Basement membrane degeneration is common in lichenoid mucositis with dysplasia

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PRACTICAL IMPLICATIONS OF THIS RESEARCH

- Dental hygienists have specific training to assess oral tissues and to differentiate between normal, healthy soft tissue and abnormal or diseased tissues.
- These assessments allow for the collection of pertinent information, such as risk habit and lesion descriptions, which should be documented and provided to pathologists along with biopsies to rule out erroneous diagnoses.
- Dysplasia seen in lichenoid lesions possesses a risk of progression to a malignancy and should be biopsied and monitored accordingly.

ABSTRACT

Background: Two subtypes of lichenoid mucositis (LM) with oral epithelial dysplasia have been proposed, with differing risks of malignant transformation. However, no research has been done to authenticate this hypothesis. The study objective was to determine whether there are 2 subcategories within this entity, one with primary lichenoid and secondary dysplastic features (L_1D_2), and the other with primary dysplastic and secondary lichenoid features (D_1L_2), and to compare the proportion of malignant progression in these groups. **Methods:** Patients with a diagnosis of lichenoid mucositis with low-grade (mild/moderate) oral epithelial dysplasia, no history of head and neck cancer, and who had at least 5 years of follow-up were eligible to participate in this nested case–control study. Cases (n = 10) were defined as lesions that progressed to severe dysplasia, carcinoma in situ or squamous cell carcinoma; controls (n = 32) were defined as those that did not progress. Immunohistochemistry was performed to assess for basement membrane (BM) degeneration using collagen IV—an integral BM protein. **Results:** Lesions that progressed to cancer exhibited a similar proportion of BM degeneration at baseline (70%) compared to non-progressors (78%), with no statistically significant difference between groups ($p = 0.69$). **Conclusion:** BM degeneration is frequently seen in LM with dysplasia and alone does not appear to be a predictor of malignant progression in lesions with both lichenoid and low-grade dysplastic features. Dysplasia should not be discounted in the presence of LM. Lesions that display any degree of dysplasia warrant clinical follow-up and continued monitoring.

RÉSUMÉ

Contexte : Deux sous-types de mucosites lichénoïdes (ML) avec dysplasie épithéliale buccale ont été proposés, avec des risques différents de transformation maligne. Cependant, aucune recherche n'a été faite pour valider cette hypothèse. L'objectif de l'étude était de déterminer s'il y a 2 sous-catégories au sein de cette entité, la première avec des caractéristiques lichénoïdes primaires et dysplasiques secondaires (L_1D_2), et l'autre avec des caractéristiques dysplasiques primaires et lichénoïdes secondaires (D_1L_2), et de comparer la proportion de progression maligne dans ces groupes. **Méthodologie :** Les patients ayant reçu un diagnostic de mucosite lichénoïde avec une dysplasie épithéliale buccale de faible intensité (faible/modérée), qui n'avaient aucun antécédent de cancer de la tête et du cou, et qui avaient eu au moins 5 ans de suivi, étaient admissible à participer à cette étude de cas-témoins emboîtés. Les cas (n = 10) étaient définis comme des lésions qui ont progressé à la dysplasie sévère, un carcinome in situ ou un carcinome squameux; les contrôles (n = 32) étaient définis comme ceux qui n'ont pas progressé. L'immunohistochimie a été effectuée pour évaluer s'il y avait eu une dégénérescence de la membrane basale (MB) en utilisant du collagène IV, une protéine MB intrinsèque. **Résultats :** Les lésions qui ont évolué en cancer ont présenté une proportion semblable de dégénérescence de MB au début (70 %) par rapport aux non-progresseurs (78 %), et aucune différence statistiquement significative entre les groupes ($p = 0,69$). **Conclusion :** La dégénérescence des MB est fréquemment constatée dans les ML avec dysplasie et seule, ne paraît pas être une variable explicative de l'évolution maligne dans les lésions à caractéristiques à la fois lichénoïdes et dysplasiques de faible intensité. Il ne faut pas sous-estimer la dysplasie en présence de ML. Les lésions qui présentent de la dysplasie, peu importe son étendue, exigent un suivi clinique et une surveillance continue.

Keywords: basement membrane; collagen type IV; epithelial neoplasms; leukoplakia; oral epithelial dysplasia; oral lichen planus; oral pathology; precancerous conditions

CDHA Research Agenda category: risk assessment and management

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INTRODUCTION

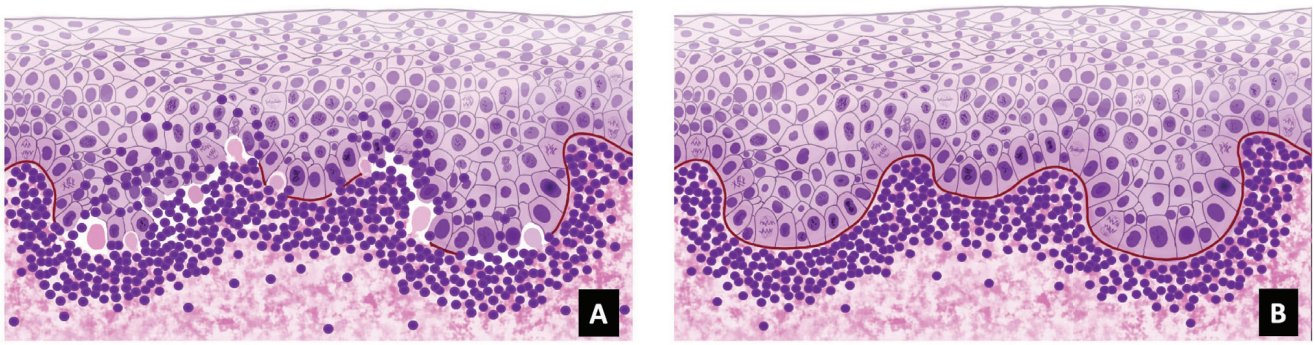
Mucosal lesions are frequently identified by frontline dental professionals at routine dental visits. Lichenoid mucositis (LM) refers to a group of mucosal lesions (including oral lichen planus [OLP] and oral lichenoid lesions [OLL] from contact with dental materials or drug reactions) that exhibit a band-like infiltrate of lymphohistiocytic inflammatory cells in the connective tissue directly beneath the epithelium.[1-3] Degeneration of the basal cell layer and basement membrane (BM) and migration of the inflammatory cells into the epithelium are other histological features common to LM.[2,4]

Oral epithelial dysplasia is a condition recognized as potentially malignant by the World Health Organization (WHO) and remains the best-known precursor lesion to oral carcinoma.[5-7] OLP is also categorized as potentially malignant by WHO, however this label is not as widely accepted.[7-9] The controversy around OLP stems from the lack of stringent, standardized reporting criteria in the literature analysing malignant transformation of this condition, the variability in diagnostic criteria used, and misdiagnosis with other conditions resembling OLP.[10,11] Some believe that OLP does not have inherent malignant predisposition, pointing to evidence drawn from retrospective analyses of OLP cases that progressed to cancer, many of which exhibited dysplasia in the initial biopsy or occurred in patients with a known history of exposure to carcinogens (i.e., tobacco use).[12-14] They hypothesize that only lichenoid lesions also exhibiting dysplasia, termed "lichenoid dysplasia," have malignant risk.

The label "lichenoid dysplasia" was first introduced in 1985 by Krutchkoff and Eisenberg[13] to describe conditions in which there is a concurrent presence of the microscopic features of lichenoid lesions, defined above, and dysplasia, within a lesion biopsy. However, in these cases, it is not always clear whether the dysplasia is a primary event, reflecting true clonal change with malignant risk, which then triggers a protective, inflammatory response, or a secondary event, as a reactionary response to the heavy inflammation. Thus, Raj et al.[15] have proposed a classification schema to differentiate the primary and secondary pathology for lesions with both lichenoid changes and dysplasia (Figure 1). They propose 2 subcategories of lesions with both lichenoid changes and dysplasia, with the status of the basal layer being the distinguishing feature between the 2 groups: primary LM with secondary dysplasia (L_1D_2) versus primary dysplasia with secondary lichenoid reaction (D_1L_2).[15,16] In L_1D_2, the hallmark inflammatory component represents a T-lymphocyte-mediated immune reaction to antigens expressed by the basal cells, leading to destruction of the BM (breakage of BM) and destruction (absence) or degeneration of the basal layer.[17] Thus, Raj et al. hypothesize that a primary lichenoid lesion will exhibit breakage of the BM and basal layer destruction or degeneration, and have a lesser risk of subsequent progression to cancer, as the dysplasia is secondary. In contrast, in D_1L_2, although the immune infiltrate will consist mostly of lymphocytes, and clinically the lesion could have a reticular appearance, the BM breakage may be absent since dysplasia is the primary event.

However, no research has been done to validate this classification schema. Since the proposed dividing line of the schema is the presence or absence of breakage of the BM, the identification of such breakage should shed light on the schema, i.e., D_1L_2 would show no BM breakage but a higher malignant transformation, whereas L_1D_2 would demonstrate BM breakage and lower malignant transformation. It is possible to visualize the epithelial–connective tissue interface, which is separated by the BM, a thin and dense sheet of interlocking collagen and protein fibers, using immunohistochemistry (IHC), a widely-used technique to stain for antigens of interest.

Figure 1. Proposed subtypes of lichenoid mucositis with dysplasia



(A) lichenoid features are primary and dysplasia is secondary; **(B)** dysplasia is primary and the lichenoid infiltrate is secondary. The difference between them is the integrity of the epithelial–connective tissue interface.

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If the presence or absence of BM breakage can classify lesions with both lichenoid changes and dysplasia into 2 lesion groups with differing risks of progression to cancer, the ability to identify and sort these lesions by subtype could have important clinical implications. The greatest predictor of oral cancer mortality and morbidity is late-stage diagnosis[18]; if lesions at risk can be identified at the premalignant stage, treatment and management approaches can be implemented early, improving patient outcomes and quality of life, and aiding in the efficient use of health services and resources. The objective of this study was to determine whether collagen IV, an integral and abundant BM component,[19] can be used to visualize the BM in lesions with both lichenoid and dysplastic features, and whether its breakage is associated with the risk of malignant progression.

METHODS

Study design and population

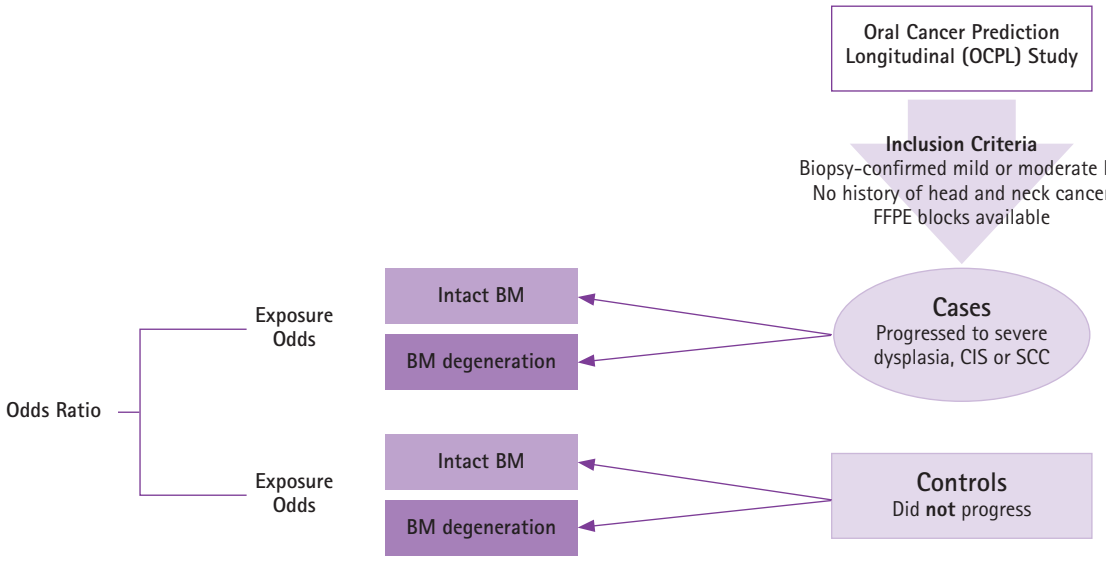
This nested case–control study design used previously collected tissue samples and data from participants in the Oral Cancer Prediction Longitudinal (OCPL) study that has been prospectively following patients from across British Columbia, Canada, with biopsy-confirmed low-grade (mild or moderate) dysplasia for more than 20 years (Figure 2). Participants in the OCPL study were identified through a centralized, population-based biopsy service—the BC Oral Biopsy Service—where community dentists and specialists across British Columbia (population 5.1 million in 2019) send biopsies for histological diagnosis. Patients with a histologically confirmed diagnosis of mild or moderate oral epithelial dysplasia (regardless of whether there is accompanying LM) were referred by community clinicians, upon recommendation from the Oral Biopsy Service, for follow-up in oral dysplasia clinics, where they were invited to participate in the OCPL study and were recruited to the study using written informed consent. Patients were followed at 6-month intervals, which allowed the study to accrue an extensive bank of tissue samples with associated demographic, clinicopathological, histological, and outcome data. Details about the OCPL cohort recruitment and participant follow-up have been published elsewhere.[20,21] Study protocol and ethical approval was obtained from the University of British Columbia BC Cancer Research Ethics Board (REB# H19-01785).

Selection of cases and controls

Sample size calculation was based on an unmatched samples design with a ratio of 3 controls to 1 case, a significance level of 5% and 80% power on 2-tailed tests (OpenEpi® Version 3.01 software).[22] As there is no previously published literature investigating this research question, sample size calculations were based on a hypothetical proportion of controls and cases with 10% and 50% exposure, respectively.

Patients with a biopsy-confirmed diagnosis of LM with low-grade (mild or moderate) dysplasia, at least 5 years of follow-up, and available formalin-fixed paraffin-embedded (FFPE) tissue sections were eligible to participate in this study. The histological diagnosis was obtained from the hospital pathology report and was confirmed by the study oral pathologist (LZ), who reviewed all slides

Figure 2. Study design



The present study employed a nested case–control design. Participants in the Oral Cancer Prediction Longitudinal (OCPL) study who met the inclusion criteria were included. Cases were lesions that exhibited both lichenoid mucositis and low-grade (mild/moderate) oral epithelial dysplasia that progressed to either severe dysplasia, carcinoma in situ (CIS) or squamous cell carcinoma (SCC). Controls were lesions that exhibited both lichenoid mucositis and low-grade (mild/moderate) oral epithelial dysplasia and did not progress after a minimum of 5 years of follow-up. Basement membrane (BM) integrity was assessed via immunohistochemistry staining of collagen IV.

using WHO histopathologic criteria for the diagnosis of dysplasia[23] and microscopic analysis for features of LM using criteria published by authorities in the field.[2,4,24] If the study pathologist's diagnosis differed from the diagnosis of the signing pathologist, discrepancies were resolved through dialogue between the pathologists. Patients with previous history of head and neck cancer were excluded from enrollment. Cases were defined as biopsy-confirmed LM with low-grade dysplasia that progressed to severe dysplasia, carcinoma in situ (CIS) or squamous cell carcinoma (SCC); controls were defined as biopsy-confirmed LM with low-grade dysplasia that did not progress to severe dysplasia, CIS or SCC after a minimum of 5 years of follow-up (no progression). A unique study ID containing no personal identifiers was assigned to each subject and used to label tissue slides. All study personnel were blinded to subject status as case or control through the entirety of the experimental and interpretation portions of the project.

Immunohistochemistry (IHC)

The BM was delineated through IHC staining of type IV collagen using monoclonal mouse anti-collagen IV antibodies (clone CIV22, Dako) on FFPE tissue. This antibody specifically binds to collagen IV in its native conformation, allowing for visualization of breaks, injury, and degeneration of the BM. The IHC protocol and antibody dilutions were optimized.

FFPE tissue blocks were cut into 4- to 5-micron sections and mounted on positively charged glass slides. Sections were deparaffinized and rehydrated in xylene and graded alcohol solutions before proteolytic-induced epitope retrieval using proteinase K was carried out. Endogenous peroxidase was blocked using hydrogen peroxide and non-specific background staining was reduced using a protein block solution. Anti-Collagen IV primary antibody (clone CIV22, Dako) in antibody diluent was applied and slides were incubated overnight at 4°C. Chromogenic signal amplification and visualization were obtained using the micropolymer Mouse and Rabbit Specific HRP/DAB IHC Detection Kit (Abcam). In brief, secondary anti-mouse antibodies conjugated to horseradish peroxidase (HRP) were applied, and visualization was achieved by incubating with 3,3'-diaminobenzidine (DAB) for 9 minutes. Washing between steps was completed using phosphate-buffered saline solution. Slides were then counterstained with hematoxylin, dehydrated, and cover slipped. Staining of normal oral mucosa was performed as a positive control, and negative control slides were created by omission of primary antibodies.

The stained slides were viewed under 400X magnification using a Zeiss Axioscope light microscope. BM integrity was assessed independently by 2 blinded clinicians, and scoring discrepancies were resolved collaboratively. An intact BM was defined as a linear, continuous band of collagen IV positivity in the epithelial–connective tissue interface. BM degeneration was defined as a staining result showing any break in collagen IV positivity in the epithelial–connective tissue interface.

Statistical analysis

Data were analysed using SPSS® Version 25.0 (Armonk, NY: IBM Corp) statistical software package. To ensure that the cases and controls were comparable, demographic (age at diagnosis, sex, and ethnicity), tobacco history, and clinical variables (risk of lesion site and grade of dysplasia) were assessed for significant differences between groups (progression/no progression) using a Chi-square analysis. The Fisher's Exact Test was used when more than 20% of cells contained expected frequencies of <5. Logistic regression was used to assess whether BM integrity predicted outcome, and the odds ratio with corresponding 95% confidence intervals was reported. The threshold for significance was set at $p < 0.05$ and all statistical tests were 2-tailed.

RESULTS

Forty-two samples were included in the study: 10 cases (progressed to severe dysplasia, CIS or SCC) and 32 controls (did not progress after a minimum of 5 years of follow-up). A comparison between groups showed no significant differences in age, sex, smoking history, and lesion site. Differences in grade of dysplasia was approaching significance ($p = 0.06$), with a greater proportion of progressors exhibiting moderate dysplasia (60%) than non-progressors (25%) (Table 1). The median length of follow-up differed between the groups ($p = 0.001$), with a longer average length of follow-up for non-progressors (85.8 months) compared to progressors (51.2 months).

Immunohistochemistry protocols for collagen IV resulted in light to dark brown staining along the epithelial–connective tissue interface (Figure 3). The results of BM analysis are shown in Table 2. Interestingly, 76% of all samples (n = 32) exhibited a broken BM (n = 25 [78%] non-progressors; n = 7 [22%] progressors), while only 10 samples (24%) presented with an intact BM (n = 7 [70%] non-progressors; and n = 3 [30%] progressors). However, the differences in BM integrity between groups failed to reach statistical significance ($p = 0.69$; OR 1.53; 95% CI = 0.3 to 7.5).

DISCUSSION

Basement membrane disruption is a common feature in lichenoid mucositis with dysplasia

The results indicated that BM disruption was a common feature in our study set—lesions showing both low-grade dysplasia and lichenoid changes—and was not by itself associated with malignant progression. Though this is the first study to investigate BM integrity in LM with dysplasia, previous research has explored this question in OLP. Researchers have found that BM degeneration is frequently seen in OLP, which is consistent with its pathogenesis.[25,26]

Table 1. Participant demographics

Total	All (%) N = 42	No progression[a] (%)[b] n = 32	Progression[c] (%)[b] n = 10	*p* value
Length of follow-up[d]; median months (range)	78.5 (16.9 to 156.3)	85.8 (28.3 to 156.3)	51.2 (16.9 to 115.8)	0.001[e]
Age at diagnosis Mean (years ± SD)	58.3 ± 12.9	58.2 ± 11.4	58.3 ± 9.1	0.98[e]
Age category				
<60 years	25 (60)	18 (56)	7 (70)	0.49[f]
>60 years	17 (40)	14 (44)	3 (30)	
Sex				
Male	13 (31)	11 (34)	2 (20)	0.47[f]
Female	29 (69)	21 (66)	8 (80)	
Smoking history[g]				
Never	19 (45)	14 (44)	5 (50)	1.00[f]
Ever	23 (55)	18 (56)	5 (50)	
Risk of lesion site[h]				
Low risk	10 (24)	9 (28)	1 (10)	0.40[f]
High risk	32 (76)	23 (72)	9 (90)	
Grade of dysplasia				
Mild	28 (67)	24 (75)	4 (40)	0.06[f]
Moderate	14 (33)	8 (25)	6 (60)	

[a]No progression = no progression to severe dysplasia, carcinoma in situ or squamous cell carcinoma after a minimum of 5 years of follow-up.
[b]Column percentage reported.
[c]Progression = progression to severe dysplasia, carcinoma in situ or squamous cell carcinoma.
[d]Months to last follow-up or progression, whichever occurred first.
[e]Independent samples T-test was used. Statistical test was 2-tailed.
[f]Fisher's Exact Test was used. Statistical test was 2-tailed.
[g]Never smoker <100 cigarettes in lifetime; Ever smoker >100 cigarettes in lifetime.
[h]High risk = floor of mouth, soft palate, and tongue; Low risk = all other sites.

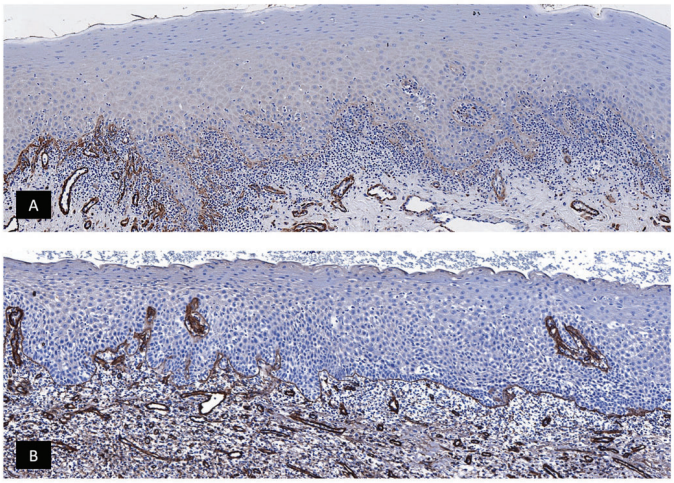
Though the antigen or antigens responsible for triggering the disease process are unknown, the basal epithelial cells appear to be the primary target of the activated inflammatory mediators, of which T-cells predominate.[27,28] These inflammatory cells release enzymes, such as matrix metalloproteinases and proteases, which cleave and degrade the BM.[28,29] Cytotoxic T-cells are seen to migrate through BM breaks to enter the epithelium, where they are believed to facilitate basal cell apoptosis.[30] Conversely, the destruction or degeneration of the basal epithelial cells, which contribute to the structure of the BM through secretion of BM components, further disrupts BM integrity.[31]

The nature of dysplasia in lichenoid lesions

This intense, chronic inflammatory environment and the simultaneous initiation of wound-healing mechanisms in OLP have been hypothesized to contribute to malignant risk[28]; reactive oxygen and nitrogen species released may lead to oxidative stress in the tissue and DNA damage.[32,33] Coupled with the increase in cellular turnover in the inflammatory and wound-healing processes, there may be risks not only of acquiring cellular mutations, but also for the expansion and survival of these mutated clones.[34-36] However, despite circumstances seemingly conducive to carcinogenesis, studies investigating the molecular events and pathways in OLP malignant transformation have not offered consistent or firm conclusions.[37,38]

In contrast, molecular studies have provided evidence to support the premalignant potential of LM with dysplasia. Using microsatellite analysis, researchers have looked for the loss of key chromosomes that contain tumour suppressor genes—a type of genetic change that is often seen in early carcinogenesis and which is a validated predictor of malignant progression for low-grade dysplasia.[21] They found that dysplastic lichenoid lesions exhibited a high frequency of these genetic changes, termed "loss of heterozygosity" (LOH), with values similar to those found in dysplastic lesions without lichenoid inflammation.[39] One of the key genes in the chromosome arms analysed in this molecular risk-prediction model is *p53*, which codes for a protein that regulates the cell cycle and DNA repair. Mutant *p53* or impairment of this gene has been associated with the development and progression of many types of cancer.[40] Researchers have investigated the expression of *p53*, as well as survivin, which triggers anti-apoptotic pathways, and found that expression levels of both proteins were greater in dysplasia with lichenoid features than in OLP, oral lichenoid reaction, and normal oral mucosa, and were

Figure 3. Microphotographs of lesions exhibiting both lichenoid mucositis and dysplasia visualized via collagen IV immunohistochemistry



(A) lesion with basement membrane degeneration; **(B)** lesion with an intact basement membrane

in the range of that expressed in epithelial dysplasia.[41,42] These conclusions were reinforced by a longitudinal study previously conducted by our group, which found no statistically significant difference between the rate, speed, and proportion of malignant progression in dysplasia with lichenoid infiltration versus dysplasia without lichenoid infiltration.[43] Taken together, these studies provide evidence that presence of dysplasia is indicative of malignant risk, irrespective of the presence of inflammation. Our current study supports these findings, with a greater proportion of cases progressing to malignancy exhibiting a moderate (versus mild) grade of dysplasia.

Strengths and limitations of the study

The strength of this research lies in the fact that it is nested within the OCPL study—a unique longitudinal cohort study in which participants are community based, rather than drawn from a high-risk population. This is the largest longitudinal study conducted to date with long-term patient follow-up and known clinical outcome. This is important as malignant progression is a relatively rare event and may take many years to occur.[44] LM with dysplasia is also a relatively rare diagnosis, thus using a case–control study allowed us to account for these factors. However, bias is an inherent limitation of all case–control studies, which being retrospective, cannot establish cause and effect. The small sample size and failure to meet sample size requirements due to scarcity of cases are additional limitations of this study, increasing the risk that a type II error may have occurred. Although we cannot make firm conclusions regarding the existence of L_1D_2 and D_1L_2 subtypes and associated malignant risks, the findings of this study provide early clues that can inform future research and introduce new questions for investigation to shed light onto this enigmatic pathology. To determine whether cases of LM with dysplasia represent primary dysplasia with a subsequent inflammatory response, or primary OLP that undergoes dysplastic change, prospective study designs with clear diagnostic criteria are needed. Additionally, such studies should aim to incorporate clinicopathological data including data on new habits, medications, new food, gum, toothpaste, and dental restorative materials (lichenoid contact reaction). In the future, larger studies incorporating molecular analyses may aid in the subtyping of LM with dysplasia and provide greater insight into its potential for malignant progression.

Translational impact

Dysplasia seen in lichenoid lesions, regardless of BM integrity, is at risk of malignant transformation. It should be monitored, and upon any clinically significant change, a comparative biopsy should be performed. Frontline dental professionals, including dental hygienists, may encounter mucosal lesions at routine dental visits. Dental hygienists have the specific training to assess oral tissues and to differentiate between normal, healthy soft tissue and abnormal or diseased tissue.[45] The dental hygiene appointment is naturally predisposed to oral cancer screening as a part of routine dental hygiene care.[46] Practice standards dictate that dental hygienists have a professional responsibility to conduct systematic,

Table 2. Basement membrane integrity and malignant outcome

Characteristic	All (%)	Basement membrane		*p* value	Odds ratio (95% CI)
		Broken (%[a])	Intact (%[a])		
No progression[b]	32 (76)	25 (78)	7 (70)	0.69[d]	1
Progression[c]	10 (24)	7 (22)	3 (30)		1.53 (0.3 to 7.5)
Total	42 (100)	32 (100)	10 (100)		

[a]Column %
[b]No progression = no malignant progression after a minimum of 5 years of follow-up.
[c]Progression = progression to severe dysplasia, carcinoma in situ or squamous cell carcinoma.
[d]Fisher's Exact Test was used. Statistical test was 2-tailed.

comprehensive hard and soft tissue assessments of the head, neck, and oral cavity.[46] The documentation of medical history, modifiable risk factors (i.e., tobacco and alcohol consumption), and lesion description (i.e., site, symptoms, duration, dimensions, colour, margins, and appearance) not only serves as a part of a client's records and provides evidence of lesion changes over time, but is also important information for histological diagnosis and risk assessment, ensuring that clients receive appropriate care. Interprofessional health care collaboration is a key area of responsibility that dental hygienists must fulfill in the process of client care. Dental professionals have an opportunity to facilitate early detection and thus early intervention, which is a means to significantly reduce the morbidity and mortality of oral cancer. In addition to referring any new, worrisome lesions for biopsy, dental hygienists should evaluate lesions that have had a previous histological diagnosis of lichenoid mucositis with any degree of dysplasia at every recare visit and re-refer for a comparative biopsy, along with relevant documentation, upon any significant clinical change.

CONCLUSION

Based on the sample size and statistical analysis in this study, BM breakage is frequently seen in LM with dysplasia (regardless of whether dysplasia is a primary or secondary event) and alone does not appear to be a predictor of malignant progression in lesions with both lichenoid and low-grade dysplastic features. The presence of strikingly lichenoid features within a histological section of LM with dysplasia may lead to a misdiagnosis of OLP or OLL. Yet, as the evidence shows, dysplasia in these lesions does have malignant risk. Careful histologic examination of these lesions, as well as consideration of risk habit engagement, history, and lesion clinical presentation, are important when making a diagnosis. Lesions that demonstrate any degree of dysplasia upon biopsy warrant careful clinical follow-up with continued monitoring.

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CONFLICTS OF INTEREST

The authors declare no known conflicts of interest.

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