e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(11); 1530-1537

Original Research Article

To Determine the Expression of E-Cadherin and Vimentin in Pre-Malignant and Malignant Oral Lesion and Their Relationship with Epithelial to Mesenchymal Transition Theory

Renu Sahay[1], Sufia Ahmad Khan[2], Madhu Singh[3], Jitendra Singh Yadav[4]

[1]Professor, Department of Pathology, M.L.B, Medical College, Jhansi, UP, India
[2]Associate Professor, Department of Pathology, M.L.B, Medical College, Jhansi, UP, India
[3]Junior Resident, Department of Pathology, M.L.B, Medical College, Jhansi, UP, India
[4]Professor, Department of Otorhinolaryngology, Maharani Laxmi Bai Medical College, Jhansi, UP, India

Received: 25-08-2024 / Revised: 23-09-2024 / Accepted: 26-10-2024
Corresponding Author: Dr. Madhu Singh
Conflict of interest: Nil

Abstract:

Background: Oral squamous cell carcinoma (OSCC) is a significant global health concern, especially in regions like the Indian subcontinent. The process of EMT, characterized by the loss of E-cadherin and gain of Vimentin, plays a crucial role in cancer metastasis and progression. Understanding the expression patterns of these markers can provide insights into the mechanisms behind EMT and OSCC progression. This study aimed to evaluate the expression of Vimentin and E-cadherin in different grades of premalignant and malignant oral lesions also determine the relationship between the expression of these markers and epithelial-mesenchymal transition (EMT).

Material and Methods: The cross-sectional study was conducted in the Department of Pathology at Maharani Laxmi Bai Medical College, Jhansi, from April 2023 to March 2024. The study included 100 cases of biopsies and resected specimens from patients with clinically diagnosed premalignant or malignant lesions of the oral cavity. Samples were fixed, processed, sectioned, and stained using Hematoxylin and Eosin. The expression of E-cadherin and Vimentin was assessed through immunohistochemistry.

Results: E-cadherin expression was found to be lower in older age groups and significantly associated with malignant lesions. High Vimentin expression was more common in older and middle-aged groups and was also significantly associated with malignant lesions. Statistical analysis revealed a significant relationship between marker expression and clinical characteristics such as age and diagnosis, with p-values <0.004 for age and <0.001 for malignant versus premalignant lesions.

Conclusion: The study indicates a potential inverse relationship between marker E-cadherin and Vimentin expression and different grades of dysplagia and OSCC. Lower E-cadherin expression and high Vimentin expression correlates with advancing age and malignancy. These findings support the role of EMT in OSCC progression and highlight the importance of E-cadherin and Vimentin as biomarkers for evaluating the malignancy potential of oral lesions.

Keywords: E-cadherin, Vimentin, Epithelial-Mesenchymal Transition (EMT), Oral Squamous Cell Carcinoma (OSCC), Premalignant Lesions.

Introduction

All clinical presentations that have a high probability of malignancy relative to healthy tissue are considered potentially malignant diseases.[1] Premalignant (precancerous) lesions are morphologically changed tissues where the risk of oral cancer is higher than in their apparently normal counterparts due to tobacco usage, human papillomavirus exposure, and betel nut chewing.

Approximately 3% of all cancers are oral cavity cancers, which is a serious global health issue. [2, 3] Premalignant diseases of the oral cavity are the root cause of many oral SCCs. [4, 5] With a high death rate, oral squamous cell carcinoma (OSCC) is the most frequent cancer affecting the oral cavity and is the sixth most common cancer globally.

Globally, there were 377,713 new cases and 177,757 fatalities recorded in 2020; this is an increase from 354,864 new cases and 177,384 deaths in 2018. [6,7,8] At an estimated yearly incidence of 75,000–80,000 new cases, OSCC is the most prevalent cancer among women and the most common among males in India [9, 10]. Oral squamous cell carcinoma most frequently occurs on the tongue, soft palate, lower lip, floor of the

mouth, and gingivo-buccal sulcus [11]. The two biggest risk factors for oral squamous cell carcinoma (OSCC) are alcohol and tobacco use [12]. It is more common in the Indian subcontinent to be linked to long-term usage of tobacco and areca nuts.[13] Because so many patients come with advanced disease stage and regional lymph node metastases, the overall 5-year survival rate is roughly 50%, making it a worldwide health burden even with the introduction of better diagnostic and therapeutic techniques [14–16]. Understanding the fundamental mechanism underlying the metastatic dissemination of oral squamous cell carcinoma is crucial for the development of anticancer treatments.

Tumor invasion is the initial phase in the complex and dynamic process of tumor metastasis, which involves multiple variables. EMT may result in a decline and loss of epithelial cell adhesion as well as the phenotypic characteristics of interstitial cells, which enhances tumor cell migration and invasiveness while also offering a possible explanation for the mechanism of tumor metastasis.

There are significant differences in the morphological traits, degree of cell differentiation, and biological behavior of tumor cells, which are more objective to assess recurrence and prognosis of the tumor. Bryne proposed the tumor invasive front (ITF) theory, which refers to the tumor host junction in the forefront of the three to six layers of tumor cells or dispersed cell group [17, 18].

The CDH1 gene, which is found on chromosome 16q21, encodes e-cadherin. An essential cell adhesion molecule and signal transduction factor, E-cadherin (Epitheia-cadhein) is a calcium-dependent transmembrane glycoprotein found in epithelial tissue. It can control the formation of protein complexes attached to the actin cytoskeleton in conjunction with beta-catenin formation, which can prevent and reduce tumor cell adhesion. Numerous studies have demonstrated that a decrease in tumor differentiation and an increase in the likelihood of metastatic dissemination occur when epithelial E-cadherin expression is lost, indicating a function for E-cadherin in tumor invasiveness and metastasis [19, 20].

Vimentin is a type III intermediate filament protein generally found in mesenchymal cells.[21] Vimentin is a cytoskeletal protein that is abundantly expressed in interstitial cells by lymphocytes, fibroblasts, and endothelial cells but not in normal epithelial cells. Vimentin expression has been found to significantly correlate with a number of OSCC prognostic variables, including tumor size, clinical stage, regional lymph node metastasis, local recurrence, and poor survival[13]. Epithelial mesenchymal transition (EMT) markers have the potential to significantly enhance patients' quality of life and cancer-free longevity by facilitating early detection and management of OSCC during its premalignant stage.[22]

The expression of vimentin and E-cadherin in oral squamous cell carcinomas is investigated in this work, and the possible contribution of the epithelial-mesenchymal transition to invasiveness is explored.

Material and Methods

The cross-sectional study was conducted in the Department of Pathology, Maharani Laxmi Bai Medical College, Jhansi (U.P.) from April 2023 to March 2024, after approval from the Institutional Ethical Committee. The study consists of 100 cases, including biopsies from various sites of the oral cavity. Samples were obtained from Department of ENT. Patients presenting with oral lesions that have been clinically diagnosed as either precancerous or cancerous were included in the study. Clinically appearing benign lesions and cases with inadequate material were excluded from the study.

Tissue preparation

To fix the tissues, 10% buffered formalin was utilized. Next, using an automated histokinette, the tissues were treated in several grades of alcohol and xylol. Sections of 4 micron thickness were cut from paraffin blocks and stained with H&E. Suitable blocks were identified for IHC.

Primary Antibodies: Primary antibodies that were particular to the targets of interest were treated with the tissue sections. The CONFIRM anti-Vimentin (V9) primary antibody and the VENTANA anti-E-cadherin mouse monoclonal primary antibody were applied, and they were incubated for 16 minutes at 37°C. The staining procedure is made more specific by the primary antibodies' binding to the appropriate antigens (Vimentin and E-cadherin) in the tissue sections.

Secondary Antibody Application: Following the incubation of the main antibody, sections were incubated for 8 minutes at 37°C with a secondary antibody. This antibody binds to the primary antibody, forming a complex that can be observed using an enzymatic reaction.

Eight drops of DAB (3,3'-diaminobenzidine) chromogen and one drop of DAB H2O2 were added, and the mixture was incubated for eight minutes to develop the color. A DAB enhancer (copper sulfate) was used to heighten the staining, and the sections were incubated for four minutes. This can boost the chromogenic signal and provide a staining that is more pronounced and sharp. Following DAB treatment, the sections were washed with xylene to get rid of any remaining staining reagents and water, and then

counterstained for 20 minutes with hematoxylin. DPX, a synthetic resin mountant, was used to finish the mounting.

Grading of E-cadherin immunostaining was based on the proportion of cells stained [19]; 1: < 10% positive cells; 2: 10 to 50% positive cells; 3: >50 to 80% positive cells; and 4: > 80% positive cells. E-cadherin grading was also assessed according to staining intensity [19, 23]. Staining levels range from 0 (absent) to 1 (weak), 2 (moderate), and 3 (strong). Vimentin was graded according to the stain intensity [21]. No staining (0), mild staining (1+), moderate staining (2+), marked staining (3+), and strong and diffuse (4+).

Statistical analysis: The statistical software, SPSS 26.0, was used to conduct the analyses. The association between the IHC markers for vimentin and E-cadherin was evaluated using bivariate analysis, which included the chi-square test and Fisher's exact test. A significant level of p-value < 0.05 was taken into consideration.

Results

The study comprised 100 cases of oral cavity biopsies from patients ranging in age from 28 to 79 years. The mean age of the cases was 50.68 years, and 96% of the cases were from individuals who were older than 30 years. There were 27 female cases and 73 male cases among these. According to the classification of lesion types, 29% of lesions are premalignant, and 71% are malignant. In this study, individuals with premalignant and malignant lesions were shown to be prevalent in both rural and urban settings. Many sites, including the buccal mucosa, dorsum of the tongue, gingivobuccal sulcus, lateral border of the tongue, lip, retromolartrigone, hard palate, and floor of the mouth, are affected in both male and female patients; however, the most frequently affected sites in both malignant and premalignant lesions are the buccal mucosa and lateral border of the tongue.

The most common type of substance addiction among people over 50 is tobacco usage. Individuals in the middle age group, notably those aged 31 to 50, are more likely to mix their use of alcohol, tobacco, and smoking (table 1).

Table 1: Association between substance abuse and age in premalignant and malignant oral cavity lesions

	Premalignant				**Malignant**			
Substance Abuse	**Age group**				**Age group**			
	≤30 years	31-50 years	>50 years	Total	≤30 years	31-50 years	>50 years	Total
Tobacco	1 (10.0)	3 (30.0)	6 (60.0)	10	0 (0.0)	10(47.6)	11(52.4)	21
Smoking	0 (0.0)	2(100.0)	0 (0.0)	2	0 (0.0)	1 (33.3)	2 (66.7)	3
Alcohol	0 (0.0)	0 (0.0)	0 (0.0)	0	0 (0.0)	0 (0.0)	2(100.0)	2
Tobacco + Alcohol	0 (0.0)	3(100.0)	0 (0.0)	3	0 (0.0)	7 (58.3)	5 (41.7)	12
Tobacco + Smoking	1 (33.3)	0 (0.0)	2 (66.7)	3	0 (0.0)	8 (57.1)	6 (42.9)	14
Smoking + Alcohol	1 (20.0)	4 (80.0)	0 (0.0)	5	0 (0.0)	3 (37.5)	5 (62.5)	8
Tobacco + Alcohol + Smoking	1 (16.7)	3 (50.0)	2 (33.3)	6	0 (0.0)	6 (54.5)	5 (45.5)	11

The degree of E-cadherin expression and the lesions' malignancy were strongly correlated. The findings indicate that premalignant lesions are more likely to have high E-cadherin expression, while malignant lesions are more likely to have low or negative expressions. As lesions advance from premalignant to malignant stages, this pattern suggests a possible decrease in E-cadherin expression (table 2).

Table 2: E-cadherin expression in oral cavity lesions

E-cadherin expression	**Premalignant (n=29)**	**Malignant (n=71)**	**p- value**
Low (Total Score 1-4)	7(13.5)	45(86.5)	<0.001
High (Total Score >4)	22(45.8)	26(54.2)	

A possible correlation between elevated expression of Vimentin and malignancy in lesions of the oral cavity is shown by table 3. Vimentin expression is generally absent in premalignant lesions but more commonly present in malignant ones.

Table 3: Vimentin expression in oral cavity lesions

Vimentin expression	**Premalignant (n=29)**	**Malignant (n=71)**	**p- value**
0	19 (63.3%)	11(36.7%)	<0.001
1+	9(26.3%)	25(73.5%)	
2+	1(5.6%)	17(94.4%)	
3+	0(0.0%)	8(100.0%)	
4+	0(0.0%)	10(100.0%)	

All dysplasia severities combined show a considerable number of patients with high E-cadherin expression. In severe dysplasia, low E-cadherin expression is more prevalent; in mild and moderate dysplasia, it is nonexistent. E-cadherin expression indicates a considerable correlation between the degree of dysplasia and its severity, with a statistically significant connection (p-value = 0.007) (table 4).

Table 4: E-cadherin Expression in Premalignant Lesions by Dysplasia Severity

E-cadherin Expression	Mild Dysplasia (n=09)	Moderate Dysplasia (n=06)	Severe Dysplasia (n=14)	Total (n=29)	P - value
High (Total Score >4)	9	6	7	22	0.007
Low (Total Score 1-4)	0	0	7	7	

Premalignant lesions primarily lack vimentin expression; most instances exhibit no staining at all dysplasia severities. Only moderate and severe dysplasia exhibits mild to moderate staining, indicating that vimentin expression in premalignant lesions is low initially but rises with severity of dysplasia. (Table 5).

Table 5: Vimentin Expression in Premalignant Lesions by Dysplasia Severity

Vimentin Expression	Mild Dysplasia (n=9)	Moderate Dysplasia (n=6)	Severe Dysplasia (n=14)	Total (n=29)	P value
0 (No Staining)	9	5	5	19	
1+ (Slight Staining)	0	1	8	9	
2+ (Moderate Staining)	0	0	1	1	0.025
3+ (Marked Staining)	0	0	0	0	
4+ (Strong and Diffuse Staining)	0	0	0	0	

There is a statistically significant correlation (p-value = 0.047) between the expression of E-cadherin and the different grades of oral squamous cell carcinoma. A substantial association between vimentin expression and OSCC grade is shown by a p-value of less than 0.001. The findings of the study demonstrate a possible inverse association between these markers and the degree of differentiation in OSCC by demonstrating a concomitant drop in E-cadherin expression and an increase in vimentin expression as the differentiation of OSCC declines (table 6).

Table 6: Grades of OSCC with E-cadherin and Vimentin expression

Grades of oscc						
Variable	**Groups**	**Well**	**Moderate**	**Poor**	**Total**	**p-value**
E- cadherin expression	**Low**	27	14	4	45	0.047
	High	11	15	0	26	
Vimentin expression	**0**	9	2	0	11	<0.001
	1+	20	5	0	25	
	2+	5	12	0	17	
	3+	4	3	1	8	
	4+	0	7	3	10	

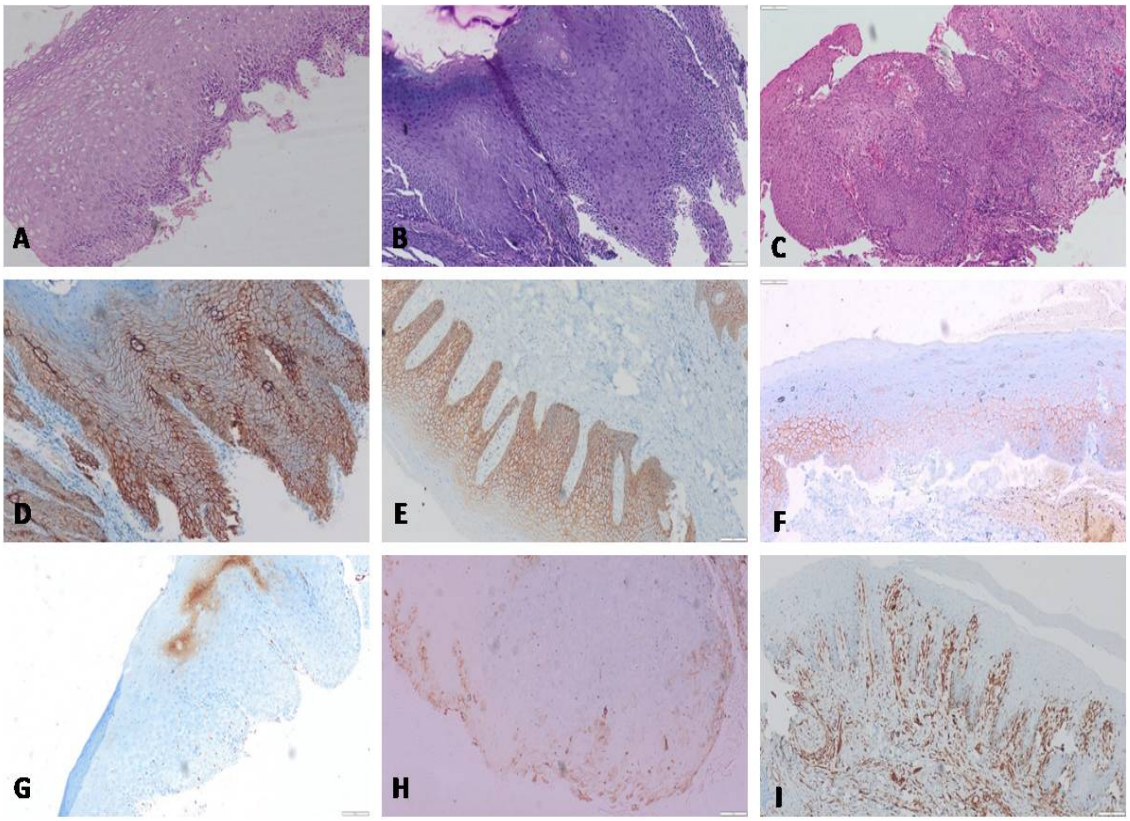

Figure 1: (A) Mild dysplasia, (B) Moderate dysplasia, (C) Severe dysplasia, (D,E,F) E-cadherin expression in different grades of dysplasia, (G,H,I) Vimentin expression in different grades of dysplasia

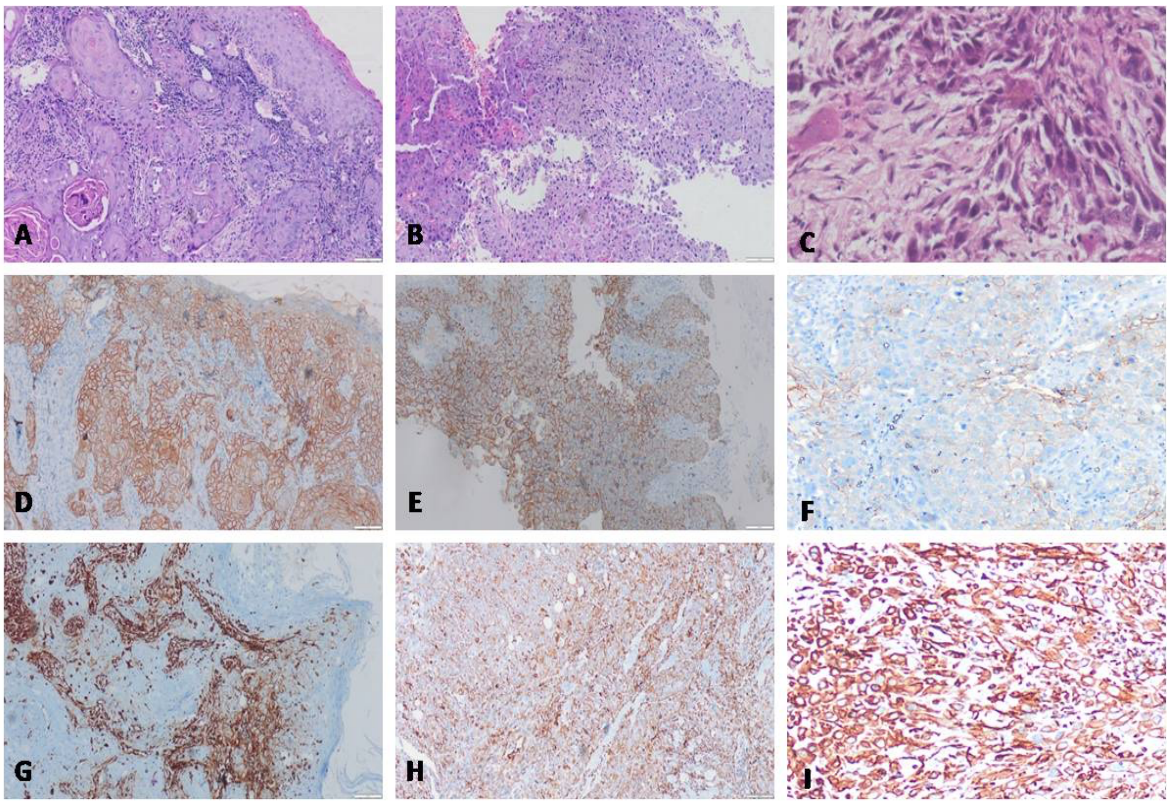

Figure 2 : (A) WD Oral squamous cell carcinoma,(B)- MD Oral squamous cell carcinoma, (C) PD Oral squamous cell carcinoma (D,E,F) E-cadherin expression in different grades of OSCC, (G,H,I) Vimentin expression in different grades of OSCC

Discussion

The aggressiveness and high metastatic potential of oral squamous cell carcinoma (OSCC) represent a major worldwide health burden [24]. Comprehending the role of molecular markers like Vimentin and E-cadherin in the pathogenesis of OSCC has become essential for clarifying the mechanisms behind tumor growth and treatment resistance [25]. 90% of head and neck cancers are squamous cell carcinomas, which begin in the oral cavity, according to published research [26,27, 28]. Similar to our analysis, a study by Balasundaram P. et al. (2014) reported that the mean age was 44 years and that there was a male predominance [19]. The results of Thankappan S. et al. (2024) [29] show similar results. The majority of patients in the study by Faiz SM et al. (2018) came from the 26–50 year age range, and 55.29% of the cases were male[30].

The lateral border of the tongue (26.7%) and buccal mucosa (38.0%) discovered in malignant lesions were found in this study. These findings were similar to those of Yamakanamardi B et al.'s study from 2023, which indicated that the tongue (21.0%) and buccal mucosa (48.0%) were the most common sites in malignant lesions [31]. According to a 2019 study by Talukdar L, the buccal mucosa is the most often occurring site in 46.51% of cases of malignant lesions [32].

According to this study, the most frequent risk factor for the development of malignant and premalignant lesions is tobacco use. Similar observation noted by Khanna S et al (2012) , Palliya SA et at (2019) and Taruna et al (2023)[33-35]. This suggests a decrease in E-cadherin expression as lesions progress from premalignant to malignant stages, supporting the findings of Yamakanamardi B. et al (2023), who observed reduced E-cadherin in malignant oral lesions [31]. Other similar studies are Kalaimani G. et al (2023.) and Silva AD et al. (2017) [36-38] Vimentin expression is generally absent in premalignant lesions but more frequently present in malignant ones, suggesting a potential association between increased Vimentin expression and malignancy in oral cavity lesions. Vimentin expression was more common in malignant lesions, with a significant proportion showing positive expression (84.5%) ($p<0.001$). Our findings are consistent with those of Mogre S et al. (2022), who observed heightened Vimentin expression in OSCC and its contribution to EMT [39].

Inverse relationship between expression of E-cadherin and Vimentin markers are strongly correlated with different severities of dysplasia. These findings are similar with the studies of Kafil Akhtar et al. (2016) in which opposite pattern of expression of both the markers was significantly associated with grades of dysplasia with $p=0.025$ and $p=0.013$ respectively [40]. Both Myong et al. (2012) and Fernandez A et al. (2011) observed a strong inverse relationship between E-cadherin and Vimentin expression, with Myong et al. reporting a statistically significant correlation ($p<0.001$). [41, 42]

The relationship between OSCC grades and the expression of E-cadherin ($p=0.047$) and Vimentin ($p<0.001$). Well-differentiated OSCC exhibited high E-cadherin and low Vimentin expression, while poorly differentiated OSCC showed the opposite pattern. This inverse relationship between both markers expression in OSCC grades is consistent with the findings of Liu K.L. et al. (2009) and Puneeta N. et al.(2022), Ali AN et al (2023) reported similar trends in their study on OSCC ($p<0.01$) [43,36,44]. Nguven et al [2011] utilized the immunohistochemical technique to validate the elevated presence of N-cadherin, which is linked to the metastasis and invasion of cancer cells in HNSCC patients [45]. Nijkamp et al (2011) research found that when E-cadherin expression is reduced in HNSCC, the cancer cells are more likely to spread (metastasize) to other parts of the body. [46].

Conclusion

Vimentin and E-cadherin are both useful biomarkers for determining the aggressiveness of OSCC and dividing up patient risk. These biomarkers provide information about tumor behavior that is essential for early diagnosis and individualized therapy planning. Comprehending the regulatory mechanisms that oversee these indicators offers prospects for focused treatments that try to reinstate E-cadherin functionality or obstruct EMT pathways, consequently potentially reducing tumor invasiveness and enhancing patient results.

According to the study's findings, E-cadherin and vimentin play important roles in the progression of OSCC and are important targets for treatment.

References

1. 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 Dec 17; 14:972. doi: 10.1186/1471-2407-14-972. PMID: 25518919; PMCID: PMC4301860.
2. Kademani D. Oral cancer. Mayo Clin Proc. 2007 Jul. 82(7):878-87.
3. Silverman S Jr. Demographics and occurrence of oral and pharyngeal cancers. The outcomes, the trends, the challenge. J Am Dent Assoc. 2001 Nov. 132 Suppl: 7S-11S.

4. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984 Feb 1. 53(3):563-8.
5. Silverman S Jr. Observations on the clinical characteristics and natural history of oral leukoplakia. J Am Dent Assoc. 1968 Apr. 76(4):772-7.
6. Solan P, Gale N, Hunter K, Lingen M, Nylander K, Reibel J, et al. Malignant surface epithelial tumors. In: El-Naggar AK, Chan JK, Grandis JR, Takata T, Slootweg PJ, editors. WHO Classification of Head and Neck Tumours. 4th ed. Switzerland: WHO Press; 2017. p. 105-16.
7. Sridevi U, Jain A, Nagalaxmi V, Kumar UV, Goyal S. Expression of E-cadherin in normal oral mucosa, in oral precancerous lesions and in oral carcinomas. Eur J Dent 2015; 9:364-72.
8. Tranby EP, Heaton LJ, Tomar SL, Kelly AL, Fager GL, Backley M, Frantsve-Hawley J. Oral Cancer Prevalence, Mortality, and Costs in Medicaid and Commercial Insurance Claims Data. Cancer Epidemiol Biomarkers Prev. 2022 Sep 2; 31(9):1849-1857.
9. Sankaranarayanan R. Oral cancer in India: An epidemiologic and clinical review. Oral Surg Oral Med Oral Pathol 1990; 69:325-30.
10. National Cancer Registry Programme, Consolidated report of the population based cancer registries, 1990-1996. Indian Council of Medical Research, New Delhi, August 2001. Available from: www.icmr.nic.in/ncrp/ncrp_p/cancer_reg.pdf [Last accessed 2003 Jan 23].
11. Krolls SO, Hoffman S. Squamous cell carcinoma of the oral soft tissues: a statistical analysis of 14,253 cases by age, sex, and race of patients. J Am Dent Assoc. 1976; 92 (3): 571-574. doi: 10.14219/jada.archive. 1976. 0556.
12. Scully C. Oral cancer aetiopathogenesis; past, present and future aspects. Med Oral Patol Oral Cir Bucal. 2011; 16(3):e306-e311. doi: 10.4317/medoral.16.e306.
13. Sawant SS, Vaidya Mm, Chaukar DA, Alam H, Dmello C, Gangadaran P, et al. Clinical significance of aberrant vimentin expression in oral premalignant lesions and carcinomas. Oral Dis 2014; 20:453-65.
14. Epstein JB, Zhang L, Rosin M. Advances in the diagnosis of oral premalignant and malignant lesions. J Can Dent Assoc. 2002; 68(10):617-621.
15. Glazer CA, Chang SS, Ha PK, Califano JA. Applying the molecular biology and epigenetics of head and neck cancer in everyday clinical practice. Oral Oncol. 2009; 45(4-5): 440-446. doi: 10.1016/j.oraloncology.2008.05.013. Epub 2008 Jul 31.
16. Molinolo AA, Amornphimoltham P, Squarize CH, Castilho RM, Patel V, Gutkind JS. Dysregulated molecular networks in head and neck carcinogenesis. Oral Oncol. 2009; 45(4-5):324-334. doi: 10.1016/j.oraloncology.2008. 07. 011. Epub 2008 Sep 19.
17. Bryne M, Boysen M, Alfsen CG, Abeler VM, Sudbø J, Nesland JM, Kristensen GB, Piffko J, Bankfalvi A. The invasive front of carcinomas. The most important area for tumour prognosis? Anticancer Res 1998; 18: 4757-64.
18. Piffkò J, Bànkfalvi A, Ofner D, Bryne M, Rasch D, Joos U, Böcker W, Schmid KW. Prognostic value of histobiological factors (malignancy grading and AgNOR content) assessed at the invasive tumour front of oral squamous cell carcinomas. Br J Cancer 1997; 75: 1543-6.
19. Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer Res. 1993; 53(14):3241-3245.
20. Deng QW, He BS, Pan YQ, Sun HL, Xu YQ, Gao TY, et al. Roles of E-cadherin (CDH1) genetic variations in cancer risk: a meta-analysis. Asian Pac J Cancer Prev. 2014; 15(8):3705-3713. doi: 10.7314/apjcp.2014.15. 8.3705.
21. Steinert PM, Roop DR. Molecular and cellular biology of intermediate filaments. Annu Rev Biochem 1988;57:593–625
22. Balasundaram P, Singh MK, Dinda AK, Thakar A, Yadav R. Study of β-catenin, E-cadherin and vimentin in oral squamous cell carcinoma with and without lymph node metastases. Diagn Pathol 2014; 9:145.
23. Deng QW, He BS, Pan YQ, Sun HL, Xu YQ, Gao TY, et al. Roles of E-cadherin (CDH1) genetic variations in cancer risk: a meta-analysis. Asian Pac J Cancer Prev. 2014; 15(8):3705-3713. doi: 10.7314/apjcp.2014.15. 8.3705.
24. Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res. 2020 Apr 2; 9:229.
25. Liu LK, Jiang XY, Zhou XX, Wang DM, Song XL, Jiang HB. Upregulation of vimentin and aberrant expression of Ecadherin/ beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. Mod Pathol. 2010; 23(2):213- 224.
26. Sung H, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71(3):209–49.

27. Gormley M, Creaney G, Schache A, Ingarfield K, Conway DI. Reviewing the epidemiology of head and neck cancer: definitions, trends and risk factors. Br Dent J. 2022 Nov; 233(9):780-786.
28. Deshpande AM, Wong DT. Molecular mechanisms of head and neck cancer. Expert Rev Anticancer Ther. 2008 May; 8(5):799-809.
29. Thankam DR, A AJ. Expression of e-cadherin and vimentin in lesions of oropharynx. Int J Adv Med 2021; 8:246-53.
30. Faiz SM, Agarwal E, Bhargava A, Varshney P, Patigaroo AR, Rizvi D. Spectrum of premalignant oral lesions in rural North Indian population at a tertiary care hospital. Int J Otorhinolaryngol Head Neck Surg2018; 4:1452-7.
31. Yamakanamardi B, Rani H, Shettar D, Parinitha SS, Rao RV. Immunohistochemical Expression of E-cadherin in Premalignant and Malignant Squamous Lesions of the Oral Cavity: A Cross-sectional Study. National Journal of Laboratory Medicine. 2023; 12(4):PO14-PO20.
32. Talukdar, L., & Goswami, A. An immunohistochemical study of e-cadherin expression in primary oral squamous cell carcinoma and metastatic lymph node in a tertiary care centre. Tropical Journal of Pathology and Microbiology, 2019; 5(11): 884-891.
33. Khanna S. The interaction between tobacco use and oral health among tribes in central India. Tob Induc Dis. 2012; 10:10–16.
34. Oral health disparities among privileged and underprivileged tribes of south India - a study on precancerous oral lesions prevalence. Palliyal SA. https://www.annalsofoncology.org/ article/S0923-753457984-4/pdf Ann Oncol. 2019;30:104–105
35. Taruna T, Singh YP, Waghmare R, Sinha A, Priya L, Sharma V, Singh UB. Analyzing the Frequency of Premalignant Lesions and Oral Malignancy in Indian Subjects Attending Outpatient Department from the Low Socioeconomic Group. Cureus. 2023 Jul 17; 15(7):e42 035.
36. Puneeta N, Santosh T, Mishra I, Gaikwad P, Sahu A. Evaluation of e-cadherin and vimentin expression for different grades of oral epithelial dysplasia and oral squamous cell carcinoma – An immunohistochemical study. J Oral Maxillofac Patho 2022; 26:285-6.
37. Kalaimani G, Rao UDK, Joshua E, Ranganathan K. E-cadherin Expression in Premalignant Lesions, Premalignant Conditions, Oral Squamous Cell Carcinoma, and Normal Mucosa: An Immunohistochemical: Study. Cureus. 2023 Aug 28; 15(8):e44266.
38. Silva AD, Maraschin BJ, Laureano NK, Daroit N, Brochier F, Bündrich L, Visioli F, Rados PV. Expression of E-cadherin and involucrin in leukoplakia and oral cancer: an immunocytochemical and immunohistochemical study. Brazilian oral research. 2017; 31(0):e19.
39. Mogre S, Makani V, Pradhan S, Devre P, More S, Vaidya M, Dmello C. Biomarker potential of vimentin in oral cancers. Life. 2022 Jan 20; 12(2):150.
40. 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 Med J 2016; 31:165-9.
41. Myong NH. Loss of E-cadherin and acquisition of vimentin in epithelial-mesenchymal transition are noble indicators of uterine cervix cancer progression. Korean J Pathol 2012; 46:341-8.
42. Fernandez A, Marshall M, Santibanez N, Martinez C, Fernandez J, Martinez R. Comparing the Immuno-Expression of Endothelial Markers in Normal Oral Mucosa, Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma: Relationship between ECadherin, Vimentin, CD31, CD117 and Epithelial-Mesenchymal Transition. MOJ Anat Physiol, 2017 Sept; 4(2): 301-6.
43. Liu, Y., & El-Naggar, A.K. (2009). E-cadherin gene inactivation in primary human oral squamous cell carcinomas and its effect on invasiveness. Cancer Research, 59(12), 2861-2865.
44. Ali AN, Ghoneim SM, Ahmed ER, Salam LO, Saleh SM. Cadherin switching in oral squamous cell carcinoma: A clinicopathological study. Journal of Oral Biology and Craniofacial Research. 2023 Jul 1; 13(4):486-94.
45. Nguyen PT, Kudo Y, Yoshida M, Kamata N, and Ogawa I, Takata T. N-cadherin expression is involved in malignant behavior of head and neck cancer in relation to epithelial-mesenchymal transition. Histology and histopathology, 2011, Vol. 26(2)
46. Nijkamp MM, Span PN, Hoogsteen IJ, van der Kogel AJ, Kaanders JH, Bussink J. Expression of E-cadherin and vimentin correlates with metastasis formation in head and neck squamous cell carcinoma patients. Radiotherapy and Oncology. 2011 Jun 1; 99(3):344-8.