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CLINICAL SUBTYPE, LESION SIZE AND ANATOMICAL SITE AS DETERMINANTS OF ORAL EPITHELIAL DYSPLASIA IN LEUKOPLAKIA: A CROSS-SECTIONAL STUDY OF 330 PATIENTS FROM AN ARECA-NUT-ENDEMIC REGION OF SOUTH INDIA

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ABSTRACT:
Background: Oral leukoplakia is the commonest oral potentially malignant disorder, and epithelial dysplasia remains its strongest histological predictor of progression. Whether chairside clinical features can identify lesions likely to harbour dysplasia has direct implications for biopsy prioritisation in resource-constrained settings.
Objectives: To identify independent clinical determinants of dysplasia in oral leukoplakia, quantify grading reproducibility, and test whether lesion laterality corresponds to habitual betel quid placement side.
Methods. Cross-sectional study of 330 consecutive patients with histopathologically confirmed oral leukoplakia at a tertiary dental hospital in Chennai, India, between January 2024 and July 2026. Lesions were graded using the WHO 2017 three-tier and WHO 2022 binary systems, with a random 20% subsample (n = 66) independently regraded by a second oral pathologist. Multivariable logistic regression identified independent predictors. Reporting follows STROBE.
Results: Median age was 56 years (IQR 48–63); 251 patients (76.1%) were men. Dysplasia was present in 133 lesions (40.3%) and high-grade dysplasia in 40 (12.1%). Independent predictors of dysplasia were non-homogeneous subtype (adjusted odds ratio [aOR] 2.45, 95% CI 1.32–4.52), lesion size per 10 mm (aOR 2.43, 1.64–3.61), tobacco use (aOR 2.21, 1.16–4.23) and age per decade (aOR 1.58, 1.23–2.02); area under the curve (AUC) 0.805. For high-grade dysplasia, lesion size (aOR 3.73, 2.00–6.97), non-homogeneous subtype (aOR 3.28, 1.24–8.70) and age (aOR 1.96, 1.31–2.93) were independent (AUC 0.867). The crude association of tongue or floor-of-mouth location with dysplasia (odds ratio 3.49) attenuated to non-significance after adjustment for size and subtype. Agreement was substantial (weighted κ 0.80 three-tier; κ 0.77 binary). Among 94 patients with a unilateral lesion and lateralised habit, the lesion arose on the quid placement side in 90 (95.7%; $\kappa = 0.91$).
Conclusions: Lesion size and clinical subtype, not anatomical site per se, independently predict dysplasia in this population. Site appears to act largely as a proxy for local carcinogen contact. Clinical assessment stratifies risk usefully but does not discriminate sharply enough to defer biopsy.

KEYWORDS: Oral leukoplakia; epithelial dysplasia; oral potentially malignant disorders; areca nut; betel quid; cross-sectional studies; India.

1. INTRODUCTION

Oral leukoplakia is defined as a predominantly white plaque of questionable risk, having excluded other known diseases or disorders that carry no increased risk for cancer [1,2]. It is the most prevalent of the oral potentially malignant disorders, with a pooled global prevalence of approximately 4% and substantially higher figures reported in South and Southeast Asian populations where areca nut and smokeless tobacco use is widespread [3,4]. Reported rates of malignant transformation vary from under 1% to more than 20% across series, reflecting differences in case definition, referral pattern, exposure profile and length of follow-up; pooled estimates from systematic reviews converge on an annual transformation rate of approximately 1% and a cumulative rate of 3–5% [5,6].

The grade of oral epithelial dysplasia remains the single strongest histological predictor of progression, and lesions showing dysplasia transform considerably more often than those that do not [6,7]. Dysplasia grading is nonetheless an imperfect instrument. Inter-observer and intra-observer agreement for the conventional three-tier system is only modest in several published series, with much of the disagreement concentrated at the threshold between reactive keratosis and mild dysplasia, and between mild and moderate grades [8,9]. This limitation prompted the development of a binary low-grade versus high-grade classification intended to improve reproducibility while preserving prognostic separation [10], an approach subsequently endorsed alongside the traditional scheme in the fifth edition of the WHO classification of head and neck tumours [11,12].

Clinical assessment necessarily precedes histopathology in every care pathway, and certain clinical features have long been regarded as signalling higher risk. Non-homogeneous lesions, whether speckled, nodular or verrucous, are reported to harbour dysplasia and to transform more frequently than homogeneous plaques [2,13]. Anatomical site is similarly held to be informative, with the tongue and floor of mouth conventionally designated high-risk subsites [7,14]. Much of the evidence underpinning that designation, however, derives from Western cohorts in which smoking and alcohol are the dominant exposures and in which floor-of-mouth leukoplakia is comparatively common [15,16].

The aetiological landscape in South Asia differs materially. Areca nut, used alone or within betel quid with or without added tobacco, is classified as carcinogenic to humans and is the dominant exposure across much of India [17,18]. Quid is characteristically retained in the buccal vestibule for prolonged periods, frequently on a habitual side, which concentrates carcinogen contact on a defined mucosal surface. If the observed site distribution of leukoplakia in such populations is driven principally by where the quid is placed rather than by intrinsic regional susceptibility of the mucosa, then anatomical site carries information primarily about local carcinogen dose. That is a mechanistically distinct proposition from the Western high-risk-subsite model, and one that has to our knowledge not been formally tested by relating lesion laterality to habit laterality within the same patients.

Most published series report either dysplasia grade or lesion distribution; relatively few examine clinical subtype, lesion size, anatomical site and habit exposure together within a single multivariable framework capable of separating their independent contributions. We therefore undertook this study with three objectives: to identify independent clinical determinants of epithelial dysplasia and of high-grade dysplasia in oral leukoplakia; to quantify the reproducibility of dysplasia grading in our setting under both the three-tier and binary systems; and to test the concordance between lesion laterality and habitual quid placement side.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was a cross-sectional study conducted in the Department of Oral Medicine and Radiology of a tertiary dental teaching hospital in Chennai, Tamil Nadu, India. Clinical and histopathological records of patients presenting between January 2024 and July 2026 were reviewed. The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [19].

2.2 Participants

Consecutive patients with a clinical diagnosis of oral leukoplakia who underwent biopsy during the study period were screened for eligibility. Inclusion criteria were a clinical diagnosis of oral leukoplakia made by a specialist in oral medicine, an available histopathology report from the same lesion, and documentation of the anatomical site and clinical subtype at first presentation. Exclusion criteria were a clinical or histopathological diagnosis of another potentially malignant disorder as the primary lesion, including oral submucous fibrosis, erythroplakia without a leukoplakic component, and oral lichen planus or lichenoid lesions; white lesions attributable to a known cause such as frictional keratosis, leukoedema, candidal infection, nicotinic stomatitis or a hereditary condition; invasive squamous cell carcinoma identified on the index biopsy; proliferative verrucous leukoplakia, now regarded as a distinct entity [12]; and incomplete documentation of site, clinical subtype or dysplasia grade. The flow of cases through screening is shown in Figure 1.

2.3 Variables and definitions

Lesions were classified clinically as homogeneous, defined as uniformly white, flat and thin with a consistent surface texture, or non-homogeneous, comprising speckled or erythroleukoplakic, nodular and verrucous variants, following WHO criteria [1,2]. Non-homogeneous lesions were further subclassified. Anatomical site was recorded as documented at presentation across thirteen categories. For multivariable analysis, sites were grouped as buccal mucosa (right, left or bilateral), labial mucosa and commissure, tongue and floor of mouth, multifocal, and other (gingiva and alveolar ridge, hard palate, soft palate and retromolar area). Buccal lesions were pooled for the risk model because laterality was considered a marker of habit behaviour rather than a distinct anatomical subsite; laterality was analysed separately against quid placement side.

Habit history was recorded as use of smoked tobacco, smokeless tobacco, areca nut in any form and alcohol, each as a binary variable, together with the duration in years and frequency per day of the dominant habit and habit status (current, former or never). Former use was defined as cessation of all habits at least six months before presentation. The side of the mouth on which quid or smokeless tobacco was habitually retained was recorded where documented. Lesion characteristics recorded were the largest dimension in millimetres, the number of lesions (solitary or multifocal), and the patient-reported duration in months.

2.4 Histopathological assessment

Haematoxylin and eosin-stained sections were assessed by a consultant oral pathologist. Epithelial dysplasia was graded using the WHO three-tier system as absent, mild, moderate or severe [12]. The binary grade (low-grade versus high-grade) was assigned independently on the basis of architectural and cytological criteria following the system described by Kujan and colleagues [10], and was not derived mechanically from the three-tier grade. Two outcomes were prespecified: the presence of epithelial dysplasia of any grade, chosen as the primary outcome because it yielded sufficient events to support a fully adjusted model, and high-grade dysplasia, analysed as a secondary outcome of greater clinical consequence but lower event frequency.

To quantify reproducibility, a random 20% subsample was independently regraded by a second consultant oral pathologist blinded to the original report and to all clinical data. Agreement was quantified using Cohen's kappa, unweighted and linearly weighted for the three-tier grade and unweighted for the binary grade, with 95% confidence intervals obtained by bootstrap resampling (3,000 replicates) and interpreted according to the benchmarks of Landis and Koch [20].

2.5 Sample size

All eligible cases within the study period were included, giving a sample of 330. The sample was evaluated for precision rather than for power to detect a prespecified effect. With 330 participants and an observed dysplasia prevalence of 40.3%, the half-width of the 95% confidence interval around that prevalence is 5.3 percentage points. The number of events constrained model complexity: the 133 dysplasia events permitted a model with up to thirteen degrees of freedom at the conventional ten events per variable [21], whereas the 40 high-grade events permitted only four, and the secondary model was specified accordingly.

2.6 Statistical analysis

Continuous variables were summarised as median with interquartile range after assessment of normality using the Shapiro–Wilk test; categorical variables were summarised as frequencies and percentages with Wilson score 95% confidence intervals [22]. Associations between categorical variables and each outcome were examined using the Pearson chi-square test, with Fisher's exact test substituted for 2×2 tables in which any expected cell count was below five. Continuous variables were compared between outcome groups using the Mann–Whitney U test, and ordered differences in the three-tier grade were assessed using the Kruskal–Wallis test.

Two multivariable binary logistic regression models were fitted. The primary model, for the presence of epithelial dysplasia, included clinical subtype, site group, age, sex, lesion size, lesion number and the three habit variables. The secondary model, for high-grade dysplasia, was restricted to clinical subtype, site group, age and lesion size in order to respect the events-per-variable constraint. Age was modelled per ten-year increment and lesion size per ten-millimetre increment. Model performance was assessed by the McFadden pseudo R^2, the Hosmer–Lemeshow goodness-of-fit test and the area under the receiver operating characteristic curve [23]. Multicollinearity was assessed using variance inflation factors, with a threshold of five. To examine whether the crude association between anatomical site and dysplasia was mediated by lesion size and clinical subtype, site-specific odds ratios were compared before and after adjustment for those variables.

Concordance between lesion laterality and habitual quid placement side was examined among patients with a unilateral buccal lesion and a documented unilateral habit, using Fisher's exact test and Cohen's kappa. A sensitivity analysis excluding the three cases of carcinoma in situ was performed for the secondary model. Missing data were not imputed and the number of observations contributing to each analysis is reported. A two-sided p value below 0.05 was considered statistically significant. Analyses were performed using Python 3.12 with the pandas, SciPy, statsmodels and scikit-learn libraries.

2.7 Ethical considerations

The study protocol was approved by the Institutional Ethics Committee of Saveetha Dental College and Hospitals (approval number [SRB/SDC/OMED-2305/25/269]. Because the study used de-identified archival records, a waiver of individual informed consent was granted. Data were extracted onto a coded proforma containing no patient identifiers and the linking key was held separately under restricted access. Written informed consent for publication was obtained for all clinical photographs.

3. RESULTS

3.1 Characteristics of the study population

Of 590 patients with a clinical diagnosis of oral leukoplakia screened over the study period, 260 (44.1%) were excluded and 330 (55.9%) met the eligibility criteria and were analysed (Figure 1). The median age was 56 years (IQR 48–63) and 251 patients (76.1%) were men. A tobacco or areca nut habit was recorded in 298 patients (90.3%): smokeless tobacco in 199 (60.3%), areca nut or betel quid in 185 (56.1%), smoked tobacco in 119 (36.1%) and alcohol in 97 (29.4%). Most patients were current users (249, 75.5%), with 49 (14.8%) former users and 32 (9.7%) reporting no habit. The median habit duration was 18 years (IQR 12–23). Demographic and habit characteristics, with their univariate associations with each outcome, are presented in Table 1.

No individual habit was associated with the presence of dysplasia on univariate analysis. Habit status was associated with both outcomes, with former users showing a higher prevalence of dysplasia (61.2%) than current users (36.5%) or never users (37.5%; $p = 0.005$). Patients with dysplasia were older than those without (median 58 versus 54 years; $p = 0.001$), and the age difference was larger for high-grade disease (median 62 years; $p < 0.001$). Habit duration and frequency were not significantly associated with either outcome. Habit duration and frequency were not recorded for 32 patients (9.7%); no other variable had missing values.

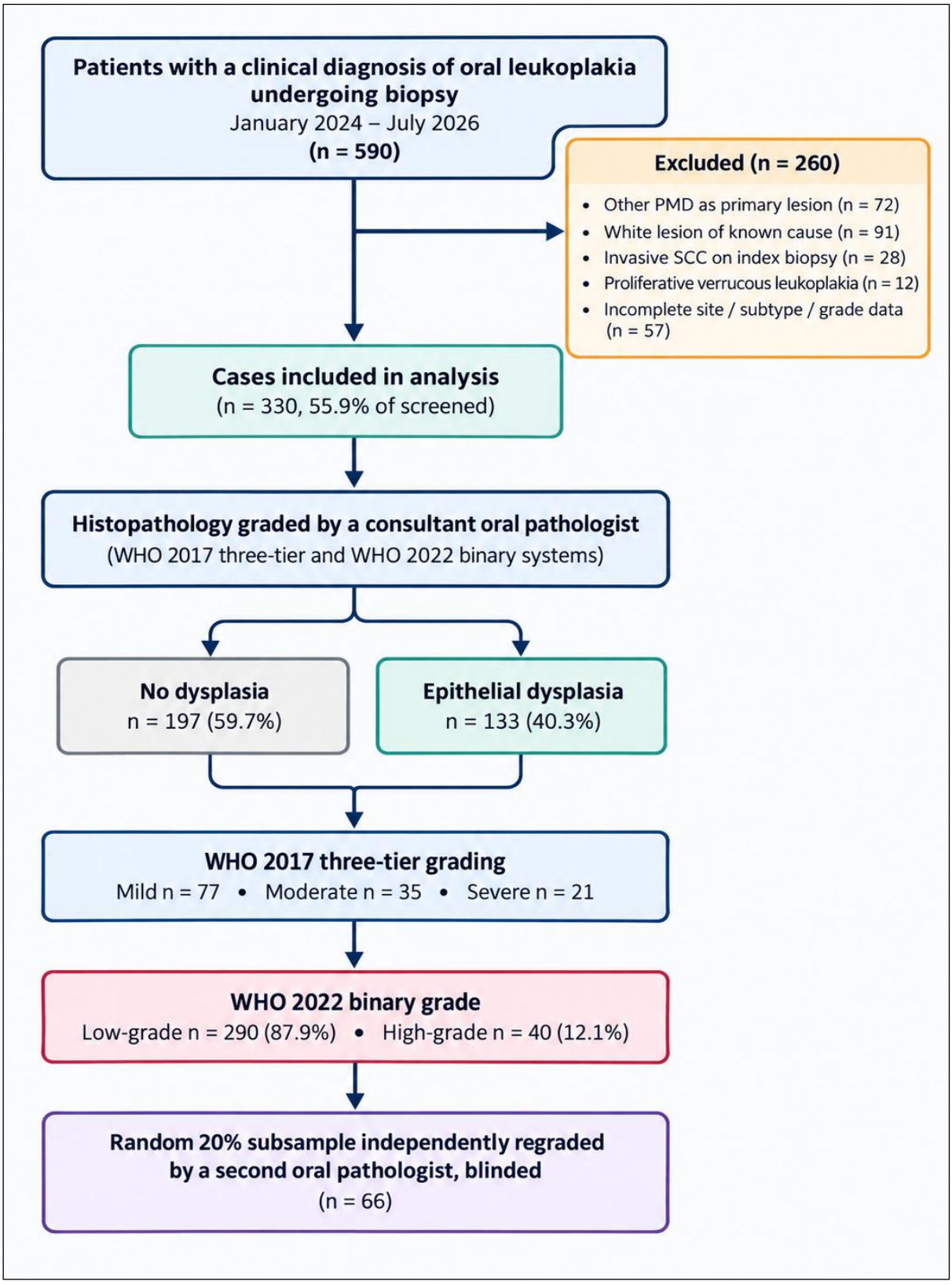

Figure 1. Flow of patients through the study, reported in accordance with the STROBE statement. Numbers screened and excluded are to be completed from the departmental screening log.

3.2 Clinical characteristics of the lesions

Lesions were homogeneous in 216 cases (65.5%) and non-homogeneous in 114 (34.5%). Among non-homogeneous lesions, 49 (43.0%) were speckled or erythroleukoplakic, 40 (35.1%) verrucous and 25 (21.9%) nodular. The buccal mucosa was the commonest site group (167, 50.6%), followed by tongue and floor of mouth (72, 21.8%). Lesions were solitary in 296 cases (89.7%). The median largest dimension was 22 mm (IQR 17–29) and the median reported duration was 10 months (IQR 7–16). Clinical characteristics are summarised in Table 2.

3.3 Histopathological findings and grading reliability

Epithelial dysplasia was present in 133 lesions (40.3%, 95% CI 35.1–45.7): mild in 77 (23.3%), moderate in 35 (10.6%) and severe in 21 (6.4%). The remaining 197 lesions (59.7%) showed hyperkeratosis without dysplasia. Three lesions (0.9%) met criteria for carcinoma in situ. Under the WHO 2022 binary system, 40 lesions (12.1%, 95% CI 9.0–16.1) were classified as high-grade and 290 (87.9%) as low-grade. Notably, the binary and three-tier classifications were not interchangeable: three lesions graded mild were assigned high-grade status, one graded severe was assigned low-grade, and the 35 moderate lesions divided almost evenly between the two binary categories (17 high-grade, 18 low-grade).

In the regraded subsample of 66 cases, raw agreement was 81.8% for the three-tier grade and 93.9% for the binary grade. Cohen's kappa was 0.71 unweighted and 0.80 linearly weighted (95% CI 0.68–0.90) for the three-tier system, and 0.77 (95% CI 0.49–0.95) for the binary system, indicating substantial agreement by the Landis and Koch benchmarks. All but one disagreement occurred at the boundary between no dysplasia and mild dysplasia (11 of 12 discordant cases); the remaining discordance lay between moderate and severe. The agreement matrix is shown in Figure 7B.

3.4 Clinical subtype and dysplasia

Dysplasia was present in 76 of 114 non-homogeneous lesions (66.7%) compared with 57 of 216 homogeneous lesions (26.4%; odds ratio 5.58, $p < 0.001$). The difference was more pronounced for high-grade disease, present in 32 non-homogeneous lesions (28.1%) and 8 homogeneous lesions (3.7%; odds ratio 10.14, $p < 0.001$). The distribution of three-tier grades across clinical subtypes is shown in Figure 2A. Within the non-homogeneous group, nodular lesions showed the highest prevalence of dysplasia (84.0%) and of high-grade dysplasia (36.0%), followed by verrucous (65.0% and 30.0%) and speckled lesions (59.2% and 22.4%), although differences between the three variants did not reach significance ($p = 0.45$), reflecting the small numbers within each (Figure 2B).

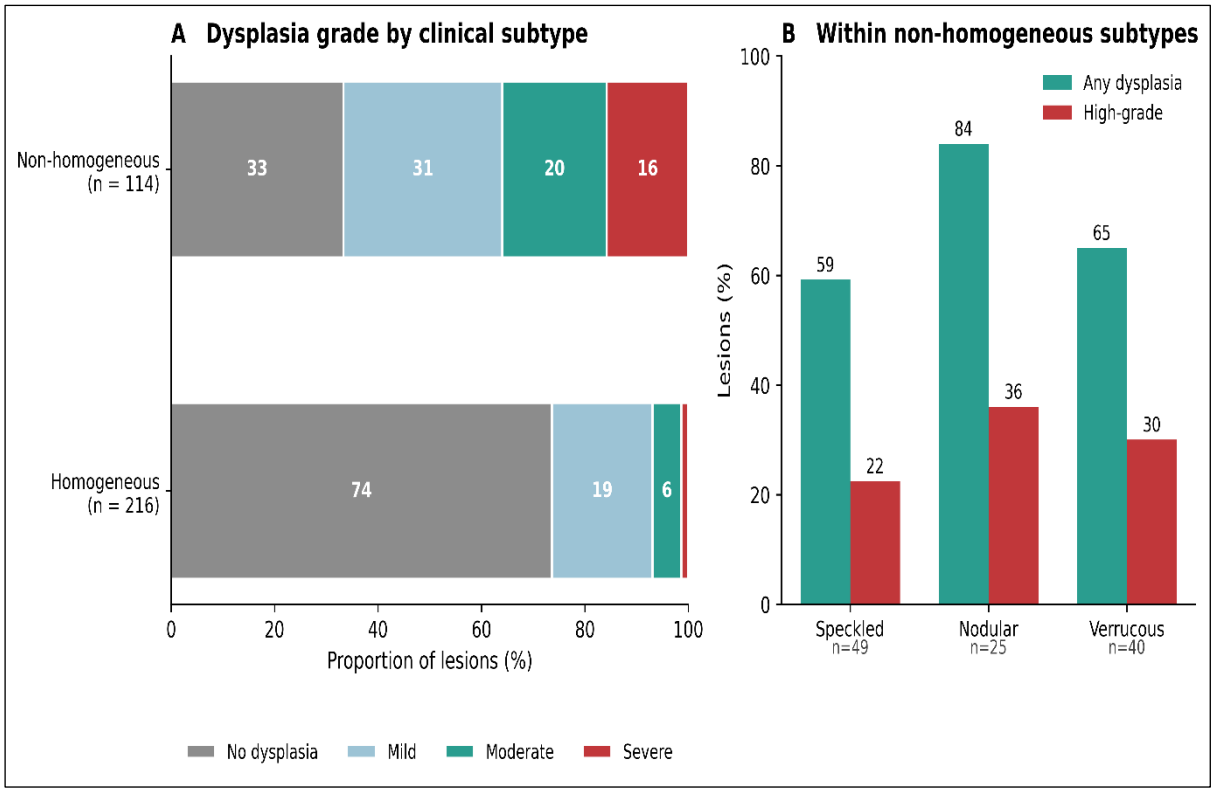

Figure 2. Clinical subtype and epithelial dysplasia. (A) Distribution of three-tier dysplasia grades within homogeneous and non-homogeneous lesions, expressed as a proportion of lesions in each subtype. (B) Prevalence of any dysplasia and of high-grade dysplasia within the three non-homogeneous variants; differences between variants were not statistically significant (p = 0.45).

3.5 Anatomical site and dysplasia

The prevalence of dysplasia differed significantly across site groups ($p < 0.001$). Lesions of the tongue and floor of mouth showed dysplasia in 45 of 72 cases (62.5%) and multifocal lesions in 7 of 12 (58.3%), compared with 54 of 167 buccal lesions (32.3%) and 7 of 22 labial or commissural lesions (31.8%). The corresponding figures for high-grade dysplasia were 19.4%, 33.3%, 8.4% and 4.5% respectively ($p = 0.017$). Site-specific proportions with Wilson confidence intervals are displayed in Figure 3, and the full thirteen-category breakdown is given in Table 3. The crude odds ratio for dysplasia associated with a tongue or floor-of-mouth location was 3.49 ($p < 0.001$). After adjustment for lesion size and clinical subtype alone, this attenuated to 1.89 ($p = 0.058$), and in the fully adjusted model to 1.93 (95% CI 0.98–3.78; $p = 0.056$). Lesions at these sites were both larger (mean 27.3 mm versus 20.7 mm at the buccal mucosa) and more often non-homogeneous (58.3% versus 22.8%), consistent with these two variables accounting for a substantial part of the apparent site effect.

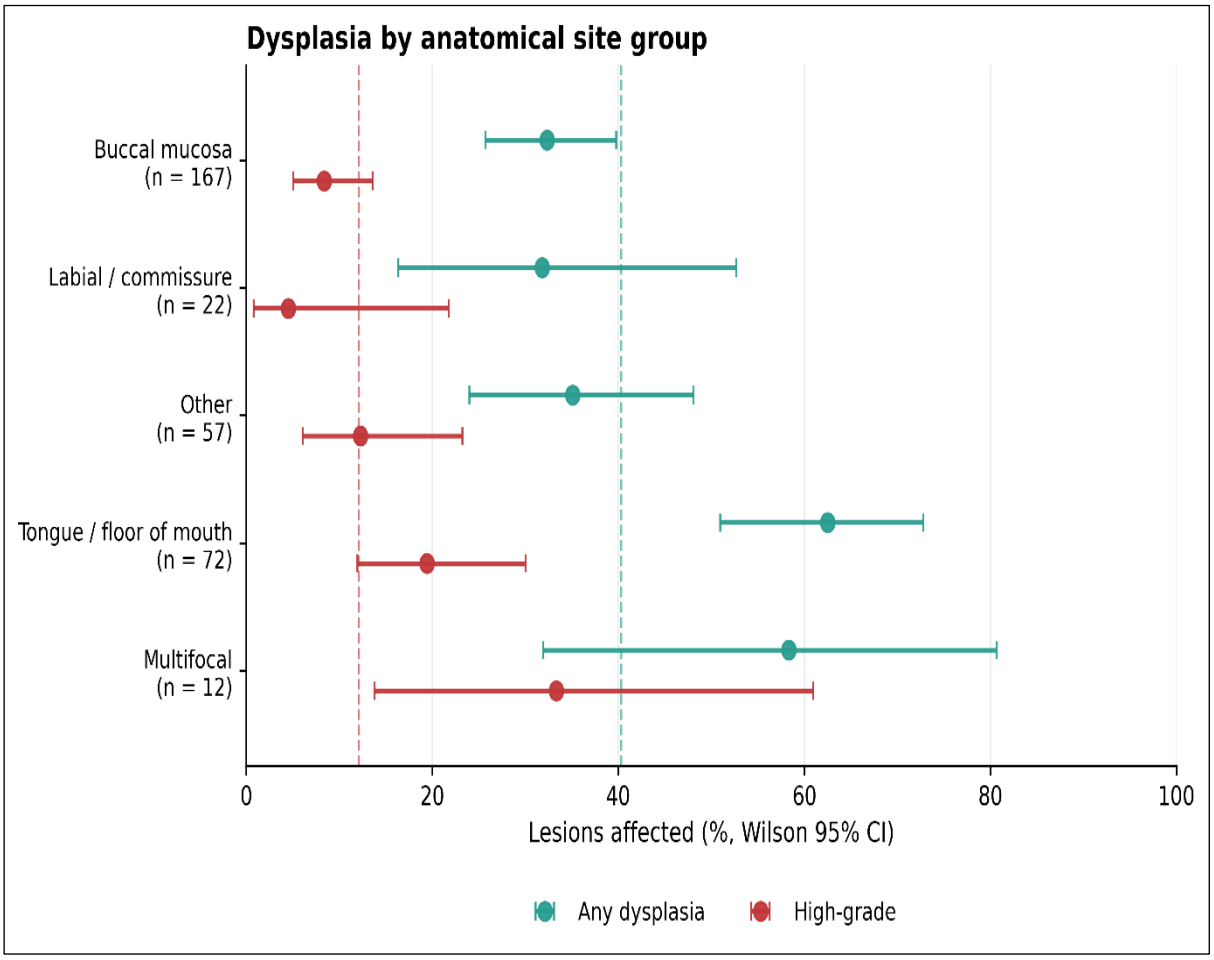

Figure 3. Prevalence of any epithelial dysplasia (teal) and high-grade dysplasia (red) by anatomical site group, with Wilson score 95% confidence intervals. Dashed vertical lines indicate the corresponding prevalence in the cohort overall.

3.6 Independent predictors of dysplasia

In the primary multivariable model (330 patients, 133 events), four variables were independently associated with the presence of epithelial dysplasia: lesion size per 10 mm increase (aOR 2.43, 95% CI 1.64–3.61; $p < 0.001$), non-homogeneous subtype (aOR 2.45, 95% CI 1.32–4.52; $p = 0.004$), tobacco use in any form (aOR 2.21, 95% CI 1.16–4.23; $p = 0.016$) and age per decade (aOR 1.58, 95% CI 1.23–2.02; $p < 0.001$). Female sex approached significance (aOR 1.89, 95% CI 1.00–3.58; $p = 0.051$). Neither areca nut use nor alcohol was independently associated with dysplasia once other factors were accounted for. The model explained 22.1% of the variance (McFadden pseudo R^2), showed acceptable calibration (Hosmer–Lemeshow $p = 0.066$) and discriminated well (AUC 0.805). No variance inflation factor exceeded 1.6. Adjusted odds ratios are presented in Table 4 and displayed in Figure 4A.

In the secondary model for high-grade dysplasia (40 events), lesion size remained the strongest predictor (aOR 3.73 per 10 mm, 95% CI 2.00–6.97; $p < 0.001$), followed by non-homogeneous subtype (aOR 3.28, 95% CI 1.24–8.70; $p = 0.017$) and age (aOR 1.96 per decade, 95% CI 1.31–2.93; $p = 0.001$). Multifocal location carried a non-significant threefold elevation (aOR 3.81, 95% CI 0.77–18.79; $p = 0.10$), and tongue or floor-of-mouth location showed no independent association (aOR 1.01). The model showed good calibration (Hosmer–Lemeshow $p = 0.446$) and discrimination (AUC 0.867; Table 5, Figures 4B and 5B). Excluding the three carcinoma in situ cases did not materially change these estimates (non-homogeneous subtype aOR 3.15, lesion size aOR 3.50, age aOR 1.99).

Lesion size alone discriminated moderately for both outcomes (AUC 0.735 for any dysplasia and 0.817 for high-grade dysplasia), outperforming clinical subtype alone (AUC 0.689 and 0.759 respectively; Figure 5). Using the Youden index, the optimal size threshold was 21 mm for the presence of dysplasia (sensitivity 78%, specificity 61%) and 33 mm for high-grade dysplasia (sensitivity 60%, specificity 91%). Lesion size increased monotonically across dysplasia grades (Kruskal–Wallis $p < 0.001$; Figure 7A).

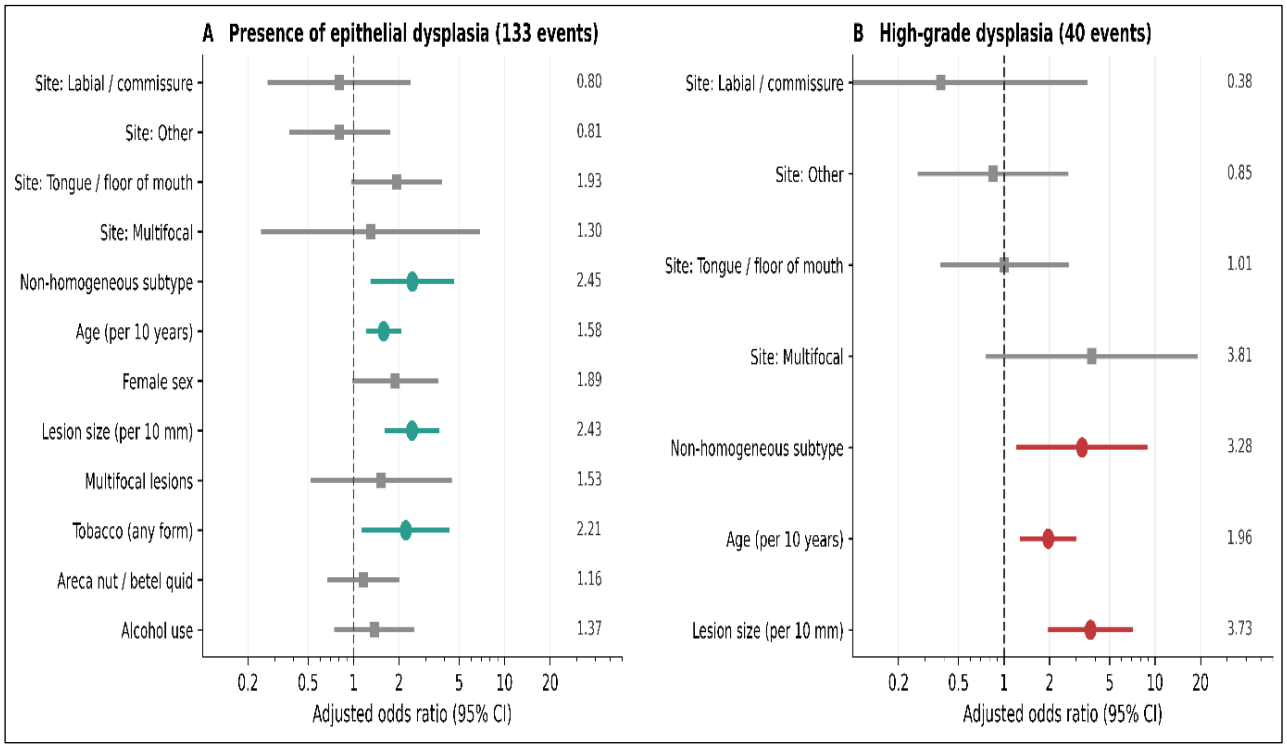

Figure 4. Adjusted odds ratios with 95% confidence intervals from the multivariable logistic regression models, plotted on a logarithmic scale. (A) Presence of epithelial dysplasia (133 events). (B) High-grade dysplasia (40 events). Filled circles denote statistically significant associations; squares denote non-significant associations. Buccal mucosa is the reference category for site.

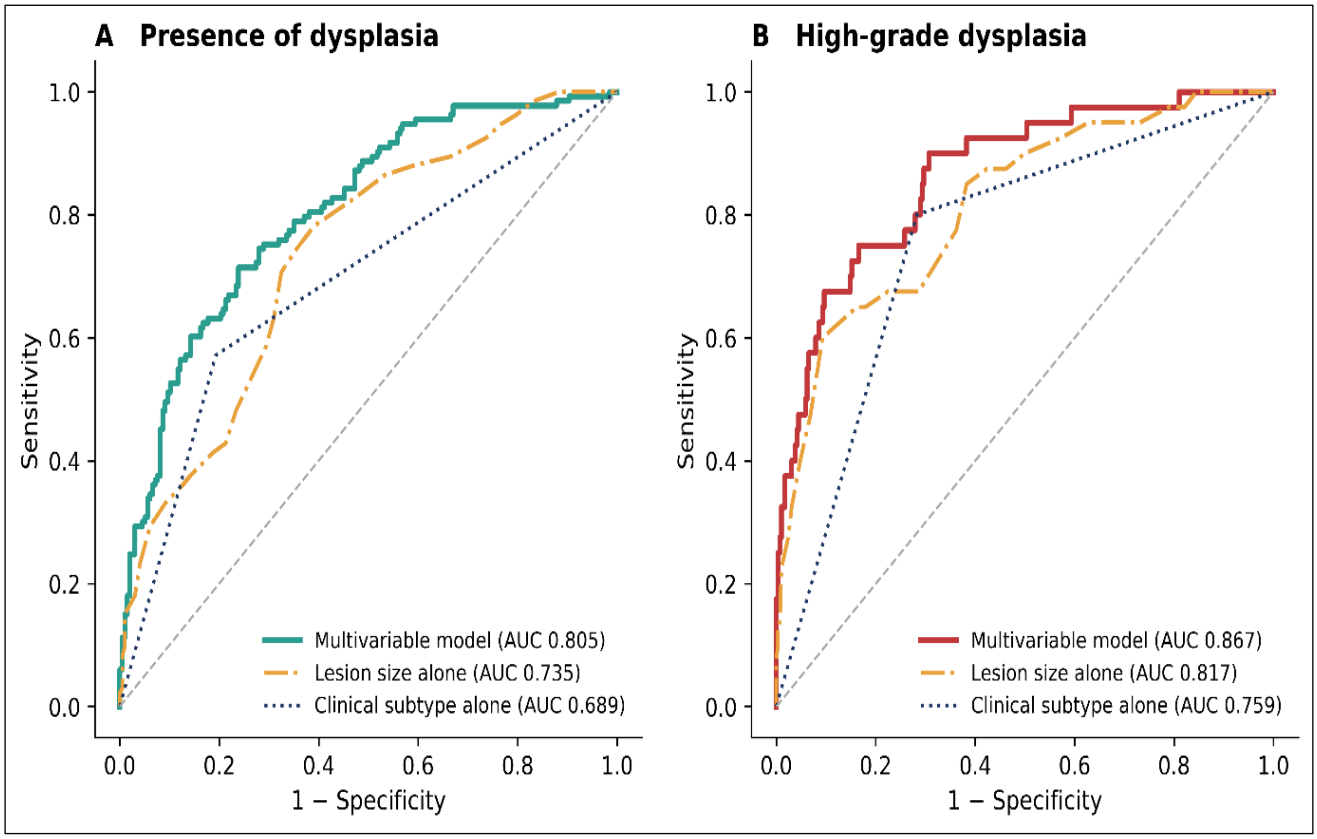

Figure 5. Receiver operating characteristic curves for (A) the presence of epithelial dysplasia and (B) high-grade dysplasia, comparing the full multivariable model with lesion size alone and clinical subtype alone.

3.7 Lesion laterality and habitual quid placement

Habitual quid placement side was documented in 269 patients: right in 85, left in 77, bilateral in 76 and alternating in 31. Among the 94 patients with both a unilateral buccal lesion and a documented unilateral habit, the lesion arose on the

side of habitual quid placement in 90 cases (95.7%, 95% CI 89.6–98.3). The association was very strong (Fisher's exact $p < 0.001$; Cohen's kappa 0.91, indicating almost perfect agreement). Among patients with bilateral or alternating habits, no corresponding lateral predilection was observed. The concordance matrix and the distribution of lesion side across habit laterality categories are shown in Figure 6 and Table 6.

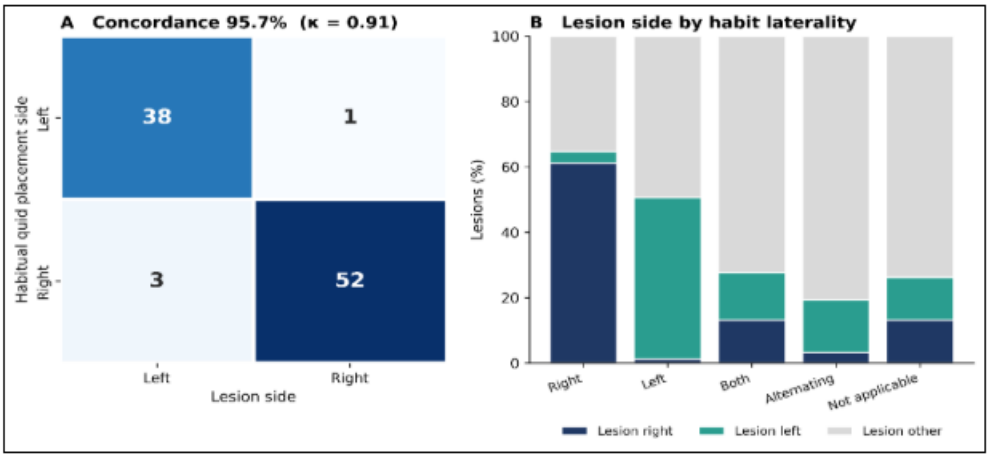

Figure 6. Lesion laterality and habitual quid placement. (A) Concordance matrix among the 94 patients with a unilateral buccal lesion and a unilateral quid habit. (B) Distribution of lesion side across all categories of habit laterality.

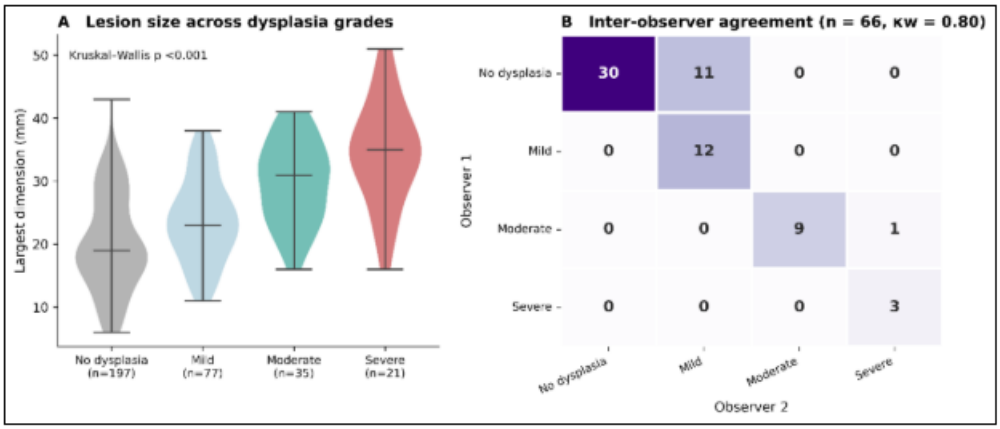

Figure 7. (A) Distribution of lesion size across three-tier dysplasia grades; horizontal bars indicate medians and ranges. (B) Agreement matrix between the two oral pathologists for the three-tier grade in the regraded subsample (n = 66).

Table 1. Demographic and habit characteristics of the study population and their univariate association with epithelial dysplasia (n = 330).

Variable	Category	n (%)	Any dysplasia n (%)	High-grade n (%)	p†	p‡
Age, years	median (IQR)	56 (48–63)	58 (51–66)	62 (56–68)	0.001	<0.001
Sex	Male	251 (76.1)	97 (38.6)	30 (12.0)	0.336	1.000
	Female	79 (23.9)	36 (45.6)	10 (12.7)		
Smoked tobacco	Yes	119 (36.1)	54 (45.4)	17 (14.3)	0.195	0.466

Variable	Category	n (%)	Any dysplasia n (%)	High-grade n (%)	p†	p‡
	No	211 (63.9)	79 (37.4)	23 (10.9)		
Smokeless tobacco	Yes	199 (60.3)	78 (39.2)	21 (10.6)	0.696	0.366
	No	131 (39.7)	55 (42.0)	19 (14.5)		
Areca nut / betel quid	Yes	185 (56.1)	77 (41.6)	24 (13.0)	0.661	0.715
	No	145 (43.9)	56 (38.6)	16 (11.0)		
Alcohol	Yes	97 (29.4)	43 (44.3)	11 (11.3)	0.401	0.924
	No	233 (70.6)	90 (38.6)	29 (12.4)		
Habit status	Current	249 (75.5)	91 (36.5)	26 (10.4)	0.005	0.055
	Former	49 (14.8)	30 (61.2)	11 (22.4)		
	Never	32 (9.7)	12 (37.5)	3 (9.4)		
Habit duration, years	median (IQR)	18 (12–23)	18 (13–24)	19 (15–24)	0.056	0.124
Habit frequency, per day	median (IQR)	7 (5–9)	7 (5–9)	7 (4–10)	0.958	0.973

IQR, interquartile range. † p value for the presence of epithelial dysplasia of any grade. ‡ p value for high-grade dysplasia. Chi-square or Fisher's exact test for categorical variables; Mann–Whitney U test for continuous variables. Habit duration and frequency were not recorded for 32 patients (9.7%).

Table 2. Clinical characteristics of the leukoplakic lesions and their univariate association with epithelial dysplasia (n = 330).

Variable	Category	n (%)	Any dysplasia n (%)	High-grade n (%)	p†	p‡
Clinical subtype	Homogeneous	216 (65.5)	57 (26.4)	8 (3.7)	<0.001	<0.001
	Non-homogeneous	114 (34.5)	76 (66.7)	32 (28.1)		

Variable	Category	n (%)	Any dysplasia n (%)	High-grade n (%)	p†	p‡
Non-homogeneous subtype	Speckled / erythroleukoplakia	49 (14.8)	29 (59.2)	11 (22.4)	<0.001	<0.001
	Nodular	25 (7.6)	21 (84.0)	9 (36.0)		
	Verrucous	40 (12.1)	26 (65.0)	12 (30.0)		
Anatomical site group	Buccal mucosa	167 (50.6)	54 (32.3)	14 (8.4)	<0.001	0.017
	Labial mucosa / commissure	22 (6.7)	7 (31.8)	1 (4.5)		
	Tongue / floor of mouth	72 (21.8)	45 (62.5)	14 (19.4)		
	Multifocal	12 (3.6)	7 (58.3)	4 (33.3)		
	Other§	57 (17.3)	20 (35.1)	7 (12.3)		
Lesion number	Solitary	296 (89.7)	117 (39.5)	35 (11.8)	0.507	0.583
	Multifocal	34 (10.3)	16 (47.1)	5 (14.7)		
Largest dimension, mm	median (IQR)	22 (17–29)	26 (21–33)	33 (25–37)	<0.001	<0.001
Lesion duration, months	median (IQR)	10 (7–16)	9 (6–14)	10 (7–16)	0.379	0.555
Biopsy type	Incisional	287 (87.0)	122 (42.5)	38 (13.2)	0.052	0.174
	Excisional	43 (13.0)	11 (25.6)	2 (4.7)		

† p value for the presence of epithelial dysplasia of any grade. ‡ p value for high-grade dysplasia. § Gingiva and alveolar ridge, hard palate, soft palate and retromolar area.

Table 3. Prevalence of epithelial dysplasia and of high-grade dysplasia by anatomical site (n = 330).

Lesion site	n	Any dysplasia n (%)	95% CI	High-grade n (%)	95% CI
Right buccal mucosa	72	22 (30.6)	21.0–42.1	8 (11.1)	5.7–20.4
Left buccal mucosa	65	20 (30.8)	20.8–43.0	5 (7.7)	3.3–16.8
Bilateral buccal mucosa	30	12 (40.0)	24.6–57.7	1 (3.3)	0.6–16.7
Commissure of lip	12	4 (33.3)	13.8–60.9	0 (0.0)	0.0–24.2
Labial mucosa	10	3 (30.0)	10.8–60.3	1 (10.0)	1.8–40.4

Lesion site	n	Any dysplasia n (%)	95% CI	High-grade n (%)	95% CI
Tongue – lateral border	42	27 (64.3)	49.2–77.0	10 (23.8)	13.5–38.5
Tongue – ventral	14	8 (57.1)	32.6–78.6	1 (7.1)	1.3–31.5
Floor of mouth	16	10 (62.5)	38.6–81.5	3 (18.8)	6.6–43.0
Gingiva / alveolar ridge	20	7 (35.0)	18.1–56.7	3 (15.0)	5.2–36.0
Hard palate	15	4 (26.7)	10.9–52.0	1 (6.7)	1.2–29.8
Soft palate	8	4 (50.0)	21.5–78.5	2 (25.0)	7.1–59.1
Retromolar area	14	5 (35.7)	16.3–61.2	1 (7.1)	1.3–31.5
Multifocal	12	7 (58.3)	32.0–80.7	4 (33.3)	13.8–60.9
Total	330	133 (40.3)	35.1–45.7	40 (12.1)	9.0–16.1

CI, confidence interval, calculated by the Wilson score method. Percentages are within-site.

Table 4. Multivariable logistic regression model for the presence of epithelial dysplasia (n = 330; 133 events).

Predictor	Adjusted OR	95% CI	p
Non-homogeneous subtype (vs homogeneous)	2.45	1.32–4.52	0.004
Lesion size, per 10 mm increase	2.43	1.64–3.61	<0.001
Tobacco use, any form	2.21	1.16–4.23	0.016
Age, per 10-year increase	1.58	1.23–2.02	<0.001
Female sex (vs male)	1.89	1.00–3.58	0.051
Multifocal lesions (vs solitary)	1.53	0.53–4.38	0.433
Areca nut / betel quid use	1.16	0.69–1.97	0.576
Alcohol use	1.37	0.76–2.48	0.293
Site: tongue / floor of mouth	1.93	0.98–3.78	0.056
Site: multifocal	1.30	0.25–6.75	0.757
Site: labial mucosa / commissure	0.80	0.28–2.34	0.686
Site: other	0.81	0.38–1.71	0.577

OR, odds ratio; CI, confidence interval. Reference category for site is buccal mucosa. McFadden pseudo $R^2 = 0.221$; Hosmer–Lemeshow p = 0.066; area under the curve 0.805. All variance inflation factors < 1.6.

Table 5. Multivariable logistic regression model for high-grade epithelial dysplasia (n = 330; 40 events).

Predictor	Adjusted OR	95% CI	p
Lesion size, per 10 mm increase	3.73	2.00–6.97	<0.001
Non-homogeneous subtype (vs homogeneous)	3.28	1.24–8.70	0.017
Age, per 10-year increase	1.96	1.31–2.93	0.001
Site: multifocal	3.81	0.77–18.79	0.100
Site: tongue / floor of mouth	1.01	0.39–2.62	0.992
Site: other	0.85	0.27–2.62	0.771
Site: labial mucosa / commissure	0.38	0.04–3.48	0.393

Reference category for site is buccal mucosa. Model restricted to four predictors to respect the events-per-variable constraint. McFadden pseudo $R^2 = 0.314$; Hosmer–Lemeshow p = 0.446; area under the curve 0.867.

Table 6. Concordance between habitual quid placement side and lesion laterality among patients with a unilateral buccal lesion and a unilateral habit (n = 94).

Habitual quid placement side	Lesion on right	Lesion on left	Total
Right	52	3	55
Left	1	38	39
Total	53	41	94

Concordance 90/94 = 95.7% (95% CI 89.6–98.3). Fisher's exact p < 0.001; Cohen's kappa 0.91.

4. DISCUSSION

In this cross-sectional study of 330 patients with oral leukoplakia drawn from an areca-nut-endemic population, epithelial dysplasia was present in 40.3% of lesions and high-grade dysplasia in 12.1%. Lesion size and non-homogeneous clinical appearance were the strongest independent determinants of both outcomes, with increasing age and tobacco use contributing further. The apparent excess risk associated with tongue and floor-of-mouth location, evident on crude analysis, was largely accounted for by the larger size and greater proportion of non-homogeneous lesions at those sites. Grading reproducibility was substantial under both the three-tier and binary systems, with disagreement concentrated almost entirely at the threshold between reactive keratosis and mild dysplasia. Finally, in patients with a unilateral quid habit, the lesion arose on the habitual placement side in 95.7% of cases.

4.1 Clinical subtype

The association between non-homogeneous appearance and dysplasia observed here accords with the established literature. Non-homogeneous leukoplakia has long been regarded as the higher-risk clinical phenotype, and systematic reviews report substantially higher transformation rates for non-homogeneous than for homogeneous lesions [2,5,6]. Our crude odds ratio of 5.58 for the presence of dysplasia, and 10.14 for high-grade dysplasia, lies within the range reported in comparable hospital-based series [13,24,25]. That these ratios attenuate to 2.45 and 3.28 respectively on adjustment is itself informative: a substantial part of the crude association is attributable to the fact that non-homogeneous lesions are also larger and occur in older patients. Studies reporting only unadjusted comparisons are therefore likely to overstate the independent contribution of clinical appearance.

Within the non-homogeneous group, nodular lesions showed the highest prevalence of dysplasia and verrucous lesions the second highest, with speckled lesions lowest. This ordering runs counter to some reports in which erythroleukoplakic change is regarded as the most ominous clinical sign [7,13]. The differences between variants were not statistically significant in our data, and with 25 to 49 lesions in each subgroup the study was not powered to resolve them; we regard this as a hypothesis-generating observation requiring confirmation in larger series.

4.2 Lesion size as the dominant predictor

The most consistent finding across both models was the gradient of risk with lesion size, which conferred an adjusted odds ratio of 2.43 per 10 mm for any dysplasia and 3.73 per 10 mm for high-grade dysplasia, and which alone discriminated better than clinical subtype alone. Lesion size has been reported as a predictor of malignant transformation in several cohorts, with lesions exceeding 200 mm^2 carrying increased risk [24,26,27], but it is frequently omitted from analyses that focus on subtype and site. Our findings suggest that size deserves at least equal emphasis. Size is trivially measurable at the chairside, requires no special equipment or training, and in our data carried more information than the anatomical subsite at which the lesion arose.

The size thresholds identified by the Youden index, 21 mm for any dysplasia and 33 mm for high-grade dysplasia, should be interpreted cautiously. They were derived and evaluated in the same dataset and are therefore optimistically biased, and the sensitivity at the high-grade threshold was only 60%. They are offered as a descriptive characterisation

of the size gradient rather than as a clinical decision rule, which would require derivation and validation in independent samples.

4.3 Anatomical site: intrinsic susceptibility or carcinogen contact?

The site distribution in this cohort differs from that typically reported in Western series, in which the floor of mouth and ventrolateral tongue predominate and carry the highest risk [7,14,15]. In our population the buccal mucosa accounted for half of all lesions, consistent with other South Asian cohorts and with the mechanics of areca nut and smokeless tobacco use [17,18,28]. Tongue and floor-of-mouth lesions were nonetheless disproportionately dysplastic on crude analysis, mirroring the conventional high-risk-subsite designation.

The attenuation of that association after adjustment for lesion size and clinical subtype suggests that the high-risk status of these subsites in our data operates substantially through lesion morphology rather than independently of it. This does not exclude a genuine biological contribution; the adjusted estimate of 1.93 remained close to conventional significance, and with a wider sample the effect might well be resolved. It does, however, caution against treating anatomical site as an independent risk marker without accounting for the morphological features that accompany it.

The concordance between lesion laterality and habitual quid placement side offers a complementary line of evidence. Among patients with a unilateral habit, 95.7% of unilateral buccal lesions arose on the habitual side, with a kappa of 0.91; among those with bilateral or alternating habits no lateral predilection was seen. Laterality of the buccal mucosa carries no plausible intrinsic biological difference, so this near-perfect correspondence is most readily explained by localised carcinogen contact. To our knowledge this relationship has not previously been quantified within a single cohort, although the clinical association between quid placement and lesion site has long been recognised descriptively [17,29]. The implication is that in areca-nut-endemic populations, lesion site should be read in the first instance as a record of where the carcinogen has been held, and the Western high-risk-subsite hierarchy should not be transplanted uncritically.

4.4 Habit exposure

No individual habit was associated with dysplasia on univariate analysis, and only composite tobacco use reached significance in the adjusted model. Two features of the cohort explain this apparent paradox. First, 90.3% of patients reported at least one habit, so the unexposed comparison group was small and heterogeneous, limiting power to detect exposure effects. Second, and more fundamentally, all patients in this series already had leukoplakia; the analysis therefore asks whether habit predicts dysplasia among those who have already developed a lesion, not whether habit causes leukoplakia. The causal role of areca nut and tobacco in the genesis of oral potentially malignant disorders is well established from population-based studies and is not contradicted by these findings [17,18,30,31].

The higher prevalence of dysplasia among former habit users (61.2%) than among current users (36.5%) deserves comment and is most plausibly explained by reverse causation. Patients are commonly counselled to cease all habits at the time a suspicious lesion is identified, so former status may in part be a consequence of a more alarming clinical presentation rather than a determinant of it. Former users may also have longer cumulative exposure histories. This finding should not be read as implying that cessation is harmful, and we would caution against reporting such associations from cross-sectional data without this qualification.

4.5 Reproducibility of grading

Inter-observer agreement in our series was substantial and rather better than that reported in several earlier studies, in which kappa values for three-tier grading have frequently fallen between 0.2 and 0.5 [8,9,32]. Two factors may contribute: both observers were consultant oral pathologists working within the same department and diagnostic tradition, which tends to inflate agreement relative to multi-institutional studies, and the linear weighting we applied credits partial agreement between adjacent grades. The unweighted kappa of 0.71 remains within the substantial range.

The distribution of disagreements is instructive. Eleven of twelve discordant cases lay at the boundary between no dysplasia and mild dysplasia, a threshold of limited clinical consequence given that both categories fall within the low-grade binary category and attract similar management. By contrast, agreement on the binary grade, which governs clinical decision-making, was 93.9%. The observation that the binary and three-tier systems were not interchangeable in our data, with moderate lesions dividing almost evenly between binary categories, supports the argument that binary grading captures information not recoverable by mechanical recoding of the three-tier grade [10,11]. This has a practical corollary: studies that derive a binary grade by collapsing an existing three-tier grade are not measuring the same construct.

4.6 Clinical implications

Both models discriminated well, with areas under the curve of 0.805 and 0.867. Clinical assessment therefore stratifies risk usefully in this population: a large, non-homogeneous lesion in an older patient carries a substantially elevated probability of dysplasia and warrants prompt biopsy and close surveillance. The corollary is more important and less comfortable. Even in the better-performing model, discrimination was insufficient to justify withholding biopsy from clinically reassuring lesions. Dysplasia was present in 26.4% of homogeneous lesions and high-grade dysplasia in 3.7%, so a policy of observing homogeneous plaques without histological confirmation would leave a material proportion of dysplastic lesions undiagnosed. The appropriate reading of these data is that clinical features should be used to prioritise the order and urgency of biopsy where capacity is constrained, not to decide whether biopsy is required at all.

4.7 Strengths and limitations

The strengths of this study include a consecutive sample of moderate size, prespecified use of the current WHO binary grading system assigned independently of the three-tier grade, formal quantification of inter-observer agreement with bootstrap confidence intervals, multivariable adjustment with explicit attention to the events-per-variable constraint, a prespecified rule for handling sparse categories, and reporting in accordance with STROBE.

Several limitations temper the interpretation. First and most importantly, the cross-sectional design means dysplasia grade is a surrogate for malignant potential rather than a measured outcome; no inference about malignant transformation can be drawn from these data, and a prospective cohort with adequate follow-up would be required for that purpose. Second, the study was conducted at a single tertiary centre, and referral patterns are likely to have enriched the sample for advanced or persistent lesions, limiting generalisability to community or screening settings. Third, habit history was self-reported and abstracted from routine clinical records, so recall bias and under-documentation are probable, and the exposure measures available were cruder than a formally quantified index; habit duration and frequency were missing for 9.7% of patients.

Fourth, the secondary model was restricted to four predictors because only 40 high-grade events were available, so residual confounding by sex, habit exposure or lesion number cannot be excluded from those estimates, and the confidence intervals are correspondingly wide. Fifth, the biopsy site within each lesion was not standardised; because dysplasia is distributed heterogeneously within a lesion, a single incisional biopsy may underestimate the highest grade present, and this may partly explain the association between lesion size and grade if larger lesions were sampled more representatively. Sixth, quid placement side was documented in 269 of 330 patients and the concordance analysis was confined to the 94 with both a unilateral habit and a unilateral lesion, so selection bias cannot be excluded if documentation was related to the clarity of the clinical picture. Finally, both models were evaluated in the data from which they were derived; the reported discrimination is therefore optimistic, and external validation is needed before any predictive use.

4.8 Future directions

The natural extension of this work is a prospective cohort in the same population with standardised biopsy protocols, quantified exposure indices and follow-up of sufficient duration to capture malignant transformation, which would allow the predictors identified here to be tested against the outcome that matters clinically. Systematic documentation of quid placement side and lesion laterality would permit the concordance finding to be confirmed and its implications for surveillance explored. A clinical risk score combining lesion size, clinical subtype and age, derived and then validated in independent samples with attention to calibration as well as discrimination, would be of practical value in settings where access to histopathology is limited. Correlation with molecular markers of progression, and with the evolving criteria for oral epithelial dysplasia in the fifth edition of the WHO classification, would further clarify how far chairside features track the underlying biology [11,33,34].

5. CONCLUSION

In this cross-sectional study of 330 patients with oral leukoplakia from an areca-nut-endemic region, lesion size and non-homogeneous clinical appearance were the strongest independent determinants of epithelial dysplasia and of high-grade dysplasia, with increasing age contributing further. The apparent excess risk of tongue and floor-of-mouth lesions was largely explained by their larger size and more frequent non-homogeneity, and lesion laterality corresponded almost perfectly to habitual quid placement side, indicating that anatomical site in this population functions substantially as a marker of local carcinogen contact. Clinical assessment stratifies risk usefully and should guide the urgency of biopsy, but it does not discriminate sharply enough to justify withholding histological confirmation from clinically reassuring lesions.

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