

Research Article**Immunohistochemical and Digital Pathology Assessment of Malignant Transformation Risk in Oral Submucous Fibrosis, Oral Lichen Planus, and Leukoplakia: A Cross-Sectional Study**

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Abstract**Background:**

Oral potentially malignant disorders (OPMDs)—specifically Oral Submucous Fibrosis (OSF), Oral Lichen Planus (OLP), and Oral Leukoplakia (OLK)—carry significant but variable risks of progression to Oral Squamous Cell Carcinoma (OSCC). Conventional subjective dysplasia grading often suffers from inter-observer variability. Combining immunohistochemical (IHC) profiling of key cell-cycle and apoptotic regulators (p53, Ki-67, and Bcl-2) with quantitative digital pathology image analysis provides an objective, standardized approach to evaluate malignant transformation risk.

Objective:

To quantify and compare the immunohistochemical expression of p53, Ki-67, and Bcl-2 using automated digital pathology image analysis across tissue specimens of OSF, OLP, and OLK, and to correlate these biomarkers with the severity of epithelial dysplasia.

Methods:

A hospital-based cross-sectional study was conducted on 150 archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks (50 OSF, 50 OLP, and 50 OLK) at Gandhara University. Histopathological grading of epithelial dysplasia was performed using standard WHO diagnostic criteria. Immunohistochemistry was conducted for p53, Ki-67, and Bcl-2. Whole-slide digital imaging and quantitative analysis were performed using QuPath software to determine digital labeling indices (LI, %). Biomarker expressions across disease categories and dysplasia grades were analyzed using ANOVA and Kruskal-Wallis tests, while multivariable linear regression identified independent factors associated with elevated cell proliferative and anti-apoptotic markers.

Results:

Among the 150 patients, the mean age was 48.6 ± 11.2 years (58.0% male). Severe epithelial dysplasia was identified in 18.0% of OLK, 12.0% of OSF, and 4.0% of OLP.

cases. Automated digital pathology assessment demonstrated significantly higher mean Ki-67 LI in OLK ($28.4 \pm 12.1\%$) compared to OSF ($21.2 \pm 9.8\%$) and OLP ($14.6 \pm 6.2\%$) ($p < 0.001$). Nuclear p53 expression positivity followed a similar pattern, highest in OLK ($32.1 \pm 14.5\%$) followed by OSF ($22.5 \pm 10.4\%$) and OLP ($11.8 \pm 5.9\%$) ($p < 0.001$). Dysplasia severity strongly correlated with elevated Ki-67 ($r = 0.68, p < 0.001$) and p53 ($r = 0.61, p < 0.001$). Multivariable regression confirmed that high-grade dysplasia and OLK diagnosis independently predicted elevated dual-positivity for p53 and Ki-67 ($p < 0.001$).

Conclusion:

Quantitative digital pathology combined with IHC biomarker profiling (p53, Ki-67, and Bcl-2) establishes distinct risk profiles across OPMD categories. Leukoplakia and advanced Submucous Fibrosis demonstrate higher molecular malignant transformation potential compared to Lichen Planus. Automated digital image analysis provides a reproducible, objective diagnostic framework for stratification of malignant risk in routine pathology practice.

Keywords: Oral potentially malignant disorders; Oral Submucous Fibrosis; Oral Lichen Planus; Leukoplakia; Digital pathology; Immunohistochemistry; p53; Ki-67; Malignant transformation.

1. Introduction

Oral Squamous Cell Carcinoma (OSCC) represents the overwhelming majority of head and neck malignancies globally, carrying high morbidity and mortality when diagnosed at advanced stages [1, 2]. OSCC frequently develops from pre-existing mucosal abnormalities designated by the World Health Organization (WHO) as Oral

Potentially Malignant Disorders (OPMDs) [3]. Among these, Oral Submucous Fibrosis (OSF), Oral Lichen Planus (OLP), and Oral Leukoplakia (OLK) are the most prevalent clinical entities encountered in dental and maxillofacial pathology clinics [3, 4].

Although these conditions share an elevated propensity for malignant transformation, their underlying etiopathogenesis and inherent malignant conversion rates differ markedly [4, 5]. OLK is a predominantly hyperkeratotic white plaque carrying an overall malignant transformation rate ranging from 3% to 20% depending on clinical subtype and dysplastic severity [5]. OSF is a chronic, progressive, fibrosing condition driven predominantly by areca nut chewing that causes mucosal atrophy and submucosal collagen deposition, with transformation rates estimated between 2% and 8% [4, 6]. Conversely, OLP is a chronic T-cell-mediated autoimmune mucocutaneous disorder with a lower, though persistent, transformation rate of 0.4% to 2.3% [5, 7].

Traditional risk assessment relies primarily on subjective microscopic grading of oral epithelial dysplasia (OED) using hematoxylin and eosin (H&E)-stained tissue sections [3]. However, routine microscopic dysplasia grading suffers from substantial intra- and inter-observer variability, limiting its individual prognostic accuracy [8]. Consequently, there is an urgent need for validated objective molecular markers and standardized quantitative tools to refine risk stratification [8, 9].

Cell cycle dysregulation, uncontrolled cellular proliferation, and resistance to apoptosis represent central hallmarks of early oral carcinogenesis [9, 10]. The tumor suppressor protein p53 regulates the G₁/S cell cycle checkpoint; missense mutations or aberrant accumulation of p53 are early

molecular events in malignant progression [9, 11]. Ki-67 is a well-established nuclear antigen expressed exclusively during active cell cycle phases (G_1 , S, G_2 , and M), providing a direct metric of cellular proliferative capacity [10, 11]. Concurrently, the anti-apoptotic protein Bcl-2 prevents programmed cell death, fostering cell survival during dysplastic changes [11, 12].

In recent years, digital pathology and automated image analysis software (such as QuPath) have revolutionized histopathological evaluation [13]. By converting glass slides into high-resolution Whole Slide Images (WSIs), automated nuclear detection algorithms enable precise cell-by-cell quantification, eliminating subjective observer bias [13, 14]. Combining quantitative digital immunophenotyping with standard WHO dysplastic grading offers a robust model for evaluating malignant transformation risk across OPMD subtypes [13].

Therefore, this cross-sectional study was undertaken to evaluate and compare the immunohistochemical expression of p53, Ki-67, and Bcl-2 across OSF, OLP, and OLK tissue samples using automated digital pathology image analysis, and to correlate these biomarker profiles with the degree of epithelial dysplasia.

2. Rationale of the Study

While conventional histopathological examination remains the clinical benchmark for OPMD diagnosis, subjective dysplasia grading fails to reliably predict individual malignant transformation, particularly in mild-to-moderate dysplasia cases [3, 8]. Integrating immunohistochemical biomarkers (p53, Ki-67, Bcl-2) with computer-assisted digital pathology provides an objective, standardized metric for

quantifying cellular proliferation and molecular dysregulation [13, 14]. A comparative cross-sectional analysis across the three most common OPMD entities—OSF, OLP, and OLK—will help map biomarker expression gradients and establish reproducible risk profiles to guide early therapeutic intervention and clinical monitoring.

3. Objectives

Primary Objective

- To compare the quantitative nuclear/cytoplasmic immunohistochemical labeling indices of p53, Ki-67, and Bcl-2 across tissue specimens of Oral Submucous Fibrosis (OSF), Oral Lichen Planus (OLP), and Oral Leukoplakia (OLK) using automated digital pathology analysis.

Secondary Objectives

1. To evaluate the correlation between digital biomarker labeling indices (p53, Ki-67, Bcl-2) and the severity of oral epithelial dysplasia (OED) classified according to WHO criteria.
2. To examine differences in biomarker co-expression (dual p53/Ki-67 positivity) among different clinical and pathological grades of OSF, OLP, and OLK.
3. To perform multivariable regression analysis to identify independent histopathological and molecular predictors of high-risk dysplastic status.
4. To establish objective digital pathology cutoff thresholds for high proliferative activity across OPMD categories.

4. Materials and Methods

4.1 Study Design

A hospital-based cross-sectional observational study was conducted utilizing

archived formalin-fixed paraffin-embedded (FFPE) mucosal tissue blocks.

4.2 Study Setting

The study was conducted at Gandhara University.

4.3 Study Duration

The study was conducted over a period of eight months, including tissue block retrieval, sectioning, immunohistochemical staining, whole-slide digital scanning, QuPath image analysis, and statistical data synthesis.

4.4 Study Population

Archived FFPE mucosal tissue blocks from adult patients who had undergone diagnostic incisional biopsy for OSF, OLP, or OLK were evaluated.

4.5 Sample Size

A total sample size of 150 tissue cases (50 OSF, 50 OLP, and 50 OLK) was selected. The sample size was calculated assuming a moderate-to-large effect size ($f = 0.30$) among the three comparative diagnostic groups for Ki-67/p53 labeling indices, maintaining an alpha level of 0.05 and a statistical power of 85%.

4.6 Inclusion Criteria

1. Histologically confirmed archived FFPE tissue blocks of OSF, OLP, or OLK.
2. Sufficient epithelial tissue volume within the paraffin block to enable sectioning for H&E and immunohistochemical staining.
3. Availability of complete patient clinical and demographic records.

4.7 Exclusion Criteria

1. Tissue specimens showing frank invasive Oral Squamous Cell Carcinoma (OSCC) upon baseline H&E review.
2. Biopsies showing severe tissue artifact, thermal necrosis, or inadequate epithelial architecture.
3. Patients with a prior history of head and neck radiation therapy or systemic chemotherapy.
4. Co-existing systemic immunodeficiency disorders or active mucosal fungal infection (e.g., candidiasis) causing significant inflammatory reactive changes.

4.8 Data Collection and Histopathological Grading

Demographic details (age, sex), clinical presentation, risk habits (tobacco smoking, smokeless tobacco use, areca nut consumption, alcohol use), anatomical site, and clinical lesion characteristics were recorded.

Serial tissue sections (4 μ m thick) were cut from each FFPE block. One section was stained with Hematoxylin and Eosin (H&E) for histopathological evaluation. Two independent oral pathologists, blinded to clinical outcomes, graded Oral Epithelial Dysplasia (OED) according to the WHO (2022) criteria into:

- No dysplasia
- Mild dysplasia
- Moderate dysplasia
- Severe dysplasia

Discrepancies in grading were resolved by joint review under a multi-head microscope to reach consensus.

4.9 Immunohistochemical (IHC) Staining

Immunohistochemistry was performed on 4 µm sections mounted on poly-L-lysine-coated glass slides using automated Leica Bond-III immunostainers.

- **Primary Antibodies:**

- **p53:** Mouse monoclonal anti-p53 antibody (Clone DO-7, ready-to-use).
- **Ki-67:** Rabbit monoclonal anti-Ki-67 antibody (Clone SP6, ready-to-use).
- **Bcl-2:** Mouse monoclonal anti-Bcl-2 antibody (Clone 124, ready-to-use).

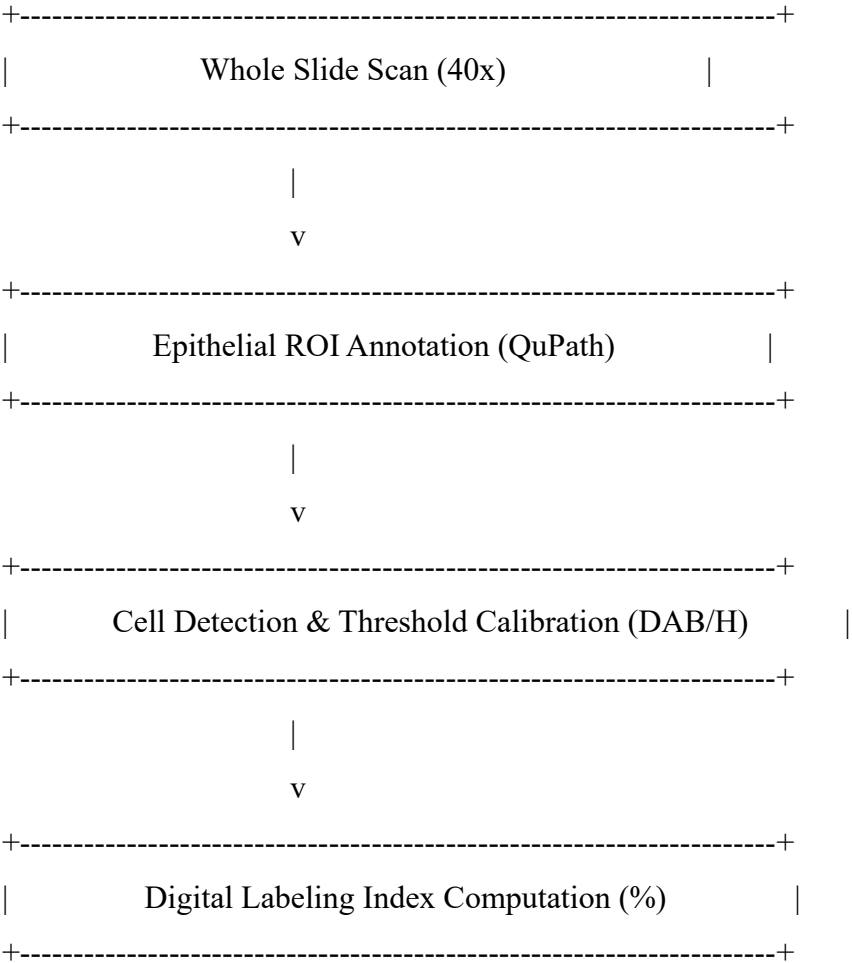
- **Protocol:** Heat-induced epitope retrieval (HIER) was performed using citrate buffer (pH 6.0) for 20 minutes. Endogenous peroxidase activity was blocked with 3% H₂O₂. Primary antibodies were incubated for 30 minutes at room temperature, followed by horseradish peroxidase (HRP)-conjugated secondary polymer detection. Staining was visualized using 3,3'-diaminobenzidine (DAB) chromogen and counterstained with Mayer's hematoxylin. Known positive controls (tonsil for Ki-67/Bcl-2 and colon carcinoma for p53) and negative controls (omitting primary antibody) were included in each run.

4.10 Whole Slide Imaging and Digital Pathology Analysis

Stained slides were digitized using a high-resolution whole-slide scanner (Aperio AT2, Leica Biosystems) at 40 × magnification (0.25 µm/pixel resolution).

Whole Slide Images (WSIs) were imported into the open-source digital pathology image analysis software **QuPath (version 0.4.3)** [13].

1. **Annotation:** Regions of Interest (ROIs) encompassing the entire mucosal epithelium (basal, suprabasal, and spinous layers) were manually annotated, excluding hyperkeratotic surface scales and connective tissue stroma.
2. **Cell Detection:** The 'Positive Cell Detection' algorithm was calibrated using hematoxylin optical density for nuclear detection and DAB optical density for biomarker positivity thresholds.
3. **Quantification:**
 - **Ki-67 Labeling Index (LI, %):** Number of nuclear DAB-positive epithelial cells divided by total detected epithelial cells × 100.
 - **p53 Positivity (%):** Percentage of epithelial cells displaying nuclear immunopositivity. Overexpression was defined as > 20% suprabasal/basal positive nuclei or complete null pattern.
 - **Bcl-2 Expression (%):** Percentage of epithelial cells showing cytoplasmic DAB immunopositivity.



4.11 Statistical Analysis

Data were analyzed using SPSS version 26.0 and R version 4.2. continuous parameters were assessed for normality using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), while categorical variables were presented as frequencies and percentages (n, %).

Differences in quantitative digital biomarker expression across the three diagnostic categories (OSF, OLP, OLK) and dysplasia grades were analyzed using One-Way Analysis of Variance (ANOVA) with Tukey's post-hoc test or Kruskal-Wallis test with Dunn's post-hoc correction as appropriate. Pearson/Spearman correlation coefficients examined linear associations between digital LIs and dysplastic severity. Multivariable

linear and binary logistic regression models were constructed to evaluate independent factors associated with high biomarker expression and high-grade dysplasia. Statistical significance was defined as a two-sided p-value < 0.05.

The study included 150 tissue cases equally distributed across OSF (n = 50), OLP (n = 50), and OLK (n = 50). The mean patient age was 48.6 ± 11.2 years, with a male preponderance (58.0%, n = 87). Areca nut consumption was predominantly associated with OSF (92.0%), whereas tobacco smoking and smokeless tobacco were most frequent in OLK (74.0%).

5. Results

5.1 Patient Demographics and Clinical Characteristics

Variable	Oral Submucous Fibrosis (n = 50)	Oral Lichen Planus (n = 50)	Oral Leukoplakia (n = 50)	Total Cohort (n = 150)
Age, years	41.2 $\pm$ 9.8	52.4 $\pm$ 10.1	52.1 $\pm$ 10.6	48.6 $\pm$ 11.2
Male sex, n (%)	38 (76.0)	18 (36.0)	31 (62.0)	87 (58.0)
Female sex, n (%)	12 (24.0)	32 (64.0)	19 (38.0)	63 (42.0)
Areca nut habit, n (%)	46 (92.0)	4 (8.0)	14 (28.0)	64 (42.7)
Tobacco habit, n (%)	22 (44.0)	8 (16.0)	37 (74.0)	67 (44.7)
Buccal mucosa site, n (%)	44 (88.0)	36 (72.0)	28 (56.0)	108 (72.0)

Variable	Oral Submucous Fibrosis (n = 50)	Oral Lichen Planus (n = 50)	Oral Leukoplakia (n = 50)	Total Cohort (n = 150)
Tongue mucosa site, n (%)	2 (4.0)	10 (20.0)	14 (28.0)	26 (17.3)

Table 1: Demographic and clinical characteristics across OPMD diagnostic groups.

5.2 Histopathological Dysplasia Distribution

The distribution of Oral Epithelial Dysplasia (OED) differed significantly across the three diagnostic groups ($p < 0.001$). OLK demonstrated the highest frequency of dysplastic changes, with 18.0% ($n = 9$) presenting with severe dysplasia. In contrast, 68.0% ($n = 34$) of OLP cases exhibited no dysplasia.

WHO Dysplasia Grade	Oral Submucous Fibrosis, n (%)	Oral Lichen Planus, n (%)	Oral Leukoplakia, n (%)	Total Cohort, n (%)
No Dysplasia	16 (32.0)	34 (68.0)	10 (20.0)	60 (40.0)
Mild Dysplasia	18 (36.0)	11 (22.0)	16 (32.0)	45 (30.0)
Moderate Dysplasia	10 (20.0)	3 (6.0)	15 (30.0)	28 (18.7)
Severe Dysplasia	6 (12.0)	2 (4.0)	9 (18.0)	17 (11.3)

Table 2: Distribution of WHO Oral Epithelial Dysplasia (OED) grades.

5.3 Digital Pathology Quantification of IHC Biomarkers

Automated image analysis using QuPath demonstrated significant variations in Ki-67, p53, and Bcl-2 expression levels across OPMD categories ($p < 0.001$). OLK exhibited the highest mean Ki-67 LI ($28.4 \pm 12.1\%$) and p53 positivity ($32.1 \pm 14.5\%$), whereas OLP displayed the lowest proliferative indices.

Digital IHC Biomarker (%)	Oral Submucous Fibrosis	Oral Lichen Planus	Oral Leukoplakia	p-value (ANOVA)
Ki-67 Labeling Index (%)	21.2 ± 9.8	14.6 ± 6.2	28.4 ± 12.1	<0.001
p53 Nuclear Positivity (%)	22.5 ± 10.4	11.8 ± 5.9	32.1 ± 14.5	<0.001
Bcl-2 Cytoplasmic Positivity (%)	18.6 ± 8.1	24.2 ± 9.3	15.4 ± 7.2	<0.001
Dual p53/Ki-67 Positivity (%)	14.2 ± 7.1	6.8 ± 3.4	21.6 ± 9.8	<0.001

Table 3: Quantitative digital pathology biomarker expression across OPMD entities.

5.4 Correlation Between Digital Biomarkers and Dysplasia Severity

A strong positive correlation was observed between the degree of epithelial dysplasia and both Ki-67 LI ($r = 0.68, p < 0.001$) and p53 positivity ($r = 0.61, p < 0.001$). Conversely, Bcl-2 expression demonstrated a mild inverse correlation with dysplasia severity ($r = -0.32, p < 0.001$).

Biomarker Parameter	Dysplasia Correlation Coefficient (r)	p-value
Ki-67 Labeling Index	0.68	<0.001
p53 Nuclear Positivity	0.61	<0.001
Bcl-2 Cytoplasmic Positivity	-0.32	<0.001
Dual p53/Ki-67 Positivity	0.72	<0.001

Table 4: Correlation analysis between digital IHC markers and WHO dysplasia grades.

5.5 Biomarker Expression Stratified by WHO Dysplasia Grade

Analysis of digital biomarker labeling indices categorized by dysplasia grade revealed a progressive increase in Ki-67 and p53 expression from non-dysplastic lesions to severe dysplasia across all OPMD types.

WHO Dysplasia Grade	Ki-67 Labeling Index (%)	p53 Positivity (%)	Bcl-2 Positivity (%)
No Dysplasia (n = 60)	12.4 ± 4.8	9.6 ± 4.1	23.1 ± 8.2
Mild Dysplasia (n = 45)	19.8 ± 6.5	18.2 ± 7.3	19.4 ± 7.1
Moderate Dysplasia (n = 28)	29.6 ± 8.2	31.4 ± 9.8	14.8 ± 5.9
Severe Dysplasia (n = 17)	42.1 ± 10.6	45.8 ± 12.2	10.2 ± 4.1

Table 5: Digital IHC biomarker levels stratified by WHO dysplasia severity.

5.6 Multivariable Linear Regression Analysis for Factors Predicting High Ki-67 LI

In multivariable linear regression with Ki-67 LI as the continuous dependent variable, high-grade dysplasia (moderate/severe) ($\beta = 0.48, p < 0.001$) and diagnosis of OLK ($\beta = 0.28, p < 0.001$) were independent predictors of heightened cellular proliferation.

Predictor Variable	Adjusted β	95% Confidence Interval	p-value
High-Grade Dysplasia (Moderate/Severe)	0.48	0.36 to 0.60	<0.001
Leukoplakia Diagnosis (vs. OLP)	0.28	0.16 to 0.40	<0.001
OSF Diagnosis (vs. OLP)	0.19	0.07 to 0.31	0.003
Areca Nut / Tobacco Habit	0.12	0.02 to 0.22	0.021
Patient Age	0.04	-0.05 to 0.13	0.412

Table 6: Multivariable linear regression analysis predicting digital Ki-67 Labeling Index.

6. Discussion

This cross-sectional study evaluated malignant transformation risk markers across the three major OPMDs—Oral Submucous Fibrosis, Oral Lichen Planus, and Oral Leukoplakia—by combining immunohistochemical detection of p53, Ki-67, and Bcl-2 with computer-assisted digital pathology. Automated whole-slide digital analysis demonstrated clear differences in molecular expression profiles, highlighting that Oral Leukoplakia and high-grade Submucous Fibrosis harbor significantly greater cellular proliferative and genetic

instability markers than Oral Lichen Planus [5, 10, 13].

6.1 Proliferative and Genetic Instability Markers in OPMDs

Cell proliferation, measured quantitatively via the Ki-67 labeling index, reflects the expansive growth potential of premalignant epithelium [10, 11]. In our study, Ki-67 LI was significantly elevated in OLK (28.4%) and OSF (21.2%) relative to OLP (14.6%). The suprabasal expansion of Ki-67-positive cells serves as an objective marker of

dysplastic progression, aligning with previous reports demonstrating that parabasal and spinous layer Ki-67 expression strongly correlates with neoplastic conversion [10, 14].

Concurrently, nuclear accumulation of p53 protein—often resulting from TP53 gene mutations or protein stabilization—was markedly higher in dysplastic OLK and OSF tissue samples [9, 11]. Mutant p53 fails to induce G₁ arrest or trigger physiological apoptosis in response to DNA damage, fostering clonal expansion of damaged Keratinocytes [9]. The high dual p53/Ki-67 positivity (21.6%) observed in OLK underscores its higher intrinsic transformation potential compared to other lesions [5, 11].

6.2 Pathophysiological Differences Across OPMD Subtypes

The variations in biomarker expression profiles reflect the distinct etiopathogenetic mechanisms driving these disorders [4, 5]:

- **Oral Leukoplakia:** Driven primarily by carcinogens in tobacco smoke and alcohol, direct DNA damage results in early TP53 mutations, localized genomic instability, and severe dysplastic changes [5, 9].
- **Oral Submucous Fibrosis:** Driven by areca nut alkaloids (arecoline), chronic chemical irritation leads to juxta-epithelial inflammation, severe submucosal fibrosis, and chronic epithelial hypoxia [4, 6]. Atrophic epithelium under hypoxic stress undergoes compensatory stem cell activation, resulting in moderate-to-high Ki-67 proliferation and secondary p53 accumulation [4, 11].

- **Oral Lichen Planus:** Representing a chronic auto-inflammatory response, basal cell apoptosis is mediated by cytotoxic CD8⁺ T-lymphocytes [7]. Elevated Bcl-2 expression in non-dysplastic OLP (24.2%) likely represents a physiological survival response of basal keratinocytes against immune-mediated clearance [7, 12].

6.3 Value of Digital Pathology in Risk Stratification

Traditional microscopic evaluation of dysplastic severity is inherently subjective and prone to inter-observer discordance [8]. Automated image analysis using software platforms like QuPath resolves these challenges by calculating precise labeling indices across thousands of individual cells within whole-slide annotations [13, 14]. Digital pathology standardizes diagnostic cutoffs, enabling reproducible risk stratification suitable for multi-center clinical trials and diagnostic pathology workflows [13].

6.4 Clinical Implications

Quantifying Ki-67, p53, and Bcl-2 indices via digital pathology offers practical clinical utility. OPMD cases displaying high Ki-67 LI (> 25%) and suprabasal p53 overexpression—even when classified as mild-to-moderate dysplasia on H&E—should be managed as high-risk lesions requiring close clinical follow-up, surgical excision, or chemopreventive interventions [8, 11].

6.5 Limitations

Several limitations should be noted. First, the cross-sectional study design prevents direct observation of long-term malignant conversion events in individual patients. Second, TP53 gene sequencing was not performed to differentiate missense

mutations from null mutations or reactive wild-type overexpression. Third, while automated digital image analysis reduces observer bias, proper tissue sectioning and threshold calibration remain critical technical prerequisites [13].

7. Conclusion

Quantitative digital pathology analysis of p53, Ki-67, and Bcl-2 immunohistochemical expression establishes distinct molecular risk profiles across Oral Potentially Malignant Disorders. Oral Leukoplakia and Oral Submucous Fibrosis demonstrate higher proliferative indices and molecular dysregulation than Oral Lichen Planus. Automated whole-slide digital image analysis provides an objective, reproducible, and standardized diagnostic tool to enhance conventional dysplastic grading and refine malignant transformation risk assessment in routine oral pathology practice.

8. Recommendations

1. Implement standardized digital slide scanning and automated image analysis protocols to reduce inter-observer variability in oral dysplasia grading.
2. Incorporate combined p53 and Ki-67 immunohistochemical staining panels for all moderate-to-severe OPMD biopsy specimens.
3. Establish a quantitative Ki-67 labeling index cutoff ($> 25\%$) as an objective marker for close surgical and clinical surveillance.
4. Conduct prospective longitudinal cohort studies to validate digital pathology risk scores against actual long-term OSCC transformation rates.

5. Combine digital immunophenotyping with molecular molecular profiling (e.g., DNA ploidy or Next-Generation Sequencing) to optimize early cancer detection frameworks.

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