Original Article

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Pathology Section

Immunohistochemical Expression of E-cadherin in Premalignant and Malignant Squamous Lesions of the Oral Cavity: A Cross-sectional Study

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ABSTRACT

Introduction: Squamous Cell Carcinoma (SCC) constitutes 90% of the malignancies of the oral cavity, making it the 6th most common cancer worldwide. Premalignant lesions of the oral cavity carry an increased risk for the development of oral SCC, which is a multistage process. Numerous molecules are involved in this process, and E-cadherin is one of them. E-cadherin is a 120 kDa glycoprotein and a calcium-dependent molecule involved in cell adhesion. Altered expression of E-cadherin has been described in preinvasive lesions and malignancies of the cervix, oesophagus, and head and neck.

Aim: The aim of this study was to observe the immunohistochemical (IHC) expression pattern of E-cadherin in premalignant and malignant lesions of the oral cavity and to correlate E-cadherin immunoexpression between different grades of dysplasia and malignancy.

Materials and Methods: This cross-sectional, ambispective study was conducted at Sri Dharmasthala Manjunatheshwara College of Medical Sciences and Hospital, Dharwad, Karnataka, India. The study duration was two and a half years, with one year being retrospective from July 2014 to June 2015, and the remaining period being prospective from July 2015 to December 2016. Systematic random sampling was done to select 60 cases. Premalignant and malignant lesions of the oral squamous epithelium were classified according to the 2017 World Health Organization (WHO) classification and subjected to E-cadherin immunohistochemistry. Grading of expression was based on the percentage of cells showing continuous homogenous membranous staining: 0 (negative)=0 to 10%, 1+ (loss)=11-25%, 2+ (weak)=26% to 50%, 3+ (strong)=51% to 75%, 4+ (intense)=>75% of cells. Results were analysed using the Statistical Package for Social Sciences version 20.0.

Results: The expression of E-cadherin between the different grades of premalignant lesions varied significantly (p-value=0.003), ranging from intense expression in mild dysplasia to weak expression in high grades of dysplasia. Immunoexpression was intense (4+) in 50% of cases, strong (3+) in 39.28%, and weak (2+) in 7.14% of cases. The expression of E-cadherin between the different grades of malignant lesions also varied significantly (p-value=0.002), ranging from negative expression in poorly differentiated SCC to strong expression in well-differentiated SCC. Immunoexpression was negative (0) in 31.25% of cases, strong (3+) in 31.25%, weak (2+) in 15.62%, and (1+) in 21.87% of cases. The pattern of expression was also significant between premalignant and malignant lesions (p-value<0.0001).

Conclusion: As the grades of dysplasia and malignancy increase, the expression of E-cadherin reduces. Hence, there is a negative correlation between E-cadherin expression and the grades of dysplasia and malignancy.

Keywords: E-cadherin, Immunohistochemistry, Oral premalignant and malignant, Pattern of expression

INTRODUCTION

Squamous cell carcinoma (SCC) constitutes 90% of the malignancies of the oral cavity. Overall, oral cancer is the 6th most common cancer worldwide. The global incidence of oral cancer is 4 cases per 100,000 population, and in India, it is more than 10 per 100,000 population due to the high prevalence of tobacco chewing [1]. The mortality rate in India due to oral cancer is 3-6.7 per 100,000 population [2].

The progression of premalignant lesions into carcinoma is a multistage process involving numerous molecules, with E-cadherin being one of the key molecules [3,4]. Human E-cadherin is encoded by the ~100kb CHD1 gene located on chromosome 16q22.1, consisting of 16 exons [5]. E-cadherin is a 120kDa glycoprotein composed of three components: extracellular, cytoplasmic, and transmembrane domains [6]. The extracellular domain contains five tandem repeats, each consisting of a 100-residue amino acid motif. The N-terminal of these tandem repeats contains adhesive activity sites. The extracellular domain also has binding sites for calcium ions in the pockets between these tandem repeats. The calcium binding site has an amino acid sequence specific to each cadherin family and different species. Cell-cell adhesion is mediated through interactions of extracellular domains in a process of lateral dimerisation [5,6]. The cytoplasmic domain consists of the Juxtamembrane Domain (JMD) and Catenin-Binding Domain (CBD), each containing around 30-35 residues. The JMD allows the clustering of cadherins and contributes to adhesive strength through p120-catenin [7]. The CBD interacts with β-catenin and γ-catenin. The α-catenin then links the bound β-catenin to the actin cytoskeleton, promoting protein clustering at adherence junctions and stabilising cell-to-cell adhesion [6].

Normally, E-cadherin stains the epithelial cells of the oral mucosa, sparing the connective tissue. It is expressed as strong, membranous, homogeneous staining of the basal, parabasal, and superficial cells [4,8,9]. The staining is absent or weak in the basal aspect of basal cells and in the most superficial cells [10].

Altered expression of E-cadherin has been described in preinvasive lesions, and loss of expression has been noted in malignancies such as the cervix, oesophagus, including the head and neck [3,5]. The diagnosis of dysplasia in premalignant lesions requires careful evaluation of cytological and architectural features.

Hence, the aim of this study was to investigate the immunohistochemical (IHC) expression pattern of E-cadherin in premalignant and malignant lesions of the oral cavity and to examine the relationship between E-cadherin IHC expression and different grades of epithelial dysplasia.

MATERIALS AND METHODS

This cross-sectional, ambispective study was carried out in the Department of Pathology at Sri Dharmasthala Manjunatheshwara College of Medical Sciences and Hospital, Dharwad, Karnataka, India. The study duration was two and a half years, with one year being retrospective from July 2014 to June 2015 and the remaining period being prospective from July 2015 to December 2016. Institutional Ethical Committee approval was obtained (SDMIEC:416:2015).

Inclusion criteria: All biopsy samples of oral mucosa showing premalignant and malignant squamous lesions were selected. All cases of oral premalignant and malignant squamous lesions received during the study period were noted, and systematic random sampling was performed to select 60 cases.

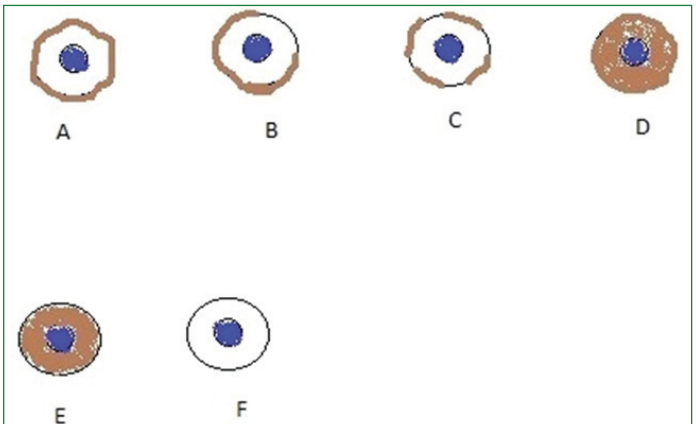
Exclusion criteria: Cases with a history of prior chemotherapy or radiotherapy and cases where the tissue sample was very scanty and IHC could not be performed were excluded from the study.

Study Procedure

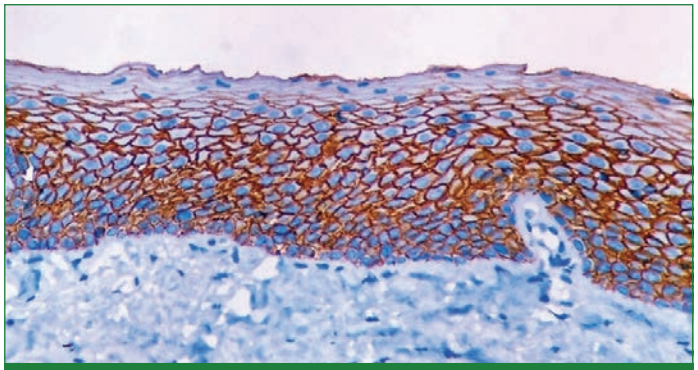
Patient details were obtained from hospital medical records. For retrospective cases, paraffin blocks and Hematoxylin and Eosin (H&E) slides were retrieved from archives. For the prospective study, oral mucosal biopsy specimens were fixed in 10% formalin, processed overnight, and embedded in paraffin. Sections taken from the paraffin blocks were subjected to H&E staining. For E-cadherin immunostaining, antigen retrieval was performed using a heat method, and primary (pathnsitu, Rabbit Monoclonal Antibody) and secondary (Anti-rabbit secondary antibodies) antibodies were added. Counterstaining with hematoxylin was done. Controls (breast carcinoma and normal oral mucosa) were run with every batch of E-cadherin IHC. The histomorphology was studied in H&E stained slides, and the findings were recorded.

Epithelial dysplasia was graded according to the 2017 WHO classification [1], into hyperplasia, mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma in-situ based on architectural and cytological parameters. Malignancies were classified and graded according to the WHO 2017 classification [1]. For E-cadherin interpretation, SCCs were graded into microinvasive, well-differentiated, moderately differentiated, and poorly differentiated.

The methodology for E-cadherin IHC study was described by Kaur G et al., Sridevi U et al., and Simionescu C et al. [8,9,11]. The pattern of expression was also noted with respect to [Table/Fig-1], including membranous staining, both membranous and cytoplasmic staining, cytoplasmic staining, and absence of staining. Only continuous homogenous membranous staining of cells was considered a normal staining pattern [Table/Fig-2]. Other patterns such as discontinuous membranous, membranous and cytoplasmic, cytoplasmic alone, and absence of staining were considered abnormal patterns [8]. The average of the cells expressing E-cadherin in three randomly selected high-power fields was calculated and expressed as a percentage. The cells with continuous homogenous membrane staining were considered positive. Based on the percentage of these positive cells, the pattern was graded [9,11].



[Table/Fig-1]: Schematic diagram of cells showing patterns of E-cadherin staining. (A-Normal, B-F abnormal staining): A) Normal continuous homogenous membranous staining; B & C- Discontinuous membrane staining; D- Cytoplasm and membrane staining; E- Only cytoplasm staining; F- No staining



[Table/Fig-2]: E-cadherin expression in normal oral mucosa showing continuous, dark, membranous staining of the cells in the basal, parabasal and prickle layer. Basal part of the basal cells and the stratum corneum does not show staining (40x).

The immunostaining was graded [9] for statistical purposes as follows:

Negative: Staining of 0 to 10% of cells

1+ (loss): Staining of 11-25% of cells

2+ (weak): Staining of 26 to 50% of cells

3+ (strong): Staining of 51% to 75% of cells

4+ (intense): Staining of more than 75% of cells.

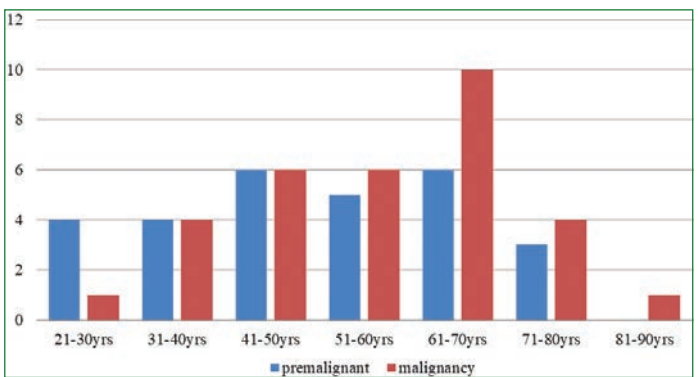
STATISTICAL ANALYSIS

Descriptive statistics, such as percentages and means, were used to analyse the data. The correlation between E-cadherin immunoexpression and microscopic features (WHO histological grading) was assessed by calculating the Pearson's coefficient (r-value). The values were tabulated, and the p-value was calculated using Statistical Package for the Social Sciences version 20.0 (SPSS v20.0) software. A p-value less than 0.05 was considered statistically significant.

RESULTS

In the present study, a total of 60 cases of premalignant and malignant squamous lesions of the oral cavity were included. Among these cases, 32 (53.33%) were malignancies of various grades, and 28 (46.66%) were premalignant lesions.

The most common age group for premalignant cases was 41-50 years, with 6 cases, and 61-70 years, with 5 cases, with a mean age of 50.39 years [Table/Fig-3]. For malignant cases, the most common age group was 61-70 years, with a total of 10 cases, and a mean age of 57.06 years. The minimum and maximum ages for premalignant lesions were 22 and 75 years, respectively, and for malignant lesions, they were 29 and 82 years.



[Table/Fig-3]: Age distribution in premalignant and malignant cases.

Among the 60 cases, 53 (88.33%) were males and 7 (11.67%) were females. Among the 28 premalignant cases, 24 (85.71%)

were males and 4 (14.29%) were females. Among the 32 malignant cases, 29 (90.63%) were males and 3 (9.37%) were females [Table/Fig-4].

Cases	Female (n,%)	Male (n,%)	Total
Premalignant	4 (14.29%)	24 (85.71%)	28
Malignant	3 (9.37%)	29 (90.63%)	32
Total	7 (11.67%)	53 (88.33%)	60

[Table/Fig-4]: Sex distribution of cases.

The most common site for both premalignant and malignant lesions in this study was the buccal mucosa, with 38 cases (63.3%), followed by the tongue, with 11 cases (18.3%) [Table/Fig-5].

Site	Premalignant (n,%)	Malignant (n,%)	Total (N,%)
Buccal mucosa	23 (82.14%)	15 (46.87%)	38 (63.34%)
Buccal vestibule	0	1 (3.12%)	1 (1.66%)
GB sulcus	0	1 (3.12%)	1 (1.66%)
Gingiva	0	1 (3.12%)	1 (1.66%)
Lower lip	0	1 (3.12%)	1 (1.66%)
Mandibular alveolus	1 (3.57%)	2 (6.25%)	3 (5.00%)
Maxillary alveolus	0	1 (3.12%)	1 (1.66%)
Palate	0	1 (3.12%)	1 (1.66%)
Retromolar trigone	0	2 (6.25%)	2 (3.33%)
Tongue	4 (14.29%)	7 (21.87%)	11 (18.33%)
Total	28	32	60

[Table/Fig-5]: Site distribution of lesions.

Among the premalignant lesions, there were 18 cases of leukoplakia, of which 3 showed only hyperplasia without dysplasia, 7 showed mild dysplasia, 4 showed moderate dysplasia, and 4 showed severe dysplasia. Among the 6 cases of oral submucous fibrosis (OSMF), one case showed moderate dysplasia, and the other 5 cases showed no dysplasia. There were 3 cases of lichen planus, none of which showed dysplasia. The study also included 1 case of carcinoma in-situ [Table/Fig-6].

Dysplasia	Leukoplakia	OSMF	Lichen planus	In-situ carcinoma
No dysplasia/ hyperplasia	3	5	3	0
Mild dysplasia	7	0	0	0
Moderate dysplasia	4	1	0	0
Severe dysplasia	4	0	0	0
In-situ carcinoma	0	0	0	1
Total	18	6	3	1

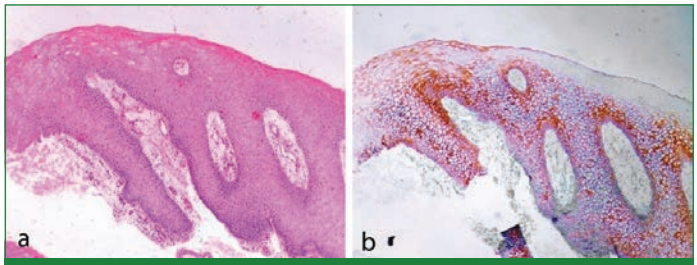
[Table/Fig-6]: Distribution of premalignant lesions (n=28).

Among the 32 malignant lesions, there were 5 cases (15.63%) of microinvasive carcinoma, 17 cases (53.12%) of well-differentiated SCC (including 11 cases of conventional SCC, 3 cases of verrucous carcinoma, and 3 cases of papillary SCC), 6 cases (18.75%) of moderately differentiated SCC, and 4 cases (12.50%) of poorly differentiated SCC [Table/Fig-7].

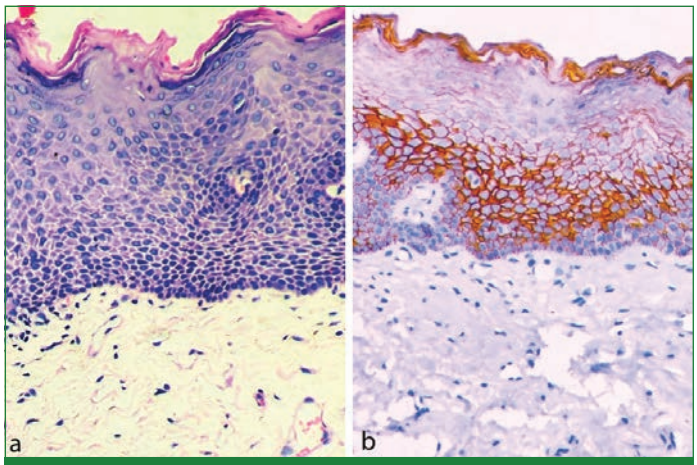
Malignant lesions	Number	Percentage
Microinvasive SCC	5	15.63%
Welldifferentiated SCC	17	53.12%
Moderately differentiated SCC	6	18.75%
Poor differentiated SCC	4	12.50%

[Table/Fig-7]: Distribution of malignant lesions (n=32).
SCC: Squamous cell carcinoma

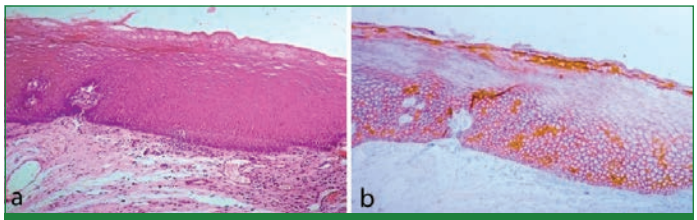
Regarding E-cadherin expression in premalignant lesions, out of the 11 premalignant lesions without dysplasia, 7 cases (63.64%) showed intense (4+) staining [Table/Fig-8,9], and 4 cases (36.36%) showed strong (3+) staining. None of the cases showed weak/loss/negative staining. In the 7 lesions of mild dysplasia, 5 cases (71.43%) showed intense (4+) staining [Table/Fig-10,11], and 2 cases (28.57%) showed strong (3+) staining.



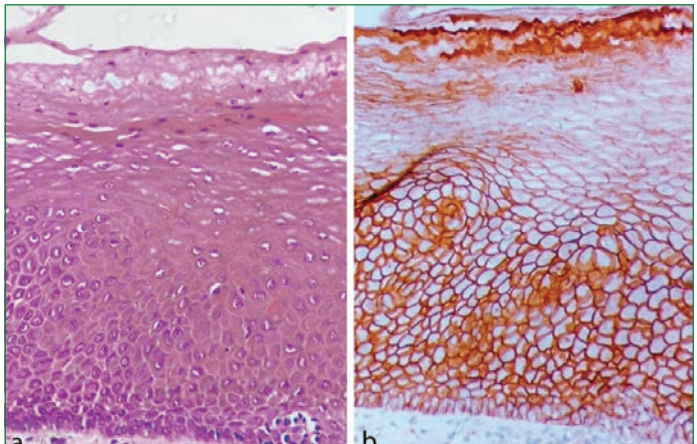
[Table/Fig-8]: a) Photomicrograph of Leukoplakia without dysplasia with epithelium showing parakeratosis, acanthosis and irregular elongated rete (Hyperplasia) (H&E, 10x). b) E-cadherin expression in Leukoplakia without dysplasia (hyperplasia) showing continuous membranous staining of cells in basal, parabasal and prickle layer, similar to normal epithelium (10x). (4+, intense)



[Table/Fig-9]: a) Photomicrograph of OSMF without dysplasia showing hyperkeratosis, acanthosis. Subepithelium shows band of collagen with sparse inflammatory cells (H&E, 40x). b) E-cadherin expression in OSMF showing continuous membranous staining of the cells in basal, parabasal and lower prickle layer, similar to normal mucosa (40x). (4+, intense)



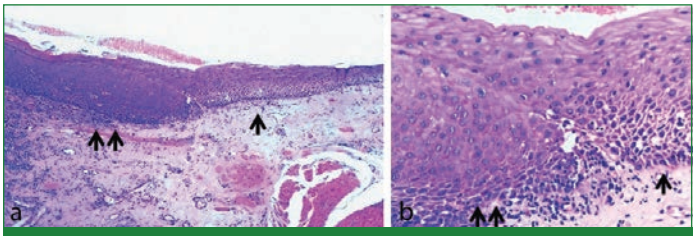
[Table/Fig-10]: a) Photomicrograph of mild dysplasia (H&E, 10x). b) Photomicrograph of E-cadherin expression in mild dysplasia (10x).



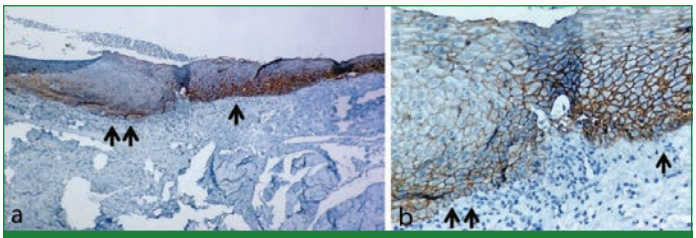
[Table/Fig-11]: a) Higher magnification of mild dysplasia showing, crowding, anisonucleosis and mild nuclear pleomorphism in lower 1/3rd of the epithelium (H&E, 40x). b) E-cadherin expression in mild dysplasia showing expression similar to normal mucosa with mild reduction of staining of cells in upper prickle layer (40x). (4+, intense)

In the 5 lesions with moderate dysplasia, 2 cases (40%) showed intense (4+) staining, 2 cases (40%) showed strong (3+) staining [Table/Fig-12,13], and 1 case (20%) showed weak (2+) staining.

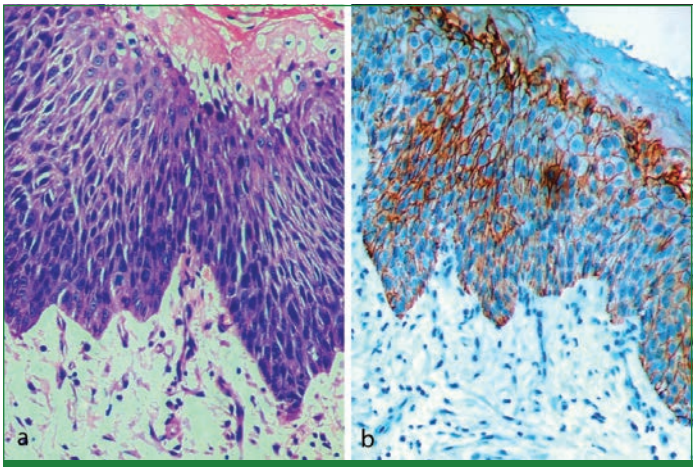
In 4 lesions of severe dysplasia, 2 cases (50%) showed strong (3+) staining [Table/Fig-14,15], 1 case (25%) showed weak (2+) staining, and 1 case (25%) showed loss of (1+) stain. In one case (100%) of carcinoma in-situ, strong (3+) staining was observed. Premalignant lesions predominantly (50%) showed an intense staining pattern,



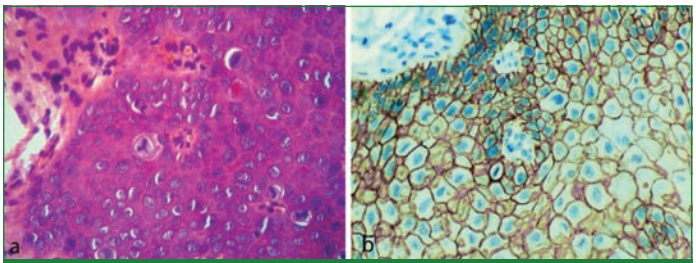
[Table/Fig-12]: a) Photomicrograph showing normal stratified squamous epithelium (single arrow) with adjacent areas showing moderate dysplasia (double arrow) (H&E, 10x). b) Higher magnification showing loss of polarity of cells, anisonucleosis, mild nuclear pleomorphism in lower 2/3rd of epithelium (H&E, 40x).



[Table/Fig-13]: a) E-cadherin expression in moderate dysplasia showing reduced staining of the cells in the basal, and parabasal layer (double arrow) compared to the adjacent normal epithelium (single arrow) (10x). b) Higher magnification of the same (40x). (3+, strong).



[Table/Fig-14]: a) Photomicrograph showing severe dysplasia. Cells in more than 2/3rd of the epithelium show loss of polarity, marked anisonucleosis, nuclear pleomorphism and hyperchromasia. The cells in superficial layer show maturation with keratinisation (H&E, 40x). b) E-cadherin expression is reduced in all the layers with only focal areas and few cells in upper prickle layer showing normal staining (40x). (3+, strong)



[Table/Fig-15]: a) Photomicrograph showing another case of severe dysplasia showing increased atypical mitosis (H&E, 40x). b) E-cadherin expression is near normal (40x). (3+, strong).

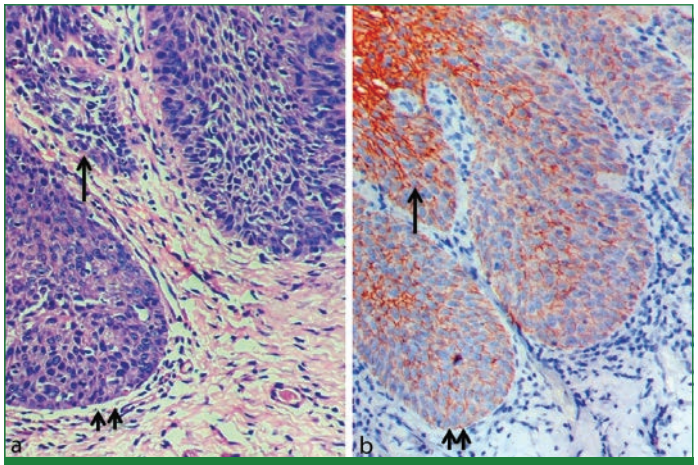
followed by strong staining (39.29%) and weak expression (7.14%). Only one case showed loss of expression (3.57%). None of the premalignant lesions showed negative expression [Table/Fig-16].

Lesions	0+(0-10%)	1+(11-25%)	2+(26-50%)	3+(51-75%)	4+(76-100%)	Total
No dysplasia	0	0	0	4 (36.36%)	7 (63.64%)	11
Mild dysplasia	0	0	0	2 (28.57%)	5 (71.43%)	7
Moderate dysplasia	0	0	1 (20%)	2 (40%)	2 (40%)	5
Severe dysplasia	0	1 (25%)	1 (25%)	2 (50%)	0	4
Carcinoma in-situ	0	0	0	1 (100%)	0	1
Total	0	1 (3.57%)	2 (7.14%)	11 (39.29%)	14 (50%)	28

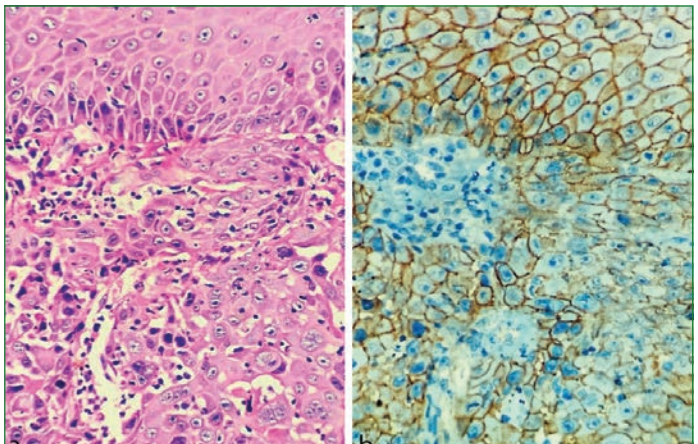
[Table/Fig-16]: Pattern of E-cadherin immunostaining in premalignant lesions.

The expression of E-cadherin varied significantly between different grades of premalignant lesions, ranging from intense expression in mild dysplasia to reduced expression in high grades of dysplasia. There was a moderate negative correlation of E-cadherin expression between different grades of premalignant lesions (Pearson's Correlation=-0.537), which was statistically significant (p-value=0.003).

Regarding E-cadherin expression in malignant lesions, out of 5 cases of microinvasive SCC, 2 cases (40%) showed strong (3+) staining, 2 cases (40%) showed weak (2+) staining [Table/Fig-17], and 1 case (20%) showed loss of (1+) staining. None of the cases showed negative (0+) staining. Out of 17 cases of well-differentiated SCC, 7 cases (41.18%) showed strong (3+) staining [Table/Fig-18], 2 cases (11.76%) showed weak (2+) staining, 5 cases (29.41%) showed loss of (1+) staining, and 3 cases (17.65%) showed negative (0+) staining.

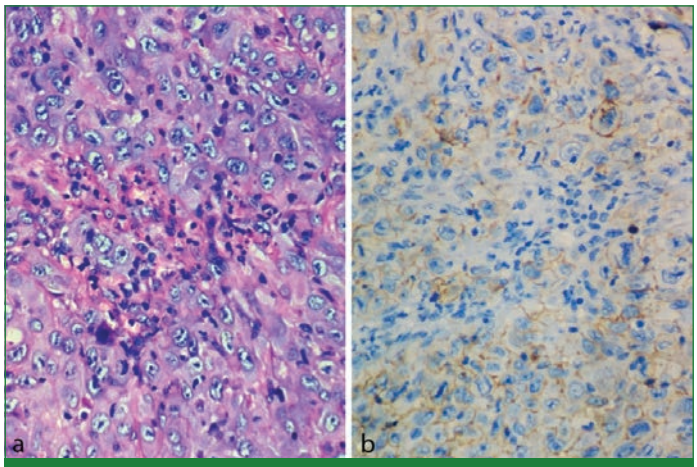


[Table/Fig-17]: a) Invasive tongues (double arrow) of microinvasive SCC (H&E, 40x). b) Reduced expression of E-cadherin in the invasive front of the tumour (double arrow) (2+, weak), in comparison with its expression in the central part of tumour (single arrow) (40x). (3+, strong)

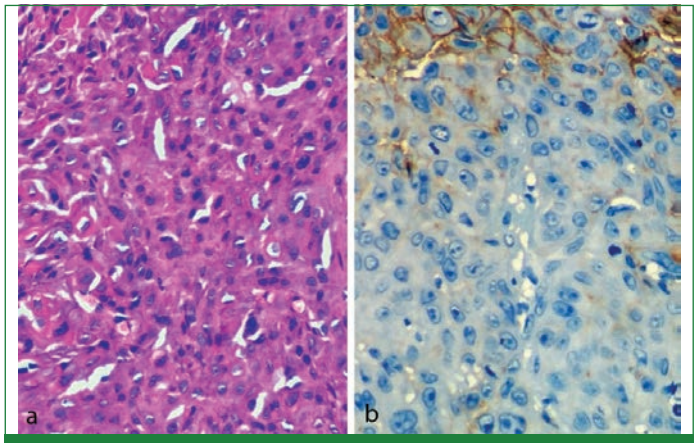


[Table/Fig-18]: a) Well-differentiated SCC with overlying epithelium (H&E, 40x). b) Tumour showing reduced expression of E-cadherin, in the form of discontinuous membranous and few with absent staining, (2+, weak). Overlying epithelium shows normal expression (40x) (3+, strong).

Out of 6 cases of moderately differentiated SCC, 1 case (16.66%) showed strong (3+) staining, 1 case (16.66%) showed weak (2+) staining, 1 case (16.66%) showed loss of (1+) staining [Table/Fig-19], and 3 cases (50.00%) showed negative (0+) staining. All 4 cases (100%) of poorly differentiated SCC showed negative (0+) staining [Table/Fig-20]. None of the malignancies expressed intense (4+) staining.



[Table/Fig-19]: a) Moderately differentiated SCC showing tumour cells in sheets (H&E, 40x). b) E-cadherin expression showing tumour cells with decreased intensity of staining and loss of expression in few cells (40x). (1+, loss)



[Table/Fig-20]: a) Poorly differentiated SCC showing tumour cells in sheets (H&E, 40x). b) E-cadherin expression showing predominantly loss of expression in tumour cells (40x). (0, negative).

The expression of E-cadherin varied significantly between different grades of malignant lesions, ranging from negative expression in poorly differentiated SCC to strong expression in well-differentiated SCC [Table/Fig-21]. There was a significant correlation of E-cadherin expression between different grades of malignancy (Pearson's correlation=-0.532), which was statistically significant (p-value=0.002).

Malignant lesions	0 (0-10%)	1+(11-25%)	2+(26-50%)	3+(51-75)	4+(76-100)	Total
Microinvasive	0	1 (20%)	2 (40%)	2 (40%)	0	5
Well differentiated	3 (17.65%)	5 (29.41%)	2 (11.76%)	7 (41.18%)	0	17
Moderately differentiated	3 (50%)	1 (16.66%)	1 (16.66%)	1 (16.66%)	0	6
Poorly differentiated	4 (100%)	0	0	0	0	4
Total	10 (31.25%)	7 (21.88%)	5 (15.62%)	10 (31.25%)	0	32

[Table/Fig-21]: Pattern of E-cadherin immunostaining in malignant lesions.

The significance of E-cadherin expression was observed through a moderately negative correlation between E-cadherin expression and grades of premalignant lesions (p-value=0.003) [Table/Fig-22]. Similarly, a moderately negative correlation was found between E-cadherin expression and grades of malignancy (p-value=0.002). When comparing E-cadherin expression among premalignant and malignant lesions, there was a moderately negative correlation (p-value≤0.0001). There was a mild negative correlation between E-cadherin expression in premalignant lesions without dysplasia and lesions with dysplasia, but it was not statistically significant.

Correlation of E-cadherin expression	R-value	p-value
With grades of premalignancy	-0.537	0.003
With grades of malignancy	-0.532	0.002
Premalignant vs malignant	-0.673	<0.0001
Non dysplastic vs dysplastic premalignant	-0.293	0.130

[Table/Fig-22]: Correlation of E-cadherin expression in premalignant and malignant squamous lesions.

DISCUSSION

The current trend in cancer research is moving towards understanding the molecular and genetic basis of cancer, rather than relying solely on morphological diagnosis. Immunohistochemistry (IHC) is an ancillary technique that is directly related to the molecular evolution of cancer. Improved understanding of the molecular basis of cancer can aid in accurate diagnosis and better treatment options [9].

The malignant transformation of tumours is associated with the loss of epithelial differentiation and the acquisition of a mesenchymal phenotype. This is characterised by increased expression of mesenchymal genes and reduced expression of epithelial genes, which leads to increased cell motility, loss of cell adhesion, and loss of polarity. These molecular changes occur early in the development of malignancy, including in dysplasia. Identification of these transition molecules, such as E-cadherin, can serve as biomarkers for identifying high-risk lesions [4].

In our study, oral premalignant lesions without dysplasia and those with mild dysplasia showed strong and intense expression of E-cadherin, similar to normal mucosa. As the grades of dysplasia increased, the intensity of E-cadherin expression decreased. Moderate dysplasia cases showed weak expression in 20% of cases, and severe dysplasia cases showed further decrease in expression, with weak and loss of expression observed in 50% of cases. This pattern of expression is consistent with previous studies by Sharma J et al., [12] and Thankam DR et al., [13]. However, Gupta A et al. reported intense expression in 14.28% of cases of severe dysplasia, which differs from our findings [Table/Fig-23] [12-14].

Study	Grading of E-cadherin expression	Present study (n=27)	Sharma J et al., [12] (n=40)	Gupta A et al., [13] (n=28)	Thankam D R et al., [14] (n=21)
Place of study		Dharwad, Karnataka	Faridabad, Haryana	Ghaziabad, New Delhi	Alappuzha, Kerala
Year of study		2023	2022	2018	2021
No dysplasia	0-50%	-	-	-	-
	51-75% (Strong)	36.36% (4)	100% (20)		18.75% (3)
	76-100% (Intense)	63.64% (7)		100% (7)	81.25% (13)
Mild dysplasia	0-50%	0%	-	-	-
	51-75% (Strong)	28.57% (2)	100% (8)	14.28% (1)	50.00% (1)
	76-100% (Intense)	71.43% (5)		85.71% (6)	50.00% (1)
Moderate dysplasia	0-25%	0%	-	-	-
	26-50% (Weak)	20.00% (1)	62.50% (3)		
	51-75% (Strong)	40.00% (2)	37.50% (5)	57.14% (4)	100% (3)
	76-100% (Intense)	40.00% (2)		42.85% (3)	

Severe dysplasia	0-10% (Negative)	0%	-		
	11-25% (Loss)	25.00% (1)	50.00% (2)		
	26-50% (Weak)	25.00% (1)	50.00% (2)		
	51-75% (Strong)	50.00% (2)	-	85.71% (6)	100% (3)
	76-100% (Intense)	0%	-	14.28% (1)	

[Table/Fig-23]: Summary of comparison of E-cadherin expression in premalignant lesions with other studies {percentage (number of cases)} [12-14].

Poorly differentiated SCC	0-10% (Negative)	100% (4)		100% (7)	
	11-25% (Loss)		100% (4)		8.33% (1)
	26-50% (Weak)				41.66% (5)
	51-75% (Strong)				50.00% (6)
	76-100% (Intense)				

[Table/Fig-24]: Summary of comparison of E-cadherin expression in malignant lesions with other studies {percentage (number of cases)} [12,13,15].

Sridevi U et al. reported weak expression of E-cadherin in 75% of cases of mild dysplasia (9). In cases of oral submucous fibrosis (OSMF), 60% of cases with moderate dysplasia showed strong staining, and 33% of leukoplakia cases with moderate dysplasia showed strong staining. However, the expression of E-cadherin in cases of dysplasia was not statistically significant.

In cases of squamous cell carcinoma (SCC), the expression of E-cadherin decreased as the grades of malignancy increased. Well-differentiated SCC showed predominantly strong expression in 41% of cases, similar to severe dysplasia. Moderately differentiated SCC showed predominantly negative expression in 50% of cases. In cases of well- and moderately differentiated SCC, IHC staining was further reduced in focal areas with high-grade cytologic atypia compared to other areas with predominantly low-grade cytological atypia within the same tumour.

In addition, in cases of well- and moderately differentiated tumours, the intensity of immunostaining for E-cadherin in tumour cells varied within the tumour, with differences between cells at the invasive front and those in other areas. Poorly differentiated SCC showed negative expression in all cases, with occasional (<10%) cells showing complete membranous staining in areas of preserved differentiation. This is consistent with the findings of Sharma J et al., Gupta A et al., and Khant HR [12,14,15]. However, Gupta A et al. reported predominantly negative expression in moderately differentiated SCC, which aligns with our findings [14]. In cases of poorly differentiated SCC, Sharma J et al., Gupta A et al., reported predominantly negative and loss of E-cadherin expression, similar to our study, while Khan HR et al. reported predominantly strong expression [12,14,15] [Table/Fig-24].

Study	Grading of E-cadherin expression	Present study (n=27)	Sharma J et al., [12] (n=20)	Gupta A et al., [14] (n=21)	Khan HR et al., [15] (n=37)
Place of study		Dharwad, Karnataka	Faridabad, Haryana	Ghaziabad, New Delhi	Sevagram, Maharashtra
Year of study		2023	2022	2018	2022
Well differentiated SCC	0-10% (Negative)	17.65% (3)			
	11-25% (Loss)	29.41% (5)			
	26-50% (Weak)	11.76% (2)	25.00% (2)	28.57% (2)	16.66% (2)
	51-75% (Strong)	41.18% (7)	75.00% (6)	57.14% (4)	83.33% (10)
	76-100% (Intense)			14.28% (1)	
Moderately Differentiated SCC	0-10% (Negative)	50.00% (3)		57.14% (4)	
	11-25% (Loss)	16.66% (1)	37.50% (3)	14.28% (1)	
	26-50% (Weak)	16.66% (1)	62.50% (5)		53.84% (7)
	51-75% (Strong)	16.66% (1)		28.57% (2)	46.15% (6)
	76-100% (Intense)				

In our study, the expression pattern of E-cadherin was statistically significant between grades of dysplasia, grades of SCC, and between dysplasia and SCC. This is consistent with the findings of Gupta A et al., Santos-García A et al., Yuwanati MB et al., and Kaur J et al., who reported a statistical correlation between E-cadherin expression and grades of dysplasia and SCC [12,16-18]. Zeidler SVV et al., Khan HR et al., Santos-García A et al., and Akhtar K et al., also reported a statistical correlation between E-cadherin expression and grades of SCC [4,15,16,19]. However, there was no statistical correlation between E-cadherin expression in premalignant conditions with dysplasia and those without dysplasia, as some cases of mild and moderate dysplasia also showed strong and intense expression similar to non-dysplastic cases.

Limitation(s)

One limitation of our study is the small sample size, which is due to the short study duration and financial constraints.

CONCLUSION(S)

The pattern of E-cadherin immunoexpression in oral premalignant lesions was very similar to that seen in normal epithelium. Therefore, it cannot be used as a marker to differentiate dysplastic from non-dysplastic lesions. It was also observed that the intensity of E-cadherin expression was inversely proportional to the grades of dysplasia and malignancy. However, there were a few cases of moderate and severe dysplasia that showed expression similar to mild dysplasia. Therefore, E-cadherin cannot be used as the sole marker to grade dysplasia. Additionally, E-cadherin alone cannot differentiate invasive lesions from severe dysplasia without invasion, as some cases of severe dysplasia, microinvasive SCC, and well-differentiated SCC showed similar expression patterns.

REFERENCES

[1] El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO classification of head and neck tumours 4th ed. Lyon: International Agency for Research on Cancer (IARC); 2017. Pp.105-131.
[2] Park K. Park's Textbook of Preventive and Social Medicine. 24th ed. Jabalpur: M/s Banarasidas Bhanot; 2017. Pp. 406.
[3] Gnepp DR. Diagnostic surgical pathology of the head and neck. Philadelphia: Saunders Elsevier; 2009.
[4] von Zeidler SV, de Souza Botelho T, Mendonça EF, Batista AC. E-cadherin as a potential biomarker of malignant transformation in oral leukoplakia: a retrospective cohort study. BMC Cancer. 2014;14:972.
[5] Pećina-Slaus N. Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell Int. 2003;3(1):17.
[6] Gall TMH, Frampton AE. Gene of the month: E-cadherin (CDH1). J Clin Pathol. 2013;66(11):928-32.
[7] Barnes L, Eveson JW, Reichart P, Sidransky D, (ed). World Health Organization. Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005. Pp.163-208.
[8] Kaur G, Carnelio S, Rao N, Rao L. Expression of E-cadherin in primary oral squamous cell carcinoma and metastatic lymph nodes: An immunohistochemical study. Indian J Dent Res. 2009;20(1):71-76.
[9] Sridevi U, Jain A, Nagalaxmi V, Kumar U, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. Eur J Dent. 2015;9(3):364-72.
[10] Downer CS, Speight PM. E-Cadherin expression in normal, hyperplastic and malignant oral epithelium. Oral Oncol Eur J Cancer. 1993;29(4):303-05.
[11] Simionescu C, Margaritescu C, Surpateanu M, Mogoanta L, Zavoi R, Ciurea R, et al. The study of E-cadherine and CD44 immunoexpression in oral squamous cell carcinoma. Rom J Morphol Embryol. 2008;49(2):189-93.

[12] Sharma J, Bhargava M, Aggarwal S, Aggarwal A, Varshney A, Chopra D. Immunohistochemical evaluation of E-cadherin in oral epithelial dysplasia and squamous cell carcinoma. Indian J Pathol Microbiol. 2022;65(4):755-60.
[13] Thankam DR, Aria Jyothi A. Expression of e-cadherin and vimentin in lesions of oropharynx. Int J Adv Med. 2021;8(2):246-53.
[14] Gupta A, Sharma S, Batra M, Abidullah M, Bhuvinder S, Katragadda P. Role of Ecadherin in progression of oral squamous cell carcinoma: A retrospective immunohistochemical study. The Journal of Contemporary Dental Practice. 2018;19(9):1105-10.
[15] Khan HR, Patil BU, Gangane NM. E-cadherin as a prognostic biomarker in oral squamous cell carcinoma: A pilot study at tertiary care hospital. Med J DY Patil Vidyapeeth. 2022;15:501-06. https://journals.lww.com/mjdy/Fulltext/2022/15040/E_cadherin_as_a_Prognostic_Biomarker_in_Oral.13.aspx
[16] Santos-García A, Abad-Hernández MM, Fonseca-Sánchez E, Julián -Gonzalez R, Galindo-Villardón P, Cruz-Hernández JJ, et al. E-cadherin, laminin and collagen IV expression in the evolution from dysplasia to oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2006;11(2):E100-05.
[17] Yuwanati MB. Expression of E-cadherin in oral epithelial dysplasia and oral squamous cell carcinoma: An in vivo study. J Clin Exp Investig. 2011;2(4):347-53.
[18] Kaur J, Sawhney M, DattaGupta S, Shukla NK, Srivastava A, Walfish PG, et al. Clinical significance of altered expression of b-catenin and e-cadherin in oral dysplasia and cancer: potential link with ALCAM expression. PLoS ONE. 2013;8(6):01-12.
[19] Akhtar K, Ara A, Siddiqui SA, Sherwani RK. Transition of immunohistochemical expression of e-cadherin and vimentin from premalignant to malignant lesions of oral cavity and oropharynx. Oman Medical J. 2016;31(3):165-69.

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