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IMMUNOHISTOCHEMICAL EVALUATION OF E-CADHERIN AND MMP-9 IN ORAL EPITHELIAL DYSPLASIA AND SQUAMOUS CELL CARCINOMA

Pathology

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC), often evolving from oral epithelial dysplasia (OED), remains a leading cause of morbidity in India due to late detection. Identifying early molecular changes could improve diagnostic precision and therapeutic outcomes. **Objectives:** This study aimed to evaluate the immunohistochemical expression of E-cadherin and MMP-9 in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma, and to correlate these findings with histological severity, tumor differentiation, and size. **Methods:** A prospective observational study was conducted on 100 formalin-fixed paraffin-embedded oral tissue samples, including benign, premalignant, and malignant lesions. Immunohistochemistry was performed using monoclonal antibodies against E-cadherin and MMP-9. Expression was graded based on staining intensity and proportion. Statistical significance was assessed using Chi-square tests. **Results:** MMP-9 expression showed a significant progressive increase ($p < 0.001$) from benign to malignant lesions, while E-cadherin exhibited a corresponding decrease ($p < 0.001$). Tumors >4 cm demonstrated higher MMP-9 and reduced E-cadherin levels. An inverse correlation between both markers was observed with increasing dysplasia and carcinoma grade. **Conclusion:** E-cadherin and MMP-9 are valuable biomarkers in assessing malignant transformation in oral lesions. Their combined evaluation can aid early diagnosis, risk stratification, and personalized management in Oral Squamous Cell Carcinoma.

KEYWORDS

E-Cadherin; MMP-9; Oral Squamous Cell Carcinoma; Dysplasia; Immunohistochemistry

INTRODUCTION

India has a significant burden of oral cancer, accounting for approximately 30% of all cancers in the country. Oral squamous cell carcinoma (OSCC) accounts for approximately 90% of all oral malignancies and is recognized for its aggressive nature and poor prognosis, especially in developing nations like India, where late-stage diagnoses are common [1]. OSCC often evolves from oral epithelial dysplasia (OED), a precursor lesion characterized by varying degrees of epithelial disorganization and atypia [2]. Early recognition of molecular alterations within dysplastic and malignant tissues is critical for improving outcomes.

E-Cadherin, a transmembrane glycoprotein, is essential for epithelial cell adhesion and tissue integrity. Its downregulation is a hallmark of epithelial-to-mesenchymal transition (EMT), a process that facilitates tumor invasion and metastasis [3-5]. Concurrently, MMP-9, a zinc-dependent matrix metalloproteinase, promotes extracellular matrix degradation, enhancing tumor infiltration, angiogenesis, and lymph node spread [6-8]. The combined alteration of these markers is implicated in the progression from dysplasia to carcinoma.

Although immunohistochemistry (IHC) has emerged as a reliable tool for detecting such molecular changes and predicting tumor behavior [9], limited studies have evaluated the co-expression of E-Cadherin and MMP-9 in OED and OSCC, particularly within the Indian demographic context. Given the distinctive etiological factors prevalent in this population, such as areca nut usage and tobacco exposure, understanding the molecular landscape of oral lesions becomes even more pertinent [10].

We aimed to assess E-cadherin and MMP-9 expression in histologically confirmed OED and OSCC cases, correlating findings with histological severity to explore their potential as early diagnostic and prognostic biomarkers.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Pathology at B.R.D. Medical College, Gorakhpur, over a duration of one year, from September 2023 to September 2024. The study included histopathologically confirmed cases of oral premalignant and malignant lesions collected from patients attending the Otorhinolaryngology OPD. A total of 100 formalin-fixed, paraffin-embedded tissue samples were evaluated, comprising 90 cases of oral lesions and 10 samples of histologically confirmed normal oral mucosa used as controls. Sample size was calculated using Cochran's formula based on a prevalence rate of 17%, an allowable error of 7.5%, and a 95% confidence interval, yielding a sample size of 100 cases.

All specimens were processed following standard histopathological protocols. Tissues were fixed in 10% neutral buffered formalin, followed by dehydration in graded alcohol, xylene clearing, and paraffin embedding. Sections of 2–3 µm thickness were obtained and stained using Hematoxylin and Eosin (H&E) for microscopic evaluation and diagnosis. Eligible cases included all oral biopsy specimens received from the Otorhinolaryngology department, provided that patients had given informed consent. Inadequate or autolysed tissues and those lacking consent were excluded.

Immunohistochemical analysis was conducted to evaluate the expression of E-cadherin and MMP-9. For this, monoclonal mouse anti-E-cadherin (Clone 36) and rabbit anti-MMP-9 (Clone EP127), both manufactured by Bio SB, were used. Antigen retrieval was performed using citrate buffer for E-cadherin and Tris-EDTA buffer for MMP-9 through a microwave-assisted method. Subsequent steps included blocking, incubation with primary antibodies, detection using polymer HRP, development with DAB chromogen, and counterstaining with hematoxylin. Tissue sections from ductal breast carcinoma and bone marrow were employed as positive controls for E-cadherin and MMP-9 respectively, while sections processed without primary antibodies served as negative controls.

Immunoreactivity was interpreted based on the percentage of stained cells and staining intensity. E-cadherin staining was graded according to criteria proposed by Gupta et al. (2022) [11], focusing on membranous expression, whereas MMP-9 expression was graded using criteria established by Jose and Mane et al. (2018) [12], focusing on cytoplasmic staining. For each slide, 1000 cells were counted across ten high-power fields in a zigzag fashion to ensure uniform sampling.

Data were entered into tables and analyzed using descriptive statistics and the Chi-square test to determine associations, with a p-value <0.05 considered statistically significant. The entire study adhered to ethical

standards and was approved by the Institutional Ethics Committee of B.R.D. Medical College, Gorakhpur.

RESULTS

In our study, premalignant lesions were more common in younger individuals (≤50 years), while malignant lesions predominated in older age groups (51–60 years), though the difference was not statistically significant (p = 0.064). Both premalignant and malignant cases showed a strong male predominance with M:F ratios of 4:1 and 5.8:1, respectively, but without significant gender-based difference (p = 0.897). Larger tumors (>4 cm) showed significantly higher MMP-9 and lower E-Cadherin expression, indicating greater invasiveness and loss of adhesion in aggressive lesions (p < 0.05), as shown in Table 1.

Table 1: Comparion of Age, Sex, Region, Risk Factors, Laterality, Site and Symptoms

Parameters	No. Of cases
Age (years)	
< 40	16
> 40	48
Sex	
Male	80
Female	20
Region	
Rural	62
Urban	38
Laterality	
Right	58
Left	42
Risk factors	
Present	96
Not Present	04
Site	
Buccal mucosa	48
Tongue	44
Lips	04
Other	04
Symptoms	
Ulceroproliferative growth	59
Ulcer	20
fungating growth	08
Swelling	08
Other	05
Histological type	
Benign	10
Pre-malignant	15
Malignant	75
Tumor size	
< 4cm	65
>4cm	10

MMP-9 expression was entirely absent in benign lesions but showed a progressive rise in both proportion and intensity from potentially malignant to malignant lesions. Malignant cases predominantly demonstrated moderate to strong MMP-9 activity, reflecting its role in tissue invasion and malignancy progression. The association was statistically significant (p < 0.001), confirming MMP-9 as a reliable marker of histopathological severity, as shown in Table 1.

Table 2: Correlation of MMP-9 Proportion and Intensity Grade with Histological Diagnosis

	Non-Neoplastic Benign Lesions		Potentially Malignant lesions		Malignant Lesions		Chi sq.= 89.35
	n	%	n	%	n	%	p-Value
MMP-9 proportion grade							
Grade 0	10	100.00	2	13.33	0	0.00	<0.001
Grade 1	0	0.00	3	20.00	25	33.33	
Grade 2	0	0.00	6	40.00	44	58.67	
Grade 3	0	0.00	4	26.67	6	8.00	
MMP-9 intensity grade							
Grade 0	10	100.00	2	13.33	0	0.00	<0.001
Grade 1	0	0.00	6	40.00	24	32.00	
Grade 2	0	0.00	6	40.00	39	52.00	
Grade 3	0	0.00	1	6.67	12	16.00	

E-Cadherin exhibited strong membranous expression in benign lesions, which declined progressively in potentially malignant and malignant tissues. Most malignant lesions showed weak or absent expression, indicating loss of cell adhesion as a key event in tumor progression. The statistically significant trend (p < 0.001) highlights E-Cadherin's inverse correlation with dysplasia severity and carcinoma grade, as shown in Table 2.

Table 2: Correlation of E-Cadherin Proportion and Intensity Grade with Histological Diagnosis

	Non- Neoplastic Benign Lesions		Potentially Malignant lesions		Malignant Lesions		Chi sq.=86.09
	n	%	n	%	n	%	p-Value
E-cadherin Proportion grade							
Grade 1	0	0.00	1	6.67	25	33.33	<0.001
Grade 2	0	0.00	3	20.00	37	49.33	
Grade 3	0	0.00	7	46.67	13	17.33	
Grade 4	10	100.00	4	26.67	0	0.00	
E-cadherin Intensity grade							
Grade 1	0	0.00	1	6.67	16	21.33	<0.001
Grade 2	0	0.00	6	40.00	55	73.33	
Grade 3	10	100.00	8	53.33	4	5.33	

Mild oral epithelial dysplasia showed high E-Cadherin and low MMP-9 expression, whereas severe dysplasia and poorly differentiated squamous cell carcinoma showed the reverse trend. A clear inverse relationship was noted between E-Cadherin and MMP-9 expression with increasing histological severity, reinforcing their complementary roles in carcinogenesis and progression, as shown in Table 2.

Table 2: A Comparative Analysis of E-cadherin and MMP-9 Expression Score with Different Grade of Oral Epithelial Dysplasia Different Grades of OSCC

		E-cad Low (0-2)	E-cad Moderate (3-6)	E-cad High (7-12)	MMP-9 Low (0-2)	MMP-9 Moderate (3-5)	MMP-9 High (6-9)
Mild OED	n	0	0	8	8	0	0
	%	0%	0%	100%	100%	0%	0%
Moderate OED	n	0	5	0	1	2	2
	%	0%	100%	0%	20%	40%	40%
Severe OED	n	1	1	0	0	0	2
	%	50%	50%	0%	0%	0%	100%
WDSCC	n	5	29	2	19	11	6
	%	13.89%	80.56%	5.55%	52.78%	30.55%	16.67%
MDSCC	n	19	14	1	13	17	4
	%	55.89%	41.17%	2.94%	38.23%	50%	11.77%
PDSCC	n	3	2	0	0	1	4
	%	60%	40%	0%	0%	20%	80%

Discussion

In this study, immunohistochemical evaluation of E-cadherin and MMP-9 was performed across 100 cases, including benign, premalignant, and malignant oral lesions. Age-wise distribution showed that premalignant lesions were more common in individuals ≤50 years, while malignant lesions peaked in the 51–60 age group. Males were predominantly affected in both lesion types. A significant correlation was observed between tumor size and biomarker expression—tumors >4 cm showed low E-cadherin and high MMP-9 expression. MMP-9 proportion and intensity increased progressively from benign to malignant lesions, while E-cadherin expression showed an inverse trend. Combined marker analysis revealed that mild dysplasia retained high E-cadherin and low MMP-9, whereas poorly differentiated SCC exhibited the opposite pattern. These findings are consistent with prior literature. Mohapatra et al. and Nayak et al. also reported higher incidence of OSCC in middle-aged males [13,14]. Similar rural predominance was noted by Sharma et al., and tobacco-related sites were commonly affected, as observed by Yasin et al. and Moore & Catlin [15,16]. Immunohistochemical results align with Bashir et al. and D. Kani et al., who reported significant E-cadherin loss with increasing dysplasia and tumor grade [17,18]. Elevated MMP-9 expression in advanced lesions was similarly reported by Deepa Jose et al. and Chandolia et al. [12,19], confirming its role in matrix degradation and invasion. The inverse relationship between E-cadherin and MMP-9 observed here supports the concept of epithelial-mesenchymal transition (EMT), as described by Liu et al. and Afrem et al. [20,21] Thus, dual assessment of E-cadherin and MMP-9 provides valuable insight into oral carcinogenesis and may enhance diagnostic and prognostic accuracy in clinical pathology.

CONCLUSION

We concluded that reduced E-cadherin and elevated MMP-9 expression correlate strongly with histological severity in oral lesions, supporting their utility as complementary biomarkers in assessing malignant transformation and guiding early intervention strategies in OSCC.

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