Assessing Oral Epithelial Dysplasia Risk for Transformation to Cancer: Comparison Between Histologic Grading Systems Versus S100A7 Immunohistochemical Signature-based Grading

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Abstract: While a 3-tier oral epithelial dysplasia grading system has been utilized for decades, it is widely recognized as a suboptimal risk indicator for transformation to cancer. A 2-tier grading system has been proposed, although not yet validated. In this study, the 3-tier and 2-tier dysplasia grading systems, and an S100A7 immunohistochemical signature-based grading system were compared to assess prediction of risk of transformation to oral cancer. Formalin-fixed, paraffin-embedded biopsy specimens with known clinical outcomes were obtained retrospectively from a cohort of 48 patients. Hematoxylin and eosin-stained slides were used for the 2- and 3-tier dysplasia grading, while S100A7 for biomarker signature-based assessment was based on immunohistochemistry. Inter-observer variability was determined using Cohen's kappa (*K*) statistic with Cox regression disease free survival analysis used to determine if any of the methods were a predictor of transformation to oral squamous cell carcinoma. Both the 2- and 3-tier dysplasia grading systems ranged from slight to substantial inter-observer agreement (*Kw* between 0.093 to 0.624), with neither system a good predictor of transformation to cancer (at least $P = 0.231$; ($P > > > 0.05$). In contrast, the S100A7 immunohistochemical signature-based grading system showed almost perfect inter-observer agreement ($Kw = 0.892$) and was a good indicator of transformation to cancer ($P = 0.047$ and 0.030). The inherent grading challenges with oral epithelial dysplasia grading systems and the lack of meaningful prediction of transformation to carcinoma highlights the significant need for a more objective, quantitative, and reproducible risk assessment tool such as the S100A7 immunohistochemical signature-based system.

Key Words: biomarkers, risk assessment, squamous cell carcinoma, immunohistochemistry, S100 calcium-binding protein A7

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Oral squamous cell carcinoma (OSCC) is a major global health problem with an annual global incidence of over 450,000 cases and a 5-year mortality rate of ~50%.[1] Although OSCC is associated with known risk factors like smoking, alcohol consumption, and use of areca nut, with or without tobacco, about one third of OSCCs develop in the absence of known etiologies.[2,3] Despite decades of research and understanding of OSCC development, oral potentially malignant disorders (OPMDs) and potentially premalignant oral epithelial lesions – a group of oral lesions and conditions that may have a predisposition to malignant transformation to OSCC – there has been little transform in reducing the incidence and mortality rate in OSCC.

The most accepted model of OSCC development is that the oral mucosa undergoes transformation from normal to an OPMD including leukoplakia and erythroplakia to invasive SCC.[4] The current standard of practice to reach an accurate diagnosis of OPMD is through histopathological diagnosis of biopsy material.[5] The histopathological assessment involves the identification of oral epithelial dysplasia (OED) followed by grading, which is based on architectural and cytomorphonuclear features. There is the assumption that grade transformation is associated with a

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higher risk of transformation to invasive SCC.[5] OED grade has limitations in clinical application as a tool for patient care stratification due to widely recognized inter- and intra-observer variation.[6] While the majority of OPMDs do not transform into cancer, distinguishing high-risk from low-risk lesions is difficult, and as a result, the management and diagnosis of OPMDs under the current standards is sub-optimal.

Over the years, many biomarkers have been proposed to be associated with OED risk of transformation to OSCC, but most of these studies have only established *qualitative* correlations with these biomarkers.[7–13] A new prognostic tool that *quantifies* the expression of the S100A7 biomarker and cytomorphometric features in biopsy specimens, and through algorithms based on clinical outcomes, has shown that this S100A7 immunohistochemical (IHC)-signature-based risk assessment to be a more effective prognostic assessment for transformation to SCC than OED grading alone.[14] S100A7, also known as Psoriasin, is a member of the S100 protein family of calcium-binding proteins, and was originally characterized as a protein secreted from psoriatic skin.[15] Since its discovery, S100A7 has been found to be overexpressed in breast ductal carcinoma in situ, epithelial ovarian cancers, gastric carcinoma, lung, bladder, oral, and head and neck SCCs, as well as serving in host defense and inflammatory processes.[16–23] The functional and molecular mechanisms of S100A7 in OED and OSCC have not been fully evaluated, but a recent study into the role of S100A7 in OSCC, elucidated S100A7 as an activator of the p38/MAPK and RAB2A signaling pathway, regulating cell growth, migration, and invasion.[24]

Since the description of the first grading system for OED in 1969,[25] many systems have been proposed in an attempt to obtain objectivity and minimize observer variation.[5,26–28] Although the 3-tier World Health Organization (WHO) grading system (mild, moderate, and severe) is the most widely accepted and used system in clinical practice, a 2-tier grading system (low grade and high grade) is gaining popularity and acceptance.[26] In this study, we investigated a retrospective cohort of 48 patients diagnosed with OED to evaluate the 2- and 3-tier grading systems, along with the S100A7 IHC-signature-based grading system[14,29] for assessing risk of transformation to cancer.

MATERIALS AND METHODS

Oral Biopsies and Tissue Processing

Formalin-fixed, paraffin-embedded (FFPE) biopsy material along with clinical data including the original dysplasia grade, sex, site of lesion, patient age in years, follow-up period (in months), and patient outcome was obtained from Northern Ireland Biobank (Queen's University Belfast, United Kingdom) approved via a master services agreement between trans-Hit Biomarkers (Laval, Quebec, Canada) and Proteocyte Diagnostics, Inc. (Toronto, Ontario, Canada), in compliance with the Helsinki Declaration. This study was conducted with the approval of the Research Ethics Board of the University of Western Ontario (WREM), study number 105952. Patient consent to publish was not required as there are no personally identifiable data included.

Two adjacent 5 μm-thick sections were cut: one prepared for hematoxylin and eosin (H&E) stain and the other for S100A7 (47C1068, Novus Biologicals) IHC. For the reassessment of dysplasia grade, each observer independently examined the H&E sections without knowledge of OED grade or clinical outcomes from the original pathology reports or grading rendered by other pathologists in this study. For OED grades, both the 2- and 3-tier dysplasia grading were used.[5] Three-tier grading was performed at the University of Toronto and Western University (UWO) by three oral pathologists (DM, MD, and CM, with collective experience >50 yr) and by a head and neck pathologist (LT). Two-tier grading was performed by two anatomical pathologists at McMaster University (JCC and SS; consensus diagnosis), an independent anatomical pathologist (LT), and two oral pathologists at UWO (MD, CM). Both H&E staining and IHC were performed at a CAP accredited laboratory within Mount Sinai Hospital, Toronto, Ontario, Canada. The S100A7 stained slides were digitally scanned at 20x magnification on a Hamamatsu Nanozoomer-XR slide scanner (Toronto Centre for Phenogenomics, Toronto, Ontario, Canada). The images of the S100A7 stained slides were imported into Visiopharm VIS according to the manufacturer's recommendation (Hoersholm, Denmark) by two independent assessors at Proteocyte Diagnostics, Inc., for S100A7 IHC signature-based risk assessment (trade name Staticyte) as previously described.[14,29]

Statistical Analysis

Statistical analysis was performed using SPSS statistical software, version 28 for Windows (IBM Corp., Armonk, New York). Inter-observer variability between the original and the oral pathologist's 3-tier OED gradings, and between the oral pathologist and anatomical pathologists' 2-tier gradings were measured with Cohen's kappa (K) statistic. Since the OED grading system produces ordinal data, both weighted and unweighted K coefficients were calculated. Unweighted K gives a correlation based on strict agreement between the observers while weighted K factors in how close the disagreement was. The observations were interpreted using the quantitative significance of K according to Landis and Koch, with K of 0 to 0.2 as slight, 0.2 to 0.4 fair, 0.4 to 0.6 moderate, 0.6 to 0.8 substantial, and 0.8 to 1.0 near perfect agreement.[30] The Cox regression disease free survival analysis was used to determine if any of the systems (2-tier, 3-tier, or S100A7 IHC signature-based risk categories) were a predictor of clinical outcome: transformation to invasive SCC.

RESULTS

Study Set Characteristics

For this retrospective study, a well-defined cohort of 48 patients with histopathologic diagnosis of oral dysplasia (based on the original sign-out report as mild,

moderate, or severe OED) at initial biopsy and known clinical outcome (transformation/no transformation to cancer) at follow-up (median 47 months) was included (Table 1). The patients in this study were 54% females and 46% males, with 65% of the biopsies from the tongue, 27% from the floor of mouth (FOM), and 8% from other parts of the oral cavity. The average age of the patients at initial biopsy was 61 years, with a median of 62 years, and a range of 25 to 88 years. Original OED grading included 33% mild, 60% moderate, and 6% severe. The overall malignant transformation rate for all OEDs in this this study was 63%, with a median follow-up of 47 months (mean 52 mo; range 5 to 144 mo).

Wide Range of Inter-Rater Variability in the 3-Tier and 2-Tier OED Gradings

To compare inter-observer variability of the 3-tier OED grading between the original sign-out report and the reassessed OED grading by the pathologists, and the 2-tier OED grading between the oral pathologists and the anatomical pathologists, Cohen's kappa statistic was employed. There was slight to fair agreement in the 3-tier OED grading between the original sign-out report and the reassessments by the pathologists (highest weighted $K = 0.239$ [95% CI 0.10; 0.38], unweighted $K = 0.170$ [95% CI 0.01; 0.33]), with the highest inter-observer exact agreement of 40%. There was an improvement from fair to substantial agreement in the 2-tier OED grading (highest weighted $K = 0.624$ [95% CI 0.47; 0.78], unweighted $K = 0.521$ [95% CI 33; 0.72]), with an increase in the inter-observer exact agreement to 75%. Thus, the 3-tier OED grading system shows limited agreement, but the 2-tier OED grading system shows substantial improvements.

TABLE 1. Retrospective Study Set Patient Characteristics Based on Original Pathology Report

			Dysplasia Grading		
			Mild	Moderate	Severe
n		48	16	29	3
Malignant transformation (%)		30 (63%)	9 (56%)	20 (69%)	1 (33%)
Gender	Male	22	8	12	2
	Female	26	8	17	1
Site	Tongue	31	9	22	0
	FOM	13	5	5	3
	Other	4	2	2	0
Age at initial biopsy (years)	Mean	61	60	61	56
	Median	62	62	62	56
	Min	25	37	25	48
	Max	88	80	88	65
Follow-up period (months)	Mean	52	50	51	63
	Median	47	51	41	66
	Min	5	5	10	37
	Max	144	144	107	85

FOM indicates floor of mouth; Other: Labial sulcus, buccal mucosa, retromolar pad, and alveolar ridge.

Low Inter-Rater Variability in the S100A7 IHC Signature-Based Assessment

To compare inter-observer variability of the S100A7 IHC signature-based grading system, two assessors, blinded to each other as well as the clinical information, performed the S100A7 IHC signature-based assessment. The results from the assessors were compared using Cohen's kappa statistic. There was near perfect agreement between the two assessors (weighted $K = 0.892$ [95% CI 0.77; 1], unweighted $K = 0.883$ [95% CI 0.75; 1]) with an inter-observer exact agreement of 94%. The computer assisted S100A7 IHC signature-based assessment is more reproducible than the WHO OED grading systems.

Correlation of the 3-Tier and 2-Tier OED Gradings and the S100A7 IHC Signature-Based Assessment to Clinical Outcomes

The distribution of the 3-tier and 2-tier OED gradings with respect to clinical outcomes is presented in Table 2. According to this 3-tier OED grading, 2 cases were classified as non-dysplastic (one hyperplasia and one chronic hyperplastic candidiasis), 5 as mild, 21 as moderate, and 18 as severe dysplasia/carcinoma in situ (CIS), and 2 OSCC. The number of cases which transformed to cancer in non-dysplastic, mild, moderate, and severe dysplasia/CIS were 1 of 2 (the case which transformed was chronic hyperplastic candidiasis), 1 of 5, 15 of 21, and 13 of 18, respectively. According to the 2-tier OED grading criteria, 2 cases were classified as non-dysplastic, 9 as low-grade and 35 as high-grade dysplasia, and 2 OSCC. The number of cases which transformed to cancer in non-dysplastic, low-grade, and high-grade dysplasia were 1 (chronic hyperplastic candidiasis) of 2, 5 of 9, and 24 of 35, respectively.

Seven cases graded as either non-dysplastic (n = 2) or mild dysplasia (n = 5) under the 3-tier OED grading system were classified as non-dysplastic or low-grade dysplasia by the 2-tier OED grading criteria, with 2 of these 7 lesions (29%) transforming to invasive OSCC. In contrast, 21 cases were graded as moderate dysplasia under the 3-tier OED grading system; of these, 17 were considered high-grade and 11 (61%) transformed into cancer; 4 were considered low-grade and all 4 (100%) transformed to invasive OSCC. Both the 3-tier and 2-tier OED grading systems failed to identify a significant proportion of at-risk cases in the non-dysplastic/mild/low-grade dysplasia categories.

The distribution of the S100A7 IHC signature-based grading with respect to clinical outcomes is presented in Table 3 with representative S100A7 IHC from low- (Fig. 1A–D) and high-risk cases (Fig. 1E–H). Two cases were classified as low-risk, 21 cases as medium-risk, and 25 cases as high-risk according to the S100A7 IHC signature-based assessment (Table 3). The cancer transformation rates for the S100A7 IHC signature-based low-, and elevated-risk (medium-, and high-risk) were 0% and 65%, respectively. This suggests that in this cohort, the S100A7 IHC signature-based assessment can identify at risk cases

TABLE 2. Correlation Between the Oral Epithelial Dysplasia Grading Systems and Clinical Outcomes

	Clinical Outcome		
OED Grade	Transformation	No Transformation	Total
Correlation Between the WHO 2-tier Oral Epithelial Dysplasia Classification and Clinical Outcomes.			
ND	1	1	2
Mild	1	4	5
Moderate	15	6	21
Severe/CIS	13	5	18
OSCC	—	—	2
Total	30	16	
Correlation Between the WHO 2-tier Oral Epithelial Dysplasia Classification and Clinical Outcomes.			
ND	1	1	2
Low Grade	5	4	9
High Grade	24	11	35
OSCC	—	—	2
Total	30	16	

The "ND" case that transformed was diagnosed as chronic hyperplastic candidiasis.

CIS indicates carcinoma in situ; ND, no dysplasia; OSCC, oral squamous cell carcinoma.

(medium- or high-risk) from those that are not (low-risk), independent of OED grade.

S100A7 IHC Signature-Based Assessment as a Predictor of Clinical Outcome

A Cox regression analysis was used to assess if any of the 3-tier and 2-tier OED grading systems performed by the various pathologists, or the S100A7 IHC signature-based low-, medium- and high-risk classes, could be used as a predictor of cancer transformation (Table 4). Of the various OED grading systems used and OED grades assigned by the pathologists, none of the OED systems or grades were a predictor of cancer transformation (p values substantially greater than 0.05 in all Predictor assessments; Table 4). In contrast, the S100A7 IHC signature-based risk classes, by either of the independent assessors, demonstrated the ability to predict cancer transformation ($P=0.047$; HR 2.068, 95% CI 1.008 to 4.240; $P=0.030$; HR 2.261, 95% CI 1.081 to 4.732; Table 4). Together, this suggests that in this cohort, the S100A7 IHC signature-based assessment can identify lesions at high-risk of transformation to cancer from those at low-risk.

TABLE 3. Correlation Between S100A7 Immunohistochemistry Signature-Based Classification to Clinical Outcomes From the Original Pathology Report

	Clinical Outcomes		
Straticyte Risk	Transformation	No Transformation	Total
Low	0	2	2
Medium	16	5	21
High	14	11	25
Total	30	18	48

DISCUSSION

The risk of transformation of OED to OSCC remains a significant management conundrum. While OED grading has been in clinical use for decades, it is widely recognized as a suboptimal indicator for assessing the risk for transformation to invasive OSCC. Coupled with the variable rates of malignant transformation reported in the literature on OED and OPMD, it has become increasingly difficult for clinicians and patients to identify and manage this debilitating, disfiguring and potentially fatal disease.

Management Approach

At the fourth meeting of the World Workshop on Oral Medicine, it was concluded that it was not possible to offer evidence-based recommendations for specific surgical interventions of dysplastic oral lesions, and that in some situations, some types of nonsurgical interventions may have some efficacy.[31] Others believe that the risk of malignant transformation of OED is reduced, but not eliminated by surgical excision, and suggest a management approach of surgical excision and continued surveillance, especially for high-grade lesions.[32] A recent review suggested that the most current treatment modality for OED is surgical intervention with excision, laser ablation, or a combination of both - despite which there is still considerable recurrence and malignant transformation.[33] These authors recommended regular and frequent follow-up, especially for lesions with moderate to severe dysplasia, taking into consideration the concept of field cancerization, unpredictable time to malignant transformation, and recurrence rate. While these studies have identified a need for continued surveillance with moderate/severe/high-grade OEDs, managing mild/low-grade OEDs, and especially lesions with no evidence of OED have become a clinical challenge. This highlights an urgent need for a more quantitative and reproducible risk indicator like the S100A7 IHC signature-based assessment seen in this study.

Three-Tier OED Grading

Generally, it is accepted that there is a poor inter- and intra-observer reproducibility for dysplasia grading, and frequently a poor association with outcome. Abbey et al concluded that accurate reproducible agreement among experienced board-certified oral pathologists diagnosing OED is difficult to achieve.[34] Dost et al suggested that the 3-tier system of mild, moderate and severe OED has poor predictive value and therefore is not reliable as a management guide.[35] The present study showed similar results with high inter-observer variability and poor predictive value of the 3-tier OED grading system.

Two-Tier OED Grading

More recently, a 2-tier grading system has been proposed as more reliable than the traditional 3-tier system. In 2006, Kujan et al proposed a new 2-tier grading system of OED, tiered into low-grade and high-grade lesions, which may have merit in helping clinicians make critical clinical decisions particularly for cases of moderate dysplasia. These authors contended that histological

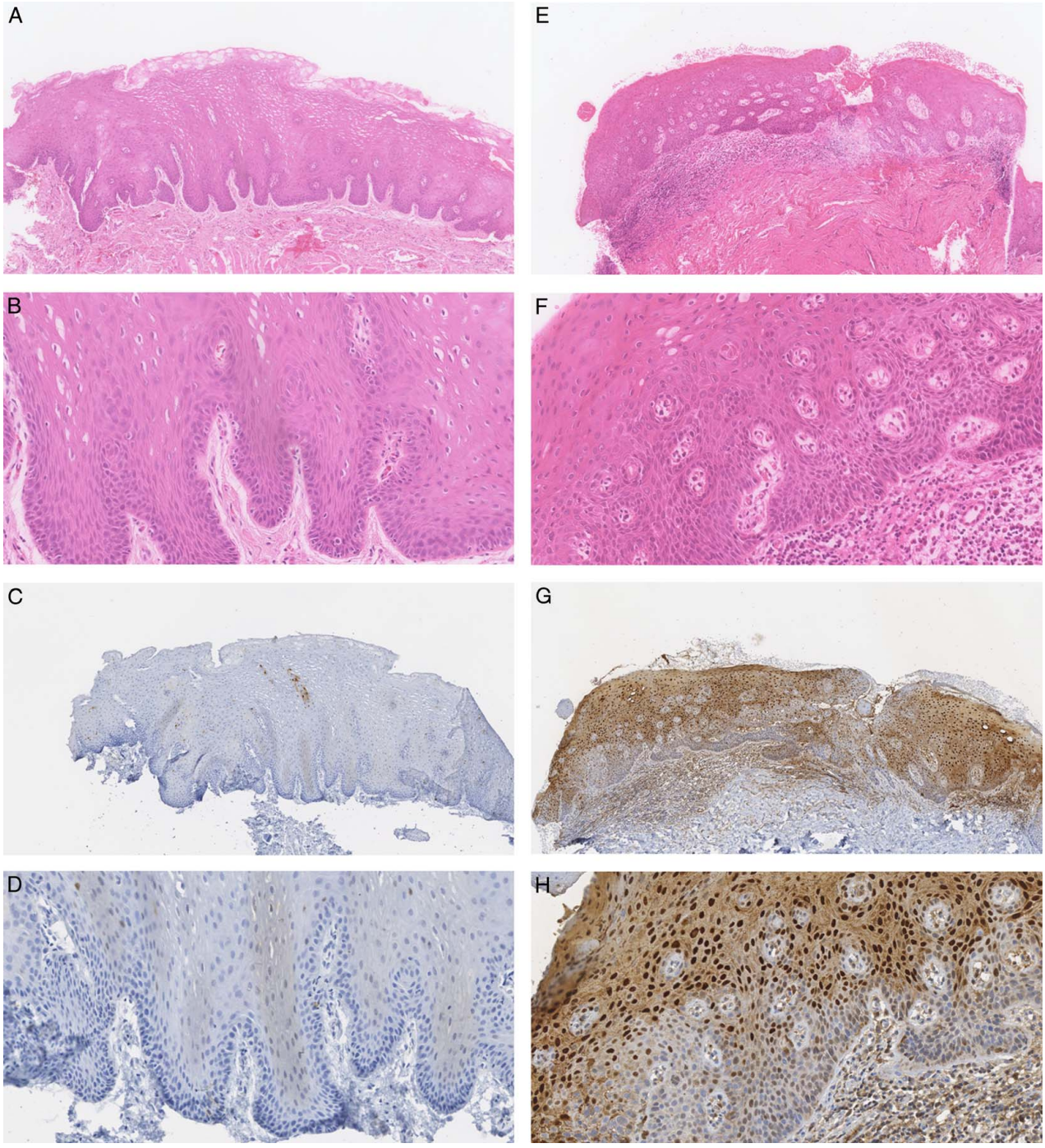

FIGURE 1. A, B, E, and F, Mild dysplasia according to H&E histological assessment and (C, and D) Low-Risk, and (G, and H) High-Risk according to S100A7 IHC signature-based risk assessment. A, C, E, and G, 25x original magnification; B, D, F, and H, 100x original magnification.

grading of dysplasia using established criteria is a reproducible prognostic indicator in OED; and that consensus scoring on the degree of dysplasia, assessment of risk, or the presence of each morphological characteristic by a panel is advisable.[26] The primary concern with moderate dysplasia is that there is a high potential for truly at-risk cases to be "down-graded" to low-grade dysplasia, based on the 2-tier grading system. In the present study, 21 cases were classified as moderate dysplasia by the 3-tier system of which 4 were considered low-grade in the 2-tier system; all 4 of these cases transformed into cancer.

TABLE 4. Cox Regression Cancer Free Survival Analysis

Predictor	*P*	Hazard ratio	95% CI lower	95% CI upper
Original (3T)	0.921	0.968	0.506	1.852
DM (3T)	0.976	1.007	0.648	1.565
MD (3T)	0.765	1.068	0.696	1.637
CM (3T)	0.815	1.047	0.712	1.539
LT (3T)	0.860	1.040	0.676	1.598
JCC/SS (2T)	0.231	1.527	0.764	3.053
MD (2T)	0.732	1.121	0.582	2.159
CM (2T)	0.878	1.415	0.684	2.927
LT (2T)	0.658	1.156	0.610	2.190
S100A7 IHC signature (Assessor 1)	0.047	2.068	1.008	4.240
S100A7 IHC signature (Assessor 2)	0.030	2.261	1.081	4.732

2T indicates 2-tier grading; 3T, 3-tier grading; IHC, immunohistochemistry.

Nankivell et al in 2013, using a minor threshold modification to Kujan's proposed system, showed that the 2-tier system has similar prognostic ability, but superior reproducibility compared with the 3-tier system. Prognostication improved by using the modified threshold.[36] In a recent systematic review of dysplasia grading systems, Yan et al concluded that 2-tier grading of OED into low-grade and high-grade categories may effectively determine malignant potential, with improved inter-observer agreement over the 3-tier OED grading. Improved grading schemes of OED may help guide management (watchful waiting vs. excision) of these OPMDs.[37] In the present study, while the 2-tier system did show a decrease in inter-observer variability over the 3-tier system, it was not a good indicator of transformation to OSCC.

S100A7 IHC Signature-Based Assessment as a Predictor of Clinical Outcome

While many studies have reviewed biomarkers for their ability to predict risk transformation to OSCC from OEDs, to date no such biomarker has emerged.[10,38–40] However, recent studies have identified S100A7 expression in the oral epithelium as a risk factor corresponding to poor prognosis of OSCC patients.[11] Overexpression of S100A7 has been observed in the majority of cases where OEDs transformed into malignancy, highlighting its potential for stratifying patients that present with OED at a significantly higher risk for malignant transformation irrespective of OED grade.[14] S100A7 overexpression has also been detected in squamous epithelial hyperplasia without dysplasia. An ideal risk for transformation to cancer biomarker would have high sensitivity to capture most lesions at risk and a low false negative rate so that the fewer number of lesions at risk would be missed. In the present study, no low-risk cases assessed by the S100A7 IHC signature-based analysis system transformed into cancer, suggesting a good negative predictive value and high sensitivity.

Limitations

The overall proportion of cases that transformed into OSCC in this study is considerably higher compared to other studies reviewed elsewhere[41] and reported in our previous evaluation.[14] This suggests that the patients in this cohort may have had more advanced disease at clinical presentation and is a likely consequence of obtaining samples through a tumor tissue bank. While the results of this study show potential for S100A7 as a candidate biomarker for assessing the risk of OED transformation to cancer, a larger study cohort with malignant transformation rates more reflective of the general community is warranted.

CONCLUSIONS

In this study, the S100A7 IHC signature-based risk assessment was the best predictor of clinical outcome; both the 3-tier and 2-tier systems of OED grading failed to adequately stratify high-risk from low-risk of transformation cases, resulting in false negatives. The S100A7 IHC signature-based analysis provides a more objective and quantitative 5-year risk assessment for transformation of OED lesions.[14,29] It has the potential to identify "at-risk" cases in the mild/low-grade OED and in those cases with no evidence of OED, and offer supplemental information to enable a more effective, customized patient management strategy. Possible improvements may be to re-structure the estimates into low- and high-risk categories only, to give clinicians a more definitive guidance with respect to patient management.

COMPLIANCE WITH ETHICAL STANDARDS

This clinical investigation was conducted in accordance and compliance with all statutes, directives, and guidelines of an Internal Review Board authorization involving retrospective data analysis of human subjects. No informed consent was required as this study was a retrospective analysis using de-identified data.

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