underlying TP53 mutation. In addition, only a limited number of biomarkers were investigated, while other molecular pathways involved in proliferation, apoptosis, cell-cycle regulation, invasion, and malignant transformation were not evaluated. Although increased Ki-67 and p53 expression was associated with higher OSCC histological grade, long-term outcomes such as recurrence, metastasis, and disease-specific survival were not assessed, limiting conclusions regarding their prognostic value. Therefore, larger multicenter prospective studies incorporating standardized immunohistochemical assessment, molecular validation, and long-term clinical follow-up are warranted to establish the diagnostic and prognostic significance of these biomarkers.

VI. Conclusion

The present study demonstrated a significant difference in Ki-67 and p53 immunohistochemical expression across oral premalignant and malignant lesions. Both markers showed progressively higher expression in OSCC compared with premalignant conditions, with the greatest expression observed in poorly differentiated tumors. A significant positive correlation between Ki-67 and p53 further suggested an association between increased proliferative activity and altered cell-cycle regulation. These findings indicate that Ki-67 and p53 may serve as useful adjunctive biomarkers for characterizing biological changes associated with oral carcinogenesis. However, their expression should be interpreted alongside conventional clinical and histopathological findings, as neither marker alone can reliably establish malignant transformation or tumor behavior. Further studies with larger sample sizes, standardized immunohistochemical protocols, molecular validation, and long-term clinical follow-up are warranted to clarify their diagnostic and prognostic significance.

Conflicts of Interest: Nil
Financial Support: None

References

[1]. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MÁ, Kerr AR, Lodi G, Mello FW, Monteiro L, Ogden GR, Sloan P, Johnson NW. Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. Oral Dis. 2021 Nov;27(8):1862-1880. doi:10.1111/odi.13704. PMID: 33128420.

[2]. Iocca O, Sollecito TP, Alawi F, Weinstein GS, Newman JG, De Virgilio A, Di Maio P, Spriano G, Pardiñas López S, Shanti RM. Potentially malignant disorders of the oral cavity and oral dysplasia: A systematic review and meta-analysis of malignant transformation rate by subtype. Head Neck. 2020 Mar;42(3):539-555. doi:10.1002/hed.26006. PMID: 31803979.

[3]. Mello FW, Melo G, Guerra ENS, Warnakulasuriya S, Garnis C, Rivero ERC. Oral potentially malignant disorders: A scoping review of prognostic biomarkers. Crit Rev Oncol Hematol. 2020 Sep;153:102986. doi:10.1016/j.critrevonc.2020.102986. PMID: 32682268.

[4]. Mondal K, Mandal R, Sarkar BC. Importance of Ki-67 Labeling in Oral Leukoplakia with Features of Dysplasia and Carcinomatous Transformation: An Observational Study over 4 Years. South Asian J Cancer. 2020 Jun;9(2):99-104. doi: 10.1055/s-0040-1721212. Epub 2020 Dec 14. PMID: 33365288; PMCID: PMC7752503.

[5]. A study of Ki-67 expression and its clinicopathological determinants in nondysplastic oral leukoplakia. J Oral Maxillofac Pathol. 2016;20(3):395-400. doi:10.4103/0976-237X.194106. PMID: 27994417.

[6]. Importance of Ki-67 Labeling in Oral Leukoplakia with Features of Dysplasia and Carcinomatous Transformation: An Observational Study over 4 Years. Anticancer Res. 2021. PMID: 33365288.
[7]. Ramos-García P, González-Moles MÁ, Warnakulasuriya S. Significance of p53 overexpression in the prediction of the malignant transformation risk of oral potentially malignant disorders: A systematic review and meta-analysis. Oral Oncol. 2022 Mar;126:105734. doi:10.1016/j.oraloncology.2022.105734. PMID: 35091134.
[8]. Sawada K, Momose S, Kawano R, Kohda M, Irié T, Mishima K, Kaneko T, Horie N, Okazaki Y, Higashi M, Tamaru JI. Immunohistochemical staining patterns of p53 predict the mutational status of TP53 in oral epithelial dysplasia. Mod Pathol. 2022 Feb;35(2):177-185. doi:10.1038/s41379-021-00893-9. PMID: 34404905.
[9]. Novack R, Zhang L, Hoang LN, Kadhim M, Ng TL, Poh CF, Ko YCK. Abnormal p53 immunohistochemical patterns shed light on the aggressiveness of oral epithelial dysplasia. Mod Pathol. 2023 Jul;36(7):100153. doi:10.1016/j.modpat.2023.100153. PMID: 36906072
[10]. Wood MW, Medina JE, Thompson GC, Houck JR, Min KW. Accumulation of the p53 tumor-suppressor gene product in oral leukoplakia. Otolaryngol Head Neck Surg. 1994 Dec;111(6):758-63. doi: 10.1177/019459989411100610. PMID: 7527509.
[11]. Langdon JD, Partridge M. Expression of the tumour suppressor gene p53 in oral cancer. Br J Oral Maxillofac Surg. 1992 Aug;30(4):214-20. doi: 10.1016/0266-4356(92)90263-i. PMID: 1380822.
[12]. Varun BR, Ranganathan K, Rao UK, Joshua E. Immunohistochemical detection of p53 and p63 in oral squamous cell carcinoma, oral leukoplakia, and oral submucous fibrosis. J Investig Clin Dent. 2014 Aug;5(3):214-9. doi: 10.1111/jicd.12038. Epub 2013 Jun 18. PMID: 23776093.
[13]. Gadbail AR, Sarode SC, Chaudhary MS, Gondivkar SM, Tekade SA, Yuwanati M, Patil S. Ki67 Labelling Index predicts clinical outcome and survival in oral squamous cell carcinoma. J Appl Oral Sci. 2021 Mar 1;29:e20200751. doi: 10.1590/1678-7757-2020-0751. PMID: 33656066; PMCID: PMC7934280.
[14]. Gonzalez-Moles MA, Ruiz-Avila I, Gil-Montoya JA, Esteban F, Bravo M. Analysis of Ki-67 expression in oral squamous cell carcinoma: why Ki-67 is not a prognostic indicator. Oral Oncol. 2010 Jul;46(7):525-30. doi: 10.1016/j.oraloncology.2010.03.020. Epub 2010 Apr 18. PMID: 20400362.
[15]. Verma R, Singh A, Jaiswal R, Chandra A, Verma R, Tak J. Association of Ki-67 antigen and p53 protein at invasive tumor front of oral squamous cell carcinoma. Indian J Pathol Microbiol. 2014 Oct-Dec;57(4):553-7. doi: 10.4103/0377-4929.142660. PMID: 25308006.