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Immunohistochemical biomarkers for predicting malignant transformation in oral leukoplakia and lichen planus: a systematic review study

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Abstract

Introduction: Oral leukoplakia (OLK) and oral lichen planus (OLP) are recognized as potentially malignant disorders with varying risks of progression to oral squamous cell carcinoma (OSCC). Immunohistochemical (IHC) biomarkers have been extensively studied to identify predictive markers for malignant transformation in these lesions.

Objectives: This systematic review synthesizes current evidence on IHC biomarkers associated with malignant progression in OLK and OLP.

Materials and Methods: In this systematic review study, a systematic literature search was conducted across databases, including PubMed, Scopus, Embase, Web of Science, Cochrane Library, and Google Scholar search engine up to April 2025. Studies included were those investigating IHC biomarkers in OLK and OLP with reported malignant transformation outcomes.

Results: This systematic review included 10 studies with a total of 549 participants. The most frequently assessed IHC markers were B-cell lymphoma 2 (Bcl-2), p53, Ki-67, Bcl-2-associated X protein (Bax), survivin, mouse double minute 2 homolog (MDM2), and proliferating cell nuclear antigen (PCNA). Bcl-2 was the predominant marker, evaluated in 9 studies, followed by p53 was examined in 7, Ki-67 in 4, and Bax in 3 studies. Less commonly studied markers included survivin, MDM2, PCNA, and p21, each reported in a single study.

Conclusion: Although Bcl-2 and p53 emerge as the most significant IHC markers for predicting malignant transformation in oral premalignant lesions due to their roles in apoptosis and tumor suppression, the Ki-67 and Bax biomarkers contribute important information on cell proliferation and apoptotic balance, while markers like survivin, MDM2, PCNA, and p21 are less commonly studied but may have specialized roles. Overall, a panel of these biomarkers, especially Bcl-2 and p53, shows strong potential for improving risk assessment and guiding clinical management of OLK and OLP.

Registration: This study has been compiled based on the PRISMA checklist, and its protocol was registered on the PROSPERO (International Prospective Register of Systematic Reviews) (ID: CRD420251063588) and Research Registry (UIN: reviewregistry2021) websites.

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Introduction

Oral leukoplakia (OLK) represents the most prevalent oral potentially malignant disorder, defined by the World Health Organization (WHO) as a predominantly white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk for cancer (1,2). This definition reflects decades of evolving terminology from "precancerous lesions" to the current nomenclature of oral potentially malignant disorders, introduced by the WHO in 2005 to encompass conditions with statistically increased risk of developing oral cancer (3). Global prevalence studies demonstrate OLK affects approximately 1.39% of the population, with a pooled estimated prevalence of 2.23% for population-based studies and 1.36% for clinic-based population studies (4). The etiology remains multifactorial, with tobacco use, alcohol consumption, viral infections (particularly human papillomavirus), and chronic mechanical irritation serving as principal risk factors (5). Malignant transformation rates vary considerably, with recent systematic reviews reporting varying annual transformation rates, depending on geographical location, study methodology, and lesion characteristics (4).

Oral lichen planus (OLP) is a chronic mucocutaneous disorder affecting 0.5% to 2.0% of the general population, characterized as a T-cell-mediated autoimmune disease involving cytotoxic CD8+ T cells that

Key point

- The most frequently assessed immunohistochemical (IHC) markers for malignant transformation of oral leukoplakia and lichen planus were the B-cell lymphoma 2 (Bcl-2), p53, Ki-67, Bcl-2-associated X protein (Bax), survivin, mouse double minute 2 homolog (MDM2), and proliferating cell nuclear antigen (PCNA).
- Bcl-2 was the predominant marker, evaluated in 9 out of 10 studies, followed by p53, assessed in 7, Ki-67, reported in 4, and Bax evaluated in 3 studies.
- Less commonly studied markers included survivin, MDM2, PCNA, and p21, each reported in only one study.

trigger apoptosis of basal epithelial cells (6). The WHO has classified OLP as a potentially malignant disorder, though its malignant transformation potential remains controversial among clinicians and researchers (7,8). Recent systematic reviews and meta-analyses using strict diagnostic criteria report substantially lower malignant transformation rates than previously believed, with studies showing 0.44% to 2.28% transformation rates (7-9). A comprehensive meta-analysis of 12,838 OLP patients found only 56 cases underwent legitimate malignant transformation (0.44%) when applying strict criteria, including proper OLP diagnosis verification and clear documentation of carcinoma developing at the same site (8). The erosive form of OLP appears to carry a higher malignant transformation risk compared to reticular patterns, with malignant transformation rates of 1.7% for erosive forms versus lower rates for non-erosive variants (6,10).

Immunohistochemical (IHC) biomarkers for predicting malignant transformation in oral potentially malignant disorders have emerged as critical research priorities, given limitations of traditional histopathological assessment in accurately predicting progression risk (11,12). Cell cycle-related biomarkers, including p53, Ki-67, cyclin D1, and epidermal growth factor receptor (EGFR), show particular promise, with studies demonstrating p53 expression correlating with dysplasia grade in 60% of oral precancerous lesions (12,13). Recent systematic reviews of tissue biomarkers in OLK identify 30 different biomarkers, with Bmi-1, CD3/CD8, Ki-67, p16, and DNA ploidy evaluated in multiple studies; in this study multivariate analyses reveal biomarkers, including Bmi-1, Tipe-2, copy number alteration, DcR2, Ki-67, PTHrP, podoplanin (PDPN), and BubR1/Mad2, demonstrate independent significant value for malignant transformation prediction (11). Combined biomarker approaches, particularly p53 and Ki-67 expression patterns, show enhanced predictive capacity with sensitivity greater than 75% for delineating high-risk from low-risk lesions (13,14).

Objectives

The objective of this systematic review study is to comprehensively evaluate and synthesize existing evidence on IHC biomarkers for their potential to predict malignant transformation in OLK and lichen planus. The study aims to identify key biomarkers associated with apoptotic regulation and proliferative activity that can serve as reliable indicators of the risk of malignant progression in these oral premalignant lesions, thereby informing clinical assessment and management strategies.

Materials and Methods

Study design

This systematic review was conducted following the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (15). The objective of this systematic review is to comprehensively evaluate and synthesize existing evidence on IHC biomarkers that predict malignant transformation in OLK and OLP, intending to identify reliable molecular indicators for early detection and risk stratification of these potentially malignant oral disorders.

Search strategy

An extensive literature search was performed across several databases, such as Embase, Cochrane Library, PubMed, Web of Science, and Scopus, covering publications up to April 2025. Additionally, Google Scholar was utilized to supplement the search. The search strategy incorporated a broad range of Medical Subject Headings (MeSH) keywords to effectively capture all pertinent studies. The applied keywords included oral squamous cell carcinoma, malignant transformation, leukoplakia, lichen planus, cell proliferation, immunohistochemistry, BCL-2, P53, and Ki-67. The search strategy for this systematic review was conducted without limitations on publication date, language, or geographic location. Titles and abstracts of the retrieved studies were independently screened by two reviewers, and full-text articles were subsequently evaluated to determine their eligibility.

As an example of database searching, the following search strategy was applied: ((((((((((((((((Oral squamous cell carcinoma[Title/Abstract]) OR (Oral leukoplakia[Title/Abstract])) OR (Oral lichen planus[Title/Abstract])) AND (Malignant transformation[Title/Abstract])) AND (Malignant progression[Title/Abstract])) AND (Immunohistochemistry[Title/Abstract])) OR (B-cell lymphoma 2[Title/Abstract])) OR (p53[Title/Abstract])) OR (Ki-67[Title/Abstract])) OR (Bcl-2-associated X protein[Title/Abstract])) OR (Bax[Title/Abstract])) OR (Bcl-2[Title/Abstract])) OR (Survivin[Title/Abstract])) OR (Mouse double minute 2 homolog[Title/Abstract])) OR (MDM2[Title/Abstract])) OR (Proliferating cell nuclear antigen[Title/Abstract])) OR (PCNA[Title/Abstract]).

PICO component

- Population (P): Patients with OLK and OLP, which are oral potentially malignant disorders.

- Intervention/Exposure (I): The IHC assessment of biomarkers in tissue samples from OLK and OLP lesions.
- Comparison (C): Comparison of biomarker expression levels between lesions that underwent malignant transformation and those that did not.
- Outcome (O): Prediction or identification of malignant transformation IHC biomarkers.

Eligibility criteria

- Inclusion criteria: Original research articles evaluating IHC biomarkers in OLK and/or OLP with follow-up data or outcomes related to malignant transformation.
- Exclusion criteria: Reviews, editorials, case reports, studies without IHC analysis, or those lacking data on malignant progression.

Quality assessment

The quality of the studies included in this systematic review was evaluated using the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist. This tool consists of 22 items that cover different aspects of observational research. Each item is scored with two points, and the cumulative score represents the overall study quality. According to this scoring system, a total score between 1 and 15 denotes poor quality, scores from 16 to 30 indicate moderate quality, and scores ranging from 31 to 44 correspond to high quality (16). We include articles of medium and high quality. Two reviewers independently evaluated the quality assessment, with any disagreements settled by discussion or, if necessary, by involving a third reviewer.

Data extraction

Two reviewers independently performed data extraction. A checklist, including of first author's names, study type, place of publication, publication year, study population, size of sample, research aim, IHC biomarker, and main results. In case of disagreements between the first two reviewers, the third reviewer was consulted to reassess the data.

Risk of bias assessment

We used the Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS-I) guideline, specifically adapted to assess the risk of bias in outcomes from non-randomized intervention studies. Unlike randomized controlled trials (RCTs), which randomly assign participants to intervention groups, non-randomized studies (NRSIs)—including cohort studies, case-control studies, controlled before-and-after studies, and interrupted time-series studies—do not use randomization. This lack of random assignment increases their susceptibility to various biases. The primary purpose of ROBINS-I is to help reviewers systematically evaluate the internal validity of NRSIs by identifying potential biases that could cause systematic differences between the observed outcomes of these studies and the expected results from a well-conducted randomized trial involving the same participant population. To assess the risk of bias in the included studies, we evaluated seven domains by this guideline: confounding, participant selection, classification of interventions, deviations from intended interventions, missing data, outcome measurement, and selection of reported results (17). Two independent reviewers conducted the bias assessments using external spreadsheets to ensure accuracy and consistency.

Results

A total of 1072 studies were initially identified through the database search. Following the removal of duplicate records, 981 unique studies remained and were subjected to title and abstract screening. Based on the predefined eligibility criteria, 951 studies were excluded at this stage, resulting in 30 records being retained for full-text review. After a thorough evaluation of the full texts, 10 studies met all inclusion criteria and were subsequently incorporated into the qualitative synthesis of this review (Figure 1).

Based on the STROBE checklist quality assessment, the 10 included studies demonstrated variable adherence to reporting standards across different methodological domains. All studies fully met criteria for title and abstract, background/rationale, objectives, participant description, variable definition, data sources/measurement, descriptive data, outcome data, key findings, interpretation, ethical approval, and funding source disclosure. However, performance was less consistent in several areas; study design reporting was adequate in 9 studies but not met in Sudha et al, while setting description was fully met in only 3 studies (18-20) and partially met in the remaining 7 studies. Bias consideration was problematic across the evidence base, with no study fully addressing this component; 8 studies partially met this criterion, while 2 (21,24) failed to address bias adequately. Study size justification was met in only 4 studies, with 3 studies not justified and 3 offering partial justification. Statistical methods were generally well-reported, except for Dwivedi et al, which partially met this criterion. In the results section, the main results were adequately reported in 9 studies, with only Pigatti et al (20) partially meeting this standard, while other analyses were poorly reported overall, with 6 studies not meeting this criterion and 4 providing partial coverage. Discussion quality varied considerably; limitations were fully discussed in only 3 studies, with 7 providing partial coverage, while generalizability was uniformly problematic, with all 10 studies only partially addressing this critical component. Overall quality scores ranged from 15/22 to 19/22 criteria met, with Chamorro-Petronacci et al (18) achieving the highest score and Sudha et al the lowest, indicating moderate to good adherence to STROBE guidelines (Table 1).

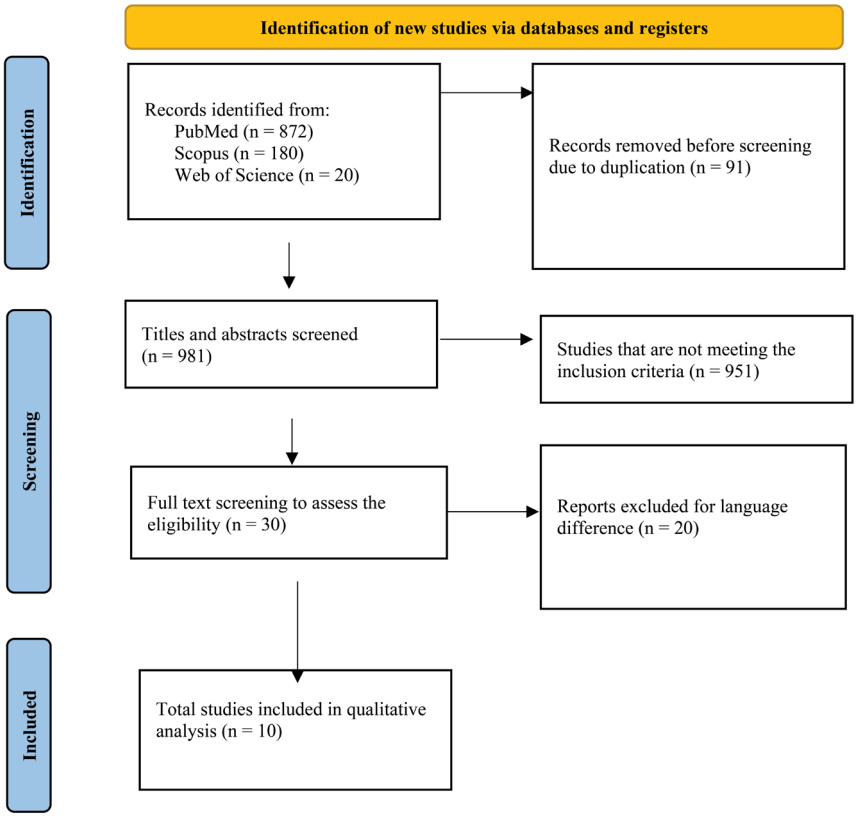

Figure 1. PRISMA flow diagram illustrating the study selection process

The risk bias assessment of the included studies indicated that across the ten included studies, the risk-of-bias profile was broadly consistent, with all studies rated as having a moderate risk of bias for confounding and participant selection, except Jane et al (25), which demonstrated a low selection risk. Measurement bias, deviations from intended interventions, outcome measurement, and selective reporting were consistently judged low risk in all studies, indicating sound methodological rigor in these domains. Missing-data bias was also low for most investigations; however, Sousa et al (23) and Nogami et al (24) exhibited a moderate risk due to incomplete data handling, suggesting some caution when interpreting their findings. Overall, the evidence base shows moderate concerns primarily in selection-related domains, whereas other methodological areas remain robust (Table 2).

This systematic review included a total sample size of 549 participants across 10 studies conducted in various countries, including Spain, India, Brazil, Japan, and Italy. The IHC markers most frequently assessed were B-cell lymphoma 2 (Bcl-2), p53, Ki-67, Bcl-2-associated X protein (Bax), survivin, mouse double minute 2 homolog (MDM2), and proliferating cell nuclear antigen (PCNA). The most common IHC marker reported in the included studies was Bcl-2, which was assessed in 9 studies, making it the most frequently used marker for evaluating apoptotic regulation and malignant transformation potential in oral premalignant and malignant lesions. Following Bcl-2, p53 was evaluated in seven studies, reflecting its critical role in tumor suppression and cell cycle regulation. Ki-67, a marker of cell proliferation, was reported in four studies, often alongside Bcl-2 and p53, to provide insight into proliferative activity within lesions. Bax, another apoptosis-related protein, was assessed in three studies, typically in combination with p53 and Bcl-2 to understand the balance of pro- and anti-apoptotic signals. Less frequently studied markers included survivin, MDM2, PCNA, and p21, each reported in one study. This frequency distribution underscores the central focus on Bcl-2 and p53 pathways in IHC investigations of oral lesion transformation risk, with Ki-67 and Bax providing complementary proliferative and apoptotic context (Table 3).

Discussion

This systematic review demonstrated that the most frequently used IHC markers for identifying malignant transformation of OLK and OLP were Bcl-2, p53, Ki-67, Bax, survivin, MDM2, and PCNA. Bcl-2 was the most common marker, followed by p53, Ki-67, and Bax, respectively. Less common IHC markers included survivin, MDM2, PCNA, and p21. The current findings' identification of Bcl-2, p53, Ki-67, Bax, survivin, MDM2, and PCNA as the most frequently studied IHC markers for malignant transformation in OLK and OLP demonstrates both consistency and divergence from previous research findings. A comprehensive meta-analysis by Normando

Table 1. Quality assessment of included studies using the STROBE checklist

STROBE component	Chamorro-Petronacci et al (2021) (18)	Dwivedi et al (2020) (19)	Pigatti et al (2015) (20)	Sudha et al (2011) (21)	de Sousa et al (2009) (22)	Sousa et al (2009) (23)	Nogami et al (2003) (24)	Jane et al (2006) (25)	Piattelli et al (2002) (26)	Tanda et al (2000) (27)
Title and Abstract	M	M	M	M	M	M	M	M	M	M
Introduction										
Background/Rationale	M	M	M	M	M	M	M	M	M	M
Objectives	M	M	M	M	M	M	M	M	M	M
Methods										
Study design	M	M	M	NM	M	M	M	M	M	M
Setting	M	M	M	PM	PM	PM	PM	PM	PM	PM
Participants	M	M	M	M	M	M	M	M	M	M
Variables	M	M	M	M	M	M	M	M	M	M
Data Sources/Measurement	M	M	M	M	M	M	M	M	M	M
Bias addressed	PM	PM	PM	NM	PM	NM	PM	PM	PM	PM
Study size justification	M	NM	NM	NM	PM	NM	PM	PM	PM	PM
Quantitative variables handled	M	M	M	M	M	M	M	M	M	M
Statistical methods	M	PM	M	M	M	M	M	M	M	M
Results										
Descriptive data	M	M	M	M	M	M	M	M	M	M
Outcome data	M	M	M	M	M	M	M	M	M	M
Main results	M	M	PM	M	M	M	M	M	M	M
Other analyses	PM	NM	PM	NM	NM	PM	NM	NM	NM	NM
Discussion										
Key findings	M	M	M	M	M	M	M	M	M	M
Interpretation	M	M	M	M	M	M	M	M	M	M
Limitations	M	M	M	PM	PM	PM	PM	PM	PM	PM
Generalisability	PM	PM	PM	PM	PM	PM	PM	PM	PM	PM
Ethical considerations										
Approval and consent	M	M	M	M	M	M	M	M	M	M
Funding										
Funding source	M	M	M	M	M	M	M	M	M	M
Overall quality	19/22 M 3/22 PM 0/22 NM	17/22 M 3/22 PM 2/22 NM	17/22 M 4/22 PM 1/22NM	15/22M 3/22 PM 4/22 NM	16/22M 5/22 PM 1/22NM	16/22 M 4/22 PM 2/22 NM	16/22 M 5/22 PM 1/22 NM	16/22 M 5/22 PM 1/22 NM	16/22 M 5/22 PM 1/22NM	16/22 M 5/22 PM 1/22NM

Ratings: Met (M), Partially met (PM), Not met (NM)

Table 2. Summary of risk bias assessment of the included studies

First author and publication year	Confounding	Participants selection	Measurement bias	Deviations from Intended Interventions	Missing data	Outcomes measurement	Selection of Reported Results
Chamorro-Petronacci et al (2021) (18)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Dwivedi et al (2020) (19)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Pigatti et al (2015) (20)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sudha et al (2011) (21)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
de Sousa et al (2009) (22)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sousa et al (2009) (23)	Moderate risk	Moderate risk	Low risk	Low risk	Moderate risk	Low risk	Low risk
Nogami et al (2003) (24)	Moderate risk	Moderate risk	Low risk	Low risk	Moderate risk	Low risk	Low risk
Jane et al (2006) (25)	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Piattelli et al (2002) (26)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk
Tanda et al (2000) (27)	Moderate risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk

et al involving 142 studies and 173 proteins revealed that p53 and Ki-67 were the most frequently investigated markers, which aligns with the current findings placing these markers among the top-ranked biomarkers. However, their analysis showed that Bcl-2 did not demonstrate significant differences between OLK and OSCC groups (OR: 1.22, 95% CI: 0.45-3.32), contrasting with the current study's identification of Bcl-2 as the most common marker (28). A previous systematic review by Monteiro et al consistently identified podoplanin and p53 as the most assessed proteins in oral potentially malignant disorders, with podoplanin showing a significant association with malignant transformation, yet podoplanin was not prominently featured in the current study's findings (29). For OLP specifically, a systematic review by Al-Jamaei et al indicated that while p53, Bcl-2, and Ki-67 were the most frequently investigated proteins, we identified PCNA and p21 as the only proteins showing consistent evidence of clinical usefulness as cancer predictors, which partially supports the current study's inclusion of PCNA among the identified markers, though with lower frequency (30).

The identification of apoptosis-related proteins (Bcl-2, Bax, survivin) and cell cycle regulatory proteins (p53, Ki-67, MDM2, PCNA, p21) as the most frequently studied biomarkers reflects the underlying molecular mechanisms of malignant transformation in oral potentially malignant disorders. The prominence of Bcl-2 as the most common marker is particularly significant given its role as an anti-apoptotic protein that promotes cell survival, which aligns with recent evidence showing that most OLP samples exhibit anti-apoptotic pathways (31). The frequent investigation of p53 and Ki-67 is consistent with established knowledge that p53 expression progressively increases from normal mucosa to OLK to OSCC, while Ki-67 serves as a reliable proliferation marker that correlates with dysplasia grade and malignant potential (20,32,33). However, the clinical utility of these markers remains limited due to methodological heterogeneity across studies, with previous systematic reviews highlighting that no biomarker has achieved benchmarks for clinical application to detect malignant transformation (34). The inclusion of MDM2 among frequently studied markers is noteworthy, as recent meta-analyses have shown a significant association between MDM2 expression and greater abundance in OSCC patients (OR: 0.44, 95% CI: 0.24-0.81) (28). The relatively lower frequency of survivin, despite its established role in oral carcinogenesis, may reflect the emerging nature of this biomarker in the field, though studies have consistently shown significant survivin expression in OLK and OLP compared to normal mucosa (33,35).

Overall, our systematic review's findings demonstrate that the research focus on IHC biomarkers for malignant transformation in OLK and OLP has concentrated on fundamental cellular processes, including apoptosis regulation, cell cycle control, and proliferation. While the identification of Bcl-2, p53, Ki-67, Bax, survivin, MDM2, and PCNA as the most frequently studied markers provides valuable insights into research priorities, significant challenges remain in translating these biomarkers into clinical practice. The discrepancy between biomarker frequency and clinical utility, as evidenced by previous meta-analyses showing limited predictive value for several commonly studied markers, underscores the need for more rigorous validation studies with standardized methodologies. Future research should prioritize prospective longitudinal studies with unified diagnostic criteria and focus on biomarker panels rather than individual markers. The emphasis on PCNA and p21 in OLP research, as identified in previous systematic reviews, suggests that condition-specific biomarker approaches may be more clinically relevant than universal markers for all oral potentially malignant disorders. Ultimately, the development of clinically applicable biomarkers requires

Table 3. Characteristics of included studies in this systematic review study

First author and publication year	Study characteristics						
	Country	Study design	Sample size	Population	IHC marker	Objective	Results
Chamorro-Petronacci et al (2021) (18)	Spain	Retrospective observational	136	The study included samples previously diagnosed with OLP (n=59), leukoplakia without dysplasia (n=15), leukoplakia with dysplasia (n=14), fibroma (n=18), and healthy controls (n=16).	Bcl-2 expression	The objective of this study is to evaluate the IHC expression of Bcl-2 in healthy oral mucosa, various oral potentially malignant disorders, and OSCC, and to assess its diagnostic significance.	Studies indicate a distinction between OLK and the expression of blc-2; hence, Blc-2 biomarkers serve as indicators for identifying malignant transformation of premalignant lesions.
Dwivedi et al (2020) (19)	India	Retrospective observational	45	Archived samples were obtained from the department, with a known tobacco usage history for >2 years. 30 patients were diagnosed with OLK and 15 with proven OSCC 5 with normal	Bcl-2 expression.	The aim of this study is to assess the usefulness of Bcl-2 in distinguishing dysplastic or malignant epithelium from non-dysplastic or normal epithelium to aid in the prediction of malignant transformation potential.	OLK with dysplasia demonstrated increased Bcl-2 expression levels relative to non-dysplastic lesions, highlighting variability based on lesion type and stage. Conversely, several studies revealed limited or negligible expression of Bcl-2 in both OLP and OSCC cases, suggesting that other apoptotic pathways or biomarkers may be more relevant for predicting malignant transformation.
Pigatti et al (2015) (20)	Brazil	Retrospective observational	37	Archived samples for patients with OLP (n=14), leukoplakia with moderate(n=8) or severe dysplasia(n=6), and normal mucosa (n=9)	Bcl-2 and Ki-67	The study aims to investigate IHC staining for Bcl-2 and Ki-67 and correlate the findings from lesions of and leukoplakia with epithelial dysplasia.	The expression of Bcl-2 may exert a dual influence on tumor growth and progression. Augmented cell proliferation in the epithelium may elevate the chance of cancer formation in OLP. Ki-67 expression can function as an auxiliary marker for evaluating proliferative activity in lesions with malignant potential.
Sudha et al (2011) (21)	India	Retrospective observational	60	Archived samples for patients with leukoplakia, oral submucous fibrosis, OLP (n=10 for each), and OSCC (n=30).	Bcl-2 expression	This study aims to examine the distribution and extent of bcl-2 overexpression in potentially malignant conditions like leukoplakia, OSMF, and OLP, OSCC and compare them to OSCC. It aims to evaluate whether bcl-2 protein could be used as a marker for tumors	Increased Bcl-2 expression was observed in OSCC compared to other potentially malignant disorders. Variability in Bcl-2 expression was noted within the potentially malignant disorders, particularly in terms of its topographical distribution and degree. .
Sousa et al (2009) (23)	Brazil	Retrospective observational	48	This study included 24 cases of OLP and 24 cases of OSCC	Expression of PCNA, p53, bax, and bcl-2 proteins.	This study aimed to assess the expression of proteins involved in cell proliferation and apoptosis by comparing oral lichen planus with OSCC.	The expression of p53, bax, and bcl-2 in OLP and OSCC did not differ statistically significantly, which could indicate that OLP has the ability to develop into malignant transformation.

Table 3. Continued

First author and publication year	Study characteristics						
	Country	Study design	Sample size	Population	IHC marker	Objective	Results
de Sousa et al, (2009) (22)	Brazil	Retrospective observational	48	This study included 24 cases of OLP and 24 cases of epithelial dysplasia (4 mild, 12 moderate, 8 severe).	Expression of p53, bax, and bcl-2	Evaluation of the potential for malignant transformation of OLP	Alteration of expression of these proteins suggests the potential for malignant transformation.
Jane et al (2006) (25)	India	Retrospective observational	55	The samples were for patients with primary OSCC (n=38) and leukoplakia (n=17).	The expression levels of Bax, Bcl-2, survivin, and p53 were assessed through IHC staining	Analyzing the expression of apoptosis-regulating genes in precancerous and cancerous lesions.	Anti-apoptotic survivin expression is upregulated in high-grade cancers, indicating that survivin probably plays a major role in apoptosis resistance to treatment.
Nogami et al (2003) (24)	Japan	Retrospective observational	18	The samples were for patients with normal mucosa (n=5) and leukoplakia (n=13).	Expression of ki-67, p53, Bcl-2, and Bax.	Evaluation of apoptosis in OLK	Expression of bcl-2 in OLK undergoing malignant transformation, accompanied by a decrease in the number of apoptotic cells.
Piattelli et al (2002) (26)	Italy	Retrospective observational	70	They included samples for patients with normal oral mucosa (n=10), leukoplakia (n=12), epithelial dysplasia (with 6 mild and 6 severe dysplasia, including carcinoma in situ), and invasive carcinoma categorized by differentiation (n=36): 12 well-differentiated (G1), 12 moderately differentiated (G2), and 12 poorly differentiated (G3).	Expression of p53, bcl-2, MIB-1, and the apoptotic index (AI) using immunohistochemistry	The study aimed to examine the expression and correlation of p53, bcl-2, MIB-1, and the apoptotic index (AI) using immunohistochemistry.	A substantial correlation was seen between AI, p53 overexpression, and cell proliferation (MIB-1). The expression of Bcl-2 was found to be inversely linked with MIB-1 and AI. The correlation between p53 and bcl-2 was demonstrated to be considerably negative. The expression of MIB-1 and AI demonstrated a robust positive correlation.
Tanda et al (2000) (27)	Japan	Retrospective observational	32	The samples were for patients with OL (n=13), OLP (n=10), and normal mucosa (n=9).	Expression of wt-p53, p21, MDM2, Bcl-2.	Description of human keratinocyte expression of several apoptotic signaling proteins in leukoplakia, in OLP, and normal oral mucosa	Elevated levels of MDM2 and Bcl-2 in leukoplakia may facilitate malignant transformation by suppressing apoptosis and impairing normal keratinocyte development. In OLP, increased levels of wt-p53 and p21WAF1/CIP1 may indicate a defensive reaction to apoptotic signaling, without considerable impairment of keratinocyte structure or function.

IHC, Immunohistochemical; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; MDM2, Mouse double minute 2 homolog; PCNA, proliferating cell nuclear antigen; OLP, Oral lichen planus; OSMF, Oral submucous fibrosis; OSCC, Oral squamous cell carcinoma; OLK, Oral leukoplakia.

large-scale collaborative studies with standardized protocols to overcome the methodological heterogeneity that currently limits the translation of promising research findings into routine clinical practice.

Conclusion

The systematic review results found that the most frequently assessed IHC markers were Bcl-2, p53, Ki-67, Bax, survivin, MDM2, and PCNA, respectively, with Bcl-2 being the predominant marker, followed by p53. In conclusion, the consistent and frequent evaluation of Bcl-2 and p53 across multiple studies underscores their significance as key indicators in the complex interplay of apoptosis regulation and tumor suppression pathways. While Ki-67 and Bax provide valuable insights into cellular proliferation and the apoptotic balance, respectively, the less frequent assessment of markers such as survivin, MDM2, PCNA, and p21 suggests an evolving understanding or more specialized utility of these biomarkers. The findings affirm that a panel of IHC markers, particularly involving Bcl-2 and p53, holds promise as reliable indicators of malignant progression risk, offering crucial information to clinicians for enhanced prognostication and more targeted management strategies for individuals with oral premalignant lesions.

Authors' contribution

Conceptualization: Sarah AlFarabi Ali and Ahoud Jazzar
Data curation: Ahoud Jazzar and Sara Akeel, and Nada A. Alhindi
Investigation: Yasmin Hisham Mair and Soulafa Adnan Almazrooa
Methodology: Maha Talal Alsharif and Hebah AlDehlawi
Project management: Sarah AlFarabi Ali
Supervision: Yasmin Hisham Mair
Visualization: Sara Akeel and Maha Talal Alsharif
Writing—original draft: All Authors
Writing—review and editing: All Authors

Conflicts of interest

The authors declared no conflict of interest

Declaration of generative artificial intelligence (AI) and AI-assisted technologies in the writing process

During the preparation of this work, the authors utilized AI (Perplexity.ai and Grammarly.com) to refine grammar points and language style in writing. Subsequently, the authors thoroughly reviewed and edited the content as necessary, assuming full responsibility for the accuracy and content of the publication.

Ethical issues

This investigation has been compiled based on the PRISMA checklist, and its protocol was registered on the PROSPERO (International Prospective Register of Systematic Reviews) website with (ID: CRD420251063588) and Research Registry (Unique Identifying Number [UIN]: reviewregistry2021). Besides, the authors have observed ethical issues (including plagiarism, data fabrication, and double publication).

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