Expression of e-cadherin and vimentin in lesions of oropharynx

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INTRODUCTION

Oral and oropharyngeal cancers are major neoplasms worldwide and accounts for most head and neck cancers. More than 90% of malignant neoplasms of the oral cavity and oropharynx are squamous cell carcinomas arising from the lining mucosae with relatively rare neoplasms arising in minor salivary glands and soft tissues.

Oral squamous cell carcinoma is a devastating disease and remains a major threat to global public health. Oral squamous cell carcinoma is the sixth most common malignancy in the world and ranks as first in males in the Indian subcontinent. It is a major cause of cancer morbidity and mortality and can develop from oral precancerous lesions such as leukoplakia and erythroplakia.

Early oropharyngeal cancer is asymptomatic, which results in delayed diagnosis. Any single ulcerated lesion persisting for more than 3 weeks should be looked in with suspicion, and a biopsy should be performed. The mnemonic RULE (red, ulcerated, lump, extending for 3 or more weeks) can be used as an aid to diagnosis. Survival rate of oral and oropharyngeal carcinomas after 5 years is

ABSTRACT

Background: Oral squamous cell carcinoma is the 6th most common malignancy in the world and ranks as first in males in Indian sub-continent. Vimentin is an intermediate filament found in mesenchymal cells and e-cadherin is an adhesion molecule found in epithelial cells. The objective of the study is to evaluate the expression of e-cadherin and vimentin in lesions of oropharynx and to assess the sensitivity, specificity and positive predictive value of e - cadherin and vimentin in oropharyngeal squamous cell carcinoma (OPSCC), against routine H and E stained histopathological diagnosis.

Methods: 100 oropharyngeal biopsy specimens taken and routine H and E stained histopathological slide diagnosis made. E-cadherin and vimentin expression studied in OPSCC, moderate to severe dysplasia, mild dysplasia and benign cases and its sensitivity, specificity, positive predictive value and negative predictive value analysed using appropriate statistical tools.

Results: Vimentin positivity was observed in 70 out of 79 OPSCC, 2 out of 3 cases of moderate - severe dysplasia, 0 out of 2 mild dysplasia and 2 out of 16 benign lesions. Out of 79 cases of OPSCC, 15 were e-cadherin negative, 27 showed low and 37 cases showed high membraneous positivity.

Conclusions: We observed a significant decrease in e-cadherin membrane expression from dysplasia to carcinoma insitu to invasive carcinoma and a significant increase in vimentin expression with progression of the tumor. E-cadherin is a good prognostic marker whereas vimentin expression indicates a poor prognosis.

Keywords: E-cadherin, Vimentin, Oropharyngeal squamous cell carcinoma, Dysplasia
≤50%. However, this can be increased when these cancers are diagnosed at an early stage.³

Squamous cell carcinoma is common in the developing world, mostly in older males. Now there is an increase in younger patients and OPSCC in women, due to human papillomavirus (HPV) infection.

The etiology of squamous cell carcinoma is multifactorial and strongly related to lifestyle, habits and diet (particularly tobacco alone or in combination with betel, and alcohol use). Other factors such as infective agents mostly Human Papilloma Virus (HPV) may also be implicated, especially in oropharyngeal cancer. Immune defects or immunosuppression, defects of carcinogen metabolism, or defects in DNA-repair enzymes underlie some cases of SCC. Sunlight exposure predisposes to lip cancer.

Vimentin is an intermediate filament found in mesenchymal cells, but not in epithelial cells. E-cadherin is a calcium dependent cell-cell adhesion molecule found in epithelial cells and serve an important role in cell adhesion and signalling pathways that regulate cell proliferation, differentiation and survival. The loss of expression of e-cadherin together with the acquisition of vimentin expression is known to be a marker of epithelial mesenchymal transition (EMT) changes in epithelial cells. Adhesion molecules play a central role in the pathogenesis and progression of malignant tumours. Therefore, it is important to evaluate the role of cell adhesion molecule, e-cadherin along with vimentin in various lesions of oropharynx.⁴

The loss of expression of e-cadherin together with the acquisition of vimentin expression is known to be a marker of EMT changes in epithelial cells. Several reports have shown the acquisition of mesenchymal marker vimentin in oral squamous cell carcinomas, with the concomitant loss of epithelial marker e-cadherin. E-cadherin is a major constituent of the adherens junctions in the process of EMT.⁴ The objectives of the study is to study the expression of e-cadherin and vimentin in lesions of oropharynx and to assess the sensitivity, specificity and positive predictive value of e-cadherin and vimentin in oropharyngeal squamous cell carcinomas against oral squamous cell carcinomas against expression of EMT.

Histopathological diagnosis of the biopsy specimens are classified into normal, mild dysplasia, moderate to severe dysplasia, or OSCC, as described by the WHO 2005 classification.⁵

Immunohistochemistry (IHC) was performed on thick sections which are made on Poly –L-lysine coated slides. Sections were dewaxed and rehydrated, then placed in Tris EDTA Borate buffer for antigen retrieval. Antigen retrieval was done by heat method in pressure cooker for 10 minutes followed by cooling to room temperature. Sections were then washed in water for 5 minutes. Endogenous peroxidase was abolished by submerging the slides in quenching solution (30 ml 30% H2O2:300 ml distilled water) for 20 minutes and then washed in tap water for 5 minutes; rinsed in distilled water for 3 minutes and kept in Tris EDTA Borate buffer for 5 minutes. Sections were dried by wiping all around. Serum blocking solution was added and kept for 10 minutes. Sections were then be blotted and primary antibody added on to sections and incubated for 1 hour in a moisture chamber; washed in PBS for 5 minutes for 3 days. Sections were treated with super enhancer and kept for 25 minutes. Secondary antibody was then added and incubated for 25 minutes. PBS wash for 2 minutes up to 3 times. Chromogen Diamino Benzidine was added (constituted by mixing 1 ml buffer, 50 microlitre liquid DAB) and kept for 5 minutes; washed in PBS for 5 minutes. Sections were rinsed in distilled water for 5 minutes; stained with Harris Hematoxylin by 10 dips and blued for 5 minutes. Sections were then be dehydrated, cleared and mounted with DPX.

Normal oral mucosa in the specimen acts as positive control for e-cadherin and vimentin. Negative controls are created by omission of primary antibody and replacement with phosphate buffered saline.

A positive membranous with or without cytoplasmic staining in more than 10% of neoplastic cells is considered as positive staining for e-cadherin and any vimentin cytoplasmic staining is account as positive for vimentin. Immunoreactivity was semiquantitatively evaluated on the basis of staining intensity and distribution using the immunoreactive score.⁶

Immunoreactive score=intensity score × proportion score.⁷

The intensity score was defined as

0= negative; 1= weak; 2= moderate; or 3= strong, and the proportion score was defined as 0= negative; 1= <10%; 2= 11–50%; 3= 51–80%; or 4= >80% positive cells.
The total score ranged from 0 to 12.

The immunoreactivity was divided into three groups on the basis of the final score: negative immunoreactivity was defined as a total score of 0, low immunoreactivity was defined as a total score of 1–4, high immunoreactivity was defined as a total score >4.

Statistical analysis

All statistical analysis were carried out using Statistical package for social sciences (SPSS).

Sensitivity = true positive / (true positive + false negative),

Specificity = true negative / (true negative + false positive),

Positive predictive values = true positive / (true positive + false positive)

Negative predictive values = true negative / (true negative + false negative) of each.

RESULTS

A total of 108 oropharyngeal biopsy specimens were received from the Surgery, ENT and OMFS departments. 8 were inadequate specimens. Hence, they were excluded from the study. A total of 100 cases which met the inclusion criteria were included in the study and analysed.

The mean age in our study was 61 years ranging from 20 years – 90 years.

Cases were divided into 7 age groups-group 1 ranging from 21-30 years, group 2 ranging from 31-40 years, group 3 from 41-50 years, group 4 from 51-60 years, group 5 from 61-70 years, group 6 from 71-80 years and group 7 from 81-90 years. There were 5 cases in group 1 (5%), 5 cases in group 2 (5%), 13 cases in group 3 (13%), 25 cases in group 4 (25%), 34 cases in group 5 (34%), 12 cases in group 6 (12%) and 6 cases in group 7 (6%).

In our study out of the 100 cases, 76 cases were males and 24 cases were females. Among this, 24 females, 15 were OPSCC cases and 9 were benign/normal cases. Among the 76 males 64 were OPSCC cases, 3 were moderate to severe dysplasia cases, 2 were mild dysplasia cases and 7 were benign / normal cases.

Of the total 100 specimens studied, oropharyngeal squamous cell carcinoma was identified in 79 cases (79%), moderate - severe dysplasia in 3 cases (3%), mild dysplasia in 2 cases (2%) and benign / normal mucosa in 16 cases (16%).

In our study maximum number of OPSCC were seen in the sixth decade. Out of 79 cases of OPSCC 31 cases were in age group 5, 21 cases were in age group 4, 11 cases were in age group 6, 7 cases were in age group 3,6 cases were in age group 7, 2 cases were in age group 1 and only 1 case was in age group 2. Out of three moderate to severe dysplasia cases one case was each in age groups 2,4 and 5. No moderate to severe dysplasia cases were seen in age groups 1,3,6 and 7. Out of 2 mild dysplasia cases, one case was seen in age group 1 and the other in age group 3. No cases of mild dysplasia was seen among other age groups.

Out of 16 benign / normal cases, no case was seen in age group 7, 2 cases were seen in age group 1, 3 cases were seen in age group 2, 4 cases were seen in age group 3, 4 cases in age group 4, 2 cases in age group 5 and only one case in age group 6.

The expression of e-cadherin and vimentin in benign/normal mucosa, in lesions with mild dysplasia, moderate - severe dysplasia and in OPSCC were analysed.

Out of 79 cases of OPSCC, 70 showed vimentin positivity and 9 showed vimentin negativity. Among 3 cases of moderate-severe dysplasia, 2 showed vimentin positivity and 1 was vimentin negative. Out of 2 cases of mild dysplasia, both 2 were vimentin negative and out of 16 benign cases 2 were vimentin positive and 14 were vimentin negative.

Table 1: sensitivity and specificity of vimentin against routine H and E stained slide diagnosis.

<table>
<thead>
<tr>
<th>Vimentin</th>
<th>OPSCC</th>
<th>Benign/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>70 (a)</td>
<td>2 (b)</td>
</tr>
<tr>
<td>negative</td>
<td>9 (c)</td>
<td>14 (d)</td>
</tr>
</tbody>
</table>

Out of 79 cases of OPSCC, 15 were e-cadherin negative, 27 showed low membraneous positivity, (that is immunoreactivity score between 0-4) and 37 cases showed high membraneous positivity (that is immunoreactivity score more than 4).

\[ Sensitivity = \frac{a}{a + c} \times 100 \]

\[ = \frac{70}{70+9} \times 100 \]

\[ = 88.6\% \]

\[ Specificity = \frac{d}{b + d} \times 100 \]

\[ = \frac{14}{2+14} \times 100 \]

\[ = 87.5\% \]

\[ Positive\ predictive\ value = \frac{a}{a + b} \times 100 \]

\[ = \frac{70}{70+2} \times 100 \]

\[ = 97.2\% \]

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Negative predictive value = \((d + c + d) \times 100\)

\[= (14/9 + 14) \times 100\]

\[= 60.9\%\]

**Table 2: Sensitivity and specificity of e-cadherin against routine H and E stained slide diagnosis.**

<table>
<thead>
<tr>
<th></th>
<th>OPSCC</th>
<th>Benign/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin negative</td>
<td>15 (a)</td>
<td>0 (b)</td>
</tr>
<tr>
<td>E-cadherin high positivity</td>
<td>37 (c)</td>
<td>13 (d)</td>
</tr>
</tbody>
</table>

In our study, we took 100 cases of oropharyngeal biopsies to study the expression of the epithelial mesenchymal transition markers like vimentin and e-cadherin and we got 88.6% sensitivity and 87.5% specificity for vimentin in detecting OPSCC against routine H and E stained histopathological slide diagnosis. The positive predictive value of vimentin was 97.2% and the negative predictive value of vimentin was 60.9%.

**Table 3: Sensitivity and specificity of e-cadherin against routine H and E stained.**

<table>
<thead>
<tr>
<th></th>
<th>OPSCC</th>
<th>Benign/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin negative</td>
<td>15 (a)</td>
<td>0 (b)</td>
</tr>
<tr>
<td>E-cadherin low positivity</td>
<td>27 (c)</td>
<td>3 (d)</td>
</tr>
</tbody>
</table>

Sensitivity=35.7%

Specificity=100%

Positive predictive value=100%

Negative predictive value=10%

In our study the immunoreactivity of E-cadherin was semi-quantitatively evaluated on the basis of staining intensity and distribution using the immunoreactive score.

**Immunoreactive score**

\[= \text{intensity score} \times \text{proportion score}\]

**Table 4: Expression of vimentin in various lesions.**

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Cases</th>
<th>Vimentin positive</th>
<th>Vimentin negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPSCC</td>
<td>79</td>
<td>70</td>
<td>9</td>
</tr>
<tr>
<td>Moderate-severe dysplasia</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Benign</td>
<td>16</td>
<td>2</td>
<td>14</td>
</tr>
</tbody>
</table>

**Figure 1: Distribution of various lesions of oral cavity in various age groups.**

**Figure 2: Vimentin expression in various lesions of oral cavity.**

The immunoreactivity was divided into three groups on the basis of the final score: negative immunoreactivity was defined as a total score of 0, low immunoreactivity was defined as a total score of 1–4, high immunoreactivity was defined as a total score >4.
When we are comparing the negative immunoreactivity of E-cadherin in OPSCC with that of high immunoreactivity in benign /normal cases, we are getting a sensitivity of 28.8%, specificity of 100%, positive predictive value of 100% and negative predictive value of 26%.

Vimentin is a cytoskeletal protein, not expressed in normal epithelial cells, but widely distributed in fibroblasts, endothelial cells, and lymphocytes in the interstitial cells. Several studies have found that the abnormal expression of vimentin was also observed in a variety of epithelial tumors, and had close relationship with differentiation, invasion and metastasis of cancer cell.10,11

The burden of oropharyngeal carcinoma falls on men who are elderly or black.13 The median age at diagnosis is approximately sixty years.14 Squamous cell carcinoma is the most common malignant neoplasm of the head and neck. It was reported that there were 633,000 new registered cases and 355,000 deaths in 2008 worldwide.14 There is a large geographic variability in the occurrence and the site of origin of head and neck squamous cell carcinoma (HNSCC), which reflects the prevalence of tobacco and alcohol consumption, and ethnic and genetic differences among populations.15 Data from the Kerala Cancer Registry 2011-2012 gives the trends in oral and pharyngeal cancers in 3 hospital based cancer registries (RCC Thrivanthapuram, AIMS Kochi and MCC Thalassery) in Kerala.16 There has been statistically significant increase in the incidence of oral and oropharyngeal cancer in all the registries.

In spite of an ever-expanding fund of knowledge about the etiology and pathophysiology of malignant neoplasms, oral and oropharyngeal squamous cell carcinoma continues to be a disfiguring and fatal disease. The 5-year survival rate for patients with squamous cell carcinoma of the oral cavity or oropharynx is a dismal 56%, which has remained relatively unchanged in recent year.17 This poor prognosis reflects the fact that most patients present with advanced-stage disease, often making a complete cure an unattainable goal. In fact, just 46% of oral cavity and 16% of oropharyngeal cancers are diagnosed when there is only local disease.18

The mean age of the 100 cases in our study was 60.11 years with a range of 21-90 years. The percentage of OPSCC in our study was 79%, moderate - severe dysplasia was 3%, mild dysplasia was 2% and benign / normal mucosa was16%. In our study maximum number of OPSCC were seen in the sixth decade. Out of 79 cases of OPSCC 31 cases were in age group 5, 21 cases were in age group 4, 11 cases were in age group 6, 7 cases were in age group 3,6 cases were in age group 7, 2 cases were in age group 1 and only 1 case was in age group 2.

In a study by Akhtar et al, the number of malignant cases were increased with advancing age. There were 64 premalignant and 23 malignant cases in there study 19. 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 lesions (n=36; 56.4%) were seen in the fourth and fifth decades of life.20

### DISCUSSION

The concept of epithelial and mesenchymal transition (EMT) was first proposed by Greenberg et al.8 EMT play a key role in tumor invasion and metastasis.9 E-cadherin (Epithelia-cadhein) is a calcium dependent transmembrane glycoprotein located in the epithelial tissue, is an important cell adhesion molecule and signal transduction factor.10

**Figure 3: E-Cadherin expression in various lesions of oral cavity.**

**Figure 4: Biopsy. A. Squamous cell carcinoma, H and E 40×, B. E-cadherin positive normal squamous cells, C. E-cadherin negative squamous cell carcinoma, D. Vimentin positive squamous cell carcinoma.**
Our study is in concordance with many other studies in the facts that both premalignant and malignant cases were more prevalent in the males and the maximum number of malignant cases were in sixth to seventh decade (91.18%).

In a study conducted by Liu et al, there was an inverse correlation between vimentin and E-cadherin expression in oral squamous cell carcinoma specimens.\textsuperscript{21} The overexpression of vimentin was closely associated with the absence or reduced expression of E-cadherin at the invasive front of tumours. E-cadherin, is well recognised for its elective expression and specific roles in epithelial cellular states.\textsuperscript{22} Vimentin expression, coupled with the reduced or lack of E-cadherin expression, is characteristic of cells of mesenchymal origin, whereas the reverse is true for cells with an epithelial phenotype.\textsuperscript{23,24} These findings support the results of previous in vivo experiments in which vimentin expression resulted in the downregulation of E-cadherin expression. This epithelial–mesenchymal transition might have an important role in oral squamous cell carcinoma carcinogenesis or progression.\textsuperscript{26,28}

In the above-mentioned study by Akhtar et al most of the premalignant lesions studied showed strong (4+) membranous immunostaining of E-cadherin. The majority of premalignant lesions also showed weak (1+ or 2+) cytoplasmic immune-expression for E-cadherin. Eighty percentage of cases of dysplasias showed strong (4+) expression of E-cadherin and fifty percentage of cases of carcinoma in situ showed strong (4+) immune expression of E-cadherin. In their study, sixty percentage cases of well differentiated squamous cell carcinoma showed moderate (3+) staining intensity of E-cadherin and thirty percentage cases showed strong (4+) immune-expression 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.

In majority of studies including the above study E-cadherin expression was significantly reduced in invasive carcinomas compared to dysplasias and carcinoma insitu.\textsuperscript{29,30} Eighty percentage cases each of dysplasias and carcinoma in situ showed either negative or weak (1+/2+) staining for vimentin. Seven carcinoma showed strong (4+), staining and four cases had moderate (3+) staining. No cases were negative for vimentin.

Our study showed positivity of different intensity of E-cadherin and vimentin expression in benign, dysplastic and OPSCC cases. 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.\textsuperscript{31,32}

In our study 70 cases (88.60%) of OPSCC expressed cytoplasmic vimentin, whereas 2 cases (66.66%) of moderate-severe dysplasia and 2 cases (12.5%) of benign cases also expressed vimentin. Therefore we observed a significant increase in vimentin expression with progression of the tumor. We also observed a significant decrease in E-cadherin expression from benign to dysplastic to carcinoma. 13 benign lesions (81.25%) showed high immunoreactivity for E-cadherin, whereas 3 cases (18.75%) showed low immunoreactivity. Fifty percentage cases of mild dysplasia showed high immunoreactivity for E-cadherin and the other fifty percentage showed low immunoreactivity. Cent percentage of moderate to severe dysplasia showed low immunoreactivity for E-cadherin. Although the numbers are small, we were able to identify a subset of tumours with low E-cadherin together with high vimentin fractions.

Changes in cell adhesion molecules have an important role in increasing the motility of tumour cells and thereby enhancing migration and the formation of metastasis.\textsuperscript{33,34} During EMT, epithelial cells transform and attain mesenchymal-like properties, such as loss of E-cadherin and gain of vimentin expression.\textsuperscript{35}

**Limitations**

In our study, the negative immunoreactivity of E-cadherin in OPSCC was compared in 2 ways depending on whether the immunoreactivity is high or low in benign or normal cases. When we compare the immunoreactivity of E-cadherin in OPSCC with that of high immunoreactivity in benign / normal cases, we got a sensitivity of 28.8%, specificity of 100%, positive predictive value of 100 and negative predictive value of 26%. But when we compare with low immunoreactivity in benign/ normal cases, we will expect less specificity. But here we are getting sensitivity of 35%, specificity of 100%, positive predictive value of 100% and negative predictive value of 10%. Vimentin expression was absent in 100% mild dysplasia, but 2 benign cases showed vimentin positivity. So, there is no absolute absence of vimentin in benign cases. Malignant cases showed high percentage of vimentin reactivity.

**CONCLUSION**

Our study showed positivity of different intensity of E-cadherin and vimentin expression in benign, dysplastic and OPSCC cases. 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.
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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES


