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A Longitudinal Study of E-Cadherin and Beta-Catenin in Progression of Oral Epithelial Dysplasia

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ABSTRACT

Background: This study explored the expression patterns of epithelial-mesenchymal transition markers E-cadherin and beta-catenin in mild and moderate oral epithelial dysplasia (OED) to determine whether their expression predicts malignant progression in oral tissue.

Methods: Formalin-fixed paraffin-embedded tissue specimens with mild or moderate dysplasia were retrieved from 87 patients. Immunohistochemistry was performed to compare E-cadherin and beta-catenin expression in tissue sections that progressed to severe dysplasia, carcinoma in situ, or squamous cell carcinoma ($n = 29$) with those that did not progress ($n = 58$). Expression patterns were observed in the basal, parabasal, lower spinous, and upper spinous epithelial layers. Expression was assessed in the cell membrane for E-cadherin and beta-catenin (low expression = absent/weak staining, high = moderate/strong) and in the cytoplasm and nucleus for beta-catenin (low = absence, high = presence). Logistic regression was used to predict progression based on the expression pattern.

Results: There were no significant differences in the progression and expression patterns of E-cadherin and beta-catenin ($p > 0.05$).

Conclusion: This study found that the expression of E-cadherin and beta-catenin was not a predictor of early malignant progression, highlighting the importance of longitudinal studies in studying progression.

1 | Introduction

Epithelial-mesenchymal transition (EMT) is a biological process that contributes to embryogenesis, tissue healing, and cancer development [1]. It is characterized by epithelial cells progressively losing epithelial traits and gaining mesenchymal traits [2]. EMT involves the activation of transcription factors, expression of cell-surface proteins and extracellular matrix-degrading enzymes, and changes in the expression of cytoskeletal proteins and microRNAs [1, 3]. As adopting mesenchymal characteristics increases the motility of cells, EMT is suggested to contribute to later cancer development (i.e., invasion and metastasis) [1]. Its role earlier in the cancer process is poorly understood.

The loss of E-cadherin is one of the main markers of key transitions during EMT [1, 2]. Based on E-cadherin's role in cellular adhesion, the loss of E-cadherin can contribute to the invasion and metastasis of malignant cells [4, 5]. Cadherins are a family of cell adhesion proteins characterized by extracellular cadherin repeats and homophilic Ca^{2+} interactions [6, 7]. Classical

cadherins, including E-cadherin, have five extracellular cadherin repeats which lock adjacent epithelial cells [8]. The cadherins bind to the Armadillo repeat protein beta-catenin, which binds to the actin cytoskeleton via alpha-catenin to maintain cell-to-cell adhesion [9]. Beta-catenin is in the cytoplasm but is attached to the cadherin complex at the cell membrane (known as "membranous beta-catenin" for this paper). In addition to its function in cell adhesion, beta-catenin has an important role in the canonical Wingless/Integrated (Wnt) pathway [4].

The Wnt pathway is involved in embryonic development and adult tissue homeostasis, but when dysregulated can contribute to diseases such as cancer [5, 10]. Regardless of the Wnt pathway's activation, tumour progression is inhibited when E-cadherin is maintained at the cell membrane, as the adhesion complex formed by E-cadherin and beta-catenin is intact [11]. When the Wnt pathway is inactive but E-cadherin is lost, beta-catenin accumulates in the cytoplasm and is destroyed afterwards ("free cytoplasmic beta-catenin"). In this context, the adhesion complex is lost, which may lead to invasion and metastasis [11, 12]. When the Wnt pathway is active but E-cadherin is lost, beta-catenin will move from the cell membrane to the cytoplasm and then translocate to the nucleus to affect Wnt target genes that contribute to cancer progression [11]. This scenario might be for early progression (i.e., dysplasia) [12].

Several immunohistochemical studies have investigated E-cadherin and beta-catenin expression in oral tissue but show conflicting results. In normal oral mucosa (NOM), E-cadherin is expressed in the cell membrane. As the severity of the tissue increases from NOM, oral epithelial dysplasia (OED), to oral squamous cell carcinoma (OSCC), there is a decrease in E-cadherin expression [13–17]; however, some studies found insignificant differences [18, 19] or only prominent decreases in high-grade dysplasia and OSCC [20, 21]. For beta-catenin, membranous expression is decreased while cytoplasmic and nuclear beta-catenin are increased from NOM to OED [13, 17, 18, 22–25], but some did not report a decrease in membrane staining [26]. Although there are cross-sectional studies on E-cadherin and beta-catenin, there is no longitudinal research on their expression in prediction of malignant progression in oral tissue. This longitudinal case–control study explored E-cadherin and beta-catenin expression in OED and determined whether their expression patterns predict oral malignant progression.

2 | Materials and Methods

2.1 | Participant Selection

Eighty-seven participants followed from January 1, 1999, to September 30, 2021, were selected from the Oral Cancer Prediction Longitudinal study (OCPL) and British Columbia Oral Biopsy Service. Participants were selected based on the availability of formalin-fixed paraffin-embedded (FFPE) tissue sections. A minimum sample size requirement of 84 was calculated based on two controls to one case, a hypothetical exposure of 20% in controls, an odds ratio of 4, a significance level of 5%, and a power of 80%. Study participants included 58 non-progressors and 29 progressors with a biopsy-confirmed baseline diagnosis of mild or moderate OED and without a history of oral cancer. Participants who progressed to severe OED (8 samples; 28%), carcinoma in situ (CIS) (3; 10%), or OSCC (18; 62%) with at least 6 months between the initial biopsy and progression date were designated as progressors or cases, and participants who did not progress and had at least 5 years of follow-up were non-progressors or controls.

In British Columbia and this study, severe OED is considered progression as surgically treated severe OED shows less transformation to cancer compared to those that were not treated [27]. Informed consent was obtained via the OCPL study. Ethical approval was obtained from the UBC BC Cancer Research Ethics Board (H21-00869).

2.2 | Histopathological Review

The study pathologist (L.Z.) confirmed the histological diagnoses of selected tissue sections using the diagnostic criteria from the World Health Organization [28]. There was no disagreement between the initial and confirmation diagnoses.

2.3 | Immunohistochemistry

Immunohistochemistry (IHC) was performed using standard IHC protocols. The protocol was repeated twice for tissue sections of each participant: once staining for E-cadherin and once for beta-catenin. Each round of IHC included a tissue section of NOM from a non-participant. The epithelium and connective tissue of the NOM served as the positive and negative controls, respectively. A secondary-only control was included to ensure the absence of nonspecific binding by the secondary antibody. FFPE tissue sections (4 μm) were heated in the oven at 60°C for 30 min, deparaffinized in xylene, then rehydrated in graded alcohol and water. The sections were placed in citrate buffer (pH 6.0, 10^{x}, Antigen Retriever, C9999; Sigma-Aldrich, Saint Louis, MO, USA) in a vegetable steamer for 20 min for heat-induced antigen retrieval. Sections were left in the steamer unplugged for 15 min and removed to room temperature for 20 min. Hydrogen peroxide and a protein block were used to reduce background staining. The sections were incubated overnight at 4°C with primary antibodies for E-cadherin (rabbit monoclonal antibody 24E10, #3195; Cell Signaling Technology, Danvers, MA, USA; 1:400 dilution) and beta-catenin (mouse monoclonal antibody E-5, sc-7963; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:250 dilution) diluted in antibody diluent with background-reducing components (S3022; Agilent Technologies, Santa Clara, CA, USA). On the second day, before incubating with a horseradish peroxidase (HRP) micro-polymer conjugated goat anti-rabbit secondary antibody, the beta-catenin sections only were incubated with an unconjugated rabbit anti-mouse antibody (also known as a mouse specifying reagent). Chromogenic detection of sections was performed using 3,3′-diaminobenzidine (DAB) chromogen diluted in DAB substrate, counterstained with hematoxylin, dehydrated, then mounted using a xylene-based mountant. Phosphate buffered saline (10X Solution, BP399; Fisher Scientific, Hampton, NH, USA) served as the wash between steps. The hydrogen peroxide block, protein block, mouse specifying reagent (complement), goat anti-rabbit HRP-conjugate, 50× DAB chromogen, and DAB

substrate were obtained from the Mouse and Rabbit Specific HRP/DAB IHC Detection Kit (Micro-polymer, ab236466; Abcam, Cambridge, UK). The mounted sections were scanned using a digital slide scanner.

2.4 | Evaluation of Immunohistochemical Expression

Manual scoring of immunohistochemical expression was performed blindly and independently by two examiners (I.Y. and Ivan Sun). For each tissue sample, the most worrisome area(s) served as the region of interest for scoring by the study pathologist. If a tissue section had areas of both mild and moderate dysplasia, the areas with moderate dysplasia were considered most worrisome and selected for scoring. Tissue sections that showed a single diagnosis (e.g., moderate dysplasia) were assessed entirely. Regions with histopathological artifacts (e.g., tears, folds, etc.), inflammatory cells, or diagnoses other than dysplasia were excluded from scoring.

Immunohistochemical expression was scored in terms of cell location and epithelial layer. E-cadherin and beta-catenin expression in the cell membrane was scored as 0 for no expression, 1 for weak staining intensity, 2 for moderate, and 3 for strong. Low membranous expression was defined as negative or weak staining (0 or 1), and high expression was defined as moderate or strong staining (2 or 3). Beta-catenin expression in the cytoplasm and nucleus was scored based on the absence (low expression) or presence (high expression) of stain. Expression was assessed in select epithelial layers, including the basal, parabasal, lower spinous, and upper spinous layers, excluding the granular layer. Expression was also assessed in the entire epithelium. For E-cadherin and beta-catenin expression in the cell membrane, low expression in any epithelial layer was considered low expression for the entire epithelium, while only high expression in all epithelial layers was considered high expression for the entire epithelium. For cytoplasmic and nuclear expression of beta-catenin, high expression (presence of staining) in any epithelial layer meant high expression for the entire epithelium, and only low expression (absence) in all epithelial layers was low expression for the entire epithelium.

2.5 | Statistical Analysis

Data analyses were conducted using SPSS Version 28.0 software. All tests were 2-tailed with a threshold for significance at $p < 0.05$. To determine whether progression was associated with low membranous expression of E-cadherin and low membranous and high cytoplasmic and/or nuclear expression of beta-catenin in the epithelium, logistic regression was performed. The Bonferroni correction adjusted the significance level to reduce the possibility of Type I errors.

To assess for potential confounding factors, demographic (age at diagnosis, age category, and sex), risk habit (smoking history), and clinicopathological variables (risk of lesion site and grade of dysplasia) were analyzed for significant differences between non-progressors and progressors using the Mann–Whitney U, Chi-square, and Fisher's exact tests. Significant differences were also assessed between expression patterns and demographic,

TABLE 1 | Comparison of progression in OED with low or high cell membrane expression of E-cadherin in different epithelial layers.

	All (%)[a]	No progression (%)[b]	Progression[c] (%)[b]	*p*	Odds ratio (95% CI[d])
Total	87	58 (67)	29 (33)	—	—
Basal epithelium: Low[e]	72 (83)	47 (65)	25 (35)	0.55[f]	1.46 (0.4 to 5.1)
Basal epithelium: High[e]	15 (17)	11 (73)	4 (27)		1
Parabasal epithelium: Low[e]	21 (24)	14 (67)	7 (33)	1.00[f]	1.00 (0.4 to 2.8)
Parabasal epithelium: High[e]	66 (76)	44 (67)	22 (33)		1
Lower spinous epithelium: Low[e]	2 (2)	1 (50)	1 (50)	0.62[f]	2.04 (0.1 to 33.8)
Lower spinous epithelium: High[e]	85 (98)	57 (67)	28 (33)		1
Upper spinous epithelium (excluding granular): Low[e]	43 (49)	27 (63)	16 (37)	0.45[f]	1.41 (0.6 to 3.5)
Upper spinous epithelium (excluding granular): High[e]	44 (51)	31 (71)	13 (30)		1
Entire epithelium: Low[g]	80 (92)	54 (68)	26 (32)	0.58[f]	0.64 (0.1 to 3.1)
Entire epithelium: High[g]	7 (8)	4 (57)	3 (43)		1

Note: Adapted from Yim, 2022 [29].
[a]Total percentage reported.
[b]Values = number (row %) unless otherwise indicated.
[c]Progression = progression to severe dysplasia, CIS, or OSCC.
[d]CI = confidence interval.
[e]Low = absent or weak intensity of stain; high = moderate or strong intensity of stain.
[f]Logistic regression was used.
[g]Low = absent or weak intensity of stain in any epithelial layer; high = moderate or strong intensity of stain in all epithelial layers.

risk habit, and clinicopathological variables using the same statistical tests.

3 | Results

3.1 | E-Cadherin in the Cell Membrane

This study found 72 (83%) samples with low membranous E-cadherin expression and 15 (17%) samples with high expression in the basal layer of the epithelium (Table 1 and Figure 1). A greater but insignificant proportion of samples with low expression in this layer progressed (OR 1.46; 95% CI: 0.4–5.1; $p = 0.55$). In the parabasal layer, 21 (24%) samples presented with low membranous E-cadherin expression and 66 (76%) samples with high expression. There was no difference in progression between low and high expression (OR 1.00; 95% CI: 0.4–2.8; $p = 1.00$). In the lower spinous layer, we found 2 (2%) samples with low expression and 85 (98%) with high expression. A greater proportion of samples with low expression progressed, but again insignificant (OR 2.04; 95% CI: 0.1–33.8; $p = 0.62$). There were 43 (49%) samples with low and 44 (51%) samples with high expression in the upper spinous layer. Similar to the basal and lower spinous layers, the upper spinous layer showed a greater but insignificant proportion of samples with low expression progressing (OR 1.41; 95% CI: 0.6–3.5; $p = 0.45$). The entire epithelium showed 80 (92%) samples with low and 7 (8%) samples with high expression. There was an insignificantly smaller proportion of samples with low expression that progressed (OR 0.64; 95% CI: 0.1–3.1; $p = 0.58$).

3.2 | Beta-Catenin in the Cell Membrane

In the basal layer, there were 75 (86%) samples with low membranous beta-catenin expression and 12 (14%) samples with high expression, with no difference in progression (OR 1.00; 95% CI: 0.3–3.6; $p = 1.00$) (Table 2 and Figure 1). In the parabasal layer, 32 (37%) samples presented with low expression, and 55 (63%) with high expression. There was an insignificantly greater proportion of samples with low expression that progressed (OR 1.34; 95% CI: 0.5–3.3; $p = 0.53$). The lower spinous layer presented 2 samples (2%) with low expression and 85 (98%) samples with high expression. Again, an insignificant but greater proportion of samples with low expression progressed (OR 2.00; 95% CI: 0.1–33.8; $p = 0.62$). The upper spinous layer had 49 (56%) samples with low expression and 38 (44%) samples with high expression. A slightly smaller but insignificant proportion of low expression progressed (OR 0.93; 95% CI: 0.4–2.3; $p = 0.88$). For the entire epithelium, we found 84 (97%) samples with low expression and 3 (3%) samples with high expression. Similarly, a smaller but insignificant proportion of low expression progressed (OR 0.24; 95% CI: 0.0–2.7; $p = 0.25$).

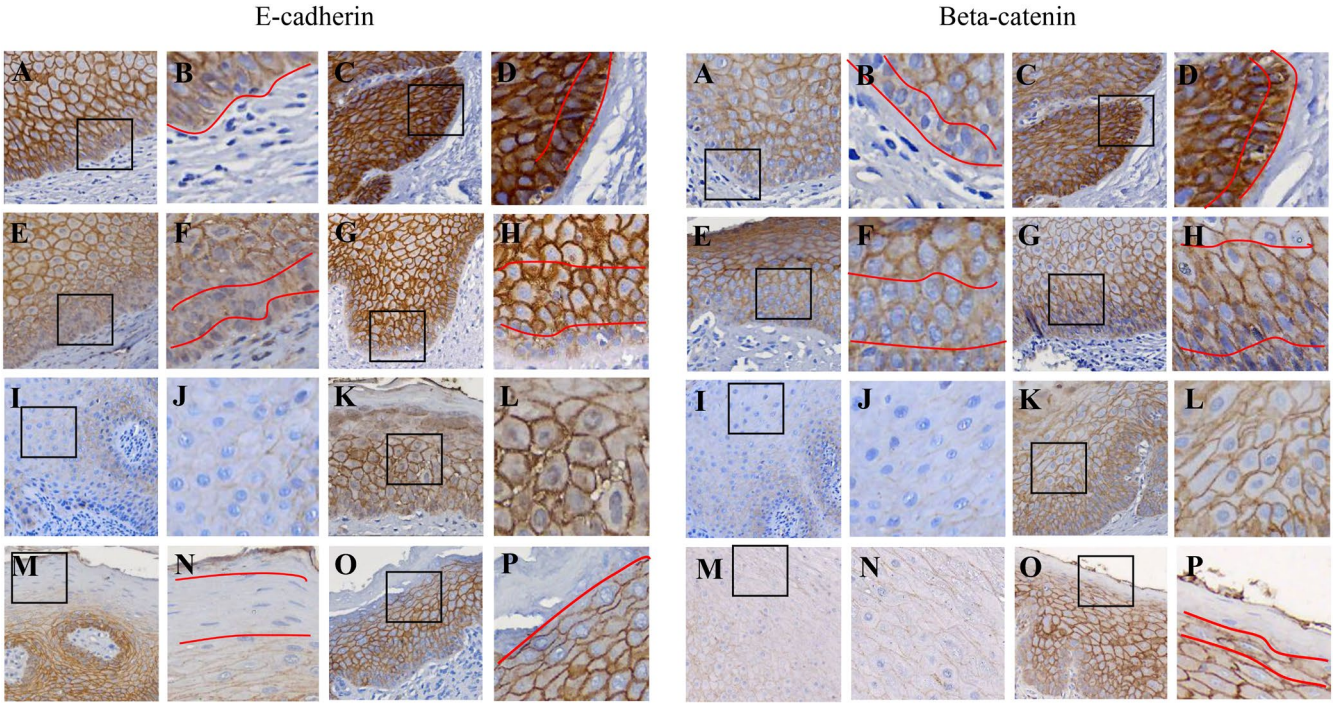

FIGURE 1 | Cell membrane expression of E-cadherin and beta-catenin in different epithelial layers of OED. Low expression is represented by an absence of or weak intensity of brown stain; high expression is represented by moderate or strong intensity of brown stain. Black squares indicate the approximate location of the specified layer, and if required, red curves further outline the specified layer (e.g., basal layer). (A, B) Low membranous expression of E-cadherin or beta-catenin in the basal layer. (C, D) High membranous expression of E-cadherin or beta-catenin in the basal layer. (E, F) Low membranous expression of E-cadherin or beta-catenin in the parabasal layer. (G, H) High membranous expression of E-cadherin or beta-catenin in the parabasal layer. (I, J) Low membranous expression of E-cadherin or beta-catenin in the lower spinous layer. (K, L) High membranous expression of E-cadherin or beta-catenin in the lower spinous layer. (M, N) Low membranous expression of E-cadherin or beta-catenin in the upper spinous layer (excluding the granular layer). (O, P) High membranous expression of E-cadherin or beta-catenin in the upper spinous layer (excluding the granular layer). Adapted from Yim, 2022 [29].

TABLE 2 | Comparison of progression in OED with low or high cell membrane expression of beta-catenin in different epithelial layers.

	All (%)[a]	No progression (%)[b]	Progression[c] (%)[b]	*p*	Odds ratio (95% CI[d])
Total	87	58 (67)	29 (33)	—	—
Basal epithelium: Low[e]	75 (86)	50 (67)	25 (33)	1.00[f]	1.00 (0.3 to 3.6)
Basal epithelium: High[e]	12 (14)	8 (67)	4 (33)		1
Parabasal epithelium: Low[e]	32 (37)	20 (63)	12 (38)	0.53[f]	1.34 (0.5 to 3.3)
Parabasal epithelium: High[e]	55 (63)	38 (69)	17 (31)		1
Lower spinous epithelium: Low[e]	2 (2)	1 (50)	1 (50)	0.62[f]	2.00 (0.1 to 33.8)
Lower spinous epithelium: High[e]	85 (98)	57 (67)	28 (33)		1
Upper spinous epithelium (excluding granular): Low[e]	49 (56)	33 (67)	16 (33)	0.88[f]	0.93 (0.4 to 2.3)
Upper spinous epithelium (excluding granular): High[e]	38 (44)	25 (66)	13 (34)		1
Entire epithelium: Low[g]	84 (97)	57 (68)	27 (32)	0.25[f]	0.24 (0.0 to 2.7)
Entire epithelium: High[g]	3 (3)	1 (33)	2 (67)		1

Note: Adapted from Yim, 2022 [29].
[a]Total percentage reported.
[b]Values = number (row %) unless otherwise indicated.
[c]Progression = progression to severe dysplasia, CIS, or OSCC.
[d]CI = confidence interval.
[e]Low = absent or weak intensity of stain; high = moderate or strong intensity of stain.
[f]Logistic regression was used.
[g]Low = absent or weak intensity of stain in any epithelial layer; high = moderate or strong intensity of stain in all epithelial layers.

3.3 | Beta-Catenin in the Cytoplasm

For cytoplasmic and nuclear staining, any presence of staining was considered high expression, while absence of staining was considered low expression. The basal and parabasal layers did not have cytoplasmic expression of beta-catenin (Table 3 and Figure 2). In the lower spinous layer, there were two (100%) non-progressors and no progressors with cytoplasmic staining. The upper spinous layer had one (100%) non-progressor and again no progressors with cytoplasmic staining. When assessing the entire epithelium, there were 85 (98%) samples with low and 2 (2%) samples with high expression. In the lower spinous, upper spinous, and entire epithelium, no samples with cytoplasmic staining progressed (OR 0.00; 95% CI: 0.0–NA; $p = 1.00$).

3.4 | Beta-Catenin in the Nucleus

Similar to cytoplasmic beta-catenin, there was no nuclear beta-catenin expression in the basal and parabasal layers (Table 3 and Figure 2). In the lower spinous layer, there were two (50%) non-progressors and two (50%) progressors with nuclear staining. In this layer, samples with nuclear staining or high expression had an insignificant but greater proportion of samples that progressed (OR 2.07; 95% CI: 0.3–15.5; $p = 0.48$). The upper spinous layer had one (100%) progressor and no non-progressors with nuclear staining. The entire epithelium showed 83 (95%) samples with low and 4 (5%) samples with high expression. There was a greater but insignificant proportion of progressors with high expression in the entire epithelium (OR 2.07; 95% CI: 0.3–15.5; $p = 0.48$).

The Bonferroni correction at a significance level of 1% showed insignificant results for all epithelial layers and cell localization of E-cadherin and beta-catenin.

3.5 | Demographic, Risk Habit, and Clinical Data Versus Progression

Demographic, risk habit, and clinical data was compared between non-progressors and progressors to identify potential confounding factors for expression pattern. We found no significant differences in age at diagnosis, age category (≤ 60 years or > 60 years), sex, ethnicity, and risk of lesion site ($p > 0.05$). Sixty-one percent of never smokers progressed, while only 22% of ever smokers progressed ($p < 0.001$). A greater proportion of samples with moderate OED progressed (41%) compared to those with mild OED (17%) ($p = 0.02$). Non-progressors (88 months) had a longer median follow-up time than progressors (46 months) ($p < 0.001$).

3.6 | Demographic, Risk Habit, and Clinical Data Versus Expression Pattern

To rule out smoking history and grade of dysplasia as potential confounding factors, bivariate analysis of demographic, risk habit, and clinical data versus expression pattern was performed. Grade of dysplasia was found to be a confounding variable in progression for membranous beta-catenin expression in the basal layer and entire epithelium ($p = 0.02$). To account for this, additional analysis for membranous beta-catenin expression in

TABLE 3 | Comparison of progression in OED with low or high cytoplasmic or nuclear expression of beta-catenin in different epithelial layers.

	All (%)[a]	No progression (%)[b]	Progression[c] (%)[b]	*p*	Odds ratio (95% CI[d])
Total	87	58 (67)	29 (33)	—	—
Basal epithelium: Low cytoplasmic/nuclear[e]	87 (100)	58 (67)	29 (33)	NA	1
Basal epithelium: High cytoplasmic/nuclear[e]	0 (0)	0 (0)	0 (0)		NA
Parabasal epithelium: Low cytoplasmic/nuclear[e]	87 (100)	58 (67)	29 (33)	NA	1
Parabasal epithelium: High cytoplasmic/nuclear[e]	0 (0)	0 (0)	0 (0)		NA
Lower spinous epithelium: Low cytoplasmic[e]	85 (98)	56 (66)	29 (34)	1.00[f]	1
Lower spinous epithelium: High cytoplasmic[e]	2 (2)	2 (100)	0 (0)		0.00 (0.0 to NA)
Upper spinous epithelium (excluding granular): Low cytoplasmic[e]	86 (99)	57 (66)	29 (34)	1.00[f]	1
Upper spinous epithelium (excluding granular): High cytoplasmic[e]	1 (1)	1 (100)	0 (0)		0.00 (0.0 to NA)
Entire epithelium: Low cytoplasmic[g]	85 (98)	56 (66)	29 (34)	1.00[f]	1
Entire epithelium: High cytoplasmic[g]	2 (2)	2 (100)	0 (0)		0.00 (0.0 to NA)
Lower spinous: Low nuclear[e]	83 (95)	56 (68)	27 (32)	0.48[f]	1
Lower spinous: High nuclear[e]	4 (5)	2 (50)	2 (50)		2.07 (0.3 to 15.5)
Upper spinous: Low nuclear[e]	86 (99)	58 (67)	28 (33)	1.00[f]	1
Upper spinous: High nuclear[e]	1 (1)	0 (0)	1 (100)		NA
Entire epithelium: Low nuclear[g]	83 (95)	56 (68)	27 (32)	0.48[f]	1
Entire epithelium: High nuclear[g]	4 (5)	2 (50)	2 (50)		2.07 (0.3 to 15.5)

Note: Adapted from Yim, 2022 [29].
[a]Total percentage reported.
[b]Values = number (row %) unless otherwise indicated.
[c]Progression = progression to severe dysplasia, CIS, or SCC.
[d]CI = confidence interval.
[e]Low = absence of stain; high = presence of stain.
[f]Logistic regression was used.
[g]Low = absence of stain in all epithelial layers; high = presence of stain in any epithelial layer.

the basal layer and entire epithelium was performed with moderate dysplasia only, excluding mild dysplasia, and results showed insignificance ($p = 0.19$, $p = 0.72$).

4 | Discussion

The molecular involvement of E-cadherin and beta-catenin in OSCC is well established in the literature [2]. This study, however, was the first to explore whether expression patterns of E-cadherin and beta-catenin predict malignant progression in oral tissue. As the risk of progression is related to the severity of dysplasia, this study investigated mild and moderate OED to assess the risk of progression early on [30]. This study provides important insight into this research, as accessing long-term follow-up data and tissue specimens, such as through the OCPL study, is needed for the longitudinal analysis of risk predictors of lesions at high risk of progression to cancer.

Although many studies have shown a reduction in membranous expression of E-cadherin and beta-catenin with the progression of oral lesions [13–17, 22–25], some studies have shown conflicting results. Williams et al. found that a reduction of E-cadherin and beta-catenin did not occur until severe dysplasia or CIS associated with adjacent infiltrating carcinoma [21]. Chaw et al. found a reduction in E-cadherin expression in dysplasia, but this result was not significant [18]. For Guo et al., there was only a significant decrease in OSCC and not dysplasia [20]. While the majority of studies support E-cadherin and beta-catenin in the progression of oral premalignant lesions, the latter studies did not suggest this role. Similarly, our study did not show a major role of E-cadherin and beta-catenin in early carcinogenesis. It

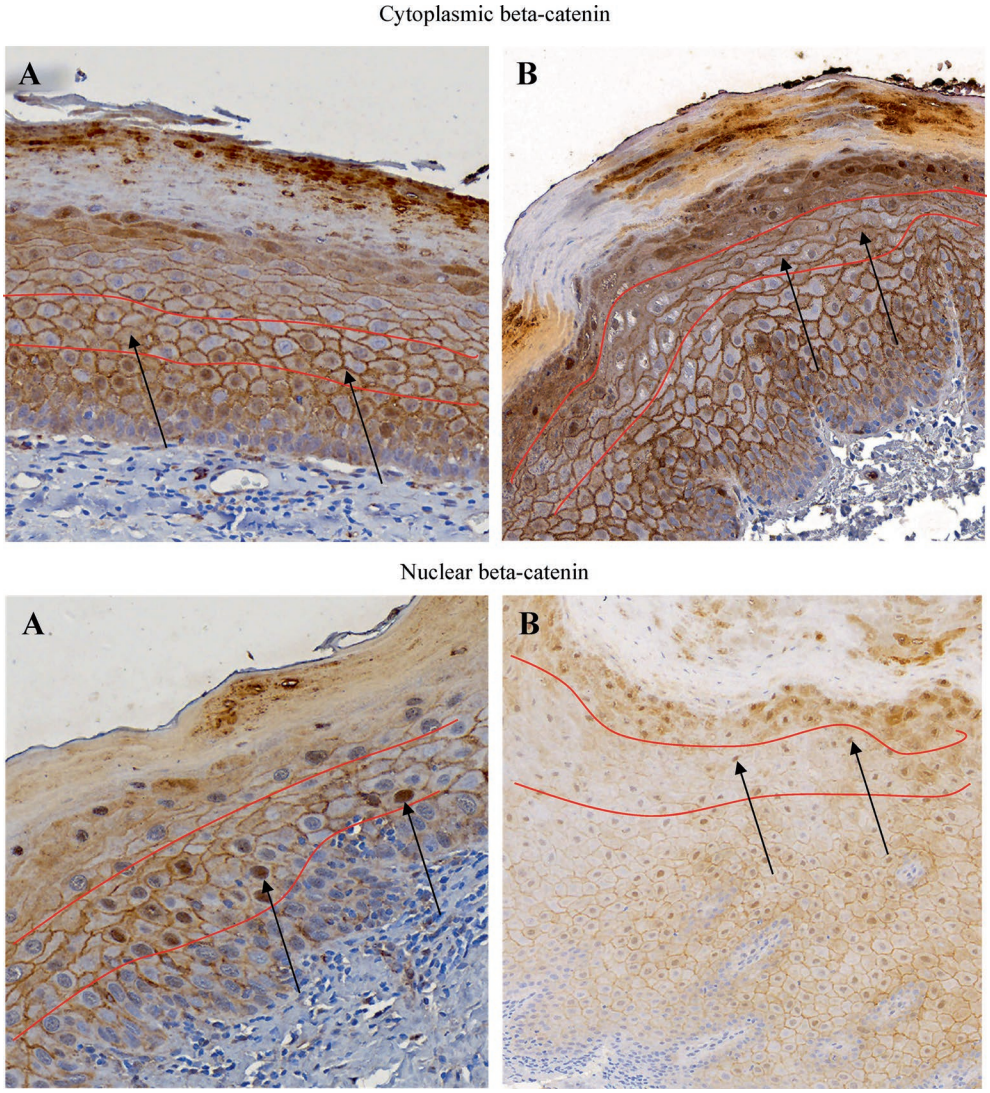

FIGURE 2 | Cytoplasmic and nuclear expression of beta-catenin in different epithelial layers of OED. Low expression is represented by an absence of brown stain; high expression is represented by the presence of brown stain. Red curves further outline the specified layer (e.g., lower spinous layer); black arrows indicate examples of cytoplasmic staining. (A) High cytoplasmic or nuclear expression of beta-catenin in the lower spinous layer. (B) High cytoplasmic or nuclear expression of beta-catenin in the upper spinous layer (excluding the granular layer). Adapted from Yim, 2022 [29].

is possible that E-cadherin and beta-catenin expression is associated with the phenotype of severe dysplasia, CIS, and OSCC; hence mild and moderate OED used in this study, even though molecularly high-risk, do not manifest such changes of reduced membrane expression.

In addition to a reduction in membranous expression of E-cadherin and beta-catenin in oral premalignant lesions, another common immunohistochemical finding is the change in localization from membranous to cytoplasmic or cytoplasmic/nuclear staining. Several studies have shown increases in both cytoplasmic and nuclear staining for beta-catenin [13, 18, 23, 26]; others have found only cytoplasmic without nuclear localization of E-cadherin and beta-catenin [17, 19, 21, 22, 25], or noticed neither cytoplasmic nor nuclear E-cadherin [14, 15, 18, 20]. Again, our study supports the latter studies and found minimal characteristics to support these proteins in the early progression of oral premalignant lesions. As tissue sections were cross-sectionally cut in this study, staining from underlying cells, which can be confused for cytoplasmic staining, may be seen in the uppermost layer. Any suspected overlapping of staining was considered overlapping and not actual cytoplasmic staining. In the hematoxylin and eosin sections, if the nucleus appeared dark or had artifacts, this was disregarded as nuclear staining in the IHC sections. This may have resulted in minimal nuclear expression of beta-catenin.

To contribute additional understanding in early malignant progression, EMT markers such as EMT-inducing transcription factors (e.g., zinc finger E-box binding homeobox [ZEB], TWIST, and SNAIL) can be studied as they activate events early in EMT [3]. Furthermore, as EMT involves many molecular processes, evaluating multiple EMT markers simultaneously is needed.

Understanding the role molecular markers play in the progression of early dysplasia can support early identification of lesions that are at high risk of becoming oral cancer. This research

studied E-cadherin and beta-catenin expression in oral tissue through longitudinal analysis, which is important for exploring the prediction of malignant progression. Expression patterns of E-cadherin and beta-catenin in OED were not found to be early predictors of malignant progression in oral tissue. Further investigation on EMT markers, including E-cadherin and beta-catenin, in early oral malignant progression is needed.

Author Contributions

Ilena S. Yim: conceptualization (equal); data curation (lead); formal analysis (lead); funding acquisition (equal); investigation (lead); methodology (lead); project administration (lead); writing – original draft preparation (lead). **Lewei Zhang:** conceptualization (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); resources (equal); supervision (equal); writing – review and editing (lead). **Leigha D. Rock:** conceptualization (equal); formal analysis (supporting); supervision (equal); writing – review and editing (equal). **Miriam P. Rosin:** conceptualization (supporting); funding acquisition (supporting); resources (equal); supervision (equal); writing – review and editing (equal). **Iris Lin:** investigation (supporting); methodology (supporting); writing – review and editing (supporting). **Denise M. Laronde:** conceptualization (supporting); funding acquisition (equal); supervision (equal); writing – review and editing (equal).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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