

# Transition of Immunohistochemical Expression of E-Cadherin and Vimentin from Premalignant to Malignant Lesions of Oral Cavity and Oropharynx

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## ABSTRACT

**Objectives:** We sought to study the expression of epithelial-to-mesenchymal transition markers E-cadherin and vimentin in precancerous lesions of the oral cavity and oropharynx and to use the specific pattern of expression to predict invasiveness. **Methods:** This cross-sectional study looked at 87 cases of oral and oropharyngeal lesions obtained between December 2012 and November 2014 in the Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, India. Fifty-three biopsies from the buccal mucosa, tongue, and pharynx and 34 resected oral specimens were evaluated for premalignant and malignant lesions using hematoxylin and eosin and immunohistochemical stains. Immunohistochemical expression of epithelial marker E-cadherin and mesenchymal marker vimentin was evaluated wherever possible. Slides were examined for staining pattern (cytoplasmic or membrane), proportion, and intensity of staining of tumor cells. Patients follow-up and therapy related changes were also studied. **Results:** There were 64 premalignant and 23 malignant cases in our study with 65 (74.7%) cases seen in males and 22 (25.3%) cases seen in females. The majority of malignant cases, (n = 15; 64.2%) were seen in the fifth and sixth decades of life while most of the premalignant lesions (n = 36; 56.4%) were seen in the fourth and fifth decade. Amongst the 64 premalignant oral lesions, leukoplakia comprised of 14 cases (21.9%), of which three cases had associated mild to moderate dysplasia. The majority of premalignant lesions showed strong E-cadherin expression and decreased expression of vimentin with negative and weak expression in both dysplasias and carcinoma in situ ( $p = 0.013$ ). E-cadherin expression was significantly reduced in invasive carcinomas compared to dysplasias and carcinoma in situ and the difference in immunoreactivity was statistically significant ( $p < 0.050$ ). Vimentin expression increased as the tumor progressed from dysplasias to carcinoma in situ to invasive carcinomas ( $p < 0.050$ ). **Conclusions:** Invasiveness of cancer can be analyzed using E-cadherin and vimentin immunohistochemical stains, which can help in predicting tumor behavior. These biomolecules can also be used as biomarkers for further research on the microinvasion of oral cancers for early diagnosis.

Leukoplakia on the floor of mouth, lateral tongue, and lower lip are most likely to show dysplastic or malignant changes. The frequency of dysplastic or malignant alterations in oral leukoplakia ranges from 15.6% to 39.2%.<sup>1</sup> In general, the thicker the leukoplakia, the greater the chance of finding dysplastic changes. Therefore, a verrucous leukoplakia is more likely to show dysplasia than a thick homogeneous leukoplakia.<sup>2</sup> All leukoplakias should be viewed with suspicion because even small, subtle lesions can manifest significant dysplasia or unsuspected carcinoma.<sup>3</sup> Additionally, all cases of erythroplakia present with some degree of epithelial dysplasia.<sup>1</sup>

True clinical erythroplakia is a much more worrisome lesion than leukoplakia.<sup>4</sup>

Oral submucosal fibrosis is well recognized as a potentially malignant disorder.<sup>5</sup> Tilakaratne et al,<sup>6</sup> found that oral submucosal fibrosis showed a malignant transformation of approximately 7%. There is considerable controversy as to the potentially malignant nature of lichen planus, with some opinion that it carries an unequivocal malignant potential and an unspecified risk.<sup>7,8</sup>

Loss of epithelial morphology and acquisition of mesenchymal characteristics, termed epithelial-to-mesenchymal transition (EMT), are typical for carcinoma cells and correlate with the local

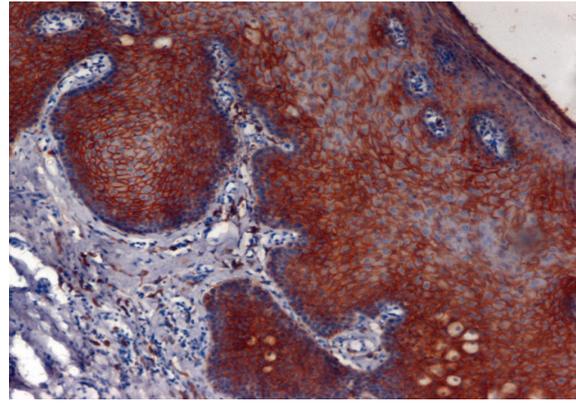
invasiveness and metastatic potential of the tumor.<sup>9</sup> In our study, we looked at the role of EMT in precancerous lesions of the oral cavity and oropharynx (specifically decreased expression of E-cadherin and increased expression of vimentin) and sought to determine if the specific pattern of E-cadherin and vimentin expression could be used to predict invasiveness.

## METHODS

This cross-sectional study looked at 87 cases of oral and oropharyngeal lesions from the Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, India, diagnosed from December 2012 to November 2014. Patients gave their informed consent and the local ethical committee approved the study.

Fifty-three biopsies from the buccal mucosa, tongue, and pharynx and 34 resected oral specimens were evaluated for all premalignant and malignant lesions using routine hematoxylin and eosin staining and E-cadherin (Novocastra ready to use mouse monoclonal antibodies for E-cadherin, Novocastra), and vimentin (with DAB (3,3'-diaminobenzidine), Dako) immunohistochemical stains, wherever possible. Immunohistochemical analysis, using rabbit and mouse antihuman polyclonal antibodies, was performed on the serial sections. Slides were examined for the staining pattern (cytoplasmic or membrane), proportion and intensity of staining of tumor cells. Patients follow-up and therapy-related changes were also studied.

Grading of E-cadherin immunostaining was based on the percentage of cells stained<sup>10</sup>; 1+: < 10% positive cells; 2+: 10 to 20% positive cells; 3+: >20 to 50% positive cells; and 4+: > 50% positive cells. Grading of E-cadherin was also evaluated based on



**Figure 1:** Leukoplakia: Photomicrograph shows strong membranous positivity of E-cadherin (4+) in acanthotic squamous epithelium, magnification = 10 ×.

the location and intensity of staining;<sup>10,11</sup> 1+: absent membrane staining; 2+: weak/heterogeneous membranous staining; and 3+: strong/homogeneous membranous staining. Grading of vimentin was done on the basis of the stain intensity:<sup>12</sup> 1+: weak and focal staining; 2+: strong and focal staining; 3+: weak and diffuse staining; and 4+: strong and diffuse staining.

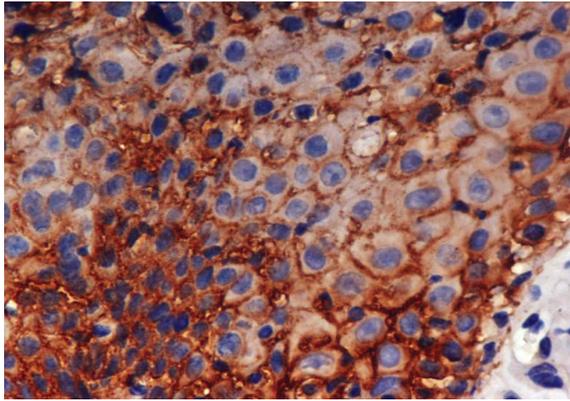
For the statistical analysis, Fischer's exact test was employed, and a *p*-value of < 0.050 was considered significant.

## RESULTS

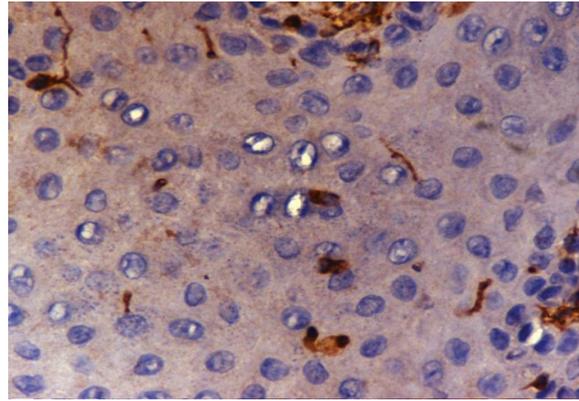
There were 64 premalignant and 23 malignant cases in our study. Sixty-five cases (74.7%) were seen in males and 22 (25.3%) in females. Both premalignant and malignant cases were more prevalent in males compared to females. The majority of malignant cases (*n* = 15; 64.2%) were seen in the fifth and sixth decades of life while most of the premalignant

**Table 1:** Expression of E-cadherin in premalignant lesions.

Lesion type	Number of cases	Degree of E-cadherin expression			
		1+ (< 10% cells)	2+ (10–20% cells)	3+ (> 20–50% cells)	4+ (> 50% cells)
Leukoplakia	5	0	0	2	3
Erythroplakia	5	0	2	1	2
Submucosal fibrosis	5	1	0	2	2
Lichen planus	5	0	1	2	2
Lichenoid dysplasia	5	0	0	3	2



**Figure 2:** Moderate dysplasia: Photomicrograph shows moderate membranous positivity of E-cadherin (3+) in squamous epithelial cells, magnification = 40 ×.



**Figure 3:** Mild dysplasia: Photomicrograph shows the absence of vimentin staining with few cells showing weak (1+) cytoplasmic positivity, magnification = 40 ×.

lesions (n = 36; 56.4%) were seen in the fourth and fifth decades of life. The number of malignant cases increased with advancing age.

Amongst the 64 premalignant oral lesions studied, leukoplakia comprised of 14 cases (21.9%), out of which three cases had associated mild to moderate dysplasia. There were 16 cases (25.0%) of different grades of dysplasias along with 11 cases (17.2%) of carcinoma in situ, seven cases (11.0%) of lichen planus, six cases (9.4%) of erythroplakia, and five cases each (7.8%) of lichenoid dysplasia and submucosal fibrosis.

Most of the premalignant lesions studied showed strong (4+) membranous immunostaining of E-cadherin ( $p = 0.853$ ) [Figure 1 and Table 1]. The majority of premalignant lesions also showed weak (1+ or 2+) cytoplasmic immune-expression for vimentin ( $p = 1.000$ ).

Eight (80%) cases of dysplasias showed strong (4+) expression of E-cadherin and five (50%) cases

of carcinoma in situ showed strong (4+) immune expression of E-cadherin [Figure 2]. In our study, six (60%) cases of well differentiated squamous cell carcinoma showed moderate (3+) staining intensity of E-cadherin and three (30%) cases showed strong (4+) immunoexpression of E-cadherin. Only two cases of moderately differentiated carcinomas showed strong (4+) expression of E-cadherin but none of the cases of poorly differentiated carcinomas showed strong (4+) expression of E-cadherin [Table 2]. We concluded that E-cadherin expression was significantly reduced in invasive carcinomas compared to dysplasias and carcinoma in situ ( $p < 0.050$ ).

Eight (80.0%) cases each of dysplasias and carcinoma in situ showed either negative or weak (1+/2+) staining for vimentin [Figure 3]. Seven (70.0%) cases of well-differentiated carcinoma showed negative/weak (1+/2+) vimentin staining, while six (60.0%) cases of poorly-differentiated

**Table 2:** Comparison of E-cadherin expression pattern in dysplasias, carcinoma in situ and invasive carcinomas.

Type of lesion	Number of cases	Degree of E-cadherin expression		
		1+/2+ (Negative/weak)	3+ (Moderate)	4+ (Strong)
Dysplasias	10	2	0	8
Carcinoma in situ	10	1	4	5
Well-differentiated squamous cell carcinoma	10	1	6	3
Moderately-differentiated squamous cell carcinoma	10	8	0	2
Poorly-differentiated squamous cell carcinoma	10	9	1	0

**Table 3:** Comparison of vimentin expression pattern in dysplasias, carcinoma in situ, and invasive carcinoma.

Type of lesion	Number of cases	Degree of vimentin expression		
		1+/2+ (Negative/weak)	3+ (Moderate)	4+ (Strong)
Dysplasias	10	8	0	2
Carcinoma in situ	10	8	2	0
Well-differentiated squamous cell carcinoma	10	7	3	0
Moderately-differentiated squamous cell carcinoma	10	2	5	3
Poorly-differentiated squamous cell carcinoma	10	0	4	6

carcinoma showed strong (4+), staining and four (40%) cases had moderate (3+) staining. No cases were negative for vimentin [Table 3]. Our study showed an increased expression of vimentin as the tumor progressed from dysplasias to carcinoma in situ to invasive carcinomas ( $p < 0.050$ ).

## DISCUSSION

Our study showed positivity of different intensity of E-cadherin and vimentin expression in premalignant and malignant lesions. We observed a significant decrease in E-cadherin membrane expression from dysplasia to carcinoma in situ to invasive carcinoma and a significant increase in vimentin expression with progression of the tumor. Loss of E-cadherin and gain of vimentin is a hallmark of tumor progression and E-cadherin is a good prognostic marker whereas vimentin expression indicates a poor prognosis.

In a study of 121 cases, Kaplanis et al,<sup>12</sup> reported that high-grade squamous intraepithelial lesion (HSIL) and invasive carcinoma showed significantly reduced E-cadherin membrane expression and increased cytoplasmic immunoreactivity compared to the low-grade SIL (LSIL), atypical squamous cell of undetermined significance, and normal groups. The study concluded that decreased E-cadherin membrane expression appears to be a useful assessment parameter of the malignant potential of cervical lesions.

Myong<sup>11</sup> also showed loss of E-cadherin membranous immunoreactivity as the tumor progressed from carcinoma in situ, micro-invasive to invasive squamous cell carcinoma (49.0%, 23.0%, and 0% respectively). The differences in the immunoreactivities were statistically significant between carcinoma in situ and micro-invasive or invasive carcinomas ( $p < 0.001$ ). Our study also

showed statistically significant E-cadherin expression between dysplasias or carcinoma in situ and invasive carcinomas. Williams et al,<sup>13</sup> found upregulation of P-cadherin expression in severe dysplasia and reduced expression of the E-cadherin and/or catenins in carcinoma in situ and infiltrating tumors. They concluded that disruption of the E-cadherin-catenin complex was a late event associated with invasion.

Myong<sup>11</sup> reported that normal control tissues exhibited almost no vimentin immunohistochemical expression, and carcinoma in situ lesions showed 8.8% immunoreactivity. Microinvasive and invasive carcinomas showed a much higher vimentin expression in 53.0% and 67.0% cases, respectively, findings concordant with our study. Thus, significantly increased vimentin expression could be used as an important EMT marker in the progression of carcinoma in situ to microinvasive or invasive squamous cell carcinoma in human cervical tissues.

Friksen et al,<sup>14</sup> suggested that the ability of carcinoma to invade and metastasize largely depends on the degree of epithelial differentiation within the tumors after their study on various human cell lines derived from bladder, breast, lung, and pancreas carcinoma. They found that carcinoma cell lines with an epithelioid phenotype were noninvasive and expressed the epithelium-specific cell-cell adhesion molecule E-cadherin whereas carcinoma cell lines with a fibroblastoid phenotype were invasive with the loss of E-cadherin expression. Fan et al<sup>15</sup> also suggested that weak E-cadherin expression was associated with a poor prognosis in oral squamous cell carcinoma.

## CONCLUSION

Invasiveness of cancer can be assessed using an immunohistochemical panel of E-cadherin and

vimentin, which could help to predict the prognosis. We can use these biomolecules as biomarkers for further research on the microinvasion of oral cancers and to develop early diagnosis, and better treatment modalities and increase patient survival.

#### Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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