Original Article

Diagnostic and Prognostic Significance of E-Cadherin and Vimentin in Oral Cancer Metastasis

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ABSTRACT

Background: To study the role of epithelial-to-mesenchymal transition by E- cadherin and vimentin expression in precancerous and cancerous lesions of oral cavity and oropharynx and to predict invasiveness by the specific pattern of E-cadherin and vimentin expression.

Methods: Biopsies/ specimens of oral cavity and oropharynx were evaluated for all premalignant lesions and invasive epithelial squamous lesions, by haematoxylin and eosin sections and by immunohistochemical expression of E-cadherin and vimentin, wherever. Patients follow up and therapy related changes were also studied during the period of study.

Results: E-cadherin staining showed 6/10 (60%) cases of well differentiated carcinoma with 4+ degree of expression while 0/10 case of poorly differentiated carcinoma showed 4+ and only a single case showed 3+ degree of expression with 8/10 (80%) cases of well differentiated carcinoma depicting strong staining intensity of E-cadherin. 6/10(60%) cases of well differentiated oral squamous cell carcinoma showed 1+ degree of expression of vimentin while 6/10(60%) cases of poorly differentiated carcinoma showed 4+ degree of expression. 1(1.6%) case of positive lymph node metastasis showed strong positive staining for E-cadherin and 4 (66.6%) cases showed absent staining pattern of E-cadherin. The differences in the immunoreactivities were statistically significant between CIS and mi-croinvasive or invasive carcinomas ($p<0.001$) in our study.

Conclusions: Invasiveness and recurrence can be analysed by the use of immunohistochemical stains of E-cadherin and vimentin, which can help in predicting the tumor behaviour, prognosis, survival and management of the patient. Also, these biomolecules can be used as biomarkers for further research on the micro-invasion of the tumor for early diagnosis and survival of the patients.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer and ranks as one of the top ten cancers worldwide. India has the dubious distinction of having the world's largest number of oral cancer patients with an annual age-standardised incidence of 12.5 per 1,00,000 cases and oral cancer accounts for 9.4 per cent of all cancers in India. [1] Oral cancer is the commonest cancer in India, accounting for 50-70% of total cancer mortality and accounts for highest incidence among Asian countries.[2]

Loss of epithelial morphology and acquisition of mesenchymal characteristics, termed as the epithelial-to-mesenchymal transition (EMT) are typical for carcinoma cells and correlate with the local invasiveness and metastatic potential of the tumor.[3] In general cells proceeding EMT exhibit down-regulation of many epithelial markers including E-cadherin, desmoplakin, cytokeratins, claudins, occluding and beta-catenin and up-regulation of mesenchymal markers, including N-cadherin, vimentin, fibronectin and Snail-1/2. [4,5]

In the present study, we studied the role of EMT by decreased expression of E- cadherin and increased expression of vimentin in precancerous and cancerous lesions of oral cavity and oropharynx. The specific pattern of E-cadherin and vimentin expression can predict invasiveness and may be used as markers for early diagnoses.

Material and Methods

The present study was carried out on oral and oropharyngeal lesions. Before the commencement of study, we considered ethical aspects and obtained ethical committee approval. Biopsies/ specimens of oral cavity and oropharynx were evaluated for all premalignant lesions and invasive epithelial squamous lesions, using routine haematoxylin and eosin sections and by immunohistochemical expression of E-cadherin and vimentin, wherever possible. Immunohistochemical analysis by using rabbit and mouse antihuman polyclonal antibodies was performed on the serial sections. The working systems for the immunohistochemical reactions were represented by Novocastra ready to use Mouse monoclonal antibodies for E-cadherin and vimentin (DAB: 3,3'- diaminobenzidine, Dako). Patients follow up and therapy related changes were also studied during the period of study.

Grading of E-Cadherin on the Basis of Percentage of the Cells Stained: 1+ < 10 % cells; 2+ 10 to 20 % cells; 3 + >20 to 50 % cells and 4+ >50 % cells. Grading of E-Cadherin on the basis of location of staining [6]: 1: Membranous staining; 2: Both membranous and cytoplasmic staining; 3: Cytoplasmic staining and 4: Absence of staining. Grading of E-Cadherin on the basis of intensity of staining [7]: Strong- Staining pattern in present in almost all tumor cells (>95%); is as strong as in the normal epithelial cells. Weak/Homogenous- All tumor cells are uniformly stained but more weakly expressed than in the normal squamous epithelium. Absent/ Heterogenous- The intensity of E-cadherin staining differs from cell to cell and the cells without immunostaining are also included. Grading of vimentin on the basis of intensity of staining: 1+ Weak and Focal staining; 2+ Strong and Focal staining; 3+ Weak and Diffuse staining; 4+ Strong and Diffuse staining.

STATISTICAL ANALYSIS TESTS: Fischer exact test showed that the probability of obtaining any such set of values was given by the hypergeometric distribution:

$$p = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} = \frac{(a+b)!\ (c+d)!\ (a+c)!\ (b+d)!}{a!\ \ b!\ \ c!\ \ d!\ \ n!}$$

P value in our study, if found to be < 0.01= highly significant; 0.01 to 0.05 = significant and > 0.05= weakly significant

Results

E-cadherin immunohistochemistry in our study, showed 6/10 (60%) cases with 4+ degree of expression and 3/10 (30%) cases with 3+ degree of expression in well differentiated carcinoma, while in poorly differentiated carcinoma 0/10 case showed 4+ and only a single case showed 3+ degree of expression. (Table 1) In our study, 8/10 (80%) cases of well differentiated carcinoma showed strong staining intensity of E-cadherin (Figure 1), while 1/10(10%) case of poorly differentiated carcinoma showed strong staining intensity and 8/10 (80%) cases showed weak or homogenous staining intensity.

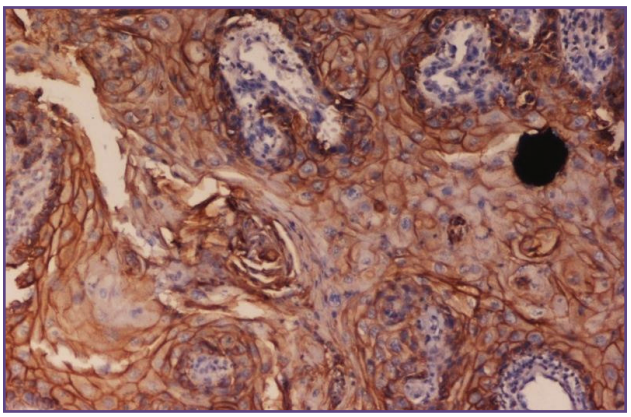

Fig. 1: Well differentiated squamous cell carcinoma: Photomicrograph shows nests of tumor cells with strong membranous positivity of E-cadherin (3+). (IHC E-cadherin x 10X)

Table 1: Expression of E-cadherin in different grades of squamous cell carcinoma cases

Grades of squamous cell carcinoma	No of cases	Degree of E-cadherin expression			
		1+ (<10%cells)	2+ (10-20% cells)	3+ (>20-50%cells)	4+ (>50% cells)
Well differentiated	10	0	1 (10%)	3(30%)	6(60%)
Moderately differentiated	10	2 (20%)	6 (60%)	0	2(20%)
Poorly differentiated	10	5 (50%)	4 (40%)	1(10%)	0

E-cadherin expression can vary between tumors of the same type and even between areas of the same tumor. Our study showed 6/10(60%) cases with membranous positivity of E-cadherin in well differentiated carcinoma and 4/10 (40%) cases showed both membranous and cytoplasmic positivity. 7/10(70%) cases of poorly differentiated carcinoma showed only cytoplasmic positivity and 2/10(20%) cases showed both cytoplasmic and membranous positivity, but none showed membranous positivity.

We found that 6/10(60%) cases of well differentiated oral squamous cell carcinoma with 1+ degree of expression of vimentin while none of the case showed 4+ staining pattern. In poorly differentiated carcinoma, 6/10(60%) cases showed 4+ degree of expression. (Figure 2 and Table 2)

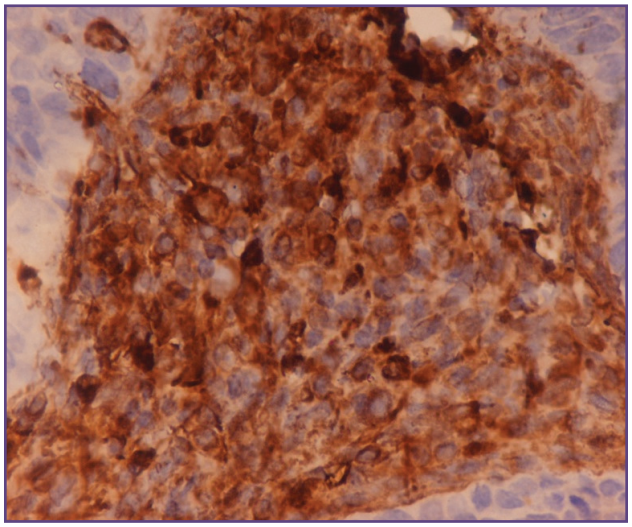

Fig. 2: Poorly differentiated squamous cell carcinoma: Photomicrograph shows strong and diffuse cytoplasmic positivity of vimentin (4+). (IHC Vimentin x 40X)

Lymph node metastasis evaluation in our study showed, 6/30 cases were positive for lymph node metastasis of squamous cell carcinoma and 24/30 cases were negative for malignant cells. 1(1.6%) case of positive lymph node metastasis showed strong positive staining for E-cadherin and 4 (66.6%) cases showed absent staining pattern of E-cadherin. It was found that 16 (66.6%) cases of negative lymph node metastasis showed strong E-cadherin staining intensity in their primary site of oral squamous cell carcinoma.

We performed immunostain of E-cadherin and vimentin on 10 cases each of dysplasias and Ca-in-Situ. 8 /10(80%) cases of dysplasias showed strong 4+ degree of expression of E-cadherin and 5/10 (50%) cases of Ca-in-Situ showed strong 4+ staining (p=0.025). Our study showed a decreased expression of vimentin with majority of cases showing negative and weak expression in both dysplasias and Ca-in-Situ (p=0.013). We concluded that E-cadherin expression was significantly reduced in invasive carcinomas as compared to dysplasias and ca- in -situ and the difference in immunoreactivity was statistically significant ($p < 0.001$). We compared the expression pattern of vimentin in dysplasias, carcinoma in situ and invasive carcinomas and concluded that vimentin expression was increased as the tumor progressed from dysplasias to ca-in-situ to invasive carcinomas, which was statistically significant ($p < 0.001$).

We also found an inverse correlation between E-cadherin and vimentin expression.18/30 cases showed strong E-cadherin expression and in these cases 15/18 (83.3%) cases showed strong vimentin negativity, while 8/11(72.7%) cases showed vimentin positivity in which E-cadherin was weakly positive, which was statistically significant ($p < 0.001$).

Table 2: Expression of vimentin immunostain in different grades of squamous cell carcinoma cases

Grades of squamous cell carcinoma	No of cases	Degree of vimentin expression			
		1+ (Weak & focal)	2+ (Strong & focal)	3+ (Weak & diffuse)	4+ (Strong & diffuse)
Well differentiated	10	6 (60%)	3(30%)	1(10%)	0
Moderately differentiated	10	0	2(20%)	5(50%)	3(30%)
Poorly differentiated	10	0	0	4(40%)	6(60%)

We followed up the known cases of oral squamous cell carcinoma, who underwent radiotherapy. Repeat biopsy was performed to analyse the post radiotherapy effects on the tumor grade and histological differentiation. Immunostaining with E-cadherin and vimentin was undertaken to determine their staining pattern and findings correlated with recurrence of tumor, metastasis and prognosis. We performed E-cadherin and vimentin immunohistochemistry on the 8 cases of recurrence. Grading the membrane staining pattern of E-cadherin, we found that majority of recurrence cases showed weak (1+ and 2+) staining pattern in all the grades of carcinoma and none showed strong (3+ or 4+) positivity. Also,1 case (50%) of poorly differentiated carcinoma, 2 cases (66.6%) of moderately differentiated carcinoma and 1 case (33.3%) of well differentiated carcinoma showed absent/ heterogenous membrane staining for E-cadherin (Figure 3) as compared to 10%, 0% and 0% respectively, in the primary cases, which signified poor prognosis in the recurrent cases.

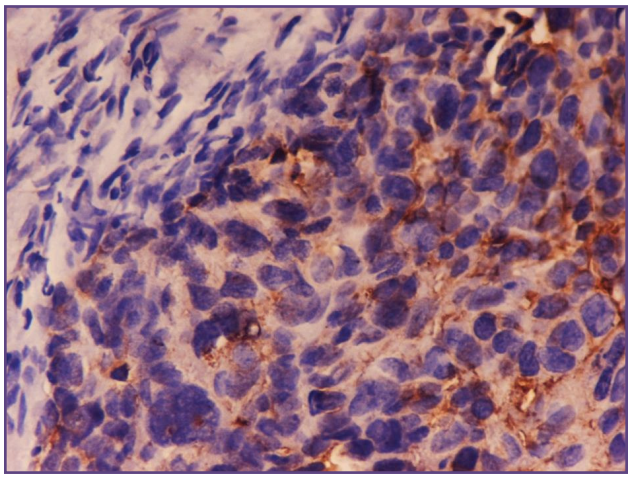

Fig. 3: Post radiotherapy recurrent poorly differentiated carcinoma: Photomicrograph shows poor cytoplasmic positivity of E-cadherin (1+) in scattered tumor cells. (IHC E-cadherin x 40X)

On immunostaining with vimentin on post radiotherapy recurrence cases, we observed that 2 cases (100%) of poorly differentiated carcinoma and 2 cases (66.6%) of moderately differentiated carcinoma showed strong (4+) cytoplasmic staining pattern as compared to 60% and 30% respectively, in primary cases. 33.3% cases of well differentiated squamous cell carcinomas depicted strong (3+ and 4+) cytoplasmic staining of vimentin in recurrence cases, as compared to 10% cases with 3+ and none with 4+ staining pattern in the primary cases, highlighting the increased cytoplasmic staining intensity in recurrent cases and poor prognosis of the patients. Our findings suggests that vimentin was more strongly expressed in recurrent cases irrespective of tumor grade and favored the tumor cells to show more invasive pattern with more tumor recurrence and metastasis. So we concluded that vimentin was a poor prognostic marker for oral squamous cell carcinomas and poor survival indicator.

Discussion

In our study, degree of E-cadherin expression decreased as the grade of the tumor was increased. Huber et al concluded in his study that the loss of E-cadherin mediated cell adhesion is prominent and overall there was a trend towards a loss of E-cadherin during carcinoma progression.[8] A study by Mehendiratta et al observed absence of staining in 0%,10%, 30%, of well, moderate and poorly differentiated oral squamous cell carcinoma, respectively.[9] Kaur et al studied E-cadherin expression in different histological grades of oral squamous cell carcinoma and reported strong expression in 90% well differentiated, 92.9% moderately differentiated and 15.4% poorly differentiated carcinoma as compared to weak and homogenous staining showed by 10%, 7.1% and 69.2% cases respectively.[6] We concluded that E-cadherin membranous positivity decreased and cytoplasmic positivity increased as the tumor grade increased from well to poorly differentiated squamous cell carcinoma, leading to poor prognosis. Kaur et al found, cytoplasmic staining in 28.6% moderately differentiated carcinoma and 61.5% cases of poorly differentiated oral squamous cell carcinoma cases.[6]

Vimentin expressions increased as the tumor progressed from well to poorly differentiated carcinoma. Na-Hye Myong et al observed that normal squamous mucosa showed no immunoreactivity for vimentin. [7] However, squamous carcinoma cells revealed a relatively dense cytoplasmic expression for vimentin, showing polygonal or multipolar appearance and heterogeneously scattered pattern. Araujo et al who reported that vimentin positivity was found in many cells of histological high grade of tumors, with poor outcome of the patients on treatment. [10]

Cavallaro and Christofori have stated that loss of E-cadherin is a hallmark of metastatic carcinoma. [11] Willipinski-Stapelfeldt et al in their study on proteonomic analysis of breast cancer have reported that circulating mammary tumor cells or those found as micrometastasis, show the evidence of mesenchymal conversion. [12] According to Ramaekers et al the vimentin cytoskeleton most likely occurs in epithelial cells only during the process of metastasis.[13]

Na-Hye Myong, showed that E-cadherin immunoreactivity to be increased in the cytoplasm or lost in the cell membranes was also observed from CIS, microinvasive and invasive

squamous cell carcinoma by either cytoplasmic (51%, 71%, and 95%, respectively) or membranous (49%, 23%, and 0%, respectively) immunoreactivities. [7] The differences in the immunoreactivities were statistically significant between CIS and microinvasive or invasive carcinomas ($p<0.001$) in our study. Na- Hye Myong, showed normal control tissues immunohistochemically presented almost no vimentin expression and the CIS lesions showed less than 10% of immunoreactivity (8.8%) and microinvasive and invasive carcinomas showed a much higher vimentin expression with 53% and 67% cases, respectively. [7] Thus, it was considered that the significantly increased vimentin expression could be used as an important EMT marker in the progression of CIS into micro-invasive or invasive SCC in the human cervical tissues.

Na- Hye Myong, have reported a statistically significant inverse correlation between E-cadherin and vimentin immuno-expression, with p value of $p <0.001$ in their study on 119 cases, a finding concordant with our study. [7]

Immunohistochemistry on recurrence cases showed more loss of E-cadherin expression, which depicted aggressive activity of the tumor. Our findings complement the evidence that movement of E-cadherin from membrane to cytoplasm was associated with increasing tumor grade and recurrence. There is loss of adhesion in the cells, increasing the metastability with more invasiveness and tumor recurrence.[14] Guzinska-Ustymowiczet al in their study on 34 colon cancer patients reported borderline significance of $p=0.06$ in the association between the recurrence at the postoperative site and change in location of E-cadherin expression in the tumor mass from membrane to cytoplasm. [14] Yasuto et al have concluded that 5 years survival rates of patients in tumor with preserved E-cadherin expression was 35.2% whereas the survival rate was 20.9% with reduced E-cadherin expression.[15]

A study was done by Yong et al, showed that vimentin and E-cadherin have a significant impact on cervical squamous cell carcinoma on patients of clinical stage 1 or 2. [16] It was found that there was down regulation of E-cadherin in metastasis and upregulation of vimentin in recurrence cases, as was seen in our study.

Hou et al on Kaplan-Meier survival analysis showed that the overexpression of EphA2 and vimentin, ectopic expression of β-catenin and down-regulation of E-cadherin indicated a poor outcome.[17] Fan et al mentioned that low E-cadherin expression was associated with poor prognosis in oral squamous cell carcinoma. [18] Liu et al studied oral squamous cell carcinoma and documented high expression of vimentin in 23/43(53%) tumors from patients who eventually developed a recurrent tumor and was associated with recurrence and death ($p <0.001$ and < 0.001respectively).[19] The combination of up regulation of vimentin and aberrant expression of E-cadherin /βcatenin complexes at the tumor invasive front may provide a useful prognostic marker in oral squamous cell carcinoma. The post radiotherapy recurrence cases showed statistically insignificant p values (> 0.05) of E-cadherin and vimentin expression in our study, because of small sample size of post radiotherapy recurrent cases. However, further studies are required with larger sample size to show statistical significant correlation.

Conclusions

Our study concludes that invasiveness and recurrence could be analysed by the immunohistochemical staining pattern of E-cadherin and vimentin, which can help in predicting the tumor behaviour, prognosis, survival and management of the patient. Also, these biomolecules can be used as biomarkers for further research on the micro-invasion of the tumor for early diagnosis and survival of the patients.

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None

Competing Interests

None declared

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