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Malignant Transformation and Biomarker Expression in Oral Potentially Malignant Disorders: A Cross-Sectional Study in Iraqi Patients

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ABSTRACT

Background: Oral potentially malignant disorders (OPMDs) constitute a heterogeneous group of lesions that frequently precede the development of oral squamous cell carcinoma (OSCC). Although tobacco use remains highly prevalent among Iraqi adult males, with smoking rates approaching 38%, there is a paucity of comprehensive data regarding the clinicopathological characteristics and biomarker expression profiles of OPMDs within the Iraqi population. Objective: This study aimed to characterize the clinicopathological features of OPMDs in Iraqi patients and to evaluate the diagnostic and predictive utility of five immunohistochemical (IHC) biomarkers—p53, Ki-67, CD44, E-cadherin, and vascular endothelial growth factor (VEGF)—in identifying high-grade epithelial dysplasia and malignant transformation. Methods: A prospective cross-sectional study was conducted at the Private dental clinics between, between January 2022 and December 2024, involving 120 patients diagnosed with OPMDs. Histopathological grading was performed according to the 2022 World Health Organization (WHO) classification criteria. Immunohistochemical expression of the selected biomarkers was quantified using H-score methodology. Statistical analyses, including chi-square testing, one-way analysis of variance (ANOVA), multivariable logistic regression, and receiver operating characteristic (ROC) curve analysis, were undertaken using SPSS version 26. Results: The mean patient age was 48.3 ± 11.2 years, with a male predominance (65%). Oral leukoplakia represented the most frequently diagnosed OPMD (45%). Erythroplakia demonstrated the highest cumulative frequency of moderate-to-severe dysplasia and OSCC (83.3%). Progressive increases in p53 expression were observed across the spectrum of dysplasia, rising from 22 ± 8 in non-dysplastic lesions to 215 ± 24 in OSCC cases. Similarly, Ki-67 expression increased from 14.8% to 74.6%, whereas E-cadherin expression exhibited a marked reduction from 248 ± 18 to 58 ± 18. Among individual biomarkers, p53 demonstrated the highest discriminatory performance for identifying high-grade lesions (AUC = 0.89; 95% CI: 0.83–0.95). Furthermore, a combined four-marker panel achieved excellent diagnostic accuracy with an AUC of 0.95. Conclusions: The findings of this study indicate that a focused immunohistochemical panel incorporating p53 and Ki-67 provides robust discriminatory capacity for the identification of high-grade OPMDs in the Iraqi clinical context. The integration of these biomarkers into routine diagnostic practice may facilitate earlier detection, improve risk stratification, and support timely therapeutic intervention, thereby potentially reducing the burden of OSCC in Iraq.

Keywords: Cadherins, Iraq, Ki-67 Antigen, Leukoplakia Oral, Lichen Planus Oral, MeSH, Mouth Neoplasms, Oral Submucous Fibrosis, Tumor Suppressor Protein p53

1 Introduction

ORAL cancer constitutes a major global public health challenge owing to its high morbidity, mortality, and socioeconomic impact. According to the GLOBOCAN 2022 database, approximately 758,000 new cases of cancers involving the lip, oral cavity, and pharynx were diagnosed worldwide in 2022, with oral squamous cell carcinoma (OSCC) accounting for nearly 90% of all oral malignancies [1]. Despite advances in diagnostic and therapeutic modalities, the prognosis of OSCC remains unsatisfactory, largely because many patients present at advanced stages of disease. In Iraq, the only available nationwide epidemiological investigation reported an OSCC incidence of 0.4 per 100,000 population between 2014 and 2018, demonstrating a pronounced male predominance and a predilection for the tongue and lip [2]. However, limitations in cancer registration systems and underreporting are likely to result in substantial underestimation of the true disease burden.

Oral potentially malignant disorders (OPMDs) comprise a heterogeneous group of clinical lesions and conditions associated with an increased risk of malignant transformation within the oral cavity [3]. The World Health Organization (WHO) 2017/2022 classification recognizes oral leukoplakia, erythroplakia, oral lichen planus (OLP), oral submucous fibrosis (OSMF), proliferative verrucous leukoplakia, and related entities as principal OPMDs [4, 5]. Histopathological assessment of oral epithelial dysplasia (OED), conventionally categorized into mild, moderate, and severe dysplasia/carcinoma in situ, continues to represent the current gold standard for evaluating malignant potential [5]. Nevertheless, morphological grading alone remains limited by substantial inter-observer variability and inconsistent predictive accuracy regarding progression to invasive carcinoma [6].

The risk of malignant transformation varies considerably among different OPMD subtypes. Oral leukoplakia demonstrates an overall pooled malignant transformation rate of approximately 9.8% (95% CI: 7.9–11.7%), according to recent meta-analytic evidence [7]. Oral lichen planus exhibits a lower pooled transformation rate of approximately 1.43%, although this increases significantly in the presence of epithelial dysplasia [8]. Similarly, oral submucous fibrosis carries an estimated malignant transformation rate of approximately 6% [9]. Erythroplakia, although relatively uncommon, exhibits the highest malignant potential among OPMDs, with reported transformation rates ranging from 12.7% to 58.3% [10]. Iraqi data regarding OPMDs and oral cancer remain limited; however, a retrospective study conducted in Sulaimani demonstrated that OSCC constituted 56.4% of all orofacial malignancies, with peak incidence observed during the seventh decade of life [11].

The limitations inherent in conventional histopathological grading have stimulated increasing interest in molecular and immunohistochemical biomarkers as adjunctive tools for risk stratification and early detection of malignant transformation [12–14]. Among these, p53, widely regarded as the "guardian of the genome," is one of the most extensively investigated tumour suppressor proteins. Aberrant p53 immunoexpression frequently reflects underlying TP53 mutations and has been associated with progression of OED independent of histological grade [15, 16]. Ki-67, a well-established marker of cellular proliferation, demonstrates progressively increased expression from mild dysplasia to invasive OSCC, thereby reflecting enhanced proliferative activity during carcinogenesis [17].

Additional biomarkers implicated in oral carcinogenesis include CD44, E-cadherin, and vascular endothelial growth factor (VEGF). CD44 functions as a cancer stem-cell marker and has been linked to tumour invasion, metastatic potential, and resistance to chemoradiotherapy [18]. E-cadherin, a critical epithelial adhesion molecule, plays a central role in maintaining epithelial integrity, and its loss contributes to epithelial–mesenchymal transition (EMT), tumour invasion, and metastasis [19, 20]. VEGF is a key mediator of tumour angiogenesis and is frequently upregulated in high-grade dysplastic lesions and OSCC, facilitating neovascularization and tumour progression [21].

The Iraqi population presents a distinctive constellation of risk factors that may contribute to oral carcinogenesis, including a high prevalence of cigarette smoking among adult males, widespread waterpipe consumption, use of smokeless tobacco products such as naswar, inadequate oral hygiene practices, and limitations in oral healthcare infrastructure secondary to prolonged socioeconomic and post-conflict challenges [22–24]. Despite these significant risk factors, there remains a notable absence of comprehensive Iraqi studies evaluating the immunohistochemical profiles of biomarkers associated with malignant transformation in OPMDs.

Accordingly, the present study was designed to investigate the clinicopathological characteristics of OPMDs in an Iraqi cohort and to evaluate the diagnostic and predictive value of a panel of five immunohistochemical biomarkers—p53, Ki-67, CD44, E-cadherin, and VEGF—in relation to dysplasia severity and malignant transformation. It was hypothesized that this biomarker panel would:

- Discriminate between varying grades of epithelial dysplasia across OPMD subtypes;
- Identify lesions exhibiting established or impending malignant transformation; and
- Demonstrate superior predictive performance compared with conventional clinical risk stratification alone.

2 Patients and Methods

2.1 Study Design and Setting

This prospective cross-sectional observational study was conducted at private dental clinics. Immunohistochemical analyses were performed at the affiliated Central Pathology Laboratory, Baghdad Medical City, Iraq. Patient recruitment and data collection were carried out over a three-year period extending from 1 January 2022 to 31 December 2024.

2.2 Ethical Considerations

The study protocol received approval from the Scientific and Ethical Committees. Written informed consent was obtained from all participants prior to enrolment. The investigation was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (2013 revision) and adhered to the recommendations of the strengthening the reporting of observational studies in Epidemiology (STROBE) guidelines [25].

2.3 Sample Size Determination

Sample size estimation was performed using an anticipated dysplasia prevalence of 60%, a confidence level of 95%, and a margin of error of 9%. The minimum calculated sample size was 114 participants. To compensate for potential inadequate or non-diagnostic biopsy specimens, a total of 120 participants were recruited.

2.4 Eligibility Criteria

Eligible participants included adults aged 18 years or older presenting with clinically suspected OPMDs, including oral leukoplakia, oral lichen planus, oral submucous fibrosis, erythroplakia, and erythroleukoplakia, confirmed independently by two oral medicine specialists. All participants provided informed consent for incisional biopsy.

Exclusion criteria comprised previous history of head and neck malignancy or radiotherapy, prior surgical excision of the lesion, current immunosuppressive therapy, pregnancy, clinically obvious traumatic or reactive lesions, and inadequate biopsy specimens containing less than 3 mm of representative epithelium.

2.5 Clinical Data Collection

Clinical and demographic data were obtained using a standardized case-record form. Variables recorded included age, sex, occupation, household income, tobacco exposure (including cigarette smoking, waterpipe smoking, and smokeless tobacco/naswar use), pack-years, alcohol consumption, betel or qat chewing habits, denture use, oral hygiene index-simplified (OHI-S), and relevant systemic medical conditions.

Lesions were clinically characterized according to anatomical site, maximum diameter, colour, surface texture (homogeneous, non-homogeneous, or speckled), and clinical subtype.

2.6 Biopsy Procedure and Histopathological Assessment

Incisional biopsies measuring at least 6 mm were obtained under local Anaesthesia from the most representative non-keratinised region of each lesion. Tissue specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at 4 µm thickness, and stained with haematoxylin and eosin.

Histopathological grading was independently performed by two experienced oral pathologists according to the WHO 2022 criteria for oral epithelial dysplasia, categorized as no dysplasia, mild dysplasia, moderate dysplasia, or severe dysplasia/carcinoma in situ [4, 5]. Cases demonstrating discordant grading were reviewed jointly using a multi-headed microscope until consensus was achieved.

2.7 Immunohistochemical Procedures

Serial 4-µm tissue sections underwent heat-induced epitope retrieval using citrate buffer (pH 6.0). The following primary antibodies were utilised: anti-p53 (clone DO-7, Dako; dilution 1:100), anti-Ki-67 (clone MIB-1, Dako; 1:150), anti-CD44 (clone DF1485, Santa Cruz Biotechnology; 1:100), anti-E-cadherin (clone NCH-38, Dako; 1:100), and anti-VEGF (clone VG1, Thermo Fisher Scientific; 1:100).

Immunodetection was performed using the EnVision FLEX+ polymer detection system (Dako) with 3,3′-diaminobenzidine (DAB) chromogen, followed by counterstaining with Mayer's haematoxylin. Appropriate external positive controls were included for each biomarker: colon adenocarcinoma for p53 and Ki-67, tonsillar tissue for CD44, normal skin for E-cadherin, and placental tissue for VEGF. Negative controls were prepared by omission of the primary antibody.

2.8 Immunohistochemical Scoring

All immunohistochemically stained slides were independently evaluated by two blinded oral pathologists. Biomarker expression was quantified using the H-score method, calculated in the following formula.

The immunohistochemical staining intensity was semi-quantitatively assessed using the histochemical score (H-score) method, which integrates both the proportion of positively stained cells and staining intensity according to the following formula:

$$H-score = \sum Pi(i+1)H\backslash text\{-\}score$$
$$=\backslash sum\ P\_i(i+1)H-score = \sum Pi(i+1)$$

where Pi represents the percentage of cells demonstrating a given staining intensity and Ⅲ corresponds to the staining intensity category (0 = negative, 1 = weak, 2 = moderate, 3 = strong). The resulting H-score ranged from 0 to 300, providing a semi-quantitative measure of biomarker expression.

The Ki-67 proliferation index was defined as the percentage of positively stained nuclei among 1,000 epithelial cells evaluated at × 400 magnification within the basal and parabasal layers. Abnormal p53 overexpression was defined as strong nuclear positivity in ≥ 10% of epithelial cells.

2.9 Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables were compared using chi-square or Fisher's exact tests. Comparisons of biomarker H-scores across dysplasia grades were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.

Multivariable logistic regression analysis was undertaken to identify independent predictors of high-grade dysplasia and OSCC. Receiver operating characteristic (ROC) curve analysis was performed to determine diagnostic performance, including calculation of the area under the curve (AUC), 95% confidence intervals (CI), optimal cut-off values using Youden's J statistic, and comparisons between ROC curves using DeLong's test. Inter-observer agreement was assessed using Cohen's kappa coefficient (κ). A two-tailed p-value < 0.05 was considered statistically significant.

3 Results

3.1 Demographic and Clinical Characteristics

A total of 120 patients were analyzed (Table 1). Mean age was 48.3 ± 11.2 years (range 30–70); 78 (65.0%) were male. Sixty-five (54.2%) resided in urban Baghdad governorate. Cigarette smoking was reported by 72 (60.0%), with a median exposure of 22 pack-years (IQR 12–34); 30 (25.0%) used smokeless tobacco (naswar); 41 (34.2%) used a waterpipe at least weekly. Any tobacco exposure was present in 86 patients (71.7%). Poor oral hygiene (OHI-S ≥3) was documented in 84 (70.0%). The buccal mucosa was the most frequently involved site (50.0%), followed by the lateral tongue (25.0%), floor of mouth (15.0%) and gingiva (10.0%). Fifty-six lesions (46.7%) were non-homogeneous/speckled.

Table 1. Demographic and clinical characteristics of the study cohort (n=120).

Variable	Value
Age, mean ± SD (range)	48.3 ± 11.2 years (30–70)
Male sex, n (%)	78 (65.0%)
Urban residence, n (%)	65 (54.2%)
Education below secondary level, n (%)	71 (59.2%)
Below-median household income, n (%)	82 (68.3%)
Cigarette smoking, n (%)	72 (60.0%)
Median pack-years (IQR)	22 (12–34)
Smokeless tobacco (naswar), n (%)	30 (25.0%)
Waterpipe use ≥ weekly, n (%)	41 (34.2%)
Alcohol consumption, n (%)	14 (11.7%)
Betel/qat chewing, n (%)	8 (6.7%)
Poor oral hygiene (OHI-S ≥3), n (%)	84 (70.0%)
Diabetes mellitus, n (%)	19 (15.8%)
Site: buccal mucosa	60 (50.0%)
Site: tongue (lateral border)	30 (25.0%)
Site: floor of mouth	18 (15.0%)
Site: gingiva / alveolar mucosa	12 (10.0%)
Non-homogeneous/speckled morphology	56 (46.7%)
Median lesion size, mm (IQR)	18 (12–28)

OHI-S = Simplified Oral Hygiene Index; IQR = interquartile range.

3.2 Distribution of OPMDs and Histopathological Findings

OPMD subtypes: oral leukoplakia 54 (45.0%), OLP 36 (30.0%), OSMF 18 (15.0%), erythroplakia 12 (10.0%) (Table 2). Histopathological grading yielded: no dysplasia 42 (35.0%), mild dysplasia 36 (30.0%), moderate dysplasia 24 (20.0%), severe dysplasia/CIS 12 (10.0%), and invasive OSCC 6 (5.0%). The aggregate high-grade lesion rate (severe/CIS + OSCC) was 15.0%. Inter-observer agreement on dysplasia grading was substantial (κ = 0.74) [11]. Erythroplakia exhibited the highest cumulative rate of moderate-to-severe dysplasia/CIS/OSCC (10/12; 83.3%), consistent with its established reputation as the highest-risk OPMD subtype [9].

Figure 1 summarizes the distribution of dysplasia grades across OPMD subtypes graphically.

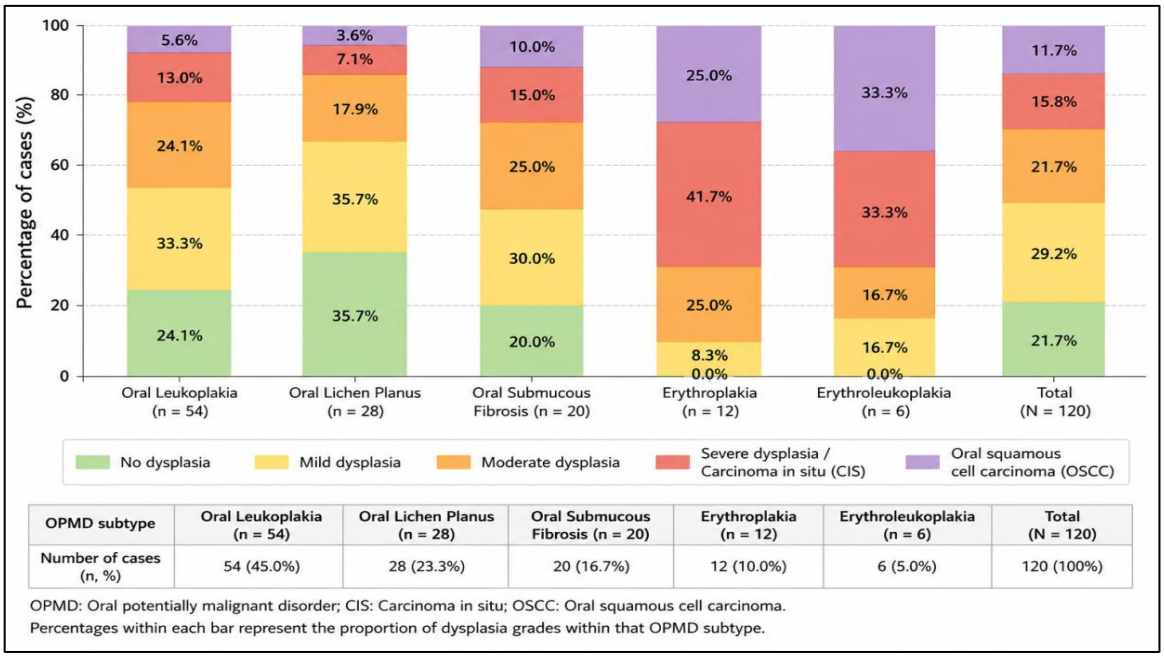
Values expressed as percentage of each OPMD subtype total. OSMF = oral submucous fibrosis.

Table 2. Distribution of oral potentially malignant disorders and histopathological dysplasia grading.

OPMD Subtype	n (%)	No Dysp.	Mild	Moderate	Severe/CIS	OSCC	Cumul. MT*

Oral leukoplakia	54 (45.0)	16 (29.6)	19 (35.2)	12 (22.2)	5 (9.3)	2 (3.7)	13.0%
Oral lichen planus	36 (30.0)	21 (58.3)	11 (30.6)	3 (8.3)	1 (2.8)	0 (0.0)	2.8%
Oral submucous fibrosis	18 (15.0)	4 (22.2)	5 (27.8)	6 (33.3)	2 (11.1)	1 (5.6)	16.7%
Erythroplakia	12 (10.0)	1 (8.3)	1 (8.3)	3 (25.0)	4 (33.3)	3 (25.0)	58.3%
Total	120	42 (35.0)	36 (30.0)	24 (20.0)	12 (10.0)	6 (5.0)	15.0%

Dysp. = dysplasia; CIS = carcinoma in situ; OSCC = oral squamous cell carcinoma; OSMF = oral submucous fibrosis; MT* = malignant transformation rate (severe/CIS + OSCC as % of subtype total). Bold row = total.



OPMD subtype	Oral Leukoplakia (n = 54)	Oral Lichen Planus (n = 28)	Oral Submucous Fibrosis (n = 20)	Erythroplakia (n = 12)	Erythroleukoplakia (n = 6)	Total (N = 120)
Number of cases (n, %)	54 (45.0%)	28 (23.3%)	20 (16.7%)	12 (10.0%)	6 (5.0%)	120 (100%)

OPMD: Oral potentially malignant disorder; CIS: Carcinoma in situ; OSCC: Oral squamous cell carcinoma.
Percentages within each bar represent the proportion of dysplasia grades within that OPMD subtype.

Fig. 1. Distribution of dysplasia grades across OPMD subtypes graphically.

3.3 Biomarker Expression by Dysplasia Grade

All five immunohistochemical biomarkers demonstrated statistically significant alterations in expression across the spectrum of dysplasia severity (all $p < 0.001$; Table 3). Progressive increases in p53 expression were observed with advancing histopathological grade, with the mean H-score rising from 22 ± 8 in non-dysplastic epithelium to 215 ± 24 in oral squamous cell carcinoma (OSCC). Abnormal p53 overexpression, defined as ≥10% strongly positive nuclei, was identified in 9.5% of non-dysplastic lesions and increased to 100% in OSCC specimens, consistent with previously reported evidence implicating TP53 dysregulation in oral carcinogenesis [16].

Similarly, the Ki-67 proliferation index demonstrated a stepwise increase with progression from low-grade dysplasia to invasive carcinoma. Immunoreactivity extended progressively from the basal epithelial compartment into the suprabasal layers, reaching a mean labelling index of 74.6% ± 7.2 in OSCC cases, thereby reflecting markedly increased proliferative activity during malignant transformation [17, 26].

CD44 expression exhibited a transition from predominantly basal and parabasal localisation in low-grade lesions to diffuse full-thickness epithelial positivity in high-grade dysplasia and OSCC, suggesting enhanced acquisition of cancer stem-cell characteristics associated with tumour progression, invasion, and therapeutic resistance [18, 27].

In contrast, E-cadherin expression demonstrated a progressive reduction in membranous staining intensity with increasing dysplasia severity, accompanied by cytoplasmic redistribution in advanced lesions. These findings are indicative of epithelial–mesenchymal transition (EMT), a critical biological process implicated in loss of epithelial cohesion, increased cellular motility, and invasive behaviour [19, 28].

VEGF expression showed significant upregulation in lesions with advanced dysplastic change and OSCC. Notably, VEGF levels were disproportionately elevated in oral submucous fibrosis (OSMF) relative to the corresponding histopathological grade, a finding that may reflect chronic tissue hypoxia and compensatory angiogenic activation characteristic of fibrotic oral mucosal disorders [21].

Figure 2. shows heat-map of biomarker expression across dysplasia grades. Green = low expression; yellow = intermediate; red = high. E-cadherin is inversely coded (green = high retention; red = loss).

Values are mean H-scores (p53, CD44, E-cadherin, VEGF) or mean labelling index % (Ki-67).

Table 3. Biomarker expression (mean H-score or labelling index ± SD) by histopathological dysplasia grade.

Biomarker	No Dysp. (n	Mild (n =	Moderate (n =	Severe/CIS (n = 12)	OSCC (n =	p-value

	= 42)	36)	24)		6)	(ANOVA)
p53 (H-score)	22 ± 8	58 ± 14	118 ± 21	178 ± 26	215 ± 24	< 0.001
Ki-67 (LI %)	14.8 ± 4.9	28.6 ± 7.1	45.3 ± 9.4	63.7 ± 8.8	74.6 ± 7.2	< 0.001
CD44 (H-score)	56 ± 18	92 ± 22	142 ± 27	198 ± 25	224 ± 22	< 0.001
E-cadherin (H-score)	248 ± 18	198 ± 21	152 ± 24	92 ± 19	58 ± 18	< 0.001
VEGF (H-score)	48 ± 16	84 ± 19	138 ± 26	188 ± 24	212 ± 22	< 0.001

LI = labelling index. H-score range 0–300. ANOVA with Tukey post hoc; all pairwise inter-grade comparisons significant (p<0.05) for each marker.

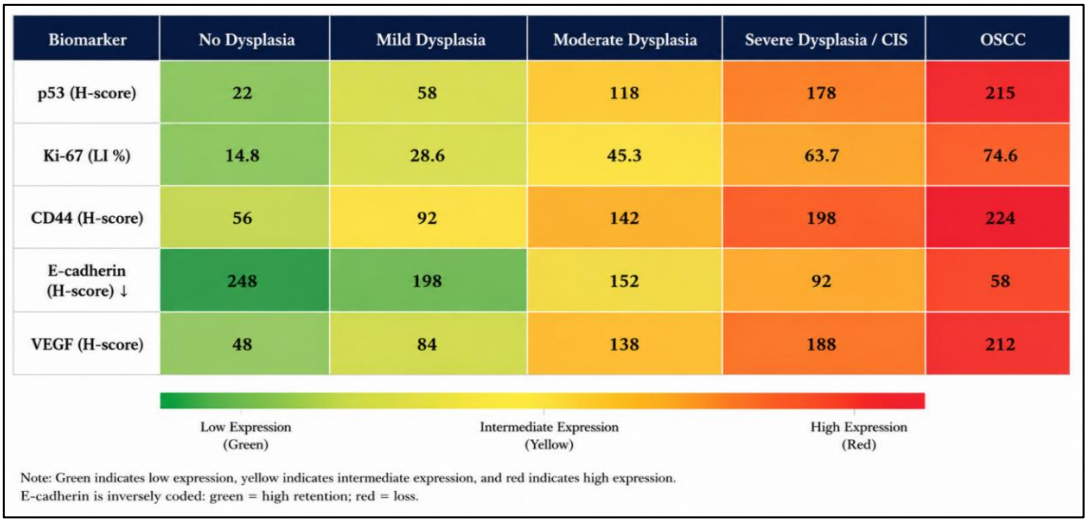


Fig. 2. Heat-map of biomarker expression across dysplasia grades.

3.4 Logistic Regression: Predictors of High-Grade Lesions

On multivariable logistic regression (Table 4), the independent predictors of high-grade disease (severe dysplasia/CIS/OSCC) after adjustment for demographic and clinical covariates were: p53 H-score, Ki-67 LI, CD44 H-score, E-cadherin loss, smokeless tobacco use, and non-homogeneous clinical morphology. VEGF lost statistical significance after adjustment (p = 0.21). The model achieved Nagelkerke R^2 = 0.71 and Hosmer-Lemeshow goodness-of-fit p = 0.62.

3.5 ROC Curve Analysis

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of individual biomarkers and combined biomarker panels in discriminating high-grade dysplasia and oral squamous cell carcinoma (OSCC) from low-grade or non-dysplastic lesions (Table 5). Among the individual biomarkers evaluated, p53 demonstrated the highest discriminatory accuracy, yielding an area under the curve (AUC) of 0.89 (95% confidence interval [CI]: 0.83–0.95). This was followed by Ki-67 (AUC = 0.85) and E-cadherin (AUC = 0.84), both of which also exhibited strong predictive performance. In contrast, VEGF showed the lowest diagnostic accuracy among the investigated markers, with an AUC of 0.79.

Table 4. Multivariable logistic regression: independent predictors of high-grade lesion (severe dysplasia/CIS/OSCC) (n = 120).

Predictor	Adj. OR	95% CI	p-value
p53 H-score (per 10-unit ↑)	1.42	1.24–1.63	< 0.001
Ki-67 LI (per 10% ↑)	1.78	1.41–2.24	< 0.001
CD44 H-score (per 10-unit ↑)	1.18	1.07–1.30	0.001
E-cadherin H-score (per 10-unit ↓)	1.31	1.16–1.48	< 0.001
VEGF H-score (per 10-unit ↑)	1.06	0.97–1.16	0.21 (NS)
Smokeless tobacco use (yes vs no)	3.21	1.32–7.80	0.010
Non-homogeneous morphology	4.05	1.66–9.88	0.002
Male sex	1.82	0.74–4.48	0.19 (NS)
Age (per 10 years)	1.34	0.94–1.91	0.10 (NS)

Adj. OR = adjusted odds ratio; CI = confidence interval; NS = not statistically significant; Nagelkerke R^2 = 0.71; Hosmer-Lemeshow p = 0.62.

Table 5. ROC curve analysis of individual biomarkers and multimarker panels for high-grade lesion (severe dysplasia/CIS/OSCC).

Biomarker / Panel	AUC	95% CI	Optimal Cut-off	Sensitivity	Specificity

p53	0.89	0.83–0.95	H-score ≥130	88.9%	86.3%
Ki-67	0.85	0.78–0.92	LI ≥45%	83.3%	81.4%
CD44	0.82	0.74–0.89	H-score ≥160	77.8%	79.4%
E-cadherin (loss)	0.84	0.77–0.91	H-score ≤120	83.3%	82.4%
VEGF	0.79	0.71–0.87	H-score ≥150	72.2%	75.5%
p53 + Ki-67	0.93	0.88–0.97	—	91.7%	88.2%
p53 + CD44	0.91	0.86–0.96	—	88.9%	87.3%
p53 + Ki-67 + CD44 + E-cadherin	0.95	0.91–0.99	—	94.4%	90.2%

AUC = area under the ROC curve; LI = labelling index; DeLong testing: four-marker panel vs each single marker, $p < 0.01$. Green AUC ≥ 0.90; blue AUC 0.85–0.89.

Combined biomarker analysis demonstrated superior predictive capability compared with individual markers alone. The dual-marker panel incorporating p53 and Ki-67 achieved an AUC of 0.93, indicating excellent discriminatory performance for identifying high-grade dysplastic lesions and malignant transformation. Furthermore, the composite four-marker panel consisting of p53, Ki-67, CD44, and E-cadherin demonstrated the highest overall diagnostic accuracy, with an AUC of 0.95. Comparative analysis using DeLong's test confirmed that the combined biomarker panels significantly outperformed all individual biomarkers (all $p < 0.01$) [14, 29].

These findings support the utility of multimarker immunohistochemical profiling as a robust adjunctive approach for risk stratification and early identification of OPMDs with increased malignant potential (Figure 3).

4 Discussion

The present investigation represents the cross-sectional study conducted in Iraq to comprehensively evaluate a five-marker immunohistochemical (IHC) panel across multiple oral potentially malignant disorder (OPMD) subtypes. The demographic profile of the study cohort, characterized by a marked male predominance (male-to-female ratio: 1.86:1), mean age within the fifth decade of life, and preferential involvement of the buccal mucosa and tongue, is broadly consistent with previously published Iraqi epidemiological studies of oral squamous cell carcinoma (OSCC), including the nationwide series reported by Alshami et al. [2] and regional observations from Sulaimani [10]. These findings further support the established demographic and anatomical trends of oral carcinogenesis within Middle Eastern populations.

Tobacco exposure emerged as the predominant modifiable risk factor within the present cohort. The prevalence of cigarette smoking (60.0%) substantially exceeded the reported national prevalence among Iraqi adult males (38%) [22], underscoring the central etiological role of tobacco use in the development and progression of OPMDs.

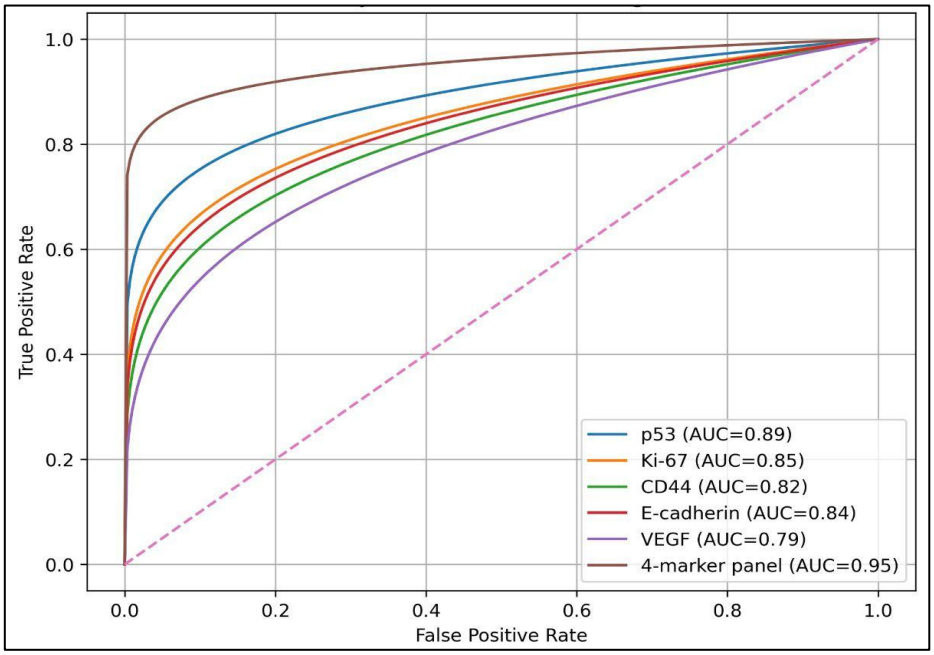


Fig. 3. ROC curve analysis of biomarkers for high grade OPMDs.

Furthermore, the relatively high prevalence of smokeless tobacco (naswar) consumption observed in the current study reflects the increasing adoption of this habit among socioeconomically disadvantaged and displaced

populations, potentially contributing to the evolving epidemiological landscape of oral cancer in Iraq.

The identification of invasive OSCC in 5.0% of lesions at the time of initial biopsy is clinically significant and corresponds closely with pooled malignant transformation estimates reported for oral leukoplakia and erythroplakia in recent meta-analyses [6, 9]. Notably, erythroplakia demonstrated an exceptionally high cumulative rate of severe dysplasia/carcinoma in situ and OSCC (83.3%), representing one of the most important findings of the present study. This observation reinforces the widely recognized aggressive biological behaviour of erythroplakia and strongly supports the recommendation that all erythroplakic lesions undergo prompt biopsy and comprehensive histopathological assessment, supplemented by immunohistochemical evaluation when feasible, particularly in resource-constrained Iraqi clinical settings.

Among the investigated biomarkers, p53 demonstrated the strongest individual discriminatory performance for identifying high-grade dysplasia and malignant transformation. The progressive increase in p53 expression across dysplasia grades, culminating in near-universal overexpression in OSCC, parallels findings reported in both Western and Asian populations [16, 30]. TP53 mutation is recognized as a pivotal early molecular event in oral carcinogenesis, and aberrant p53 immunostaining patterns have been shown to predict progression independently of conventional histopathological grading [15]. The high diagnostic accuracy observed in the present study (AUC = 0.89) highlights the practical value of p53 IHC as a cost-effective adjunctive marker in Iraqi pathology laboratories, where access to advanced molecular diagnostic techniques, including next-generation sequencing, remains limited. Furthermore, longitudinal evidence demonstrating a markedly increased progression-free hazard ratio among p53-abnormal oral epithelial dysplasia lesions further emphasizes the prognostic significance of this biomarker [16].

Ki-67 expression also demonstrated a significant stepwise increase with advancing dysplasia severity, rising from 14.8% in non-dysplastic lesions to 74.6% in OSCC. These findings are in agreement with recent meta-analytic evidence confirming the strong association between Ki-67 proliferative activity and malignant progression in OPMDs [17, 26]. Biologically, the combined utilization of p53 and Ki-67 may provide complementary information by simultaneously reflecting genomic instability and dysregulated cellular proliferation, two fundamental hallmarks of carcinogenesis. This likely explains the enhanced discriminatory performance achieved by the dual-marker panel (AUC = 0.93).

The progressive expansion of CD44 expression from focal basal staining in low-grade lesions to diffuse full-thickness epithelial positivity in severe dysplasia and OSCC supports the cancer stem-cell hypothesis in oral carcinogenesis [18, 27, 31]. Increased CD44 expression has been associated with tumour invasion, metastatic potential, and resistance to therapy, suggesting that this marker may contribute not only to risk stratification but also to identification of biologically aggressive lesions.

Conversely, E-cadherin expression exhibited progressive loss of membranous staining accompanied by cytoplasmic redistribution with increasing dysplasia severity. These alterations are characteristic features of epithelial–mesenchymal transition (EMT), a critical process underlying tumour invasion and metastatic dissemination in oral cancer [20, 28]. The inclusion of E-cadherin within the composite biomarker panel contributed substantially to the excellent overall diagnostic performance achieved by the four-marker model (AUC = 0.95). Interestingly, polymorphisms involving the CDH1 gene encoding E-cadherin have also been implicated in susceptibility to OSCC within neighboring Iranian populations, further supporting the biological relevance of this pathway in regional oral carcinogenesis [32].

Although VEGF expression increased significantly with dysplasia severity, its expression profile differed somewhat from the other investigated biomarkers. In particular, disproportionately elevated VEGF expression was observed in oral submucous fibrosis (OSMF), even in lesions with comparatively lower histopathological grades. This finding likely reflects chronic tissue hypoxia and compensatory angiogenic activation associated with progressive fibrosis and vascular compromise in OSMF [21, 33]. However, the reduced independent predictive significance of VEGF following multivariable adjustment suggests that its utility may be more subtype-specific rather than universally applicable across all OPMDs [12].

Direct comparison of the present findings with previous Iraqi studies is limited due to the scarcity of comprehensive molecular investigations in this field. Previous Iraqi studies have primarily focused on descriptive epidemiology [10], inflammatory immune-cell profiles in oral lichen planus [23], or limited application of p53 and Ki-67 in other head and neck lesions [24]. To date, no published Iraqi study has systematically evaluated this five-marker IHC panel across multiple OPMD subtypes, thereby highlighting the novel contribution of the present work. Additionally, emerging evidence from neighboring countries regarding SOX2 expression [34], as well as CD44 and E-cadherin alterations [32], provides complementary regional support for the molecular pathways identified in this study.

Several limitations should be acknowledged. First, the single-centre cross-sectional design precludes determination of true longitudinal malignant transformation rates and limits causal inference. Second, although inter-observer agreement was acceptable (κ = 0.74), moderate variability in histopathological grading remains an inherent limitation of dysplasia assessment. Third, molecular confirmation of TP53 mutation status and

evaluation of p16/HPV-associated dysplasia, as recommended in the 2022 WHO classification, were not performed because of resource limitations [4]. Additionally, the predominance of participants from urban Baghdad may reduce the generalizability of the findings to rural Iraqi populations.

Future multicentre longitudinal studies incorporating patients from Baghdad, Mosul, Basra, and Sulaimani are therefore warranted to validate these findings and establish robust national risk-stratification models. Emerging technologies, including salivary microRNA profiling, artificial intelligence-assisted digital pathology, and polygenic risk scoring systems, may provide promising avenues for improving early detection and prognostic prediction in Iraqi patients with OPMDs [35, 36].

5 Conclusion

Within this cohort of 120 Iraqi patients with oral potentially malignant disorders (OPMDs), oral leukoplakia represented the most prevalent clinical diagnosis, whereas erythroplakia exhibited the highest malignant potential and oncological risk. Immunohistochemical analysis demonstrated a progressive and statistically significant increase in the expression of p53, Ki-67, CD44, and VEGF across the spectrum from non-dysplastic epithelium to oral squamous cell carcinoma (OSCC), accompanied by a corresponding reduction in E-cadherin expression. These findings support the biological relevance of dysregulated proliferation, genomic instability, angiogenesis, cancer stem-cell activity, and epithelial–mesenchymal transition in oral carcinogenesis.

Among the investigated biomarkers, p53 demonstrated the highest individual diagnostic performance, achieving an area under the receiver operating characteristic curve (AUC) of 0.89. Furthermore, the combined biomarker panel comprising p53, Ki-67, CD44, and E-cadherin achieved excellent discriminatory accuracy (AUC = 0.95), significantly outperforming individual biomarkers alone. The synergistic diagnostic utility of these markers highlights the value of multimarker immunohistochemical profiling as an adjunctive tool for risk stratification in OPMDs.

Based on the present findings, routine implementation of a minimum dual-marker immunohistochemical panel incorporating p53 and Ki-67 is strongly recommended within Iraqi oral pathology practice, particularly for lesions demonstrating equivocal or high-risk histopathological features. CD44 and E-cadherin may provide additional confirmatory value in diagnostically challenging or biologically aggressive lesions.

Given the substantial burden of tobacco exposure and the limited availability of advanced molecular diagnostics in Iraq, the establishment of a structured national OPMD surveillance and referral pathway incorporating biomarker-supported risk stratification represents an important and urgent public health priority. Such an approach may facilitate earlier detection of high-risk lesions, optimize clinical decision-making, and ultimately contribute to reducing the morbidity and mortality associated with OSCC in the Iraqi population [37, 38].

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